PRAGUE EUROMAR 2015

EUROMAR 2015

July 5–10, 2015

Prague Congress Centre, Prague
Czech Republic

Programme & Abstract Book

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ACKNOWLEDGEMENTS

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WELCOME WORD FROM EUROMAR 2015 CHAIR

Welcome to EUROMAR 2015,

After thirteen years, the largest European congress on magnetic resonance returns to Prague, city of a hundred spires, a UNESCO monument, and one of the most beautiful European capitals. In 2002, Prague hosted the 16th European Experimental NMR Conference – EENC. This year, EUROMAR 2015, a joint congress established in 2004 as a unified forum of its predecessors (EENC, Congress Ampere, and International Meetings of the British NMR Discussion Group), is back to the Prague Congress Centre, a place with stunning panorama view of Prague Castle. The Congress Centre is close to the Prague downtown and Old Town Square, the heart of its historic core, with colorful baroque buildings, gothic churches, and scenic bridges crossing the Vltava River. Prague as a cosmopolitan city is used to welcoming foreigners. Please, experience and enjoy.

EUROMAR 2015, gathering leading scientists from all around the world, is offering a unique opportunity to report and witness the latest scientific breakthroughs in magnetic resonance in a broad range of scientific fields, stretching from physics and chemistry to biology and medicine. The congress will present new methods and instrumentation developments in established areas, as well as novel technologies and applications in emerging fields. The event is prepared to provide a stimulating forum for sharing experience, exchanging ideas, and establishing fruitful collaborations.

On behalf of the Local Organising and Programme Committees and the EUROMAR Board, I am delighted to cordially welcome you to EUROMAR 2015 in Prague. We will be very happy if you help us to make this congress a memorable scientific and personal event.

Vladimír Sklenář
EUROMAR 2015 Chair

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CEITEC Central European Institute of Technology BRNO | CZECH REPUBLIC

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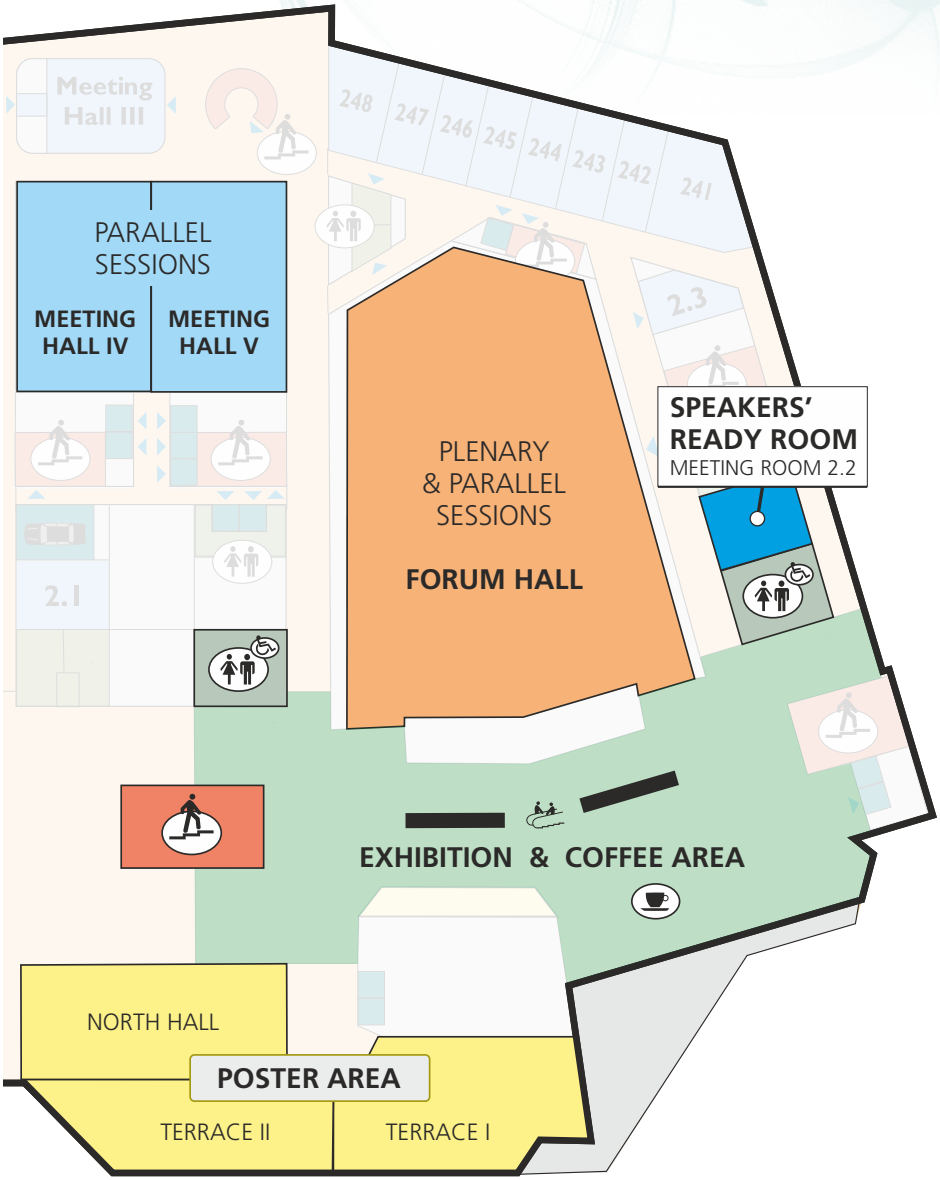
CONGRESS VENUE FLOORPLAN

2nd floor



SOCIAL EVENTS

Sunday, 5 July 2015

Welcome Mixer
Exhibition area, Forum Hall Foyer (2nd floor)
18:30–21:00

Monday, 6 July 2015

Bruker Innovation Night
National House of Vinohrady
Address: Náměstí Míru 9, Prague 2
(conveniently located 1 meto station from the Prague Congress Centre – go from "Vyšehrad" and exit at "I. P. Pavlova")
As of 19:30
Free and open to all delegates.

Tuesday, 7 July 2015

JEOL Reception
Exhibition area, Forum Hall Foyer (2nd floor)
19:00–21:00.
Free and open to all delegates.

Thursday, 9 July 2015

EUROMAR 2015 Congress Dinner
Restaurant Žofín Garden
Address: Slovanský ostrov 226; Praha 1
19:30–23:00

Admission ticket needed!
Dress Code: Smart Casual

This covered outdoor restaurant is located on the Slovanský island on the Vltava River in the heart of the city, next to the Žofín Palace (almost in front of the National Theatre).

No transfers will be provided to/from the dinner. Attendees are kindly asked to use taxis or public transportation. Please check with the Registration Staff in PCC or the Congress website/SOCIALS section.

Admission to this dinner is subject to fee of 60 EUR/pp incl. VAT; a limited number of tickets may be available at registration (onsite requests cannot be guaranteed).

Please make sure you bring your admission ticket and the actual menu voucher which shall be placed in front of you at the table, thank you.

EUROMAR is a subdivision of Groupement Ampere, that organizes annual meetings on magnetic resonance, usually in the first week of July. The meetings are held in different locations within Europe, and cover all aspects of magnetic resonance, including NMR, ESR and MRI.

Prior to 2005, three large European magnetic resonance meetings were organized:

The European Experimental NMR Conference (EENC), a body loosely inspired by the very successful ENC held annually in the USA, which met every 2 years; the **Ampere Congress**, which held meetings every two years and focused more on physics oriented aspects of magnetic resonance; and the **International Meeting** organized by **the British NMR Discussion Group** in alternation with **EENC**, which focused more on chemical aspects. In 2004, the three meetings decided to merge into a single major annual congress covering all aspects of magnetic resonance – **EUROMAR** (**EURO**pean **MA**gnetic **R**esonance).

EUROMAR congresses have taken place in the following venues:

1st 2005 Veldhoven, The Netherlands
2nd 2006 York, UK
3rd 2007 Tarragona, Spain
4th 2008 St Petersburg, Russia
5th 2009 Göteborg, Sweden
6th 2010 Florence, Italy
7th 2011 Frankfurt, Germany
8th 2012 Dublin, Ireland
9th 2013 Hersonissos, Crete
10th 2014 Zürich, Switzerland
11th 2015 Prague, Czech Republic

The 12th meeting is scheduled to take place in July 2016 in Aarhus, Denmark. Notice that Euromar tries to alternate east/west and north/south locations for its venue; proposals to host future meetings are always welcome.

As of July 2015, the EUROMAR Board of Trustees is made up by

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Fore more info visit http://www.euromar.org

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Atomes et Molécules Par Études Radio-Électriques

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AMPERE

The Groupement AMPERE is an association of scientists active in the fields of Magnetic Resonances, Optics, Dielectrics, Magnetic Resonance Imaging, as well as in the development of the related methodologies and technologies.
The society started in France 1951 and was incorporated as a European organisation in Switzerland in 1956. Although the roots and the basic activities are in Europe, members are from all over the world.

Today it is the largest organization in Europe dedicated to promoting Magnetic Resonance in Physics, Chemistry and related fields.

www.ampere-society.org

CEITEC | Central European Institute of Technology
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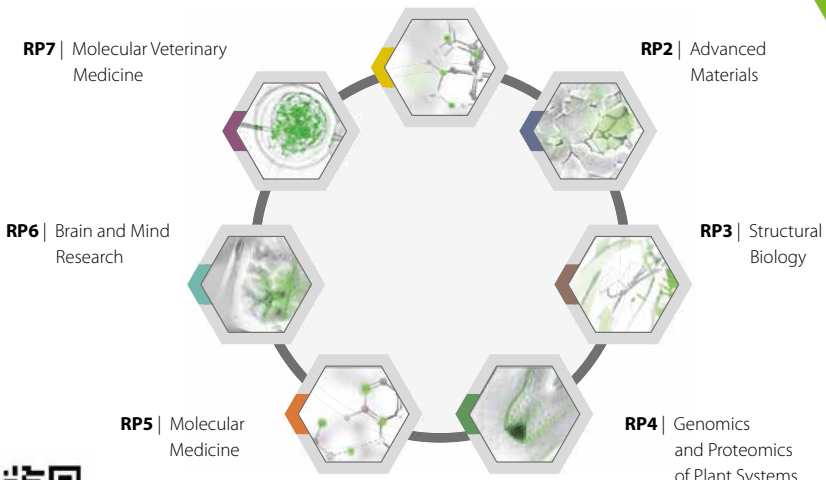
CEITEC is a scientific centre in the fields of the life sciences, advanced materials and technologies which aims to establish itself as a recognized centre for basic as well as applied research. CEITEC offers a state-of-the-art infrastructure and excellent conditions for the employment of outstanding researchers. It is a consortium of partners that include the most prominent universities and research institutes in Brno, Czech Republic: Masaryk University, Brno University of Technology, Mendel University in Brno, Institute of Physics of Materials of the Academy of Sciences of the Czech Republic, University of Veterinary and Pharmaceutical Sciences Brno and the Veterinary Research Institute. CEITEC works closely with the Region of South Moravia and the City of Brno to help increase local innovative capacity.

BASIC OVERVIEW
6 partners | 7 research programmes
61 research groups | 557 researchers (2015)
25 000 m² of new laboratories
10 core facilities

RESEARCH PROGRAMMES



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Masaryk University (MU), established in 1919 and located in Brno, Czech Republic, is a key leader and influencer in higher education in Central Europe. Scientific research, one of the top priorities since its foundation, has helped Masaryk University in attaining a leading position in both national and international competitions for research grants resulting in considerable financial investments at its new university campus to enhance its research and teaching capacity and is developing tools for the transfer of knowledge and improved support for research and innovation.

At present, with more than 1 300 study programmes in the field of natural sciences, medicine, informatics as well as humanities, social studies, law and economics, MU may be considered a pioneering institution in the practical application of research results, particularly in biotechnologies, plasma technologies and information&communication technologies.

The University currently operates a number of units and facilities which may be considered unique in the Czech Republic and even internationally, both in terms of their concept and of their equipment. These include e.g. the Central European Institute of Technology, specializing in the fields of life sciences, advanced materials and technologies and combining basic as well as applied research; the The Centre for the Study of Toxic Substances, covering the development of chemical and toxic tools monitoring the quality of environment; National Centre for Biomolecular Research, studying macromolecules, their complexes and behaviour, involved in immunological, oncological, haematological and paediatric research.

Other units conducting research in a wide array of fields have likewise produced internationally recognized results with hundreds of projects, including analyses of information and communication system security, research into the economics and management of companies in a globalized environment and studies of the impact of traumatic childhood on further development.

UNIVERSITAS MASARYKIANA BRUNENSIS

SCIENTIFIC PROGRAMME

PROGRAMME AT A GLANCE

	Saturday 4 July	Sunday 5 July	Monday 6 July	Tuesday 7 July	Wednesday 8 July	Thursday 9 July	Friday 10 July
8:45–10:05			Plenary Session 1	Plenary Session 3	Plenary Session 5	Plenary Session 7	
10:05–10:30			Coffee Break	Coffee Break	Coffee Break	Coffee Break	
10:30–12:30	10:00–12:00 pNMR Scientific Meeting	09:30–11:30 pNMR Scientific Meeting 11:30–12:30 pNMR Brunch	Parallel Session 1	Parallel Session 3	Parallel Session 5	Parallel Session 7	
12:30–13:30	Lunch	From 12:00 Registration Opens	Lunch	Lunch JEOL Symposium	Lunch JEOL Symposium	Lunch	09:00–16:00 IOCB Workshop
13:30–15:30			Poster Session I with Coffee	Poster Session II with Coffee	Poster Session III with Coffee	Parallel Session 8	
15:30–17:30	14:00–15:45 pNMR Scientific Meeting Coffee Break	14:00–16:15 Tutorial Lectures 16:15–17:00 Coffee Break	Parallel Session 2	Parallel Session 4	Parallel Session 6	15:30–16:00 Coffee Break 16:00–17:50 Wiley Award International EPR (ESR) Society Poster Awards	
17:30–17:45			Break	Break	Break		
17:45–18:25	16:15–18:00 pNMR Scientific Meeting	17:00–18:30 Opening and Prize Session	Plenary Session 2	Plenary Session 4	Plenary Session 6	Plenary Session 8 Closing	
18:30		from 18:30 Welcome Mixer					
19:00				JEOL Reception from 19:00			
19:30			from 19:30 Bruker Innovation Night			from 19:30 Congress Dinner	
20:00	from 20:00 pNMR / Bruker Sponsored Dinner						

DETAILED PROGRAMME
Sunday, 5 July 2015

Forum Hall

From 12:00	**Registration Opens**
14:00–16:15	**Tutorial Lectures** **Chair: R. Andrew Byrd** (USA)
	14:00 MODERN PURE SHIFT NMR: PROS AND CONS ***Teodor Parella*** *(Spain)*
	14:45 DETECTION SENSITIVITY IN MAGNETIC RESONANCE ***Aharon Blank*** *(Israel)*
	15:30 APPLICATIONS OF NMR SPIN RELAXATION TO CONFORMATIONAL DYNAMICS OF PROTEINS ***Arthur Palmer*** *(USA)*
16:15–17:00	**Coffee Break**
17:00–18:30	**Opening and Prize Session**
17:00	**Welcome Word**
	17:15 **Raymond Andrew Prize** FROM SLOW TO ULTRA-FAST MAS: STRUCTURAL DETERMINATION OF TYPE-THREE SECRETION SYSTEM BACTERIAL NEEDLES AND INORGANIC MATERIALS BY SOLID-STATE NMR ***Jean-Philippe Demers*** *(Germany)*
	17:45 **Russell Varian Prize** COMPOSITE PULSES: REINVENTING THE WHEEL ***Malcolm Levitt*** *(UK)*
	18:30 **Welcome Mixer**

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Abstract Book:

available for download at the EUROMAR 2015 Congress website.

	Forum Hall	Meeting Hall V	Meeting Hall IV
08:45–10:05	**Plenary Session 1 (Forum Hall)** **Chair: Lucio Frydman** (Israel)		
	08:45 NEW METHODS FOR MEASURING AND ORIENTING ORGANIC COMPOUNDS FOR RDC STRUCTURAL ANALYSIS, *Christina Thiele (Germany)* 09:25 MODERN ESR: APPLICATIONS TO PROTEIN STRUCTURE AND DYNAMICS, *Jack Freed (USA)*		
10:05–10:30	Coffee Break		
10:30–12:30	**S 01: Biosolids** **Chair: Perunthiruthy Madhu** (India)	**S 02: Small Molecules and Pharmaceuticals** **Chair: Antonín Lyčka** (Czech Republic)	**S 03: NMR - High and Low, Sparse and Dense** **Chair: Wiktor Koźmiński** (Poland)
	10:30 O 001: CONFORMATIONAL HETEROGENEITY AND INTRINSIC DISORDER IN MEMBRANE PROTEINS USING STATIC, MAS, & OS SOLID STATE NMR ***Timothy Cross*** *(USA)*	10:30 O 006: RECENT ADVANCES IN COMPUTATIONAL NMR OF THE MEDIUM-SIZED ORGANIC MOLECULES ***Leonid Krivdin*** *(Russia)*	10:30 O 011: EXTREME HIGH-PRESSURE NMR APPLICATIONS IN PHYSICS AND CHEMISTRY ***Jürgen Haase*** *(Germany)*
	11:00 O 002: ALTERNATIVE SALT BRIDGE FORMATION IN AB – A HALLMARK OF EARLY-ONSET ALZHEIMER'S DISEASE? ***Anja Böckmann*** *(France)*	11:00 O 007: J-CONTROLLED ULTRAFAST 2D NMR ***Patrick Giraudeau*** *(France)*	11:00 O 012: HIGH FIELD NMR EXPERIMENTS USING A ZERO FIELD NMR SPECTROMETER ***Michael Tayler*** *(USA)*
	11:20 O 003: BIOMOLECULAR SOLID-STATE NMR AT 111 KHZ MAS: A REVOLUTION THROUGH FASTER REVOLUTIONS ***Daniela Lalli*** *(France)*	11:20 O 008: SEALED SIRNAS: STRUCTURE BASED DESIGN OF NOVEL THERAPEUTIC SIRNA MOLECULES ***Marcel Blommers*** *(Switzerland)*	11:20 O 013: (VERY!) BROADBAND DOSY ***Jane Power*** *(UK)*
	11:40 O 004: DYNAMIC AND STRUCTURAL INVESTIGATIONS OF PRGI NEEDLE PROTEIN BY PROTON DETECTED MAS NMR ***Veniamin Shevelkov*** *(Germany)*	11:40 O 009: LONG-LIVED STATES OF PAIRS OF FLUORINE-19 NUCLEI: A NEW TOOL FOR LIGAND-PROTEIN SCREENING ***Roberto Buratto*** *(Switzerland)*	11:40 O 014: NMR BEYOND 200,000 ATMOSPHERES OF PRESSURE ***Thomas Meier*** *(Germany)*
	12:00 O 005: STRUCTURAL STUDIES OF MEMBRANE-EMBEDDED PROTEIN MACHINES BY NMR: FROM LIPID BILAYERS TO CELLS ***Marc Baldus*** *(The Netherlands)*	12:00 O 010: USING NMR TO UNRAVEL METABOLIC MECHANISMS IN CANCER CELLS ***Ulrich Günther*** *(UK)*	12:00 O 015: NEW ALGORITHMS FOR RECONSTRUCTING SPECTRA FROM NON-UNIFORMLY SAMPLED DATA: SPARSE FAST FOURIER TRANSFORM AND LOW-RANK RECONSTRUCTION ***Vladislav Orekhov*** *(Sweden)*
12:30–13:30	Lunch		

13:30–15:30	**Poster Session 1 with Coffee (Poster Area)**		
15:30–17:30	**S 04: NMR + EPR** Chair: Gunnar Jeschke (Switzerland)	**S 05: Materials NMR** Chair: Janez Stepišnik (Slovenia)	**S 06: Protein Relaxation and Dynamics** Chair: Fred Damberger (Switzerland)
	15:30 0 016: STRUCTURES OF PROTEIN-RNA COMPLEXES INVOLVED IN TRANSLATION REGULATION BY NMR and EPR *Frederic Allain (Switzerland)*	15:30 0 021: GROUP 13 (AL, GA) STUDIES OF METAL-OXIDE CLUSTERS AND THIN FILMS *Sophia Hayes (USA)*	15:30 0 026: KINETIC COOPERATIVITY, LOOP DYNAMICS, AND ALLOSTERY *Rafael Brüschweiler (USA)*
	16:00 0 017: RIDME-BASED DISTANCE MEASUREMENTS IN Gd(III) SPIN PAIRS *Maxim Yulikov (Switzerland)*	16:00 0 022: HIGH–ENTROPY ALLOYS *Janez Dolinšek (Slovenia)*	16:00 0 027: INCREASING THE SENSITIVITY AND SPEED OF RELAXATION-DISPERSION NMR IN PROTEINS AND NUCLEIC ACIDS *Sergio Gil Caballero (France)*
	16:20 0 018: PELDOR ON TRIMERIC BETAINE SYMPORTER BETP *Burkhard Endeward (Germany)*	16:20 0 023: INTERFACE SELECTIVE SOLID-STATE NMR IN INORGANIC-ORGANIC HYBRID MATERIALS *Ulrich Scheler (Germany)*	16:20 0 028: CONFORMATION NETWORKS FROM EXACT NOE ANALYSIS *Beat Vögeli (Switzerland)*
	16:40 0 019: THE FIELD DEPENDENCE OF CROSS-EFFECT DYNAMIC NUCLEAR POLARIZATION UNDER MAGIC ANGLE SPINNING: THEORY VS. EXPERIMENT *Deni Mance (The Netherlands)*	16:40 0 024: NUCLEAR SPIN CIRCULAR DICHROISM IN FULLERENES *Michal Straka (Czech Republic)*	16:40 0 029: WHAT CAN AND CANNOT BE SAID ABOUT PROTEIN DYNAMICS USING NMR: COMBINING PERSPECTIVES AND TIME SCALES *Daniel Abergel (France)*
	17:00 0 020: DYNAMIC NUCLEAR POLARIZATION OF PARAMAGNETIC BIOMOLECULES *Björn Corzilius (Germany)*	17:00 0 025: CHARACTERIZATION OF CATALYTIC MATERIALS BY CONVENTIONAL AND DNP-ENHANCED SOLID-STATE NMR METHODS *Marek Pruski (USA)*	17:00 0 030: A CONSISTENT PERSPECTIVE OF PROTEIN DYNAMICS IN THE SOLID-STATE FROM NMR LINESHAPES: THE ROLE OF SYMMETRY *Eva Meirovitch (Israel)*
17:30–17:45	Break		
17:45–18:25	**Plenary Session 2 (Forum Hall)** Chair: David Neuhaus (UK)		
	THE EVOLUTION OF KINASE DYNAMICS OVER 1 BILLION YEARS REVEALS A MODERN CANCER DRUG'S MECHANISM, *Dorothee Kern (USA)*		
19:30	**Bruker Innovation Night**		

	Forum Hall	Meeting Hall V	Meeting Hall IV
08:45–10:05	**Plenary Session 3 (Forum Hall)** **Chair: Geoffrey Bodenhausen** (France) 08:45 **SOLID-STATE NMR STUDIES OF MEMBRANE PROTEINS IN SYNTHETIC LIPIDS AND CELL MEMBRANES,** *Vladimir Ladizhansky (Canada)* 09:25 **A STEPPING STONE TO NEW EXPERIMENTS: FAILURE AND NEW TECHNOLOGIES,** *Kiyonori Takegoshi (Japan)*		
10:05–10:30	Coffee Break		
10:30–12:30	**S 07: Large biomolecular complexes** **Chair: Rolf Boelens** (The Netherlands)	**S 08: NMR Physics** **Chair: Ulrich Scheler** (Germany)	**S 09: In-vivo and In-cell NMR** **Chair: Marielle Wälti** (Switzerland)
	10:30 **O 031:** NMR ILLUMINATES TERNARY COMPLEX FORMATION AS IkBa STRIPS NFkB FROM DNA ***Jane Dyson*** *(USA)*	10:30 **O 036:** NMR AS A LOCAL PROBE IN NANOSTRUCTURED STRONGLY CORRELATED MATERIALS ***Roberto De Renzi*** *(Italy)*	10:30 **O 041:** IN-CELL NMR OF LARGER PROTEINS AND COMPLEXES ***Volker Dötsch*** *(Germany)*
	11:00 **O 032:** PROBING THE SUPRAMOLECULAR STRUCTURE OF THE 200KDA β-BARREL ASSEMBLY MACHINERY COMPLEX BY SOLID-STATE NMR ***Cecilia Pinto*** *(The Netherlands)*	11:00 **O 037:** DEVELOPMENT AND APPLICATION OF A NOVEL THZ MAGNETIC RESONANCE SPECTROMETER WITH FIELD – AND FREQUENCY-SWEEP CAPABILITIES ***Petr Neugebauer*** *(Germany)*	11:00 **O 042:** MAGNETIC RESONANCE DETECTION OF LYMPHATIC BREAST CANCER METASTASIS IN A XENOGRAFT MODEL BY HYPERPOLARIZED 13C-PYRUVATE ***Anne Fages*** *(Israel)* Magnetic Resonance in Chemistry Award For Young Scientists Prize Winner
	11:20 **O 033:** HIV-1 ENVELOPE MEMBRANE PROTEIN GP41: AN NMR STUDY OF DODECYL PHOSPHOCHOLINE EMBEDDED GP41 REVEALS A DYNAMIC PRE-FUSION INTERMEDIATE CONFORMATION ***Nils-Alexander Lakomek*** *(Switzerland)*	11:20 **O 038:** FOLLOWING LITHIATION FRONTS IN PARAMAGNETIC BATTERY ELECTRODES WITH IN SITU MAGNETIC RESONANCE SPECTROSCOPIC IMAGING ***Michael Deschamps*** *(France)*	11:20 **O 043:** ONLINE SPECTROSCOPY OF 13C-LABELLED METABOLITES IN MICRODIALYSATE UTILIZING A MICROCOIL ***Stefan Gloeggler*** *(France)*
	11:40 **O 034:** AUTOMATED NMR RESONANCE ASSIGNMENT STRATEGIES FOR NUCLEIC ACIDS USING THROUGH-BOND AND THROUGH-SPACE HIGH-DIMENSIONAL EXPERIMENTS ***Gerhard Wider*** *(Switzerland)*	11:40 **O 039:** Co METAL BASED CATALYSTS PROBED BY FERROMAGNETIC 59Co NMR ***Andrey Andreev*** *(Russia)*	11:40 **O 044:** PROBING PROTEIN QUINARY STRUCTURES BY IN-CELL NMR ***Alexander Shekhtman*** *(USA)*
	12:00 **O 035:** PHYSIOLOGY AND PATHOLOGY OF TAU BY NMR ***Guy Lippens*** *(France)*	12:00 **O 040:** HETERO-NUCLEAR CROSS EFFECTS DURING DNP ON SOLIDS ***Shimon Vega*** *(Israel)*	12:00 **O 045:** A BIOREACTOR FOR NMR OVSERVATION OF BIOLOGICAL EVENTS INSIDE LIVING CELLS ***Ichio Shimada*** *(Japan)*
12:30–13:30	Lunch		
	JEOL Symposium (13:00–14:00, Meeting Hall V) 13:00 **ULTRAFAST MAS OF 120 KHZ AND ULTRAHIGH MAGNETIC FIELD OF 1020 MHZ,** *Yusuke Nishiyama (Japan)* 13:30 **STATE OF THE ART JEOL PRODUCT LINES,** *Katsuo Asakura (Japan)*		

13:30–15:30	**Poster Session 2 with Coffee (Poster Area)**		
15:30–17:30	**S 10: Biomacromolecular Folding and Dynamics** Chair: Bernhard Brutscher (France)	**S 11: New Approaches to the MR Measurement** Chair: Beat Meier (Switzerland)	**S 12: Exotica** Chair: Helena Štěpánková (Czech Republic)
	15:30 **O 046:** NEW NMR METHODS TO STUDY RNA FOLDING AND DYNAMICS ***Harald Schwalbe*** *(Germany)*	15:30 **O 051:** MULTI-FREQUENCY PROTON NMR RELAXATION FOR THE STUDY OF PROTEIN ROTATIONAL DIFFUSION: APPLICATION TO CROWDED SOLUTIONS ***Kay Saalwächter*** *(Germany)*	15:30 **O 056:** NUCLEAR SPIN NOISE AND RADIATION DAMPING – NEW INSIGHTS AND APPLICATIONS ***Norbert Müller*** *(Austria)*
	16:00 **O 047:** NMR STUDIES OF GUANINE-RICH TETRAHELICAL DNA STRUCTURES ***Janez Plavec*** *(Slovenia)*	16:00 **O 052:** DYNAMIC NON-UNIFORM SAMPLING ***Krzysztof Kazimierczuk*** *(Poland)* Magnetic Resonance in Chemistry Award For Young Scientists Prize Winner	16:00 **O 057:** ELECTRICAL DETECTION OF ORTHO-PARA CONVERSION IN ENCAPSULATED WATER ***Benno Meier*** *(UK)*
	16:20 **O 048:** THE WWPDB NMR VALIDATION REPORTS AND UNIFIED NMR DATA REPRESENTATION ***Aleksandras Gutmanas*** *(UK)*	16:20 **O 053:** A NEW APPROACH TO ESTIMATE TISSUE ELECTRICAL PROPERTIES BY VARYING THE RF DISTRIBUTION USING HIGH PERMITTIVITY MATERIALS ***Rita Schmidt*** *(The Netherlands)*	16:20 **O 058:** SPIN NOISE GRADIENT ECHO IN STUDYING RELAXATION AND DYNAMICS OF PURE LIQUIDS AND BULK MIXTURES ***Victor Rodin*** *(Austria)*
	16:40 **O 049:** CONFORMATIONAL DYNAMICS AS A KEY FACTOR OF ACTIVATION OF THE RECEIVER DOMAIN OF SENSOR HISTIDINE KINASE CKI1 FROM ARABIDOPSIS THALIANA ***Lukáš Žídek*** *(Czech Republic)*	16:40 **O 054:** MAGNETIC RESONANCE SPECTROSCOPY AND IMAGING USING A CMOS FREQUENCY DIVISION MULTIPLEXER ***Mazin Jouda*** *(Germany)*	16:40 **O 059:** MEASUREMENT OF UNTRUNCATED NUCLEAR SPIN INTERACTIONS VIA ZERO- TO ULTRA-LOW-FIELD NMR ***John Blanchard*** *(Germany)*
	17:00 **O 050:** THE HETEROGENEOUS STRUCTURAL BEHAVIOUR OF VIRAL INTRINSICALLY DISORDERED PROTEINS REVEALED BY NMR SPECTROSCOPY ***Roberta Pierattelli*** *(Italy)*	17:00 **O 055:** LOW-FIELD NMR STUDY OF HEALTHY AND DEGRADED ARTICULAR CARTILAGE AND THE INFLUENCE OF MECHANICAL LOADING ***Siegfried Stapf*** *(Germany)*	17:00 **O 060:** DYNAMIC NUCLEAR POLARIZATION IN SILICON ***Chandrasekhar Ramanathan*** *(USA)*
17:30–17:45	Break		
17:45–18:25	**Plenary Session 4 (Forum Hall)** Chair: Frederic Allain (Switzerland)		
	DEVELOPMENT OF 13C-BASED METABOLOMICS, *Arthur Edison (USA)*		
19:00	**JEOL Reception**		

	Forum Hall	Meeting Hall V	Meeting Hall IV
08:45–10:05	**Plenary Session 5 (Forum Hall)** Chair: Christina Redfield (UK) 08:45 **A HYBRID METHODS APPROACH TO DETERMINE THE STRUCTURE OF TETRAHYMENA TELOMERASE HOLOENZYME,** *Juli Feigon (USA)* supported by CEITEC Central European Institute of Technology BRNO | CZECH REPUBLIC 09:25 **MICRO-MANUFACTURING TECHNOLOGIES FOR NMR MICRO-DETECTION SYSTEMS,** *Jan Korvink (Germany)*		
10:05–10:30	Coffee Break		
10:30–12:30	**S 13: Solid State NMR Techniques** Chair: Anja Böckmann (France)	**S 14: Biomolecular Polarization and Relaxation** Chair: Richard Hrabal (Czech Republic)	**S 15: NMR Imaging** Chair: Milan Hájek (Czech Republic)
	10:30 **O 061:** PULSED ELECTRICALLY DETECTED MAGNETIC RESONANCE: METHODOLOGICIAL ADVANCES AND TOPICAL APPLICATIONS ***Martin Brandt*** *(Germany)*	10:30 **O 066:** CHARACTERIZATION OF PROTEIN DYNAMICS BY NMR RELAXATION ***Peter E. Wright*** *(USA)*	10:30 **O 071:** POTENTIALS AND CHALLENGES OF IN VIVO 1H NMR SPECTROSCOPY OF BRAIN AT HIGH MAGNETIC FIELDS ***Ivan Tkáč*** *(USA)*
	11:00 **O 062:** 1H/1H HOMONUCLEAR MIXING AT ULTRAFAST MAS > 120 KHZ: 1H/1H, 1H CSA/CSA, 15N/15N, 14N/14N CORRELATIONS ***Yusuke Nishiyama*** *(Japan)*	11:00 **O 067:** HYPERPOLARIZED PARA-ETHANOL ***Daniele Mammoli*** *(Switzerland)*	11:00 **O 072:** RECENT ADVANCES WITH HYPERPOLARIZED 83Kr MRI AND SURFACE QUADRUPOLAR RELAXATION (SQUARE) T1 CONTRAST ***Thomas Meersmann*** *(UK)*
	11:20 **O 063:** NEW METHODS FOR THE DETECTION OF 14N NUCLEI IN SOLID STATE NMR ***Jean Paul Amoureux*** *(France)*	11:20 **O 068:** NMR WITH NANOPARTICLES: MOLECULAR SENSING AND CHROMATOGRAPHY ***Federico Rastrelli*** *(Italy)*	11:20 **O 073:** TRANSPORT IN PHLOEM TISSUE ASSESSED WITH MRI ***Alena Prusova*** *(The Netherlands)*
	11:40 **O 064:** CRYSTAL STRUCTURE DETERMINATION USING POWDER NMR CRYSTALLOGRAPHY ***Maria Baias*** *(Israel)*	11:40 **O 069:** KINETIC ISOTOPE EFFECTS ON EXCHANGE RATES OF HN AND DN IN TRYPTOPHAN ***Estel Canet Martinez*** *(Switzerland)*	11:40 **O 074:** RF EFFECTS IN THE STUDY OF METAL SURFACES: INSIGHTS INTO THE MECHANISMS OF BATTERY FAILURE ***Nicole Trease*** *(UK)*
	12:00 **O 065:** DISTANCE MEASUREMENTS IN MAS NMR USING PHASE MODULATED PULSES: THEORETICAL INSIGHTS, DIFFICULT SPINS AND A SMALL TRICK FOR A SPIN-1 ***Amir Goldbourt*** *(Israel)*	12:00 **O 070:** TRANSIENT COMPLEXES OBSERVED BY PARAMAGNETIC RELAXATION ENHANCEMENT BETWEEN ENZYME 1NTR AND NPR GOVERN SPECIFICITY AND PREVENT CROSS-OVER BETWEEN PHOSPHORYLATION PATHWAYS ***Nico Tjandra*** *(USA)*	12:00 **O 075:** IMAGING TREATMENT RESPONSE AND THE TUMOUR MICROENVIRONMENT USING METABOLIC IMAGING WITH HYPERPOLARIZED C-13-LABELLED CELL SUBSTRATES ***Kevin Brindle*** *(UK)*
12:30–13:30	Lunch		
	JEOL Symposium (13:00–14:00, Meeting Hall V) 13:00 **ULTRAFAST MAS OF 120 KHZ AND ULTRAHIGH MAGNETIC FIELD OF 1020 MHZ,** *Yusuke Nishiyama (Japan)* 13:30 **STATE OF THE ART JEOL PRODUCT LINES,** *Katsuo Asakura (Japan)*		

13:30–15:30	**Poster Session 3 with Coffee (Poster Area)**		
15:30–17:30	**S 16: Biomacromolecules** Chair: Julien Orts (Switzerland)	**S 17: Sensitivity Enhancement I** Chair: Christian Hilty (USA)	**S 18: Computation and Processing** Chair: Rafael Brüschweiler (USA)
	15:30 0 076: TRANSMEMBRANE SIGNALING THROUGH A BACTERIAL HEME TRANSPORTER *Muriel Delepierre (France)*	15:30 0 081: STORING 13C SPIN ORDER FOR MORE THAN 1 HOUR IN A ROOM TEMPERATURE LIQUID. *Malcolm Levitt (UK)*	15:30 0 086: RELATIVISTIC QUANTUM CHEMISTRY APPLIED TO NMR SHIFTS OF DIA- AND PARAMAGNETIC SYSTEMS *Martin Kaupp (Germany)*
	16:00 0 077: STRUCTURAL INSIGHTS INTO THE DYNAMIC PROCESS OF β2-ADRENERGIC RECEPTOR SIGNALING *Tae Hun Kim (Canada)*	16:00 0 082: ENHANCING SABRE WITH MICROTESLA FIELDS: BROADLY APPLICABLE, >10,000 FOLD DIRECT HETERONUCLEAR SIGNAL ENHANCEMENT WITH >20 MINUTE SIGNAL LIFETIMES *Thomas Theis (USA)* Magnetic Resonance in Chemistry Award For Young Scientists Prize Winner	16:00 0 087: TWO-DIMENSIONAL LINESHAPE ANALYSIS: METHOD DEVELOPMENT AND APPLICATIONS TO MULTISTATE PROTEIN - LIGAND INTERACTIONS AND ULTRAFAST CO-TRANSLATIONAL PROTEIN FOLDING *Christopher Waudby (UK)*
	16:20 0 078: TOWARDS A MECHANISTIC UNDERSTANDING OF THE OPIOID MY RECEPTOR ACTIVATION BY LIQUID-STATE NMR SPECTROSCOPY *Hélène Déméné (France)*	16:20 0 083: RF-SABRE MAKES FEASIBLE CONTINUOUS HYPERPOLARIZATION AT HIGH MAGNETIC FIELD *Andrey Pravdivtsev (Russia)*	16:20 0 088: NESTA-NMR: EFFICIENT AND GENERALIZED PROCESSING OF MULTI-DIMENSIONAL NUS NMR DATA *R. Andrew Byrd (USA)*
	16:40 0 079: FROM SEQUENCE TO PROTEIN STRUCTURE USING THREE 4D NMR SPECTRA: OLD TRICKS WITH NEW BRICKS *Jiří Nováček (Czech Republic)*	16:40 0 084: EFFICIENT DYNAMIC NUCLEAR POLARIZATION AT 800 MHZ WITH TRITYL-NITROXIDE BIRADICALS *Guinevere Mathies (USA)*	16:40 0 089: GRADIENT-ENCODED NMR: FROM THEORY TO PRACTICE *Bertrand Plainchont (France)*
	17:00 0 080: ADVANCING STRUCTURE DETERMINATION OF MEMBRANE PROTEINS IN LIPID BILAYER MEMBRANES *Francesca Marassi (USA)*	17:00 0 085: PHOTO-CIDNP MAS NMR *Jörg Matysik (Germany)*	17:00 0 090: MOLECULAR DYNAMICS – NMR EXPERIMENTS AND SIMULATIONS *Martin Dračínský (Czech Republic)*
17:30–17:45	Break		
17:45–18:25	**Plenary Session 6 (Forum Hall)** Chair: Carlos Geraldes (Portugal)		
	ULTRAHIGH FIELD MRI – ALZHEIMER'S DISEASE, EYE TUMOURS, DIELECTRICS AND PLASMAS, *Andrew Webb (The Netherlands)*		

	Forum Hall	Meeting Hall V	Meeting Hall IV
08:45–10:05	**Plenary Session 7 (Forum Hall)** **Chair: Bernhard Blümich** (Germany)		
	08:45 APPLICATION OF DISSOLUTION DYNAMIC NUCLEAR POLARIZATION IN CHEMISTRY, *Christian Hilty (USA)* 09:25 STRUCTURE AND DYNAMICS OF MICROTUBULE-ASSOCIATED PROTEIN ASSEMBLIES: INSIGHTS FROM MAS NMR AND MD SIMULATIONS, *Tatyana Polenova (USA)*		
10:05–10:30	Coffee Break		
10:30–12:30	**S 19: Emerging Techniques** **Chair: Janez Dolinšek** (Slovenia)	**S 20: Metabolomics and Small molecules** **Chair: Christina Thiele** (Germany)	**S 21: Relaxation and Transport Phenomena** **Chair: Eva Meirovitch** (Israel)
	10:30 0 091: TRANSIENT-COMPENSATED SOLID-STATE NMR ***Matthias Ernst*** *(Switzerland)*	10:30 0 096: NMR METABOLOMICS IN MICROBIOLOGY AND MOLECULAR EPIDEMIOLOGY ***Benedicte Elena-Herrmann*** *(France)*	10:30 0 101: NMR RELAXATION IN SOLIDS ***Danuta Kruk*** *(Poland)*
	11:00 0 092: SINGLE-PROTEIN SPIN RESONANCE SPECTROSCOPY UNDER AMBIENT CONDITIONS ***Fazhan Shi*** *(China)*	11:00 0 097: RESIDUAL DIPOLAR COUPLINGS AND RESIDUAL CHEMICAL SHIFT ANISOTROPIES FOR THE STRUCTURAL DISCRIMINATION OF SMALL MOLECULES ***Nilamoni Nath*** *(Germany)*	11:00 0 102: SOLID-STATE NMR STUDIES OF A SUPER IONIC CONDUCTOR, Li7P3S11 ***Miwa Murakami*** *(Japan)*
	11:20 0 093: ELECTRON SPIN COHERENCE NEAR ROOM TEMPERATURE IN MAGNETIC QUANTUM DOTS ***Fabrizio Moro*** *(UK)*	11:20 0 098: DISSOLUTION DNP IN LDH ACTIVITY AND CCL39 MURINE CANCER CELLS METABOLISM NMR MEASUREMENT ***Riccardo Balzan*** *(France)*	11:20 0 103: ULTRAFAST MULTIDIMENSIONAL LAPLACE NMR FOR A RAPID AND SENSITIVE CHEMICAL ANALYSIS ***Ville-Veikko Telkki*** *(Finland)*
	11:40 0 094: UTOPIA NMR: RECOVERING LOST MAGNETIZATION USING INTERLEAVED LOW-GAMMA DETECTION ***Aldino Viegas*** *(Germany)*	11:40 0 099: NMR STUDIES OF AN OXYGEN SENSING OXYGENASE ***Ivanhoe Leung*** *(New Zealand)*	11:40 0 104: DIFFUSION AND TRANSPORT VIA SINGLET TAGGING ***Giuseppe Pileio*** *(UK)*
	12:00 0 095: FASTER MAS SPINNING FREQUENCIES AND HIGHER MAGNETIC FIELDS: NEW OPPORTUNITIES IN SOLID-STATE NMR ***Beat Meier*** *(Switzerland)*	12:00 0 100: PHOSPHOLIPID AND STEROL INTERACTIONS BY SOLID-STATE NMR: ROLES IN BLOOD CLOTTING AND ANTIFUNGAL DRUG MECHANISMS ***Chad Rienstra*** *(USA)*	12:00 0 105: NMR RELAXATION AND MOLECULAR DYNAMICS IN HOST-GUEST COMPLEXES: CHLOROMETHANES@ CRYPTOPHANES AS AN EXAMPLE ***Jozef Kowalewski*** *(Sweden)*
12:30–13:30	Lunch		

13:30–15:30	**S 22: Disordered proteins** Chair: Juli Feigon (USA)	**S 23: Paramagnetic Systems** Chair: Norbert Müller (Austria)	**S 24: Sensitivity enhancement II** Chair: Bela Bode (UK)
	13:30 **O 106:** DISORDER REGULATION OF AN INSTRINSICALLY DISORDERED PROTEIN: BEYOND THE STRUCTURE-FUNCTION PARADIGM ***Miquel Pons*** *(Spain)*	13:30 **O 111:** PARAMAGNETIC NMR TOOLS TO STUDY PROTEINS AND PROTEIN COMPLEXES ***Marcellus Ubbink*** *(The Netherlands)*	13:30 **O 116:** HYPERPOLARIZATION OF NUCLEAR SPINS BY PARAHYDROGEN FOR CATALYTIC AND IMAGING APPLICATIONS ***Igor Koptyug*** *(Russia)*
	14:00 **O 107:** THE CONFORMATIONAL ENSEMBLE OF INTRINSICALLY DISORDERED WIP: BIOLOGICAL AND BIOPHYSICAL INSIGHTS FROM 13C-DETECTED SPECTROSCOPY ***Jordan Chill*** *(Israel)*	14:00 **O 112:** RADICALS AND RADICAL PAIR IN LIGHT-ACTIVE FLAVOPROTEINS ***Erik Schleicher*** *(Germany)*	14:00 **O 117:** MEASURING ABSOLUTE SPIN POLARIZATION IN DISSOLUTION-DNP BY SPIN POLARIMETRY MAGNETIC RESONANCE (SPY-MR) ***Basile Vuichoud*** *(Switzerland)*
	14:20 **O 108:** STRUCTURAL AND ENERGETIC DETAILS OF THE UNFOLDING LANDSCAPE OF STAPHYLOCOCCAL NUCLEASE FROM HIGH-PRESSURE NMR ***Christian Roumestand*** *(France)*	14:20 **O 113:** RIGID, HIGH-AFFINITY LANTHANIDE CHELATING TAGS MONITOR PROTEIN-LIGAND INTERACTIONS BY NMR (PCS, PRE AND RDC), DEER-EPR AND FRET ***Daniel Häussinger*** *(Switzerland)*	14:20 **O 118:** NUCLEAR DEPOLARIZATION AND ABSOLUTE SENSITIVITY IN MAS-DNP: AMUPOL VS TOTAPOL WHO IS WINNING? ***Frederic Mentink-Vigier*** *(France)*
	14:40 **O 109:** VISUALIZING THE MOLECULAR RECOGNITION TRAJECTORY OF AN INTRINSICALLY DISORDERED PROTEIN USING MULTINUCLEAR RELAXATION DISPERSION NMR ***Robert Schneider*** *(France)*	14:40 **O 114:** DYNAMICS OF CHARGE SEPARATION IN POLYMER-FULLERENE BULK-HETEROJUNCTIONS VS PHOTOSYNTHESIS AS REVEALED BY TIME-RESOLVED EPR/ENDOR/DFT STUDY ***Oleg Poluektov*** *(USA)*	14:40 **O 119:** SOLID-STATE DYNAMIC NUCLEAR POLARIZATION AT HIGH-TEMPERATURE, HIGH-FIELD & FAST MAS ***Moreno Lelli*** *(France)*
	15:00 **O 110:** NMR CONTRIBUTIONS TO STRUCTURAL DYNAMICS OF INTRINSICALLY DISORDERED PROTEINS ***Robert Konrat*** *(Austria)*	15:00 **O 115:** TRIPLET STATE DELOCALISATION IN LINEAR AND CYCLIC PORPHYRIN ARRAYS ***Christiane Timmel*** *(UK)*	15:00 **O 120:** TOWARDS SUPER-HIGH FIELD AND ULTRA-COMPACT SIZE NMR MAGNETS OPERATED BEYOND 1 GHZ (REVIEW) ***Hideaki Maeda*** *(Japan)*
15:30–16:00	Coffee Break		
16:00–16:10	Wiley Award Ceremony (Forum Hall)		
16:10–16:20	International EPR(ESR) Society Poster Awards (Forum Hall)		
16:20–17:40	**Plenary Session 8 (Forum Hall)** Chair: Thomas Vosegaard (Denmark)		
	16:20 SOLID-STATE DYNAMIC NUCLEAR POLARIZATION AT 263 TO 527 GHZ: INSTRUMENTATION DESIGN AND POLARIZING AGENTS, *Melanie Rosay (USA)* 17:00 EXPLOITING CHEMICAL SHIFTS AND RDCS IN THE STUDY OF STRUCTURED AND INTRINSICALLY DISORDERED PROTEINS, *Ad Bax (USA)*		
17:40–17:50	Closing (Forum Hall)		
19:30	**Congress Dinner**		

Friday, 10 July 2015

Institute of Chemical technology, building B, lecture room B1

09:00 – 16:00	**IOCB Workshop**

SPECIAL PROGRAMME SESSIONS

Saturday, 4 July 2015 & Sunday, 5 July 2015

pNMR Workshop

pNMR: from rags to riches – Challenges and potentials of NMR on paramagnetic molecules

Club D

EUROMAR 2015 satellite event
(www.ens-lyon.fr/crmn/pnmr/events/satelite-event-prague-2015).

Sunday, 5 July 2015

Tutorial Lectures

Forum Hall, 14:00–16:15

The Tutorial Lectures offer you a more in-depth view on the selected topics from the field and comprise of three different talks:

Dr. Teodor Parella
(Universitat Autònoma Barcelona, Spain)

Prof. Aharon Blank
(Technion – Israel Institute of Technology, Israel)

Dr. Arthur Palmer
(Columbia University Medical Center, USA)

Opening and Prize Session

Forum Hall, 17:00–18:30

The Opening Ceremony will be followed by the acknowledgement of the laureates of the Raymond Andrew Prize and the Russell Varian Prize and their lectures:

The Raymond Andrew Prize laureate

Dr. Jean-Philippe Demers
(Leibniz-Institut für Molekulare Pharmakologie (FMP), Molecular Biophysics, Berlin, Germany)

The Russell Varian Prize laureate

Prof. Malcolm H. Levitt
(Head of Magnetic Resonance, Department of Chemistry, University of Southhampton, UK)

Tuesday, 7 July 2015 & Wednesday, 8 July 2015

JEOL Symposia

Meeting Hall V, 13:00–14:00

1) "Ultrafast MAS of 120 kHz and ultrahigh magnetic field of 1020 MHz" by Dr. Yusuke Nishiyama
2) "State of the art JEOL Product lines" by Dr. Katsuo Asakura

Both talks will be given during each session and repeated on both days.

All delegates are welcome to attend, no pre-registration required. Sushi lunch boxes are offered to the 50 first attendees. Limited capacity of the room: 160 persons.

For more details please ask at the JEOL booth in the Exhibition area.

Thursday, 9 July 2015

Wiley Award Ceremony

Forum Hall, 16:00–16:10

The MRC Award for Young Scientists, established in 2006, honours the outstanding researchers under the age of 40 working with NMR spectroscopy in analytical chemistry within industry or academia in any part of the world.

During the Ceremony, three awardees will receive a certificate of merit and a cheque for 500 Euro.

This year's Awardees:

Dr. Anne Fages
(Weizmann Institute of Science, Chemical and Physics, Rehovot, Israel)

Dr. Krzysztof Kazimierczuk
(University of Warsaw, Centre of New Technologies, Warsaw, Poland)

Dr. Thomas Theis
(Duke University, Chemistry, Durham, USA)

Friday, 10 July 2015

IOCB Workshop

Institute of Chemical Technology, building B – lecture room B1

Satellite one-day symposium – ceremonial inauguration of a new high-field NMR facility by The Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.
(http://www.euromar2015.org/iocb-workshop.htm)

PRIZES

The Russell Varian Prize 2015

The Russell Varian prize honours the memory of the pioneer behind the first commercial Nuclear Magnetic Resonance spectrometers and co-founder of Varian Associates. The prize is awarded to a researcher based on a single innovative contribution (a single paper, patent, lecture, or piece of hardware) that has proven of high and broad impact on state-of-the-art NMR technology.

Selected contribution:

M. H. Levitt and R. Freeman: NMR population inversion using a composite pulse (J. Magn. Reson. 33, 473-476 (1979)).

Presentation details
Session: Opening and Prize Session
Date: Sunday, 5 July
Time: 17:45–18:30
Location: Forum Hall

The Raymond Andrew Prize 2015

The Raymond Andrew Prize acknowledges the outstanding PhD thesis in the field of magnetic resonance.

Laureate:

Dr. Jean-Philippe Demers
(Leibniz-Institut für Molekulare Pharmakologie (FMP), Molecular Biophysics, Berlin, Germany)

From Slow to Ultra-Fast Mas: Structural Determination of Type-Three Secretion System Bacterial Needles and Inorganic Materials by Solid-State NMR

Presentation details
Session: Opening and Prize Session
Date: Sunday, 5 July
Time: 17:15–17:45
Location: Forum Hall

The Wiley Award 2015 – Magnetic Resonance in Chemistry

The MRC Award for Young Scientists, established in 2006, honours the outstanding researchers under the age of 40 working with NMR spectroscopy in analytical chemistry within industry or academia in any part of the world.

Awardees:

Dr. Anne Fages

(Weizmann Institute of Science, Chemical and Physics, Rehovot, Israel)

Magnetic Resonance Detection of Lymphatic Breast Cancer Metastasis in a Xenograft Model by Hyperpolarized 13C-Pyruvate

Presentation details

Session: S 09 – In-vivo and In-cell NMR (presentation O 042)

Date: Tuesday, 7 July

Time: 11:00–11:20

Location: Meeting Hall IV

Dr. Krzysztof Kazimierczuk

(University of Warsaw, Centre of New Technologies, Warsaw, Poland)

Dynamic Non-Uniform Sampling

Presentation details

Session: 11 – New Approaches to the MR Measurement (presentation O 052)

Date: Tuesday, 7 July

Time: 16:00–16:20

Location: Meeting Hall V

Dr. Thomas Theis

(Duke University, Chemistry, Durham, USA)

Enhancing Sabre with Microtesla Fields: Broadly Applicable, >10,000 Fold Direct Heteronuclear Signal Enhancement with >20 Minute Signal Lifetimes

Presentation details

Session: S 17 – Sensitivity Enhancement I (presentation O 082)

Date: Wednesday, 8 July

Time: 16:00–16:20

Location: Meeting Hall V

The Wiley Award Ceremony will take place on Thursday, 8 July, in the Forum Hall (16:00–16:10).

International EPR (ESR) Society Poster Awards

The International EPR/ESR Society (IES) will award two poster prizes (with certificate and 200$ prize money) to posters on ESR or ESR-related subjects.

The Poster Awards Ceremony will take place on Thursday, 8 July, in the Forum Hall (16:10–16:20).

TUTORIAL SPEAKERS

Blank, Aharon	Technion – Israel Institute of Technology, Israel
Palmer, III, Arthur G.	Columbia University, USA
Parella, Teodor	Universitat Autònoma Barcelona, Spain

PLENARY SPEAKERS

Bax, Ad	NIH Bethesda, USA
Edison, Arthur S.	University of Florida, USA
Feigon, Juli	University of California, Los Angeles, USA
Freed, Jack	Cornell University, USA
Hilty, Christian	Texas A&M University, USA
Kern, Dorothee	Brandeis University, USA
Korvink, Jan	Karlsruhe Institute of Technology, Germany
Ladizhansky, Vladimir	University of Guelph, Canada
Polenova, Tatyana	University of Delaware, USA
Rosay, Melanie	Bruker BioSpin, Billerica, USA
Takegoshi, Kiyonori	Kyoto University, Japan
Thiele, Christina	Technische Universität Darmstadt, Germany
Webb, Andrew	Leiden University, The Netherlands

INVITED SPEAKERS

Allain, Fred	ETH Zürich, Switzerland
Baldus, Marc	Utrecht University, The Netherlands
Brandt, Martin	TU München, Germany
Brindle, Kevin	University of Cambridge, UK
Brüschweiler, Rafael	Ohio State University, USA
Corzilius, Björn	Frankfurt University, Germany
Cross, Tim	Florida State University, NHMFL, USA
De Renzi, Roberto	University of Parma, Italy
Delepierre, Muriel	Institut Pasteur, Paris, France
Dötsch, Volker	Frankfurt University, Germany
Dračínský, Martin	Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic
Dyson, Jane	The Scripps Research Institute, La Jolla, USA
Elena-Herrmann, Benedict	University of Lyon, France
Ernst, Matthias	ETH Zürich, Switzerland
Goldbourt, Amir	Tel Aviv University, Israel
Günther, Ulrich	University of Birmingham, UK
Haase, Jürgen	University of Leipzig, Germany
Hayes, Sophia E.	Washington University, St. Louis
Kaupp, Martin	Technische Universität Berlin

Konrat, Robert	University of Vienna, Austria
Koptyug, Igor	International Tomography Center, SB RAS, Novosibirsk, Russia
Kowalewski, Jozef	Stockholm University, Sweden
Krivdin, Leonid B.	Irkutsk Institute of Chemistry, Russia
Kruk, Danuta	University of Warmia and Mazury, Olsztyn, Poland
Levitt, Malcolm H.	University of Southampton, UK
Lippens, Guy	Institut Pasteur de Lille, Université Lille Nord de France, France
Maeda, Hidaeki	RIKEN, Japan
Marassi, Francesca	Sanford-Burnham Medical Research Institute, La Jolla, USA
Matysik, Jörg	University of Leipzig, Germany
Meier, Beat	ETH Zürich, Switzerland
Meirovitch, Eva	Bar-Ilan University, Israel
Müller, Norbert	Johannes Kepler University Linz, Austria
Orekhov, Vladislav	University of Gothenburg, Sweden
Pierattelli, Roberta	CERM Florence, Italy
Pons, Miquel	Universitat de Barcelona, Spain
Pruski, Marek	Iowa State University, USA
Ramanathan, Chandrasekhar	Dartmouth College, Hannover, USA
Rienstra, Chad	University of Illinois, Urbana, USA
Saalwächter, Kay	Martin Luther University Halle-Wittenberg, Germany
Schwalbe, Harald	Johann Wolfgang Goethe University, Frankfurt, Germany
Shimada, Ichio	The University of Tokyo, Japan
Stapf, Siegfried	Technische Universität Ilmenau, Germany
Timmel, Christiane	University of Oxford, UK
Tjandra, Nico	National Institutes of Health, NHLB, Bethesda, USA
Tkáč, Ivan	University of Minnesota, USA
Ubbink, Marcellus	Leiden University, Institute of Chemistry, The Netherlands
Vega, Shimon	Weizmann Institute of Science, Rehovot, Israel
Wright, Peter	The Scripps Research Institute, La Jolla, USA

POSTERS

Almost 370 posters have been successfully accepted for this year's EUROMAR Congress. Posters will be displayed continuously from Sunday, 5 July 2015 to Wednesday, 8 July in the Poster Area on the 2nd floor (Terrace I + Terrace II + North Hall).

Three poster sessions have been scheduled to the Congress programme:

Poster Session 1 – Monday, 6 July; 13:30–15:30
Poster Session 2 – Tuesday, 7 July; 13:30–15:30
Poster Session 3 – Wednesday, 8 July; 13:30–15:30

Pins/Velcro for mounting posters will be available at the Poster desk located in the Poster Area. Assistance for poster mounting/dismantling will be available.

Presenting authors are kindly requested to be present throughout the official poster viewing time in order to explain their research and to answer questions from the delegates. There will be no guided formal discussion.

Poster mounting:

Sunday, 5 July; 16:00–20:30

(all posters must be set up by Monday, 6 July; 12:00)

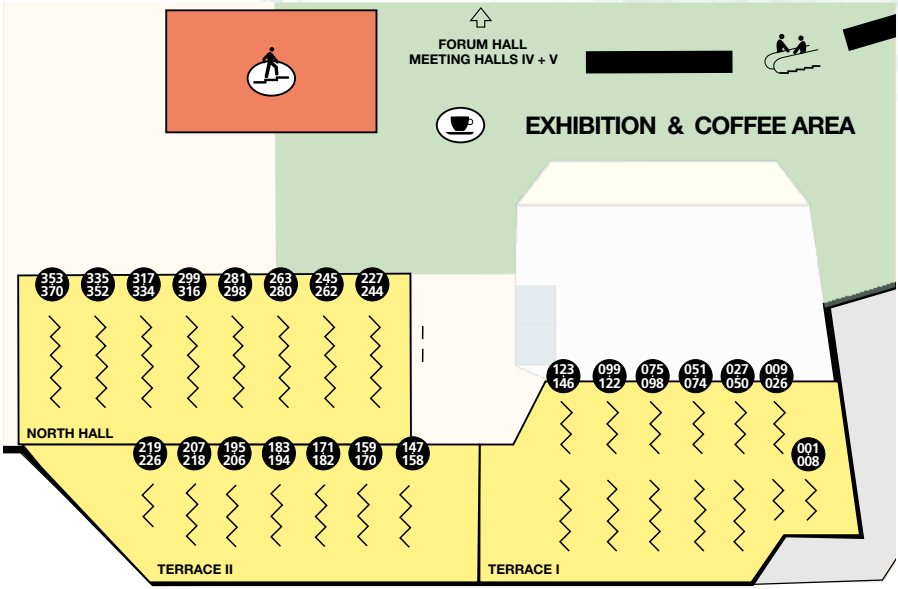
Please make sure to **mount your poster on the poster board with the number corresponding to the number assigned to your poster presentation** (e. g. P 001, P 002 etc…).

Poster dismantling:

Wednesday, 8 July; 16:00–17:45

Posters which are **not dismantled by the end of the above specified time period will be automatically discarded**. The Congress organizers cannot accept responsibility for any material left behind.

POSTERS FLOORPLAN



TERRACE I

Posters **P 001 – P 146**

TERRACE II

Posters **P 147 – P 226**

NORTH HALL

Posters **P 227 – P 370**

LIST OF POSTERS

Poster Session 1

Monday, 6 July; 13:30–15:30 (Poster Area)

P 001 **APPLICATIONS OF 19F-NMR TO STUDY PROTEIN-LIGAND INTERACTIONS AND PROTEIN CONFORMATIONAL CHANGES IN SOLUTION**
Martine I. Abboud *(UK)*

P 004 **CURCUMIN BINDING TO AMYLOID-BETA OLIGOMERS**
Oleg Antzutkin *(Sweden)*

P 007 **IN-SITU SOLID STATE NMR ON UNIFORMLY 13C-15N LABELED C. REINHARDTII THYLAKOID MEMBRANES**
Fatemeh Azadi Chegeni *(The Netherlands)*

P 013 **INSIGHTS INTO THE STRUCTURE AND DYNAMICS OF THE N-TERMINAL FRAGMENT OF THE HUNTINGTIN PROTEIN**
Maria Baias *(Israel)*

P 016 **THE STRUCTURE OF NANODISCS: IMPLICATIONS FOR HIGH-DENSITY LIPOPROTEIN PARTICLES**
Stefan Bibow *(Switzerland)*

P 019 **EGFR TRANSMEMBRANE DOMAIN PACKING DIVERSITY SUGGESTS THAT COUPLED PROTEIN-PROTEIN AND PROTEIN-LIPID INTERACTIONS UNDERLIE IN THE SIGNAL TRANSDUCTION ACROSS MEMBRANE**
Eduard Bocharov *(Russia)*

P 022 **BACTERIAL AND CELL-FREE PRODUCTION OF TRANSMEMBRANE FRAGMENTS OF HER/ERBB RECEPTOR TYROSINE KINASES FOR STRUCTURAL STUDIES OF SIGNAL TRUNSDUCTION MECHANISM**
Eduard Bocharov *(Russia)*

P 025 **NMR STUDIES ON A MEMBRANE-EMBEDDED DOMAIN OF THE LYSOSOMAL PEPTIDE TRANSPORTER TAPL**
Christoph Bock *(Germany)*

P 028 **STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF NADH BINDING TO THE HUMAN VOLTAGE-DEPENDENT ANION CHANNEL (VDAC)**
Raphael Böhm *(Switzerland)*

P 031 **NMR SOLUTION STRUCTURE OF A DNA QUADRUPLEX CONTAINING ALS AND FTD RELATED GGGGCC REPEAT**
Jasna Brčić *(Slovenia)*

P 034 **STRUCTURE OF MICROTUBULE-BOUND(296-321)**
Yunior Cabrales Fontela *(Germany)*

P 037 **FASTER PROBES AND HIGHER PROTON CONTENTS: WHEN RESOLUTION MEETS SENSITIVITY IN BIOMOLECULAR MAS NMR**
Diane Cala *(France)*

P 040 **STRUCTURAL BASIS FOR THE CONSERVED BINDING MECHANISM OF MDM2-INHIBITING PEPTIDES AND ANTI-APOPTOTIC BCL-2 FAMILY PROTEINS**
Seung-Wook Chi *(South Korea)*

P 043 **GLOBAL FOLD OF THE TRANSMEMBRANE DOMAINS OF HEPATITIS C VIRUS GLYCOPROTEINS E1 AND E2 IN LPPG MICELLES**
Jordan Chill *(Israel)*

P 046 **STRUCTURE AND DYNAMICS OF THETA-DEFENSINS; ANTIMICROBIAL CYCLIC PEPTIDES FROM PRIMATES**
Anne Conibear *(Austria)*

P 049 **INTERACTION OF A HISTONE CHAPERONE INVOLVED IN DNA REPAIR WITH CORE HISTONES**
Ivan Corbeski *(The Netherlands)*

P 052 **SOLUTION STRUCTURE OF PRIC, A SCAFFOLD PROTEIN FOR DNA, SSB, AND DNAB/DNAC BINDING DURING REPLICATION RESTART**
Claudia Cornilescu *(USA)*

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Riccardo Balzan *(France)*

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David Ban *(USA)*

P 331 **CONFORMATIONAL DYNAMICS OF DOTA-LIKE Eu(III) COMPLEX**
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Marc Brinkkötter *(Germany)*

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Fred Damberger *(Switzerland)*

P 340 **THE DYNAMICS OF TETHERED POLYISOPRENE CHAINS IN ASYMMETRIC POLY(STYRENE-B-ISOPRENE) DIBLOCK COPOLYMER: THE CASE OF PI BLOCKS CONFINED IN SPHERICAL DOMAINS**
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Mikyung Lee *(South Korea)*

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Christina Helmling *(Germany)*

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Daniela Hofmann *(Switzerland)*

P 008 **AN EQUILIBRIUM-BASED MODEL FOR -1 PROGRAMMED RIBOSOME FRAME-SHIFT STIMULATOR**
Shang-Te Danny Hsu *(Taiwan)*

P 011 **THE DYNAMICS OF THE G PROTEIN-COUPLED NEUROPEPTIDE Y2 RECEPTOR IN PHOSPHOLIPID MEMBRANES INVESTIGATED BY SOLID-STATE NMR SPECTROSCOPY**
Daniel Huster *(Germany)*

P 014 **THE SOLUTION STATE NMR INSIGHTS IN THE CYTOSOLIC TAIL OF THE TUMOR MARKER PROTEIN-TROP2**
Gregor Ilc *(Slovenia)*

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Andreea Iorgu *(UK)*

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Severine Jansen *(Czech Republic)*

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Zuzana Jasenakova *(Czech Republic)*

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Gunnar Jeschke *(Switzerland)*

P 029 **A NOVEL APPROACH TO PROTEIN ASSIGNMENT IN SOLID STATE NUCLEAR MAGNETIC RESONANCE**
Michael Jolly *(UK)*

P 032 **RECOMBINANT EXPRESSION, REFOLDING AND INITIAL NMR STUDIES OF THE EXTRINSIC PHOTOCHEMICAL PROTEIN PSBO**
Michal Kamenický *(Austria)*

P 035 **STUDY OF CISPLATIN DERIVATIVE BY NUCLEAR MAGNETIC RESONANCE (NMR), RAMAN SPECTROSCOPY, SMALL ANGLE X-RAY SCATTERING (SAXS) AND THEORETICAL APPROACHES**
Magdalena Krejcikova *(Czech Republic)*

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Ulrike Krug *(Germany)*

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Flemming Hofmann Larsen *(Denmark)*

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Weontae Lee *(South Korea)*

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Christofer Lendel *(Sweden)*

P 056 **PROTEIN ASSIGNMENT USING PARAMAGNETIC EFFECTS WITH PARASSIGN**
Mathilde Lescanne *(The Netherlands)*

P 059 **COMBINING SPECIFIC-METHYL LABELING, 3D & 4D NUS, SAXS AND ADVANCED COMPUTATION TO UNDERSTAND ARFS AND ARFGAPS IN THE RAS SUPERFAMILY**
Yifei Li *(USA)*

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Juan Lopez *(France)*

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Petr Louša *(Czech Republic)*

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Peter Josef Lukavsky *(Czech Republic)*

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Kateřina Melková *(Czech Republic)*

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Konstantin Mineev *(Russia)*

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Flavia Mercurio *(Italy)*

P 083 **STRUCTURAL INSIGHTS INTO EGCG-INDUCED AMYLOID-B OLIGOMERS**
Vanessa Morris *(Germany)*

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Mikhail Myshkin *(Russia)*

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Kirill Nadezhdin *(Russia)*

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Romain Navarro *(France)*

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Philipp Neudecker *(Germany)*

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Oliver Ohlenschlaeger *(Germany)*

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Gyula Palfy *(Hungary)*

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Galina Pavlovskaya *(UK)*

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Sina Kazemi *(Germany)*

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Mikhail M. Kucherov *(Russia)*

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Mikhail Kudryavtsev *(Germany)*

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Carlos Geraldes *(Portugal)*

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Julia Hollenbach *(Germany)*

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Yuji Horiuchi *(Japan)*

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Samih Isber *(Lebanon)*

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Anu M Kantola *(Finland)*

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Rafał Konefał *(Czech Republic)*

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Philipp Korntner *(Austria)*

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Petr Kříšťan *(Czech Republic)*

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Valentyn Laguta *(Czech Republic)*

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Nikolay Le *(Russia)*

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Sang-Yun Lee *(Germany)*

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Chun-Rong Lin *(Taiwan)*

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Francesca Martini *(Italy)*

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Ewa Jawień *(Poland)*

P 179 **RECONSTRUCTION OF TOP-RESOLUTION SPECTRA USING SPECTRAL ALIASING. APPLICATION TO 2D HSQC AND 1D HOMODECOUPLED PROTON SPECTRA WITH SCALAR COUPLING INFORMATION**
Damien Jeannerat *(Switzerland)*

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Matthias Köck *(Germany)*

P 185 **A CLEAN IN-PHASE COSY-EXPERIMENT**
Martin Koos *(Germany)*

P 188 **EVALUATION OF REPORTED 13C NMR DATA AND CHEMICAL STRUCTURES BY USING CAST/CNMR SHIFT PREDICTOR AND STRUCTURE ELUCIDATOR**
Hiroyuki Koshino *(Japan)*

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Hana Kouřilová *(Czech Republic)*

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Eriks Kupce *(UK)*

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Eriks Kupce *(UK)*

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Raffaele Lamanna *(Italy)*

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Toru Miura *(Japan)*

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Piotr Mlynarz *(Poland)*

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Asakura, Katsuo	P 175	Poster Session 1	06/07/2015	13:30	Poster Area
Asakura, Katsuo		JEOL Symposium	07/07/2015	13:30	Meeting Hall V
Asakura, Katsuo		JEOL Symposium	08/07/2015	13:30	Meeting Hall V
Azadi Chegeni, Fatemeh	P 007	Poster Session 1	06/07/2015	13:30	Poster Area
B					
Bachmann, Sebastian	P 244	Poster Session 1	06/07/2015	13:30	Poster Area
Badea, Valentin	P 178	Poster Session 1	06/07/2015	13:30	Poster Area
Bader, Katharina	P 319	Poster Session 1	06/07/2015	13:30	Poster Area
Baias, Maria	P 013	Poster Session 1	06/07/2015	13:30	Poster Area
Baias, Maria	S 13	Solid State NMR Techniques	08/07/2015	11:40	Forum Hall
Bailac, Laura	P 250	Poster Session 1	06/07/2015	13:30	Poster Area
Baldus, Marc	S 01	Biosolids	06/07/2015	12:00	Forum Hall
Ballaschk, Martin	P 322	Poster Session 1	06/07/2015	13:30	Poster Area
Balzan, Riccardo	P 325	Poster Session 1	06/07/2015	13:30	Poster Area
Balzan, Riccardo	S 20	Metabolomics and Small Molecules	09/07/2015	11:20	Meeting Hall V
Ban, David	P 328	Poster Session 1	06/07/2015	13:30	Poster Area
Baranowski, Mikołaj	P 256	Poster Session 1	06/07/2015	13:30	Poster Area
Barskaya, Irina	P 133	Poster Session 1	06/07/2015	13:30	Poster Area
Barskiy, Danila	P 355	Poster Session 1	06/07/2015	13:30	Poster Area
Bax, Ad		Plenary Session 8	09/07/2015	16:50	Forum Hall
Becker, Johanna	P 181	Poster Session 1	06/07/2015	13:30	Poster Area
Bertarello, Andrea	P 298	Poster Session 1	06/07/2015	13:30	Poster Area
Bibow, Stefan	P 016	Poster Session 1	06/07/2015	13:30	Poster Area
Bielejewski, Michal	P 136	Poster Session 1	06/07/2015	13:30	Poster Area

Name	Session		Date	From	Room
Biljan, Ivana	P 184	Poster Session 1	06/07/2015	13:30	Poster Area
Blahut, Jan	P 331	Poster Session 1	06/07/2015	13:30	Poster Area
Blanchard, John	S 12	Exotica	07/07/2015	16:40	Meeting Hall IV
Blank, Aharon		Tutorial Lectures	05/07/2015	14:45	Forum Hall
Blommers, Marcel	S 02	Small Molecules and Pharmaceuticals	06/07/2015	11:20	Meeting Hall V
Bocharov, Eduard	P 022	Poster Session 1	06/07/2015	13:30	Poster Area
Bocharov, Eduard	P 019	Poster Session 1	06/07/2015	13:30	Poster Area
Bock, Christoph	P 025	Poster Session 1	06/07/2015	13:30	Poster Area
Böckmann, Anja	S 01	Biosolids	06/07/2015	11:00	Forum Hall
Bode, Bela	P 301	Poster Session 1	06/07/2015	13:30	Poster Area
Bodenhausen, Geoffrey	P 358	Poster Session 1	06/07/2015	13:30	Poster Area
Böhm, Raphael	P 028	Poster Session 1	06/07/2015	13:30	Poster Area
Brandt, Martin	S 13	Solid State NMR Techniques	08/07/2015	10:30	Forum Hall
Brauckmann, Ole	P 139	Poster Session 1	06/07/2015	13:30	Poster Area
Bräuer, Maria	P 187	Poster Session 1	06/07/2015	13:30	Poster Area
Brčić, Jasna	P 031	Poster Session 1	06/07/2015	13:30	Poster Area
Brezina, Vaclav	P 190	Poster Session 1	06/07/2015	13:30	Poster Area
Brijith, Thomas	P 142	Poster Session 1	06/07/2015	13:30	Poster Area
Brindle, Kevin	S 15	NMR Imaging	08/07/2015	12:00	Meeting Hall IV
Brinkkötter, Marc	P 334	Poster Session 1	06/07/2015	13:30	Poster Area
Brückner, Stephan Ingmar	P 145	Poster Session 1	06/07/2015	13:30	Poster Area
Brunner, Eike	P 361	Poster Session 1	06/07/2015	13:30	Poster Area
Brüschweiler, Rafael	S 06	Protein Relaxation and Dynamics	06/07/2015	15:30	Meeting Hall IV
Bruschweiler-Li, Lei	P 193	Poster Session 1	06/07/2015	13:30	Poster Area
Budesinsky, Milos	P 196	Poster Session 1	06/07/2015	13:30	Poster Area
Buratto, Roberto	S 02	Small Molecules and Pharmaceuticals	06/07/2015	11:40	Meeting Hall V
Buryi, Maksym	P 148	Poster Session 1	06/07/2015	13:30	Poster Area
Byrd, R. Andrew	S 18	Computation and Processing	08/07/2015	16:20	Meeting Hall IV
		C			
Cabrales Fontela, Yunior	P 034	Poster Session 1	06/07/2015	13:30	Poster Area
Cai, Shuhui	P 199	Poster Session 1	06/07/2015	13:30	Poster Area
Cala, Diane	P 037	Poster Session 1	06/07/2015	13:30	Poster Area
Calligari, Paolo	P 109	Poster Session 1	06/07/2015	13:30	Poster Area
Canet Martinez, Estel	S 14	Biomolecular Polarization and Relaxation	08/07/2015	11:40	Meeting Hall V
Castañar Acedo, Laura	P 202	Poster Session 1	06/07/2015	13:30	Poster Area
Cespedes-Camacho, Isaac F.	P 364	Poster Session 1	06/07/2015	13:30	Poster Area
Chandra, Kousik	P 259	Poster Session 1	06/07/2015	13:30	Poster Area
Chi, Seung-Wook	P 040	Poster Session 1	06/07/2015	13:30	Poster Area
Chill, Jordan	P 043	Poster Session 1	06/07/2015	13:30	Poster Area
Chill, Jordan	S 22	Disordered proteins	09/07/2015	14:00	Forum Hall

Name	Session		Date	From	Room
Chlan, Vojtěch	P 154	Poster Session 1	06/07/2015	13:30	Poster Area
Chlan, Vojtěch	P 151	Poster Session 1	06/07/2015	13:30	Poster Area
Conibear, Anne	P 046	Poster Session 1	06/07/2015	13:30	Poster Area
Corbeski, Ivan	P 049	Poster Session 1	06/07/2015	13:30	Poster Area
Cornilescu, Claudia	P 052	Poster Session 1	06/07/2015	13:30	Poster Area
Cornilescu, Gabriel	P 205	Poster Session 1	06/07/2015	13:30	Poster Area
Corzilius, Björn	S 04	NMR + EPR	06/07/2015	17:00	Forum Hall
Cross, Timothy	S 01	Biosolids	06/07/2015	10:30	Forum Hall
Czechowski, Tomasz	P 262	Poster Session 1	06/07/2015	13:30	Poster Area
		D			
Damberger, Fred	P 337	Poster Session 1	06/07/2015	13:30	Poster Area
Dames, Sonja Alexandra	P 061	Poster Session 1	06/07/2015	13:30	Poster Area
Dammers, Christina	P 064	Poster Session 1	06/07/2015	13:30	Poster Area
Dass, Rupashree	P 265	Poster Session 1	06/07/2015	13:30	Poster Area
De Renzi, Roberto	S 08	NMR Physics	07/07/2015	10:30	Meeting Hall V
Deleanu, Calin	P 208	Poster Session 1	06/07/2015	13:30	Poster Area
Delepierre, Muriel	S 16	Biomacromolecules	08/07/2015	15:30	Forum Hall
Déméné, Hélène	S 16	Biomacromolecules	08/07/2015	16:20	Forum Hall
Demers, Jean-Philippe		Opening and Prize Session	05/07/2015	17:15	Forum Hall
Deschamps, Michael	S 08	NMR Physics	07/07/2015	11:20	Meeting Hall V
Díaz-Quintana, Antonio J.	P 067	Poster Session 1	06/07/2015	13:30	Poster Area
Dobies, Maria	P 340	Poster Session 1	06/07/2015	13:30	Poster Area
Dötsch, Volker	S 09	In-vivo and In-cell NMR	07/07/2015	10:30	Meeting Hall IV
Dolinšek, Janez	S 05	Materials NMR	06/07/2015	16:00	Meeting Hall V
Domagalska, Joanna	P 070	Poster Session 1	06/07/2015	13:30	Poster Area
Domján, Attila	P 157	Poster Session 1	06/07/2015	13:30	Poster Area
Dračínský, Martin	S 18	Computation and Processing	08/07/2015	17:00	Meeting Hall IV
Drescher, Malte	P 160	Poster Session 1	06/07/2015	13:30	Poster Area
Dudás, Erika	P 073	Poster Session 1	06/07/2015	13:30	Poster Area
D'Ursi, Anna Maria	P 058	Poster Session 1	06/07/2015	13:30	Poster Area
D'Ursi, Anna Maria	P 055	Poster Session 1	06/07/2015	13:30	Poster Area
Dyson, Jane	S 07	Large biomolecular complexes	07/07/2015	10:30	Forum Hall
		E			
Edison, Arthur		Plenary Session 4	07/07/2015	17:45	Forum Hall
Elena-Herrmann, Benedicte	S 20	Metabolomics and Small molecules	09/07/2015	10:30	Meeting Hall V
Elliott, Stuart	P 367	Poster Session 1	06/07/2015	13:30	Poster Area
Endeward, Burkhard	S 04	NMR + EPR	06/07/2015	16:20	Forum Hall
Ernst, Matthias	S 19	Emerging Techniques	09/07/2015	10:30	Forum Hall
		F			
Fages, Anne	P 370	Poster Session 1	06/07/2015	13:30	Poster Area
Fages, Anne	S 09	In-vivo and In-cell NMR	07/07/2015	11:00	Meeting Hall IV
Farjon, Jonathan	P 214	Poster Session 1	06/07/2015	13:30	Poster Area
Favier, Adrien	P 268	Poster Session 1	06/07/2015	13:30	Poster Area

Name	Session		Date	From	Room
Feigon, Juli		Plenary Session 5	08/07/2015	08:45	Forum Hall
Feintuch, Akiva	P 304	Poster Session 1	06/07/2015	13:30	Poster Area
Felli, Isabella Caterina	P 076	Poster Session 1	06/07/2015	13:30	Poster Area
Ferreira, Antonio	P 220	Poster Session 1	06/07/2015	13:30	Poster Area
Ferreira, Antonio	P 217	Poster Session 1	06/07/2015	13:30	Poster Area
Fiala, Radovan	P 079	Poster Session 1	06/07/2015	13:30	Poster Area
Finch, Graeme	P 271	Poster Session 1	06/07/2015	13:30	Poster Area
Foroozandeh, Mohammadali	P 274	Poster Session 1	06/07/2015	13:30	Poster Area
Förster, Eva	P 163	Poster Session 1	06/07/2015	13:30	Poster Area
Fredersdorf, Maic	P 223	Poster Session 1	06/07/2015	13:30	Poster Area
Fredi, André	P 226	Poster Session 1	06/07/2015	13:30	Poster Area
Freed, Jack		Plenary Session 1	06/07/2015	09:25	Forum Hall
Fuhrer, Erwin	P 127	Poster Session 1	06/07/2015	13:30	Poster Area
		G			
Gajan, David	P 166	Poster Session 1	06/07/2015	13:30	Poster Area
Galer, Petra	P 229	Poster Session 1	06/07/2015	13:30	Poster Area
Galvosas, Petrik	P 277	Poster Session 1	06/07/2015	13:30	Poster Area
Gargallo Garriga, Albert	P 232	Poster Session 1	06/07/2015	13:30	Poster Area
Gavrilova, Tatiana	P 307	Poster Session 1	06/07/2015	13:30	Poster Area
Geraldes, Carlos	P 125	Poster Session 2	07/07/2015	13:30	Poster Area
Gerolin, Marco	P 112	Poster Session 1	06/07/2015	13:30	Poster Area
Gil Caballero, Sergio	S 06	Protein Relaxation and Dynamics	06/07/2015	16:00	Meeting Hall IV
Ginthör, Stephan	P 115	Poster Session 1	06/07/2015	13:30	Poster Area
Giraudeau, Patrick	S 02	Small Molecules and Pharmaceuticals	06/07/2015	11:00	Meeting Hall V
Gloeggler, Stefan	P 373	Poster Session 1	06/07/2015	13:30	Poster Area
Gloeggler, Stefan	S 09	In-vivo and In-cell NMR	07/07/2015	11:20	Meeting Hall IV
Gloge, Thomas	P 235	Poster Session 1	06/07/2015	13:30	Poster Area
Gmeiner, Christoph	P 082	Poster Session 1	06/07/2015	13:30	Poster Area
Goldbourt, Amir	P 085	Poster Session 1	06/07/2015	13:30	Poster Area
Goldbourt, Amir	S 13	Solid State NMR Techniques	08/07/2015	12:00	Forum Hall
Goncharuk, Marina	P 088	Poster Session 1	06/07/2015	13:30	Poster Area
Goncharuk, Sergey	P 091	Poster Session 1	06/07/2015	13:30	Poster Area
Goradia, Nishit	P 094	Poster Session 1	06/07/2015	13:30	Poster Area
Gouverneur, Martin	P 280	Poster Session 1	06/07/2015	13:30	Poster Area
Gowda, Vasantha	P 169	Poster Session 1	06/07/2015	13:30	Poster Area
Gränz, Markus	P 310	Poster Session 1	06/07/2015	13:30	Poster Area
Grudziąż, Katarzyna	P 097	Poster Session 1	06/07/2015	13:30	Poster Area
Günther, Ulrich	S 02	Small Molecules and Pharmaceuticals	06/07/2015	12:00	Meeting Hall V
Guijarro, Iñaki	P 100	Poster Session 1	06/07/2015	13:30	Poster Area
Guthausen, Gisela	P 287	Poster Session 2	07/07/2015	13:30	Poster Area
Gutmanas, Aleksandras	S 10	Biomacromolecular Folding and Dynamics	07/07/2015	16:20	Forum Hall

Name	Session		Date	From	Room
		H			
Ha, Ji-Hyang	P 103	Poster Session 1	06/07/2015	13:30	Poster Area
Haase, Jürgen	S 03	NMR - High and Low, Sparse and Dense	06/07/2015	10:30	Meeting Hall IV
Håkansson, Pär	P 121	Poster Session 1	06/07/2015	13:30	Poster Area
Handwerker, Jonas	P 283	Poster Session 1	06/07/2015	13:30	Poster Area
Harpreet, Singh	P 123	Poster Session 3	08/07/2015	13:30	Poster Area
Häussinger, Daniel	S 23	Paramagnetic Systems	09/07/2015	14:20	Meeting Hall V
Hayes, Sophia	P 172	Poster Session 1	06/07/2015	13:30	Poster Area
Hayes, Sophia	S 05	Materials NMR	06/07/2015	15:30	Meeting Hall V
Hein, Christopher	P 106	Poster Session 1	06/07/2015	13:30	Poster Area
Helmling, Christina	P 002	Poster Session 2	07/07/2015	13:30	Poster Area
Hilty, Christian		Plenary Session 7	09/07/2015	08:45	Forum Hall
Hirschinger, Jérôme	P 110	Poster Session 2	07/07/2015	13:30	Poster Area
Hirschinger, Jérôme	P 376	Poster Session 1	06/07/2015	13:30	Poster Area
Hofmann, Daniela	P 005	Poster Session 2	07/07/2015	13:30	Poster Area
Hollenbach, Julia	P 131	Poster Session 2	07/07/2015	13:30	Poster Area
Hook, James	P 238	Poster Session 1	06/07/2015	13:30	Poster Area
Horiuchi, Yuji	P 134	Poster Session 2	07/07/2015	13:30	Poster Area
Horník, Štěpán	P 173	Poster Session 2	07/07/2015	13:30	Poster Area
Hrebik, Dominik	P 343	Poster Session 1	06/07/2015	13:30	Poster Area
Hsu, Shang-Te Danny	P 008	Poster Session 2	07/07/2015	13:30	Poster Area
Huster, Daniel	P 011	Poster Session 2	07/07/2015	13:30	Poster Area
		I			
Ichijo, Naoki	P 286	Poster Session 1	06/07/2015	13:30	Poster Area
Icker, Maik	P 350	Poster Session 2	07/07/2015	13:30	Poster Area
Ilc, Gregor	P 014	Poster Session 2	07/07/2015	13:30	Poster Area
Iorgu, Andreea	P 017	Poster Session 2	07/07/2015	13:30	Poster Area
Isber, Samih	P 137	Poster Session 2	07/07/2015	13:30	Poster Area
		J			
Jansen, Severine	P 020	Poster Session 2	07/07/2015	13:30	Poster Area
Jasenakova, Zuzana	P 023	Poster Session 2	07/07/2015	13:30	Poster Area
Jawień, Ewa	P 176	Poster Session 2	07/07/2015	13:30	Poster Area
Jeannerat, Damien	P 179	Poster Session 2	07/07/2015	13:30	Poster Area
Jeschke, Gunnar	P 026	Poster Session 2	07/07/2015	13:30	Poster Area
Jolly, Michael	P 029	Poster Session 2	07/07/2015	13:30	Poster Area
Jouda, Mazin	S 11	New Approaches to the MR Measurement	07/07/2015	16:40	Meeting Hall V
		K			
Kalendra, Vidmantas	P 290	Poster Session 2	07/07/2015	13:30	Poster Area
Kaltschnee, Lukas	P 239	Poster Session 2	07/07/2015	13:30	Poster Area
Kamberger, Robert	P 242	Poster Session 2	07/07/2015	13:30	Poster Area
Kamenický, Michal	P 032	Poster Session 2	07/07/2015	13:30	Poster Area
Kantola, Anu M	P 140	Poster Session 2	07/07/2015	13:30	Poster Area
Karlsson, Göran	P 245	Poster Session 2	07/07/2015	13:30	Poster Area

Name	Session		Date	From	Room
Kaupp, Martin	S 18	Computation and Processing	08/07/2015	15:30	Meeting Hall IV
Kazemi, Sina	P 113	Poster Session 2	07/07/2015	13:30	Poster Area
Kazimierczuk, Krzysztof	S 11	New Approaches to the MR Measurement	07/07/2015	16:00	Meeting Hall V
Keizer, David	P 248	Poster Session 2	07/07/2015	13:30	Poster Area
Keller, Katharina	P 293	Poster Session 2	07/07/2015	13:30	Poster Area
Kern, Dorothee		Plenary Session 2	06/07/2015	17:45	Forum Hall
Khan, Arafat Hossain	P 296	Poster Session 2	07/07/2015	13:30	Poster Area
Kim, Tae Hun	S 16	Biomacromolecules	08/07/2015	16:00	Forum Hall
Köck, Matthias	P 182	Poster Session 2	07/07/2015	13:30	Poster Area
Koczor, Balint	P 356	Poster Session 2	07/07/2015	13:30	Poster Area
Konefał, Rafał	P 143	Poster Session 2	07/07/2015	13:30	Poster Area
Konrat, Robert	S 22	Disordered proteins	09/07/2015	15:00	Forum Hall
Koos, Martin	P 185	Poster Session 2	07/07/2015	13:30	Poster Area
Koptyug, Igor	S 24	Sensitivity enhancement II	09/07/2015	13:30	Meeting Hall IV
Korntner, Philipp	P 149	Poster Session 2	07/07/2015	13:30	Poster Area
Korvink, Jan		Plenary Session 5	08/07/2015	09:25	Forum Hall
Koshino, Hiroyuki	P 188	Poster Session 2	07/07/2015	13:30	Poster Area
Kouřilová, Hana	P 191	Poster Session 2	07/07/2015	13:30	Poster Area
Kowalewski, Jozef	S 21	Relaxation and Transport Phenomena	09/07/2015	12:00	Meeting Hall IV
Krejcikova, Magdalena	P 035	Poster Session 2	07/07/2015	13:30	Poster Area
Kříšťan, Petr	P 152	Poster Session 2	07/07/2015	13:30	Poster Area
Krivdin, Leonid	S 02	Small Molecules and Pharmaceuticals	06/07/2015	10:30	Meeting Hall V
Kroupa, Tomas	P 038	Poster Session 2	07/07/2015	13:30	Poster Area
Krug, Ulrike	P 041	Poster Session 2	07/07/2015	13:30	Poster Area
Kruk, Danuta	S 21	Relaxation and Transport Phenomena	09/07/2015	10:30	Meeting Hall IV
Kuban, Vojtech	P 251	Poster Session 2	07/07/2015	13:30	Poster Area
Kubicki, Dominik	P 362	Poster Session 2	07/07/2015	13:30	Poster Area
Kucherov, Mikhail M.	P 116	Poster Session 2	07/07/2015	13:30	Poster Area
Kudryavtsev, Mikhail	P 119	Poster Session 2	07/07/2015	13:30	Poster Area
Kulminskaya, Natalia	P 201	Poster Session 3	08/07/2015	13:30	Poster Area
Kupce, Eriks	P 197	Poster Session 2	07/07/2015	13:30	Poster Area
Kupce, Eriks	P 254	Poster Session 2	07/07/2015	13:30	Poster Area
Kupce, Eriks	P 194	Poster Session 2	07/07/2015	13:30	Poster Area
		L			
Ladizhansky, Vladimir		Plenary Session 3	07/07/2015	08:45	Forum Hall
Laguta, Valentyn	P 158	Poster Session 2	07/07/2015	13:30	Poster Area
Lai, Angel	P 044	Poster Session 2	07/07/2015	13:30	Poster Area
Lakomek, Nils-Alexander	S 07	Large biomolecular complexes	07/07/2015	11:20	Forum Hall
Lalli, Daniela	S 01	Biosolids	06/07/2015	11:20	Forum Hall
Lamanna, Raffaele	P 200	Poster Session 2	07/07/2015	13:30	Poster Area
Lang, Jan	P 346	Poster Session 1	06/07/2015	13:30	Poster Area

Name	Session		Date	From	Room
Lantto, Perttu	P 299	Poster Session 2	07/07/2015	13:30	Poster Area
Larsen, Flemming Hofmann	P 047	Poster Session 2	07/07/2015	13:30	Poster Area
Le, Nikolay	P 161	Poster Session 2	07/07/2015	13:30	Poster Area
Lee, Mikyung	P 349	Poster Session 1	06/07/2015	13:30	Poster Area
Lee, Sang-Yun	P 164	Poster Session 2	07/07/2015	13:30	Poster Area
Lee, Weontae	P 050	Poster Session 2	07/07/2015	13:30	Poster Area
Lehmkuhl, Sören	P 365	Poster Session 2	07/07/2015	13:30	Poster Area
Lelli, Moreno	S 24	Sensitivity enhancement II	09/07/2015	14:40	Meeting Hall IV
Lendel, Christofer	P 053	Poster Session 2	07/07/2015	13:30	Poster Area
Lescanne, Mathilde	P 056	Poster Session 2	07/07/2015	13:30	Poster Area
Lesot, Philippe	P 203	Poster Session 2	07/07/2015	13:30	Poster Area
Leung, Ivanhoe	S 20	Metabolomics and Small molecules	09/07/2015	11:40	Meeting Hall V
Levitt, Malcolm		Opening and Prize Session	05/07/2015	17:45	Forum Hall
Levitt, Malcolm	S 17	Sensitivity Enhancement I	08/07/2015	15:30	Meeting Hall V
Li, Yifei	P 059	Poster Session 2	07/07/2015	13:30	Poster Area
Li, Zhaokai	P 257	Poster Session 2	07/07/2015	13:30	Poster Area
Lin, Chun-Rong	P 167	Poster Session 2	07/07/2015	13:30	Poster Area
Lin, Yulan	P 260	Poster Session 2	07/07/2015	13:30	Poster Area
Lippens, Guy	S 07	Large biomolecular complexes	07/07/2015	12:00	Forum Hall
Lopez, Juan	P 065	Poster Session 2	07/07/2015	13:30	Poster Area
Louša, Petr	P 068	Poster Session 2	07/07/2015	13:30	Poster Area
Lukavsky, Peter Josef	P 071	Poster Session 2	07/07/2015	13:30	Poster Area
Luy, Burkhard	P 111	Poster Session 3	08/07/2015	13:30	Poster Area
		M			
Madhu, Perunthiruthy	P 263	Poster Session 2	07/07/2015	13:30	Poster Area
Maeda, Hideaki	S 24	Sensitivity enhancement II	09/07/2015	15:00	Meeting Hall IV
Makarova, Katerina	P 206	Poster Session 2	07/07/2015	13:30	Poster Area
Makrocka-Rydzyk, Monika	P 314	Poster Session 2	07/07/2015	13:30	Poster Area
Maloň, Michal	P 266	Poster Session 2	07/07/2015	13:30	Poster Area
Mammoli, Daniele	S 14	Biomolecular Polarization and Relaxation	08/07/2015	11:00	Meeting Hall V
Mance, Deni	S 04	NMR + EPR	06/07/2015	16:40	Forum Hall
Marassi, Francesca	S 16	Biomacromolecules	08/07/2015	17:00	Forum Hall
Martini, Francesca	P 170	Poster Session 2	07/07/2015	13:30	Poster Area
Mathies, Guinevere	P 368	Poster Session 2	07/07/2015	13:30	Poster Area
Mathies, Guinevere	S 17	Sensitivity Enhancement I	08/07/2015	16:40	Meeting Hall V
Matysik, Jörg	S 17	Sensitivity Enhancement I	08/07/2015	17:00	Meeting Hall V
McFeeters, Robert	P 269	Poster Session 2	07/07/2015	13:30	Poster Area
Meersmann, Thomas	S 15	NMR Imaging	08/07/2015	11:00	Meeting Hall IV
Meier, Beat	S 19	Emerging Techniques	09/07/2015	12:00	Forum Hall
Meier, Benno	S 12	Exotica	07/07/2015	16:00	Meeting Hall IV
Meier, Thomas	S 03	NMR - High and Low, Sparse and Dense	06/07/2015	11:40	Meeting Hall IV

Name	Session		Date	From	Room
Meirovitch, Eva	S 06	Protein Relaxation and Dynamics	06/07/2015	17:00	Meeting Hall IV
Melková, Kateřina	P 074	Poster Session 2	07/07/2015	13:30	Poster Area
Mentink-Vigier, Frederic	S 24	Sensitivity enhancement II	09/07/2015	14:20	Meeting Hall IV
Mercurio, Flavia	P 080	Poster Session 2	07/07/2015	13:30	Poster Area
Mineev, Konstantin	P 077	Poster Session 2	07/07/2015	13:30	Poster Area
Minnekhanov, Anton	P 135	Poster Session 3	08/07/2015	13:30	Poster Area
Mitrev, Yavor	P 272	Poster Session 2	07/07/2015	13:30	Poster Area
Miura, Toru	P 209	Poster Session 2	07/07/2015	13:30	Poster Area
Mizuno, Takashi	P 275	Poster Session 2	07/07/2015	13:30	Poster Area
Mlynarz, Piotr	P 212	Poster Session 2	07/07/2015	13:30	Poster Area
Mohoric, Ales	P 321	Poster Session 3	08/07/2015	13:30	Poster Area
Möller, Christina	P 317	Poster Session 2	07/07/2015	13:30	Poster Area
Mollica, Giulia	P 215	Poster Session 2	07/07/2015	13:30	Poster Area
Monteagudo, Eva	P 371	Poster Session 2	07/07/2015	13:30	Poster Area
Montouillout, Valérie	P 138	Poster Session 3	08/07/2015	13:30	Poster Area
Moro, Fabrizio	S 19	Emerging Techniques	09/07/2015	11:20	Forum Hall
Morris, Vanessa	P 083	Poster Session 2	07/07/2015	13:30	Poster Area
Müller, Norbert	S 12	Exotica	07/07/2015	15:30	Meeting Hall IV
Murakami, Miwa	S 21	Relaxation and Transport Phenomena	09/07/2015	11:00	Meeting Hall IV
Myshkin, Mikhail	P 086	Poster Session 2	07/07/2015	13:30	Poster Area
		N			
Nadezhdin, Kirill	P 089	Poster Session 2	07/07/2015	13:30	Poster Area
Nagashima, Toshio	P 278	Poster Session 2	07/07/2015	13:30	Poster Area
Nartowski, Karol	P 218	Poster Session 2	07/07/2015	13:30	Poster Area
Nath, Nilamoni	S 20	Metabolomics and Small molecules	09/07/2015	11:00	Meeting Hall V
Navarro, Romain	P 092	Poster Session 2	07/07/2015	13:30	Poster Area
Neudecker, Philipp	P 095	Poster Session 2	07/07/2015	13:30	Poster Area
Neufeld, Roman	P 281	Poster Session 2	07/07/2015	13:30	Poster Area
Neugebauer, Petr	S 08	NMR Physics	07/07/2015	11:00	Meeting Hall V
Niesteruk, Anna	P 098	Poster Session 2	07/07/2015	13:30	Poster Area
Niklas, Thomas	P 284	Poster Session 2	07/07/2015	13:30	Poster Area
Nishiyama, Yusuke	S 13	Solid State NMR Techniques	08/07/2015	11:00	Forum Hall
Nishiyama, Yusuke		JEOL Symposium	07/07/2015	13:00	Meeting Hall V
Nishiyama, Yusuke		JEOL Symposium	08/07/2015	13:00	Meeting Hall V
Noda, Yasuto	P 221	Poster Session 2	07/07/2015	13:30	Poster Area
Nováček, Jiří	S 16	Biomacromolecules	08/07/2015	16:40	Forum Hall
Novikov, Valentin	P 302	Poster Session 2	07/07/2015	13:30	Poster Area
Nowacka, Agnieszka	P 144	Poster Session 3	08/07/2015	13:30	Poster Area
		O			
Offer, Patrick	P 320	Poster Session 2	07/07/2015	13:30	Poster Area
Ohlenschlaeger, Oliver	P 101	Poster Session 2	07/07/2015	13:30	Poster Area
Olaru, Alexandra M.	P 374	Poster Session 2	07/07/2015	13:30	Poster Area

Name	Session		Date	From	Room
Orekhov, Vladislav	S 03	NMR - High and Low, Sparse and Dense	06/07/2015	12:00	Meeting Hall IV
Orlova, Anna	P 240	Poster Session 3	08/07/2015	13:30	Poster Area
Orts, Julien	P 224	Poster Session 2	07/07/2015	13:30	Poster Area
		P			
Paczwa, Mateusz	P 147	Poster Session 3	08/07/2015	13:30	Poster Area
Paik, Younkee	P 227	Poster Session 2	07/07/2015	13:30	Poster Area
Palfy, Gyula	P 104	Poster Session 2	07/07/2015	13:30	Poster Area
Palisi, Angelica	P 230	Poster Session 2	07/07/2015	13:30	Poster Area
Palmer, Arthur		Tutorial Lectures	05/07/2015	15:30	Forum Hall
Palomino Schätzlein, Martina	P 233	Poster Session 2	07/07/2015	13:30	Poster Area
Parella, Teodor	P 180	Poster Session 3	08/07/2015	13:30	Poster Area
Parella, Teodor	P 177	Poster Session 3	08/07/2015	13:30	Poster Area
Parella, Teodor	P 174	Poster Session 3	08/07/2015	13:30	Poster Area
Parella, Teodor		Tutorial Lectures	05/07/2015	14:00	Forum Hall
Pavlovskaya, Galina	P 107	Poster Session 2	07/07/2015	13:30	Poster Area
Pecher, Oliver	P 243	Poster Session 3	08/07/2015	13:30	Poster Area
Pell, Andrew	P 305	Poster Session 2	07/07/2015	13:30	Poster Area
Pianet, Isabelle	P 183	Poster Session 3	08/07/2015	13:30	Poster Area
Piechatzek, Timo	P 003	Poster Session 3	08/07/2015	13:30	Poster Area
Pierattelli, Roberta	S 10	Biomacromolecular Folding and Dynamics	07/07/2015	17:00	Forum Hall
Pigliapochi, Roberta	P 308	Poster Session 2	07/07/2015	13:30	Poster Area
Pileio, Giuseppe	S 21	Relaxation and Transport Phenomena	09/07/2015	11:40	Meeting Hall IV
Pinto, Cecilia	S 07	Large biomolecular complexes	07/07/2015	11:00	Forum Hall
Plainchont, Bertrand	S 18	Computation and Processing	08/07/2015	16:40	Meeting Hall IV
Plavec, Janez	S 10	Biomacromolecular Folding and Dynamics	07/07/2015	16:00	Forum Hall
Polenova, Tatyana		Plenary Session 7	09/07/2015	09:25	Forum Hall
Poluektov, Oleg	S 23	Paramagnetic Systems	09/07/2015	14:40	Meeting Hall V
Polyhach, Yevhen	P 006	Poster Session 3	08/07/2015	13:30	Poster Area
Pons, Miquel	S 22	Disordered proteins	09/07/2015	13:30	Forum Hall
Popenda, Lukasz	P 186	Poster Session 3	08/07/2015	13:30	Poster Area
Power, Jane	S 03	NMR - High and Low, Sparse and Dense	06/07/2015	11:20	Meeting Hall IV
Prade, Elke	P 189	Poster Session 3	08/07/2015	13:30	Poster Area
Pravdivtsev, Andrey	S 17	Sensitivity Enhancement I	08/07/2015	16:20	Meeting Hall V
Prchal, Jan	P 009	Poster Session 3	08/07/2015	13:30	Poster Area
Prestel, Andreas	P 012	Poster Session 3	08/07/2015	13:30	Poster Area
Pribitzer, Stephan	P 246	Poster Session 3	08/07/2015	13:30	Poster Area
Procházková, Eliška	P 192	Poster Session 3	08/07/2015	13:30	Poster Area
Pruski, Marek	S 05	Materials NMR	06/07/2015	17:00	Meeting Hall V
Prusova, Alena	S 15	NMR Imaging	08/07/2015	11:20	Meeting Hall IV
		Q			

Name	Session		Date	From	Room
Qureshi, Nusrat	P 015	Poster Session 3	08/07/2015	13:30	Poster Area
		R			
Rachocki, Adam	P 323	Poster Session 2	07/07/2015	13:30	Poster Area
Radecki, Marek	P 326	Poster Session 2	07/07/2015	13:30	Poster Area
Ramanathan, Chandrasekhar	S 12	Exotica	07/07/2015	17:00	Meeting Hall IV
Ramirez Cohen, Marie	P 311	Poster Session 2	07/07/2015	13:30	Poster Area
Rantaharju, Jyrki	P 288	Poster Session 3	08/07/2015	13:30	Poster Area
Rastrelli, Federico	S 14	Biomolecular Polarization and Relaxation	08/07/2015	11:20	Meeting Hall V
Rathner, Adriana	P 018	Poster Session 3	08/07/2015	13:30	Poster Area
Rathner, Petr	P 021	Poster Session 3	08/07/2015	13:30	Poster Area
Redfield, Christina	P 024	Poster Session 3	08/07/2015	13:30	Poster Area
Reinsperger, Tony	P 377	Poster Session 2	07/07/2015	13:30	Poster Area
Řezníček, Richard	P 153	Poster Session 3	08/07/2015	13:30	Poster Area
Ribot, Francois	P 329	Poster Session 2	07/07/2015	13:30	Poster Area
Rieger, Stefan	P 249	Poster Session 3	08/07/2015	13:30	Poster Area
Rienstra, Chad	S 20	Metabolomics and Small molecules	09/07/2015	12:00	Meeting Hall V
Rodin, Victor	S 12	Exotica	07/07/2015	16:20	Meeting Hall IV
Rogov, Vladimir	P 027	Poster Session 3	08/07/2015	13:30	Poster Area
Rong, Xing	P 252	Poster Session 3	08/07/2015	13:30	Poster Area
Rosay, Melanie		Plenary Session 8	09/07/2015	16:10	Forum Hall
Rösch, Paul	P 030	Poster Session 3	08/07/2015	13:30	Poster Area
Rossini, Aaron	P 354	Poster Session 3	08/07/2015	13:30	Poster Area
Rouf, Syed Awais	P 114	Poster Session 3	08/07/2015	13:30	Poster Area
Roumestand, Christian	S 22	Disordered proteins	09/07/2015	14:20	Forum Hall
		S			
Saalwächter, Kay	S 11	New Approaches to the MR Measurement	07/07/2015	15:30	Meeting Hall V
Salinas, Roberto	P 033	Poster Session 3	08/07/2015	13:30	Poster Area
Salvia, Marie-Virginie	P 255	Poster Session 3	08/07/2015	13:30	Poster Area
Samoson, Ago	P 036	Poster Session 3	08/07/2015	13:30	Poster Area
Saxena, Saurabh	P 258	Poster Session 3	08/07/2015	13:30	Poster Area
Scheler, Ulrich	S 05	Materials NMR	06/07/2015	16:20	Meeting Hall V
Schleicher, Erik	S 23	Paramagnetic Systems	09/07/2015	14:00	Meeting Hall V
Schmidt, Peter	P 039	Poster Session 3	08/07/2015	13:30	Poster Area
Schmidt, Rita	S 11	New Approaches to the MR Measurement	07/07/2015	16:20	Meeting Hall V
Schneider, Robert	S 22	Disordered proteins	09/07/2015	14:40	Forum Hall
Schubeis, Tobias	P 042	Poster Session 3	08/07/2015	13:30	Poster Area
Schubert, Mario	P 261	Poster Session 3	08/07/2015	13:30	Poster Area
Schulze-Sünninghausen, David	P 195	Poster Session 3	08/07/2015	13:30	Poster Area
Schwalbe, Harald	S 10	Biomacromolecular Folding and Dynamics	07/07/2015	15:30	Forum Hall
Segawa, Takuya	P 291	Poster Session 3	08/07/2015	13:30	Poster Area

Name	Session		Date	From	Room
Sharma, Swati	P 156	Poster Session 3	08/07/2015	13:30	Poster Area
Shchukina, Alexandra	P 198	Poster Session 3	08/07/2015	13:30	Poster Area
Shekhtman, Alexander	S 09	In-vivo and In-cell NMR	07/07/2015	11:40	Meeting Hall IV
Shenkarev, Zakhar	P 048	Poster Session 3	08/07/2015	13:30	Poster Area
Shenkarev, Zakhar	P 045	Poster Session 3	08/07/2015	13:30	Poster Area
Shevelkov, Veniamin	S 01	Biosolids	06/07/2015	11:40	Forum Hall
Shi, Fazhan	P 264	Poster Session 3	08/07/2015	13:30	Poster Area
Shi, Fazhan	S 19	Emerging Techniques	09/07/2015	11:00	Forum Hall
Shih, Kun-Yauh	P 236	Poster Session 2	07/07/2015	13:30	Poster Area
Shilova, Irina	P 294	Poster Session 3	08/07/2015	13:30	Poster Area
Shimada, Ichio	S 09	In-vivo and In-cell NMR	07/07/2015	12:00	Meeting Hall IV
Shkop, Eva	P 051	Poster Session 3	08/07/2015	13:30	Poster Area
Shubhajit, Paul	P 054	Poster Session 3	08/07/2015	13:30	Poster Area
Shukla, Matsyendra Nath	P 335	Poster Session 2	07/07/2015	13:30	Poster Area
Simenas, Mantas	P 297	Poster Session 3	08/07/2015	13:30	Poster Area
Sitnik, Malgorzata	P 057	Poster Session 3	08/07/2015	13:30	Poster Area
Skovpin, Ivan	P 363	Poster Session 3	08/07/2015	13:30	Poster Area
Skovpin, Ivan	P 360	Poster Session 3	08/07/2015	13:30	Poster Area
Smith, Colin	P 060	Poster Session 3	08/07/2015	13:30	Poster Area
Smolarkiewicz, Iga	P 159	Poster Session 3	08/07/2015	13:30	Poster Area
Soleilhavoup, Anne	P 338	Poster Session 2	07/07/2015	13:30	Poster Area
Šoltésová, Mária	P 341	Poster Session 2	07/07/2015	13:30	Poster Area
Spadaccini, Roberta	P 063	Poster Session 3	08/07/2015	13:30	Poster Area
Splith, Tobias	P 344	Poster Session 2	07/07/2015	13:30	Poster Area
Srb, Pavel	P 347	Poster Session 2	07/07/2015	13:30	Poster Area
Stapf, Siegfried	P 318	Poster Session 3	08/07/2015	13:30	Poster Area
Stapf, Siegfried	P 315	Poster Session 3	08/07/2015	13:30	Poster Area
Stapf, Siegfried	S 11	New Approaches to the MR Measurement	07/07/2015	17:00	Meeting Hall V
Stepišnik, Janez	P 324	Poster Session 3	08/07/2015	13:30	Poster Area
Stevanato, Gabriele	P 366	Poster Session 3	08/07/2015	13:30	Poster Area
Straasoe, Lasse Arnt	P 066	Poster Session 3	08/07/2015	13:30	Poster Area
Straka, Michal	S 05	Materials NMR	06/07/2015	16:40	Meeting Hall V
Strotz, Dean	P 253	Poster Session 1	06/07/2015	13:30	Poster Area
Su, Xun-Cheng	P 300	Poster Session 3	08/07/2015	13:30	Poster Area
Suvorina, Svetlana	P 303	Poster Session 3	08/07/2015	13:30	Poster Area
Sykora, Jan	P 330	Poster Session 3	08/07/2015	13:30	Poster Area
Szczesniak, Katarzyna	P 333	Poster Session 3	08/07/2015	13:30	Poster Area
		T			
Takahashi, Masato	P 267	Poster Session 3	08/07/2015	13:30	Poster Area
Takegoshi, Kiyonori		Plenary Session 3	07/07/2015	09:25	Forum Hall
Taşdemir, Halil Uğur	P 306	Poster Session 3	08/07/2015	13:30	Poster Area
Tate, Shin-ichi	P 069	Poster Session 3	08/07/2015	13:30	Poster Area
Tayler, Michael	S 03	NMR - High and Low, Sparse and Dense	06/07/2015	11:00	Meeting Hall IV
Telkki, Ville-Veikko	S 21	Relaxation and Transport Phenomena	09/07/2015	11:20	Meeting Hall IV

Name	Session		Date	From	Room
Teymouri, Yadollah	P 162	Poster Session 3	08/07/2015	13:30	Poster Area
Theis, Thomas	P 369	Poster Session 3	08/07/2015	13:30	Poster Area
Theis, Thomas	S 17	Sensitivity Enhancement I	08/07/2015	16:00	Meeting Hall V
Thiele, Christina		Plenary Session 1	06/07/2015	08:45	Forum Hall
Timmel, Christiane	S 23	Paramagnetic Systems	09/07/2015	15:00	Meeting Hall V
Tjandra, Nico	S 14	Biomolecular Polarization and Relaxation	08/07/2015	12:00	Meeting Hall V
Tkáč, Ivan	S 15	NMR Imaging	08/07/2015	10:30	Meeting Hall IV
Tkach, Igor	P 273	Poster Session 3	08/07/2015	13:30	Poster Area
Tkach, Igor	P 270	Poster Session 3	08/07/2015	13:30	Poster Area
Todokoro, Yasuto	P 072	Poster Session 3	08/07/2015	13:30	Poster Area
Tošner, Zdeněk	P 276	Poster Session 3	08/07/2015	13:30	Poster Area
Trautwein, Christoph	P 204	Poster Session 3	08/07/2015	13:30	Poster Area
Trease, Nicole	S 15	NMR Imaging	08/07/2015	11:40	Meeting Hall IV
Tritt-Goc, Jadwiga	P 336	Poster Session 3	08/07/2015	13:30	Poster Area
Tsiafoulis, Constantinos	P 210	Poster Session 3	08/07/2015	13:30	Poster Area
Tsiafoulis, Constantinos	P 207	Poster Session 3	08/07/2015	13:30	Poster Area
Tzvetkova, Pavleta	P 213	Poster Session 3	08/07/2015	13:30	Poster Area
		U			
Ubbink, Marcellus	S 23	Paramagnetic Systems	09/07/2015	13:30	Meeting Hall V
Uddin, Jalal	P 216	Poster Session 3	08/07/2015	13:30	Poster Area
Ude, Johanna	P 075	Poster Session 3	08/07/2015	13:30	Poster Area
Umegawa, Yuichi	P 309	Poster Session 3	08/07/2015	13:30	Poster Area
Urban, Anatoly	P 078	Poster Session 3	08/07/2015	13:30	Poster Area
Urbanczyk, Mateusz	P 339	Poster Session 3	08/07/2015	13:30	Poster Area
		V			
Vallance, Cara	P 219	Poster Session 3	08/07/2015	13:30	Poster Area
van Rijn, Joep	P 081	Poster Session 3	08/07/2015	13:30	Poster Area
Varghese, Sabu	P 084	Poster Session 3	08/07/2015	13:30	Poster Area
Vega, Shimon	S 08	NMR Physics	07/07/2015	12:00	Meeting Hall V
Venancio, Tiago	P 342	Poster Session 3	08/07/2015	13:30	Poster Area
Verasdonck, Joeri	P 087	Poster Session 3	08/07/2015	13:30	Poster Area
Viegas, Aldino	S 19	Emerging Techniques	09/07/2015	11:40	Forum Hall
Viennet, Thibault	P 372	Poster Session 3	08/07/2015	13:30	Poster Area
Vitorge, Bruno	P 090	Poster Session 3	08/07/2015	13:30	Poster Area
Vögeli, Beat	S 06	Protein Relaxation and Dynamics	06/07/2015	16:20	Meeting Hall IV
Vostrikov, Vitaly	P 093	Poster Session 3	08/07/2015	13:30	Poster Area
Vrzal, Lukas	P 222	Poster Session 3	08/07/2015	13:30	Poster Area
Vuichoud, Basile	S 24	Sensitivity enhancement II	09/07/2015	14:00	Meeting Hall IV
		W			
Wälti, Marielle	P 345	Poster Session 3	08/07/2015	13:30	Poster Area
Wang, Ying-Hui	P 096	Poster Session 3	08/07/2015	13:30	Poster Area
Warnet, Xavier	P 126	Poster Session 3	08/07/2015	13:30	Poster Area
Waudby, Christopher	S 18	Computation and Processing	08/07/2015	16:00	Meeting Hall IV
Webb, Andrew		Plenary Session 6	08/07/2015	17:45	Forum Hall

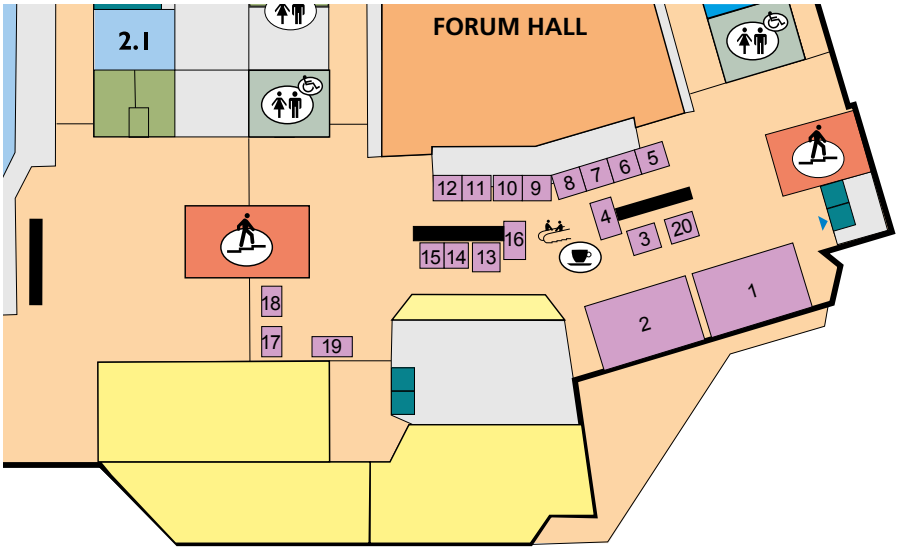
Name	Session		Date	From	Room
Weber, Julia	P 117	Poster Session 3	08/07/2015	13:30	Poster Area
Weickert, Sabrina	P 099	Poster Session 3	08/07/2015	13:30	Poster Area
Wessig, Martin	P 348	Poster Session 3	08/07/2015	13:30	Poster Area
Wider, Gerhard	S 07	Large biomolecular complexes	07/07/2015	11:40	Forum Hall
Wilman, James	P 312	Poster Session 3	08/07/2015	13:30	Poster Area
Wisser, Dorothea	P 165	Poster Session 3	08/07/2015	13:30	Poster Area
Wittwer, Matthias	P 225	Poster Session 3	08/07/2015	13:30	Poster Area
Wojtowicz, Wojciech	P 228	Poster Session 3	08/07/2015	13:30	Poster Area
Woods, Jason	P 129	Poster Session 3	08/07/2015	13:30	Poster Area
Wright, Peter E.	S 14	Biomolecular Polarization and Relaxation	08/07/2015	10:30	Meeting Hall V
		Y			
Yamada, Kazuhiko	P 279	Poster Session 3	08/07/2015	13:30	Poster Area
Yao, Shenggen	P 102	Poster Session 3	08/07/2015	13:30	Poster Area
Yilmaz, Ali	P 231	Poster Session 3	08/07/2015	13:30	Poster Area
Yoshimura, Yuichi	P 282	Poster Session 3	08/07/2015	13:30	Poster Area
Yulikov, Maxim	S 04	NMR + EPR	06/07/2015	16:00	Forum Hall
		Z			
Ząbek, Adam	P 234	Poster Session 3	08/07/2015	13:30	Poster Area
Zachrdla, Milan	P 105	Poster Session 3	08/07/2015	13:30	Poster Area
Zahan, Marufa	P 168	Poster Session 3	08/07/2015	13:30	Poster Area
Zapletal, Vojtěch	P 351	Poster Session 3	08/07/2015	13:30	Poster Area
Zawada, Katarzyna	P 237	Poster Session 3	08/07/2015	13:30	Poster Area
Zawadzka-Kazimierczuk, Anna	P 108	Poster Session 3	08/07/2015	13:30	Poster Area
Zerbetto, Mirco	P 120	Poster Session 3	08/07/2015	13:30	Poster Area
Żerko, Szymon	P 285	Poster Session 3	08/07/2015	13:30	Poster Area
Zhang, Jie	P 171	Poster Session 3	08/07/2015	13:30	Poster Area
Zhivonitko, Vladimir	P 378	Poster Session 3	08/07/2015	13:30	Poster Area
Zhivonitko, Vladimir	P 375	Poster Session 3	08/07/2015	13:30	Poster Area
Žídek, Lukáš	S 10	Biomacromolecular Folding and Dynamics	07/07/2015	16:40	Forum Hall

EXHIBITION

Exhibition Opening Hours

5 July:	13:00–21:00
6 July:	08:00–19:00
7 July:	08:00–21:00
8 July:	08:00–19:00
9 July:	08:00–17:30

EXHIBITION FLOOR PLAN



1 BRUKER
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8 NANALYSIS CORP.
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12 MAGIC ANGLE
11 INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY
13 WILEY
14 CORTECNET
15 MTÜ NMR INSTITUUT
16 MAGRITEK
17 MESTRELAB
18 ADANI SYSTEMS
19 WAKO PURE CHEMICAL INDUSTRIES
20 THERMO SCIENTIFIC

LIST OF EXHIBITORS / PROFILES

Booth number: 18

ADANI

ADANI designs and manufactures a range of compact tools for quality control and molecular research. ADANI's research grade bench-top CMS 8400 ESR(EPR) Spectrometer is competitively priced instrument specifically designed to meet the needs of scientific research and application-oriented tasks in materials science, analytical chemistry, process control, medical and pharmaceutical research.

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Booth number: 7

ARMAR AG (ARMAR Chemicals)

ARMAR AG is a producer of NMR-Solvents and Deuterated Compounds with a worldwide distribution structure. Additional we are retailer for NMR and EPR products of Wilmad LabGlass, USA. ARMAR AG was founded 1986 and since 2006 we have a subsidiary company in Germany, ARMAR (Europa) GmbH.

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Booth number: 1

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CIL is the world leader in the manufacture and separation of stable isotopes and stable isotope-labeled compounds for use in traditional and biomolecular NMR. Our large inventory includes NMR solvents and reference standards, cell growth media and reagents for labeling recombinant protein expressed in E. coli, insect and mammalian cells, amino acids, deuterated buffers and nucleotide-5'-triphosphates for labeling RNA and DNA.

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Booth number: 14

CortecNet SAS

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INNOVATION ACCELERATOR IN LIFE SCIENCE

Since more than 15 years, CortecNet is known as one if the most reliable supplier of NMR consumables and stable isotope enriched products. CortecNet is composed of a team of analytical engineers capable to understand and fulfill all your needs. Over the years, CortecNet has developed a strong professional network including thousands of academic labs and industrial companies all around the world.

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Booth number: 10

Cryogenic Ltd.

CRYOGENIC

Magnets for Magnetic Resonance experiments require high homogeneity; Cryogenic manufactures magnets types using cryogen free technology including EPR/ESR, NMR, MRI and magnets for Gyrotron applications.

With the power supplies provided by Cryogenic it is possible to set and measure the current accurately at the ppm level and allow for accurate and smooth ramping of the magnet current.

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Booth number: 4

EURISO-TOP

Euriso-top is a European leading producer of deuterated solvents and stable isotope labeled compounds for various applications dealing with NMR field of studies. With a permanent stock of more than 1000 products, Euriso-top can supply you with an exceptional technical service and a quick product delivery.

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Booth number: 2

JEOL

For more than 50 years, JEOL has been known for its wide range of NMR solutions tailored to meet the requests of academic, industrial and government customers; from routine experiments to advanced research.

We recently introduced the new ECZ-S and ECZ-R NMR Series. These systems incorporate the latest digital and high frequency technologies and offer both flexibility and expandability.

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Booth number: 12

Magic Angle

Magic Angle GmbH & Co. KG is a company which specialises in LOGS: Lab Organisation Group Sites. LOGS is a spectrum repository which allows scientific workgroups to automatically save and link relevant data. At this time, LOGS is focused on groups working in the field of magnetic resonance, i.e. NMR and EPR.

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Booth number: 16

Magritek

Founded in 2004, Magritek is a world leading company creating cryogen-free, compact NMR and MRI systems that work on the benchtop.

Magritek takes great pride in the superior technical performance and quality of the products, and in the comprehensive after sales care provided to customers.

The most revolutionary product is the Spinsolve benchtop NMR spectrometer that provides exceptional performance in a low-cost and compact package.

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Booth number: 17

MESTRELAB RESEARCH, S.L.

Mestrelab Research develops Mnova: multipage, multivendor and multiplatform analytical chemistry software suite designed for NMR & LC/GC/MS Processing, Analysis, Databasing, Managing and Reporting.

Our mission has been to deliver top quality software tools for the scientific community and to push the state-of-the-art in Graphical User Interfaces, software integration and algorithmia.

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Booth number: 8

NANALYSIS CORP.

Nanalysis was established in 2009. The company develops and manufactures compact Nuclear Magnetic Resonance (NMR) spectrometers for the laboratory instrumentation market. In the fall of 2012, the first product was launched: NMReady™, the first fully featured portable NMR spectrometer in a single compact enclosure requiring no liquid helium or any other cryogens. The NMReady is used by chemical professionals in all types of industries (oil & gas, chemical, pharma, biotech, food processing) as well as government and university labs.

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Booth number: 15

NMR Institute

Nuclear Magnetic Resonance Institute is a non-profit association, founded by national innovation award team. NMRI promotes analytical methods, equipment and applications, most notably very fast and “smart” magic angle spinning. Present application focus is on energy storage, cell-environment interaction and personal lifestyle optimization. Precision mechanics, radio-frequency engineering and metabolome based virtual biomarker study are our main competences.

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Website: www.nmri.eu

Booth number: 15

NMR/Bio

NMR-Bio distributes patented labelled precursors for the production of methyl-specific labelled proteins with stable isotopes.

NMR-Bio offers also a wide range of customized NMR services such as:

- Customized synthesis of isotopically labelled protein;
- High-field NMR data acquisition and analysis.

NMR-Bio is located in Grenoble, France.

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Website: www.nmr-bio.com

Booth number: 10

Phoenix NMR LLC

PhoenixNMR provides a complete line of solid state NMR probes including frequencies from 400–900 MHz, 1.6–6 mm spinning systems, double, triple and quadruple resonance and options such as low gamma capability and lock channel. PhoenixNMR also provides repair services for recent Varian and all Agilent solid state NMR probes.

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Website: www.phoenixnmr.com

Booth number: 5

Pure Devices GmbH

Pure Devices GmbH is a manufacturer of state-of-the-art portable and bench-top MRI scanners for education and research. Furthermore Pure Devices provides external gradient and RF amplifiers especially for applications in bench-top MRI. All our products are designed and made in Germany.

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Website: www.pure-devices.com

Booth number: 10

Revolution NMR LLC

Revolution NMR supplies spinning systems, spinning system components, and specialty probes for solid state NMR. Revolution components are compatible with Chemagnetics, Varian and Agilent solid state probes. Revolution also provides repair services and upgrades for Chemagnetics and older Varian probes.

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Booth number: 7

Rototec-Spintec

Rototec-Spintec (Originally Spintec), founded in December 1983 in Southern Germany, opened it's doors in March of 1984 and are specialised suppliers of NMR/EPR spectroscopy consumables, partial or complete NMR/MRI/EPR systems, and accessories.

With our products we offer solutions to routine problems encountered in the day to day activities of both Liquids and Solids NMR as well as EPR, including some novel solutions to both old problems, and new ones as they are encountered.

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Booth number: 3

ISOTEC/Sigma-Aldrich

Sigma-Aldrich, a leading Life Science and High Technology company focused on enhancing human health and safety, manufactures and distributes more than 230,000 chemicals, biochemicals, stable isotopes and other essential products to more than 1.4 million customers globally in research labs as well as in industrial and commercial markets.

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Website: www.sigmaaldrich.com/chemistry/stable-isotopes-isotec.html

Booth number: 6

Silantes GmbH

Silantes activities are focused on the production and marketing of compounds labelled with stable isotopes (SI: 2H, 13C, 15N) used in NMR structural analysis and quantitative mass spectrometry. Silantes is specialized on SILAC/SILAM-products.

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Booth number: 20

Thermo Scientific

Thermo Fisher Scientific Inc. is the world leader in serving science, with revenues of $17 billion and approximately 50,000 employees in 50 countries. Our mission is to enable our customers to make the world healthier, cleaner and safer.

Our compact and affordable NMR spectrometers help customers to solve their complex analytical challenges either in the lab or in the classroom.

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Website: www.thermoscientific.com/nmr

Booth number: 11

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic

The mission of the Institute is independent basic research in organic chemistry and biochemistry with strong aspects of application of the results in praxis. The research is oriented mainly to the following fields: nucleic acid components, proteins, peptides, natural products, synthetic functional molecules and molecular modelling. The aim of the Institute is to reach excellence in the international competition and to keep this position in the long term.

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Website: www.uochb.cz

Booth number: 19

Wako Pure Chemical Industries, Ltd.

As Japan's leading reagent company Wako Pure Chemical Industries, Ltd., we believe in supporting your R&D endeavors and addressing next generation needs through continued excellence in technology and quality.

Please Visit Wako's Online Catalog! www.e-reagent.com.

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Booth number: 13

MRC/A Wiley journal

MRC is a leading international journal devoted to publishing research and development of magnetic resonance techniques and their applications in any field of chemistry. Published by Wiley, and available via an app, the journal is part of a wide resource of magnetic resonance information spanning books, journals, databases, web-portals and workflow tools.

Contact Person: Paul Trevorrow
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Website: www.wileyonlinelibrary.com/journal/mrc

MEDIA PARTNER

www.technologynetworks.com

TECHNOLOGY NETWORKS

CONGRESS INFORMATION A–Z

A

Abstract Book
Will be available in electronic version only for download from EUROMAR 2015 website.

Airport
Reserve 2 hours to travel to Václav Havel Airport Prague from the PCC. There are good connections between the airport and the city centre by public transport.

Airport information

Tel.: +420 220 111 888

www.prg.aero

B

Badges
Name badge must be worn at all times when attending the sessions and official programme.

C

Cash Points/ATMs
Komercni Banka cash point is located right between the entrances No. 5 and 6.

Ceska sporitelna cash point is located next to the underground station Vyšehrad entrance.

Certificate of Attendance
All registered delegates present on-site are entitled to receive a Certificate of Attendance. Certificates can be requested at the Registration Desk as of Wednesday, 8 July afternoon.

Cloakroom
Cloakroom is located on the 1st floor besides the registration desk; service is provided free of charge to all registered participants during 5–10 July 2015.

Congress Language
The congress language is English, no translation is provided.

Currency/Exchange
The Czech currency is called the Czech crown (CZK). Exchange offices are located all around the city centre (exchange offices, banks, post offices). All rates given in the program are in EUROS (€).

D

Disclaimer
The Congress Organisers have taken all reasonable care in making arrangements for the Congress. In the event of unforeseen disruptions, neither the organisers nor their agents can be held responsible for any losses or damages incurred by delegates. The programme is correct at the time of printing, but organisers reserve the right to alter the programme if and when deemed necessary. The Congress Organisers act as agents only in securing hotels, transport and travel services, and shall in no event be liable for acts or commissions in the event of injury, damage, loss, accident delay or irregularity of any kind whatsoever during arrangements organised through contractors or by the employees of such contractors. Hotel and transportation services are subject to the terms and conditions under which they are offered to the general public. Delegates should make their own arrangements with respect to personal insurance. The Congress Organisers reserve the right to make changes as and when deemed

necessary, without prior notice to the parties concerned. All disputes are subject to resolution under Czech Law.

Doctor / First Aid

Poliklinika Budejovicka is located at the station Budejovicka 3 underground stations from the station Vysehrad (location of the venue).

MEDICON a.s. – Poliklinika Budejovicka

Address: Antala Staska 1670/80, 140 46 Praha 4

Tel.: +420 261 006 111

Fax: +420 261 006 210

Email: info@mediconas.cz

www.mediconas.cz

E

Emergency call	112
Police	158
Fire Department	150
Medical Service	155

F

First Aid

No first aid is available at the Prague Congress Centre. In case of emergency, dial 112 to get specialised help.

Food and Beverages

Coffee breaks and Lunches are included in the registration fee and will be served within the Exhibition area (Foyer 2nd floor) in allocated times. There is a Cafetería/ Bistro in the Holiday Inn hotel (1 minute from PCC entrance No 5) to buy refreshments.

G

Guided Tours

Accompanying person guided tour

Tuesday, 7 July in 9:00–13:00 (duration 4 hours)

Make sure you are at the Registration desk, 1st floor of PCC 15 min prior to scheduled departure.

Old Town & Jewish Quarter

Friday, 10 July in 9:00–13:00 (duration 4 hours)

Grand Tour of Prague

Friday, 10 July in 13:00–17:00 (duration 4 hours)

For tours on 10 July please wait in front of Entrance No. 5 of PCC 15 min prior to scheduled departure

or call +420 727 803 210 (no registration desk on this day).

Please note these are WALKING TOURS, transfers from PCC to Prague Castle/ city centre and back to PCC is included. No refreshments are included. English speaking guide.

I

Insurance and Liability

The Organisers will accept no liability for personal injuries sustained by or for loss or damage to property belonging to Congress participants, accompanying persons either during or as a result of the Congress or during all tours and events. Participants are strongly recommended to seek insurance coverage for health and accident, lost luggage and trip cancellation.

Internet

Free Wi-Fi internet connection is available in the 2nd Floor foyer only for all delegates.

Network name:	EUROMAR2015
Password:	EUROMAR2015

L

Lost & Found

A lost and found service is available at the information desk at the registration.

M

Mobile Phones

Participants are kindly requested to keep their mobile phones in the off position in the meeting rooms while sessions are being held.

P

Pharmacy

Pharmacy is located in the shopping centre Arkady Pankrac – 2 underground stations from the Vysehrad station (venue location).

Shopping Centre Arkady Pankrac – ground floor

Na Pankraci 86, 140 00 Praha 4.

Tel.: +420 225 111 211
www.lekarnapankrac.cz

Opening hours:
Monday – Sunday in 9:00–21:00

Poster Area

Poster area is placed on the 2nd floor in the Terrace I + II and the North Hall. For details please see page 4.

Programme Changes

The organisers cannot assume liability for any changes in the programme due to the external or unforeseen circumstances.

Public Transport

Each delegate gets a Public Transport Pass valid in all means of public transportation system (metro, tram, bus) **during the Congress dates of July 5–10, 2015**. There is no need to stamp/validate the ticket upon entering the journey. Please make sure to have it with you at all times when using Prague public transport. The ticket is not transferable. Please check with the Registration desk for actual usage.

Prague has sophisticated underground, tram and bus transportation system. During the peak hours, underground trains run every 1 or 2 minutes, and during off-peak hours at least every 10 minutes.

For more information about Prague public transportation visit www.dpp.cz

R

Registration Opening Hours

Sunday, July 5	12:00–19:30
Monday, July 6	07:45–19:30
Tuesday, July 7	08:30–19:30
Wednesday, July 8	08:30–19:00
Thursday, July 9	08:30–18:00

Telephone number to registration desk: +420 727 803 210

Registration desk is located on the first floor of the Prague Congress Centre.

S

Shopping

Most shops in Prague are open from 9:00 to 18:00, Monday through Saturday. Shops in the city centre are usually open from 9:00 – 20:00, Monday through Sunday.

Smoking Policy

Please note that smoking is not permitted in the venue.

Speakers' Ready Room

Is located in Room 2.2 on the 2nd floor. Please make sure to hand in your presentation at least 120 minutes prior to the start of your assigned session. Our staff in the

speakers' ready room will be happy to assist you during the opening hours:

Sunday, July 5	12:00 – 19:30
Monday, July 6	08:00 – 19:30
Tuesday, July 7	08:00 – 19:30
Wednesday, July 8	08:00 – 18:00
Thursday, July 9	08:00 – 16:00

T

Taxi

In the city centre taxis are easy to hail from the street but we strongly recommend that you use hotel taxis or obtain taxis by phone through the radio taxi service e.g. AAA (+420 14 014), City taxi (+420 257 257 257) or Speed cars (+420 224 234 234).

Boarding charge: approximately 40 CZK.

Journeys within the city: approximately 28 CZK/ 1 kilometre.

Tipping

Service is usually included in the bill in bars and restaurants but tips are welcome. If you consider the service good enough to warrant a tip, suggested level is around 10 %.

V

VAT

The Czech legislation requires that all congress costs includes the Czech VAT (21 % or 15 %). In case the VAT rate changes, the change will automatically apply to the service ordered.

Venue

Prague Congress Centre
5. května 65
140 21 Prague 4
Czech Republic

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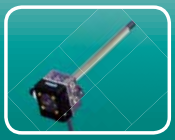

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Abstract Book

Contents

Tutorial Lectures

TL 1
MODERN PURE SHIFT NMR: PROS AND CONS

T. Parella1
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In recent years, a great interest in the development of new broadband 1H homonuclear decoupled techniques providing **simplified JHH multiplet patterns has emerged again in the field of small m**olecule NMR. The resulting highly resolved 1H NMR spectra display resonances as collapsed singlets, therefore minimizing signal overlap and expediting spectral analysis. This tutorial aims at presenting the most recent advances in pure shift NMR spectroscopy, with a particular emphasis to the Zangger–Sterk experiment. A detailed discussion about the most relevant practical aspects in terms of pulse sequence design, selectivity, sensitivity, spectral resolution and performance will be provided. The implementation of the different reported strategies into traditional 1D and 2D NMR experiments will be described while several practical applications will also be reviewed.

TL 2
DETECTION SENSITIVITY IN MAGNETIC RESONANCE

A. Blank[1]
[1]*Technion - Israel Institute of Technology, Chemistry, Haifa, Israel*

Magnetic resonance is a very powerful analytical technique, but notorious for its lack of sensitivity compared to other analytic approaches, such as mass spectrometry and fluorescence. The detection capability of a small number of electron or nuclear spins is of significant importance to many field of science, medicine, and technology. Therefore over the years various methods and approaches were pursued in an effort to overcome this limitation. In this tutorial talk I will first provide the basic expressions and discuss the factors affecting the sensitivity of conventional induction detection NMR and ESR. This would enable to better realize the reasons for the limitations of conventional detection approaches. Following that I will present some modern "non-conventional" approaches that involve, for example, force, electrical and optical detection methodologies and briefly discuss their pros and cons. In the third and last part of the talk I will go back to the "good old" induction-detection approach and check what can still be done to greatly improve detection capability without abandoning it.

TL 3
APPLICATIONS OF NMR SPIN RELAXATION TO CONFORMATIONAL DYNAMICS OF PROTEINS

A. Palmer[1]
[1]*Columbia University Medical Center, Biochemistry and Molecular Biophysics, New York, USA*

Relaxation processes restore the nuclear spin magnetization to Boltzmann equilibrium and consequently have a fundamental role in all applications of NMR spectroscopy from physics to medicine. Longitudinal and transverse relaxation rate constants, the nuclear Overhauser effect, and cross-correlated relaxation rate constants manifest complex phenomena in NMR experiments and provide detailed information on molecular structure and dynamics. This tutorial lecture will discuss the principal experimental techniques used to characterize macromolecular dynamics on time scales from picoseconds to seconds, including laboratory frame relaxation, relaxation dispersion, and CEST methods. The examples utilized will emphasize applications to proteins, but similar approaches have been applied to RNA and other biological macromolecules.

Opening and Prize Session

RAP 1
Raymond Andrew Prize
FROM SLOW TO ULTRA-FAST MAS: STRUCTURAL DETERMINATION OF TYPE-THREE SECRETION SYSTEM BACTERIAL NEEDLES AND INORGANIC MATERIALS BY SOLID-STATE NMR

J.-P. Demers[1]
[1]*Leibniz-Institut für Molekulare Pharmakologie (FMP), Molecular Biophysics, Berlin, Germany*

This work on solid-state NMR (ssNMR) methodologies and applications is divided in three parts. In the first part, methods employing ultra-fast Magic-Angle Spinning (MAS) are introduced: a set of completely low-power experiments for protein chemical shift assignment, a tailored strategy for broadband cross-polarization using low-power RF only, and an approach for sensitivity enhancement by recovery of bulk proton magnetization.

The second part presents the characterization of 11 inorganic compounds containing silicon, phosphorus and tin atoms using ssNMR techniques ranging from very slow to very fast MAS. A new strategy employing ultra-fast MAS to characterize compounds with extremely large chemical shift anisotropy is introduced.

The third part consists in the structure determination of the extracellular needle of the Type-Three Secretion System (T3SS) of *Shigella* bacteria. The T3SS is large supra-molecular assembly used by Gram-negative bacteria to deliver toxic effector proteins into eukaryotic host cells during bacterial infection. The secondary structure of the needle subunit protein was determined from ssNMR secondary chemical shifts, and the orientation of the subunit was assessed from immuno-gold labeling experiments. These results revealed a common architecture for *Shigella* and *Salmonella* T3SS needles. A hybrid structure determination approach combining ssNMR and cryo-electron microscopy (cryo-EM) was designed to

produce the atomic-resolution structure of the T3SS needle of *Shigella flexneri*. A cryo-EM density map at 7.7-Å resolution defines the placement of subunits and the symmetry of the assembly. The ssNMR secondary chemical shifts and 996 distance restraints define the local secondary structure, protein fold and inter-subunit interactions. The atomic structures, validated using an independent data set of 691 distance restraints, have a heavy-atom RMSD of 0.7 Å and resolve a controversy in the field of bacterial secretion.

RVP 1
Russell Varian Prize
COMPOSITE PULSES: REINVENTING THE WHEEL

M.H. Levitt[1]
[1]*University of Southampton, Southampton, UK*

In 1978 I was an undergraduate project student in Ray Freeman's NMR lab learning the basics of NMR, and how magnetization vectors move around in three dimensions. One of the first things I learnt about was field inhomogeneity, and how the effect of that can be reversed using a spin echo. Then I learnt about radiofrequency field inhomogeneity and the problems that could cause. Next up were phase shifts and how to visualise them. So I put these ingredients together and came up with a three-pulse trick that had the effect of a single pulse but with a pretty good compensation for radiofrequency inhomogeneity. Ray liked it and called it a "composite pulse".

I had absolutely no idea that this simple trick would launch me on a long scientific journey and that many years later it would earn me one of the most prestigious prizes in NMR.

Plenary Session 1

PL 01
NEW METHODS FOR MEASURING AND ORIENTING ORGANIC COMPOUNDS FOR RDC STRUCTURAL ANALYSIS

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Residual dipolar couplings (RDCs), which belong to the class of anisotropic NMR-parameters, can yield information complementary to ^{3}J couplings and NOE parameters for the determination of the three dimensional structure of organic or organometallic compounds by high-resolution solution state NMR spectroscopy.

For anisotropic NMR parameters to be observed the compound in question needs to be oriented with respect to the magnetic field. Some of our latest developments in anisotropic media for water insoluble organic compounds (lyotropic liquid crystals and stretched polymer gels) will be discussed together with some prerequisites and limits for the measurement of RDCs and their use in the structure determination of organic compounds.

PL 02
MODERN ESR: APPLICATIONS TO PROTEIN STRUCTURE AND DYNAMICS

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Pulse dipolar electron-spin resonance (PDS-ESR) has emerged as a powerful methodology for the study of protein structure and function. This technology, in the form of double quantum coherence (DQC)–ESR and double-electron-electron resonance (DEER) in conjunction with site-directed spin-labeling will be described. It enables the measurement of distances and their distributions in the range of 1-10 nm between pairs of spins labeled at two sites in the protein.

Concentrations from micromolar to tens of millimolar are amenable, requiring only small amounts of biomolecules. The distances are quite accurate, so a relatively small number of them are sufficient to reveal structures and functional details. Several examples will be shown. The first is defining the protein complexes that mediate bacterial chemotaxis, which is the process whereby cells modulate their flagella-driven motility in response to environmental cues. It relies on a complex sensory apparatus composed of transmembrane receptors, histidine kinases, and coupling proteins. PDS-based models have captured key architectural features of the receptor kinase arrays and the flagellar motor, and their changes in conformation and dynamics that accompany kinase activation and motor switching. Another example will be determining the conformational states and cycling of a membrane transporter, GltPh, which is a homotrimer, in its apo, substrate-bound, and inhibitor-bound, states in membrane vesicles providing insight into its energetics. In a third example the structureless (in solution) proteins alpha-synuclein and tau, which are important in Parkinson's disease and in neurodegeneration will be described and the structures they take on in contact with membranes will be described. We show how the very high sensitivity of ACERT PDS enabled the determination of the assembly pathway of a tetramer membrane protein. Also the relevance of the protein SOD-1 to Lou Gehrig's disease will be described using Cu2+-Cu2+ PDS. The relative virtues of DQC and DEER in these studies are compared, and

the technique of 5-pulse DEER, which can double acquisition times, will be described. A new denoising method for PDS-ESR will be introduced.

Whereas the structural studies by DQC and DEER are primarily performed in frozen solution at low (ca. 50°K) temperatures, protein dynamics should be studied at or near physiological or room temperature.

We describe how multi-frequency cw-ESR enables one to deconvolute complex protein dynamics in terms of the different time scales of the respective processes. Probably the most informative ESR method for dynamics in the pico-second to sub-millisecond range is the time-domain technique of 2D-ELDOR (two-dimensional electron-electron double resonance). This will be illustrated with several examples in the 9 - 17 GHz ESR range. We have been combining the respective advantages of both multi-frequency and 2D-ELDOR by extending this approach to the higher frequency of 9 GHz. The challenges and our initial results will be described.

S 01 - Biosolids

O 001

CONFORMATIONAL HETEROGENEITY AND INTRINSIC DISORDER IN MEMBRANE PROTEINS USING STATIC, MAS, & OS SOLID STATE NMR

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To understand the subtleties of membrane protein biophysics it is essential to characterize these proteins in an appropriate lipid bilayer environment. For the M2 protein from Influenza A, a great deal of structural biology from multiple research groups has been performed in various detergent and lipid environments, but the chemical and functional properties of this protein are strikingly dependent on the lipid composition of the protein's environment. Here, we report on the dramatic conformational heterogeneity, hydrogen bonding, dynamics and proton exchange of the His37 tetrad in the full length M2 protein that gives rise to the unique proton transport system of this protein in DOPC/DOPE lipid bilayers. We speculate on the unique source of the heterogeneity and its implications for a detailed functional mechanism. Numerous distance restraints have now been obtained for the transmembrane and amphipathic helix regions of the full length protein complementing the extensive oriented sample (OS) spectroscopy from the conductance domain.

We also report on the structure of a key protein, CrgA, from the divisome of Mycobacterium tuberculosis, the causative agent for TB characterized in liquid crystalline POPC/POPG lipids. CrgA appears to be recruited to the divisome by FtsZ, a protein that is responsible for forming the Z ring in dividing cells. CrgA is responsible for recruiting four other proteins to the divisome. This 93 residue protein has two transmembrane helices, an interhelical loop and a 30 residue N-terminus. The structure of the transmembrane domain was characterized using a combination of MAS and OS ssNMR and hints to the structure of the loop and N-terminus have been obtained from orientational restraints. While the first 19 residues of the protein shows only nascent structure residual anisotropies in the backbone, typical of intrinsic disorder, there appears to be a short amphipathic a-helix prior to transmembrane helix 1. The 20 residue interhelical loop appears to be mostly structured with a pair of short b-strands on either side of a proline residue and at the C-terminus of the loop there is a short disordered domain prior to the second transmembrane helix and C-terminus.

O 002

ALTERNATIVE SALT BRIDGE FORMATION IN AB – A HALLMARK OF EARLY-ONSET ALZHEIMER'S DISEASE?

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We recently reported a 3D fibril structure of the Aβ1- 40 peptide with the Osaka mutation (E22Δ)[1,2], associated with early-onset Alzheimer disease. This structure is based on a large number of unambiguous intra- and intermolecular solid-state NMR distance restraints, and differs substantially from all previously proposed models. We here compare the NMR chemical-shift of this Osaka mutant form with the published shifts of a brain-seeded form of wild-type Aβ, and suggest that the determined mutant fold is accessible to the wild-type protein as well, with small conformational adaptations which accommodate the E22 residue missing in the Osaka mutant[3]. In addition, we illustrate how other mutants could also conform to this model. The stabilization of the N-terminal part of the protein via an intermolecular salt bridge to Lys28 may represent a common structural motif for the mutants which are related to early-onset Alzheimer disease. This feature might connect to the observed increased toxicity of the mutant forms compared to wild-type Aβ1-40, where the salt bridge involving Lys28 is intramolecular.

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O 003

BIOMOLECULAR SOLID-STATE NMR AT 111 KHZ MAS: A REVOLUTION THROUGH FASTER REVOLUTIONS

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Over the last few years, the availability of probes capable of faster and faster magic-angle spinning has revolutionized biomolecular NMR, notably allowing the efficient detection of ^{1}H resonances in solid samples.
We describe here our first experiments on a new Bruker 0.7 mm MAS probe where samples spin at 111 kHz at magnetic field of 1 GHz. This new probe requires less than 0.5 mg of protein with a detection sensitivity comparable to the previously available 1.3 mm probe, thus reducing by a factor of four the sample quantities necessary for solid-state NMR studies. Most importantly, the new spinning regime halves the homogeneous contribution to the ^{1}H line-widths in fully-protonated protein samples. We show that triple-resonance experiments can be efficiently acquired on fully protonated microcrystalline GB1, yielding HCN correlations in minutes [e.g. 2D (H)NH or (H)CH] to hours [e.g. 3D (H)CANH]. ^{1}H,^{15}N and ^{13}C coherence lifetimes are significantly lengthened, allowing the design of improved schemes for rapid unambiguous backbone and side-chain ^{1}H, ^{13}C and ^{15}N assignment, yielding rapid access to important structural parameters, such as ^{1}H-^{1}H distances between side-chains.
These findings increase the impact of solid-state NMR to samples that cannot easily be deuterated, and for samples that can only be produced in sub milligram quantity, and we show the example of proteorhodopsin in a native-like membrane environment.

O 004

DYNAMIC AND STRUCTURAL INVESTIGATIONS OF PRGI NEEDLE PROTEIN BY PROTON DETECTED MAS NMR

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Employing deuteration we could obtain high resolution and sensitivity proton-detected solid-state MAS NMR spectra of insoluble, non-crystalline biological assembly, the Salmonella typhimurium type iii secretion system (T3SS) needle[1]. This supramolecular assembly is formed by 80 residue PrgI protein. We developed two methods for backbone resonance assignment of highly deuterated proteins at MAS rates of 20-28 kHz at an external magnetic field of 600 MHz. A previously unidentified second set of resonances in the N-terminal helix of PrgI was observed. The doubled resonances are close to Tryptophan-5, which indicates that signal doubling originates from different ring-current shift induced by aromatic ring of W5 which adopts two orientations. This effect provides intermolecular spatial constraints [2] up to 10A in T3SS. Additional information about intermolecular contacts up to 9 A was obtained from proton-proton magnetization transfers in 3D and 4D spectra. Comparison of apparent 15N T1 in fully protonated and deuterated samples allowed for identification of 15N longitudinal relaxation rates and amide hydrogen exchange rates for most of residues[3]. Also, proton flip-back approach was implemented to preserve and reuse remain water magnetization for sensitivity enhancement in proton detected 2D and 3D MAS NMR experiments [4].

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O 005

STRUCTURAL STUDIES OF MEMBRANE-EMBEDDED PROTEIN MACHINES BY NMR: FROM LIPID BILAYERS TO CELLS

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Membrane proteins and their complexes are an important class of biological molecules whose association with cellular compartments and their intrinsic molecular mobility can complicate their structural study by high-resolution methods [1]. Our group has developed NMR-based approaches to obtain structural and dynamical information under such conditions. For example, we have recently determined how the gating cycle of membrane-embedded potassium channels is influenced by small molecules, protein plasticity and the lipid bilayer (see, e.g., Ref. [2-4]).
Moreover we have characterized the influence of protein motion and protein-protein interactions for protein insertion and translocation machines in bacteria. In case of the BamA, the central component of the beta-barrel assembly machine, we have identified protein-specific dynamics that may be critical for substrate insertion [5-7]. Finally, we have devised experiment protocols [8-11] that allow us to study membrane proteins in the cellular context, including the use of mammalian cells. In our contribution, we describe recent progress in these areas of research.

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S 02 - Small Molecules and Pharmaceuticals

O 006
RECENT ADVANCES IN COMPUTATIONAL NMR OF THE MEDIUM-SIZED ORGANIC MOLECULES

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Recently, a vast amount of interest has been focused on structural and stereochemical applications of various computational schemes used to calculate chemical shifts and spin-spin coupling constants. Among a variety of theoretical approaches used for this purpose there are two groups of methods, namely those based on the Density Functional Theory (DFT) and those stemming from the wave function formalism within the Möller-Plesset Perturbation Theory. Within the latter, two most promising approaches are those based on the Coupled Clusters theory (CC2, EOM-CCSD, CCSD, CCSD(T) and CC3) and, on the other hand, those based on the general Polarization Propagator theory (SOPPA, SOPPA(CC2), SOPPA(CCSD). In this laboratory, extensive studies of the stereochemical behavior and stereochemical applications of spin-spin coupling constants of different types including ^{1}H-^{1}H, ^{13}C-^{1}H, ^{13}C-^{13}C, ^{31}P-^{1}H and ^{77}Se-^{1}H were performed in the recent decade in a large number of saturated carbocycles, nitrogen-containing heterocycles, organic phosphines and phosphine chalcogenides, selanylalkenes, five- and six-membered selenium-containing heterocycles, and even in much larger molecular systems like selenosugars.

The second line of our studies is the calculation of magnetic shieldings (chemical shifts) of 'heavy nuclei' like ^{29}Si, ^{31}P, ^{77}Se and ^{125}Te to reveal intra- and intermolecular coordination effects at the ZORA-GIAO-DFT level of approximation demonstrating that the contribution of the relativistic spin-orbit interaction mechanism was of crucial importance in the calculation of ^{29}Si, ^{31}P, ^{77}Se and ^{125}Te NMR chemical shifts. Also,

it has been found that the main three factors affecting the accuracy and computational cost of the GIAO-DFT calculation of NMR chemical shifts are as follows: the geometrical factor, the most efficient functionals and basis sets and the use of the Locally Dense Basis Set approximation. It has been demonstrated that in the calculation of NMR chemical shifts the best result has been achieved with the KT3 GGA functional of Keal and Toezer in combination with Jensen's pcS-3 basis set (GIAO-DFT-KT3/pcS-3), and this is indeed a very encouraging result for the future studies in this field.

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O 007
J-CONTROLLED ULTRAFAST 2D NMR

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Ultrafast (UF) NMR is capable of delivering arbitrary homo- or hetero-nuclear 2D spectra in a single scan.[1] During the last decade, the performance of this sub-second approach has been greatly improved, and UF 2D NMR rapidly became a powerful analytical tool experiencing an expanded scope of applications, from the real-time monitoring of reaction mechanisms to high-throughput metabolomics, including the coupling with hyphenated techniques such as HPLC-NMR or dissolution DNP.[2] Still, UF NMR suffers from its intrinsic low sensitivity, not only due to its single-scan nature but also to the scaling of the SNR by the square root of the spectral width and by potential molecular diffusion effects. Another major source of sensitivity losses is the J-modulation of the cross-peak intensities, arising from the constant-time nature of most UF experiments. To the best of our knowledge, this effect has neither been described nor optimized, which further limits the applicability of UF 2D NMR.

In order to better describe and understand the sources of sensitivity losses in UF 2D NMR, **we developed two general platforms for the numerical simulation of spatially-encoded experiments**, by customizing two widely used spin dynamic packages, Spinach[3] and SpinDynamica.[4] The joint analysis of simulations, theoretical formalism and experimental results provides detailed insight into the properties of UF 2D experiments. The results obtained by simulations perfectly match those obtained from product operator calculations and from

experiments performed at different magnetic fields. Thanks to this approach, we were able to describe in details the effects of J-modulation in UF spectra of complex spin systems, including those impacted by second order couplings. We found that **the sensitivity of UF experiments can be optimized depending on the targeted spin system** by the fine-tuning of a mixing period prior to the spatial encoding step. When the sample of interest contains multiple spin systems (for example in the case of complex mixtures), a compromise can be found to optimize the sensitivity of experimental spectra by preliminary numerical simulations. Moreover, we developed**a new excitation block for UF experiments**, based on spectrally selective pulses and capable of optimizing J-modulation effects for multiple spin-systems within a single scan.

This optimization strategy significantly improves the sensitivity of UF experiments, and also offers the option to perform spectral editing to remove unwanted signals (eg. diagonal peaks) while maximizing the intensity of those of interest. Such "J-controlled" UF spectra recorded on a variety of small molecule samples (small organic compounds, metabolic mixtures) will be shown, recorded with different UF pulse sequences, such as UF COSY or the UF DQS experiment that we recently described.[5]

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O 008
SEALED SIRNAS: STRUCTURE BASED DESIGN OF NOVEL THERAPEUTIC SIRNA MOLECULES

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siRNAs are 21-nucleotide duplex oligonucleotides that down regulate complementary target messenger RNA. For their potential use as therapeutics, the pharmacological properties of siRNA need to be improved, both by chemical modification of the siRNAs themselves and by proper formulation. siRNAs lead to target mRNA cleavage by association of one RNA strand with the Argonaute-2 protein (Ago-2) in the siRNA silencing complex (RISC). The ends of the guide strand are recognized by distinct domains of Ago-2: the 5'-end binds in the MID domain, the two 3'-terminal nucleotides are associated in a pocket of the PAZ domain. In order to improve siRNA stability as well as to prevent immune stimulation, we explored the possibility of substituting the 3'-terminal dinucleotide by a mimic that still interacts with the PAZ domain. To this end, the crystal structure of the human Ago-2 PAZ domain was obtained, a fragment library was designed by molecular modeling using this structure, and the fragments were screened for binding to the PAZ domain by NMR. Hits were further characterized by NMR spectroscopy. Novel PAZ domain ligands could be identified this way. About 300 analogues were synthesized and measured in an 19F reporter assay and ranked for improved affinity. From the best ligands, building blocks for RNA synthesis were derived and the correponding conjugates were prepared. The most potent RNA conjugates display enhanced chemical stability, without compromising PAZ domain binding. The structure and dynamics of the oligonucleotide

conjugates were characterized by NMR. The structure of the complex of a conjugate and the PAZ domain was obtained by X-ray crystallography. These activities resulted in novel therapeutic molecules, sealed siRNAs, that have significantly enhanced potency and duration of action as demonstrated by in vitro and in vivo assays.

O 009

LONG-LIVED STATES OF PAIRS OF FLUORINE-19 NUCLEI: A NEW TOOL FOR LIGAND-PROTEIN SCREENING

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NMR is routinely used in pharmaceutical industry for screening drug candidates by ranking their affinities for protein targets. We have exploited the enhanced contrast afforded by Long-Lived States (LLS) for drug screening [1]. Because $T_{LLS}>T_1$, the contrast between the signal intensities of free and bound ligands is dramatically improved, which allows one to reduce the concentrations of both ligands and protein targets. LLS can be used to screen and determine the dissociation constants K_D of molecular fragments that bind weakly to protein targets. We used our LLS method for screening weakly binding fragments against Heat Shock Protein Hsp90, a target for cancer treatment. By exploiting the LLS behavior of a spy molecule, we demonstrated that it is possible to measure dissociation constants K_D as large as 12 mM [2]. This corresponds to a very weak binding regime, where most other biophysical techniques fail, including other NMR methods based on the observation of ligands. This weak binding regime is crucial for fragment-based binding studies (FBB).

More recently, we have combined LLS for drug screening with ^{1}H dissolution-DNP to enhance the sensitivity. This allows one to reduce the concentrations of ligands and the protein trypsin to 120 µM and 1.4 µM respectively [3]. We observed dramatic differences between the spectra of ligands in the presence or absence of trypsin. The presence of a stronger ligand displaces a weaker "spy" ligand, which allows one to determine the dissociation constants K_D of the strong ligand by monitoring the signals of the spy ligand. We demonstrated that it is possible to perform experiments with a very dilute 12 µM solutions of ligands, obtaining similar signal-to-noise ratios in one-shot DNP experiments as after several hours of acquisition using conventional experiments without DNP.

We are now exploring LLS involving pairs of ^{19}F nuclei to study binding phenomena. The ^{19}F spectra do not suffer from any overlapping signals. In a custom-designed fluorinated ligand that binds trypsin, we have observed T_{LLS} = 2.6 s and T_1 = 0.6 s, hence a promising ratio T_{LLS}/T_1 > 4. We found a dramatic effect on the LLS lifetime T_{LLS} of 800 µM of the fluorinated ligand: an 85% contrast has been observed between signals derived from T_{LLS} with/without 2µM trypsin. The titration of the fluorinated ligand against trypsin gave a dissociation constant K_D = 106 µM. This fluorinated ligand can be used as spy molecule in competition experiments, which allows one to rank the affinities of arbitrary ligands that do not contain any fluorine.

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O 010
USING NMR TO UNRAVEL METABOLIC MECHANISMS IN CANCER CELLS

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Metabolic transformation is increasingly understood as a hallmark of cancer. This was first recognised by Otto Warburg who associated increased glycolysis and lactate production with cancer transformation. Today it is well known that simple metabolic pathways such as glycolysis and the Krebs cycle are controlled by cell signalling. This opens new opportunities for cancer diagnosis and treatment. Cancer metabolomics picks up altered metabolism in cancer, whether from blood or tissue samples. For mechanistic studies we started to use tracer based methods using labelled metabolic precursors such as glucose or glutamine. This has been used to study metabolic transformation in haematological and in breast cancer cell lines. The results show that different cancers reveal a different pattern of metabolic adaptation. In the case of leukaemia cells we also observe rapid adaptation to niche conditions using primary cancer cells in real-time experiments. We have also used NMR metabolic analysis in the context of drug discovery to study the mechanism of inhibitors used for myeloma. The key advantage of NMR in this context is its ability to study site-specific label in corporation which is not possible by mass spectrometry. This can be used to derive specific pathway activities -including anaplerotic pathways and the pentose phosphate pathway- in cancer cells.

S 03 - NMR - High and Low, Sparse and Dense

O 011
EXTREME HIGH-PRESSURE NMR APPLICATIONS IN PHYSICS AND CHEMISTRY

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Fundamental and applied research in solid state chemistry and physics benefit greatly from NMR, but also from very high pressure studies that could hardly be followed with NMR, until now, since the high energy density from bonds in solids requires pressures well into the Giga-Pascal range. Such high pressures can only be achieved with anvil-type pressure cells, and the secluded tiny sample space sealed in between the anvil vises prohibited high-sensitivity NMR. We show that radio-frequency micro-coils can be placed in the high pressure region for the construction of high-sensitivity anvil cell NMR probes that can easily be used with commercial NMR systems. In particular, with our new gasket design we report pressures beyond 200,000 atmospheres, i.e., well in excess of what was used with NMR before. Examples with 1H, 17O, 27Al, 63Cu, 71Ga, 115In NMR of liquids, and solids as powders and single crystals, at various magnetic fields are shown, and reveal how powerful the new NMR tool is in detecting changes of the electronic as well as chemical structure of various materials.

O 012

HIGH FIELD NMR EXPERIMENTS USING A ZERO FIELD NMR SPECTROMETER

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Zero or ultra-low field (|B| < 1 microgauss) and high-field (|B| > 0.1 gauss) NMR are complementary techniques in the molecular spectroscopist's toolkit. Characteristics of high-field NMR are spin evolution under a "truncated" spin Hamiltonian in the interaction frame of the Larmor precession, and isotope-selective manipulations via resonant AC fields. In zero magnetic field, absence of such truncation means the spin-spin coupling tensors are exposed in their full form, and it is more customary to manipulate the total angular momentum of the strongly coupled spin network, rather than the angular momentum of individual isotopes. Selection rules may also significantly differ – for instance, zero-field spectroscopy is primarily sensitivity to hetero-nuclear spin couplings.

The above is a reminder that the appearance (and interpretation) of an NMR spectrum depends strongly on the magnetic field at which it is recorded!

In this presentation I will discuss how the "best of both worlds" may be obtained by combining high-field NMR methodology with zero-field detection:

I will describe experiments performed using a zero-field NMR instrument based on an optically pumped rubidium-87 SERF magnetometer. This instrument is sensitive to magnetic fields above 1 fT / root Hz with spectral resolution circa 20 mHz (FWHM). The apparatus is equipped with electromagnetic coils along X, Y and Z directions for supplying controlled time-dependent magnetic fields of strengths up to 40 gauss (approximately 17 kHz proton frequency), making accessible the realm of high-field spin dynamics.

We have used these coils to perform isotope-selective AC pulses, swept-frequency adiabatic inversion pulses, and other basic high-field spin manipulations. Such AC pulses can be used to precisely measure the DC magnetic fields produced by the coils.

We have performed a two-dimensional experiment in which the sample is polarised in a strong DC field, allowed to evolve following a sudden (nonadiabatic) 90-degree switch of the DC field direction, and finally the NMR signal detected after switching to zero field. 2D Fourier transformation gives both high-field and zero-field spectral projections. This technique appears in some respect an "inside-out" version of the high field-zero field correlation experiments performed in the 1980s by Suter, Pines, et al. An interesting feature is that the high-field spectral dimension is completely broadband, since only DC magnetic fields are used. The 2D experiment is useful for cross-checking zero-field spectral assignment.

O 013
(VERY!) BROADBAND DOSY

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Resonance Offset Effects

Nuclei such as ^{13}C, ^{19}F and ^{31}P have large chemical shift ranges, e.g. for ^{19}F NMR the range can be up to ± 500 ppm. Limited radiofrequency power available for pulsed excitation makes it very challenging to excite such a wide range of frequencies, especially for multiple pulse methods. Even the best conventional composite pulse methods fall far short of such bandwidths[1], so swept-frequency "chirp" pulses are needed. Broadband DOSY sequences were built in stages by developing broadband excitation and refocusing elements that could be combined to generate broadband spin echo and stimulated echo sequences.

Broadband Excitation and Refocusing

In principle, a pair of chirp pulses of appropriate relative amplitude can be used to excite very wide bandwidths[2]. Unfortunately, gross phase errors build up towards the edges of the frequency range, reducing the bandwidth of the method. A further, insidious, problem is that the signal phase is extremely sensitive to B_1 amplitude, so that B_1 inhomogeneity causes large (> 30%) losses in signal even with good modern instrumentation. The double chirp pulse sequence element was therefore adapted and extended (a) to compensate for B_1-sensitivity and (b) to correct for the phase errors. A uniform, constant-phase broadband excitation was achieved, allowing a 300 kHz bandwidth with maximum 15 kHz RF amplitude, with no undue B_1 sensitivity and no loss in sensitivity. Broadband refocusing can be achieved, using an adiabatic composite 180°180°180° chirp pulse[3],

which is self-refocusing without phase distortion and is therefore B_1 insensitive.

Broadband DOSY

A broadband DOSY Oneshot[4] sequence, built using the elements described, produces uniform performance over almost a full 300 kHz bandwidth, with no loss in sensitivity, allowing DOSY to be performed over very wide spectral widths (e.g. 600 ppm for ^{19}F at 500 MHz). In comparison, simulation shows that with the same RF amplitude at half height excitation bandwidth, the standard uncompensated DOSY sequence will excite only 30 kHz.

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O 014
NMR BEYOND 200,000 ATMOSPHERES OF PRESSURE

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Pressure-induced changes in the chemical or electronic structure of solids require pressures well into the Giga-Pascal (GPa) range due to the strong bonding. Anvil cell designs can reach such pressures, but their small secluded sample chamber has hampered NMR experiments. With a new cell design that has a radio frequency (RF) micro-coil in the high pressure chamber, NMR experiments beyond 20 Giga-Pascal are reported for the first time. 1H NMR of water shows sensitivity and resolution obtained with the cells. 115In NMR of the ternary chalcogenide AgInTe2 discovers a insulator-metal transition with shift and relaxation measurements. The pressure cells can be mounted easily on standard NMR probes that fit commercial wide-bore magnets with regular cryostats for field- and temperature-dependent measurements ready for many applications in physics and chemistry

O 015
NEW ALGORITHMS FOR RECONSTRUCTING SPECTRA FROM NON-UNIFORMLY SAMPLED DATA: SPARSE FAST FOURIER TRANSFORM AND LOW-RANK RECONSTRUCTION

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Increasing the dimensionality of NMR experiments strongly enhances the spectral resolution and provides invaluable direct information about atomic interactions. However, the price is high: long measurement time and heavy requirements on the computation power and data storage. We introduce two new methods for addressing these challenges: Sparse Fast Fourier Transform (SFFT) and Low-Rank (LR) reconstruction. The SFFT algorithm [1], which harnesses the spectral aliasing and discrete projections, represents a rapidly developing field of signal processing methods for obtaining spectra from minimal number of sampled data points and with minimal computations. We show that SFFT is capable of producing high quality NMR spectra of essentially unlimited size and dimensionality with short measurement times, faster computations than the Fast Fourier Transform, and minimal storage for processing and handling of sparse spectra. The SFFT method is the most useful for high-resolution spectra with four and more dimensions.

The LR algorithm [2] is based on recent developments in the field of signal processing theory [3]. It utilizes the so far unexploited general property of the NMR signal, its low rank. Using experimental and simulated data, we demonstrate that for non-uniformly sampled data, the LR reconstruction is a viable alternative to the current state-of-the-art technique compressed sensing. In particular, the low rank method

is good in preserving low intensity broad peaks, and thus it increases the effective sensitivity in the reconstructed spectra.

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S 04 - NMR + EPR

O 016
STRUCTURES OF PROTEIN-RNA COMPLEXES INVOLVED IN TRANSLATION REGULATION BY NMR and EPR

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RRMs are the most common types of RNA recognition modules, being present in about 1% of all human proteins. They are a typical babbab fold although N- and C-terminal extensions of these domains have been observed. We have recently characterized the NMR structure of CPEB1 and CPEB4 (Cytoplasmic polyadenylation element binding protein) which are regulators of translation (Afroz et al, Gene & Dev, 2014). In CPEB1 and 4, the two RRMs from a V-shape surface in the free form which is used to bind the RNA in its center. The fold is unusual with several additional secondary structure elements. RRM1 binds the 5'end of the RNA while RRM2 binds only the 3'-terminal nucleotide. This binding arrangement is unprecedented among RRM-RNA structures and RNA binding induce closure of the two RRM towards the RNA reminding a "Venus fly trap" mechanism. The functional consequences of this binding mode will be presented.

In bacteria, sRNAs (small regulatory/ non-coding RNAs) coordinate global changes in gene expression. The most important global post-transcriptional regulatory system responsible for bacterial virulence is the Csr/Rsm system, in which a sRNA (CsrB/RsmZ) activates translation initiation by sequestering a homo-dimeric protein (CsrA/RsmE) that is binding to the ribosome binding site of a subset of mRNAs. However, the mechanism of translation derepression is only partially understood at the molecular and atomic level. We elucidated the 70 kDa solution structure of RsmZ bound to three RsmE protein-dimer using a combination of methods NMR and EPR (Duss et al, Nature Com, 2014) as well as multiple segmental isotope labeling of the RNA (Duss et al, NAR 2010). The structure reveals how the non-coding RNA RsmZ

sequesters, stores and releases (with the help of RNase E) the translation repressor RsmE, therefore acting as a protein "sponge" (Duss et al, Nature, 2014).

O 017
RIDME-BASED DISTANCE MEASUREMENTS IN Gd(III) SPIN PAIRS

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A currently very active application field for site directed spin labeling technique in conjunction with pulsed EPR is the determination of long-range site-to-site distances in biomacromolecules and biomolecular complexes. The Gd(III)-based spin labels demonstrate a particularly advantageous behavior in pulse EPR measurements at high magnetic fields/high microwave frequencies. Importantly for combined structure determination approaches, these spin labels can be used in paramagnetic NMR experiments more efficiently than the conventional nitroxide spin labels. Unfortunately, the informative signal in Gd(III)-Gd(III) double electron-electron resonance (DEER) experiments is typically limited to about 5% of the detected spin echo intensity, thus affecting the sensitivity and imposing the requirement of high spin labelling efficiency. In contrast to DEER, the measurement of Gd(III)-Gd(III) distances via the relaxation induced dipolar modulation enhancement (RIDME) experiment results in a depth of dipolar modulation that is comparable to the DEER modulation depth for nitroxide spin labels.[1] In this report we extend the cited proof of principle publication and present a systematic overview of the dependence of Gd(III)-Gd(III) RIDME signal on mixing time and temperature. Furthermore, we discuss the appearance of dipolar frequency overtones due to the high-spin nature of Gd(III) centers, the procedure of distance extraction in such situation, as well as the factors determining the shape of the intermolecular background decay in RIDME experiment. The discussion includes the results of experiments performed on a series of model compounds with two

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O 018
PELDOR ON TRIMERIC BETAINE SYMPORTER BETP

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PELDOR (pulsed electron electron double resonance [1]) is a magnetic resonance method for distance, orientation, and dynamic measurements of two or more paramagnetic centers in macromolecules like proteins, RNA, or DNA as well as polymers. Here we apply this method to analyze the different states of the trimeric betaine symporter BetP [2-3]. This symporter does activate at osmotic stress and transports betaine and sodium through the membrane. BetP cycles through several states during the transport. From the periplasmic open via an occluded to a cytoplasmic open state. One open question on the trimeric transport is whether it occurs on all three monomers synchronously or in a cyclic sequence. By PELDOR and site-directed spin labeling we probe the changes on activation as well as the occurring different states. We will report on the current status a this ongoing project.

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O 019

THE FIELD DEPENDENCE OF CROSS-EFFECT DYNAMIC NUCLEAR POLARIZATION UNDER MAGIC ANGLE SPINNING: THEORY VS. EXPERIMENT

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Dynamic Nuclear Polarization (DNP) has become a popular method to enhance Nuclear Magnetic Resonance (NMR) signals by orders of magnitude. In order to perform a DNP experiment it is necessary to dope the sample in a paramagnetic polarizing agent from which it is possible to exploit a polarization transfer from the electronic to nuclear spins by irradiating the Electron Paramagnetic Resonance (EPR) transitions. By doing so it allows to study heterogeneous molecular systems including peptide-binding membrane vesicles[1] or cell embedded proteins[2] including large complexes such as the Megadalton bacterial type IV secretion system core complex (T4SScc)[3]. Ideally such experiments take place at high magnetic field strengths where the spectral resolution is maximized. However this creates a problem for Magic Angle Spinning (MAS)-DNP as the enhancement is expected to decrease as the field strength increases. Nonetheless it is still possible to obtain significant enhancement by using the Cross-Effect DNP mechanism at high magnetic fields (800 MHz/527 GHz). Adapting theoretical frameworks introduced earlier[4,5] we have examined in detail the magnetic field dependence of MAS-DNP and compared our theoretical results to experimental data obtained at high field.

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O 020
DYNAMIC NUCLEAR POLARIZATION OF PARAMAGNETIC BIOMOLECULES

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Magic angle spinning (MAS) NMR has become a powerful and indispensable technique in structural biology as well as materials science. Nevertheless, the inherently low sensitivity of nuclear spins is still one of the limiting factors in its application: the small thermal spin polarization often leads to prohibitively long acquisition times. Dynamic nuclear polarization (DNP) has been introduced as a tool to overcome this problem by transferring the significantly larger spin polarization of unpaired electrons to the nuclei of interest during a typical MAS NMR experiment.

Most commonly polarizing agents are introduced by dissolution of small paramagnetic molecules within the sample solution to be polarized. Electron polarization is then transferred to the surrounding protons by DNP and further spread throughout the sample by ^{1}H–^{1}H spin-diffusion which allows for a uniform enhancement of all NMR signals. Direct DNP of low-γ nuclei such as ^{13}C, ^{15}N, or ^{17}O is increasingly inefficient due to lacking spin-diffusion pathways between the polarizing agent and the analyte. Therefore, by constraining the distance between the polarizing agent and the molecular moiety of interest, DNP becomes active and also provides additional geometric selectivity and information. For example, by using an endogenous paramagnetic center as polarizing agent, or by covalently or non-covalently introducing paramagnetic tags to the macromolecule, certain nuclei in the vicinity of the paramagnetic site can be selectively polarized. By analysis of site-specific signal enhancement factors and polarization build-up rates long-range constraints could be extracted to aid in structural modelling.

The utilization of endogenous radicals in biomolecules has already been demonstrated for ^{1}H DNP by Maly et al. (J. Phys. Chem. B, 116 (2012) 7055). However, due to the strongly coupled dipolar network of protons, their polarization will equalize almost instantaneously during DNP build-up. The introduction of paramagnetic metal complexes as polarizing agents has opened many possibilities towards DNP using endogenous paramagnetic sites of biomolecules. In contrast to other polarizing agents, metal ions such as Mn^{2+} and Gd^{3+} allow for a highly efficient direct DNP transfer to ^{13}C and ^{15}N via the solid effect at high magnetic field. Spin-diffusion can then be controlled by isotope labeling strategies and restricted to the molecule or moiety of interest. Furthermore, these metals are stable under physiological conditions which makes them potential polarizing agents for in-cell applications. In this presentation the chemical and spectroscopic properties of suitable metal ions (i.e., Mn^{2+}, Gd^{3+}) are reviewed and several routes for the labeling of biomolecules with paramagnetic metals will be discussed. These routes include direct metal binding and covalent linking with chelating tags. Several biomolecular systems containing paramagnetic metal ions including proteins and nucleic acids will be presented. We show electron paramagnetic resonance (EPR) and magic angle spinning (MAS) NMR experiments in order to investigate metal binding, electron spin properties, as well as electron–nuclear interactions such as paramagnetic relaxation and signal quenching. Finally we will demonstrate the direct, intramolecular DNP of ^{13}C from endogenous metal sites in biomolecules.

S 05 - Materials NMR

O 021
GROUP 13 (AL, GA) STUDIES OF METAL-OXIDE CLUSTERS AND THIN FILMS

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We have been characterizing special group 13 metal-oxide clusters that contain hydroxo-bridged ligands and have a structural formula of $[M_{13}(\mu_3\text{-}OH)_6(\mu_2\text{-}OH)_{18}(H_2O)_{24}](NO_3)_{15}$, where the metal, M, is typically Al or Ga. We call these "M_{13}" clusters, as a shorthand name. There are mixed metal analogues with substitution of specific sites by In of the same type: $[M_{13-x}In_x(\mu_3\text{-}OH)_6(\mu_2\text{-}OH)_{18}(H_2O)_{24}](NO_3)_{15}$ with compositions that span the range from $M_{12}In_1$, $M_{11}In_2$, to M_7In_6. The clusters are capable of being prepared on a gram scale from aqueous solution syntheses.[1]

The clusters all possess a flat tridecameric metal-oxide arrangement where the metal atoms are in (distorted) six-coordinate geometry. Since the clusters are composed of quadrupolar nuclei (such as aluminum or gallium, nuclear spin I=5/2 and 3/2, respectively), these can present a substantial challenge in acquiring high resolution NMR spectra.

Combining high field magnets (13.9T and 21.1T) with ultra-fast spinning (33kHz and 62.5kHz), the characterization of the pure gallium $[Ga_{13}(\mu_3\text{-}OH)_6(\mu_2\text{-}OH)_{18}(H_2O)_{24}](NO_3)_{15}$ cluster was recently finished.[3] This study has now been augmented with CASTEP calculations to examine the EFG's of the metal sites. The isostructural aluminum cluster $[Al_{13}(\mu_3\text{-}OH)_6(\mu_2\text{-}OH)_{18}(H_2O)_{24}](NO_3)_{15}$, currently under investigation, has presented a more unique and difficult challenge to characterize. High field multiple-quantum MAS (MQMAS) and first principle calculations

have been employed to help assign some of the observed resonances. Heterometallic clusters containing gallium and indium are currently underway and will also be presented. With the possibility of multiple isomers, the use of first principles calculations has been employed to help elucidate the resulting NMR lineshape of the heterometallic clusters.

The cluster solutions can be easily spin-coated onto substrates to produce high-k-dielectric thin films.[2] Under certain conditions, the resulting films have an almost atomically flat surface which contains little to no defects, making them strong candidates for thin film applications. Monitoring the coordination environment and characterizing the metal sites allows for important structural information to be gained on understanding the transformation of the cluster precursors to thin films. Preliminary thin film data will be presented on this system as well.

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managed by the University of Ottawa (http://nmr900.ca). EMSL, a DOE Office of Science User Facility sponsored by the Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory, provided access to the 850MHz NMR spectrometer (Richland, WA).

O 022
HIGH–ENTROPY ALLOYS

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Traditionally, metallic alloy systems have been based mainly on one principal chemical element as the matrix, even though a substantial amount of other elements was incorporated for property/processing enhancement. To date there has been about thirty practical alloy systems developed including Fe (steels), Al, Cu, Ti, Mg, and Ni-based alloys and 1970s are regarded as the period when the high-performance one-principal-element alloys had reached their maturity. The attempts to produce alloys with more than one principal element have led to the development of intermetallic compounds, quasicrystals and bulk amorphous alloys (also termed metallic glasses). All these metallic systems are again based mainly on one principal chemical element.

Within the past several years, a new approach to metallic alloy design with multiple principal elements in equimolar or near-equimolar ratios, termed high-entropy alloys (HEAs), has been proposed. According to this concept, the high entropy of mixing can stabilize disordered solid solution phases with simple structures like a body-centered cubic (bcc) or a face-centered cubic (fcc) with small unit cells of the edge length about three angstroms only, in competition with ordered crystalline intermetallic phases that often contain structurally complex giant unit cells of the edge length of several tens of angstroms. The HEA structure is characterized by a topologically ordered lattice with an exceedingly high chemical (substitutional) disorder, so that a HEA can be conveniently termed as a 'metallic glass on an ordered lattice'. In order to achieve high entropy of mixing, the alloys must be composed typically of five or more (up to thirteen) major elements in similar concentrations, ranging from 5 to 35 at. % for each element, but do not contain any element whose concentration exceeds 50 at. %. Examples of HEAs are alloys derived within the systems Al-Si-Co-Cr-Cu-Fe-Mn-Ni-Ti, W-Nb-Mo-Ta-V, and Ta-Nb-Hf-Zr-Ti.

Since many different elements can be alloyed, and the concentration of each element can vary significantly, the number of possible HEAs is virtually unlimited. Astonishingly, only about ten different HEAs have been studied up to date, making the field practically empty. Most existing studies focus on the relationship between phase, microstructure and mechanical properties. The physical properties of HEAs remain largely unexplored. In 2014, a breakthrough has been made by discovering the first superconducting HEA within the Ta-Nb-Hf-Zr-Ti system [1], showing a high superconducting transition temperature (for a metallic system) of 7.3 K and a large upper critical field of 8.2 T. The superconducting phase was demonstrated to be close to a BCS–type, which employs an electron–phonon coupling to create Cooper pairs. The presence of phonons in a highly chemically disordered lattice of a HEA is surprising as the chemical disorder is expected to suppress the lattice vibrations. Niobium NMR was used to determine the size of the superconducting gap in the electronic density of states.

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O 023
INTERFACE SELECTIVE SOLID-STATE NMR IN INORGANIC-ORGANIC HYBRID MATERIALS

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The interface between organic and inorganic components is the crucial part in natural and synthetic composites.

To study molecular dynamics in thin polymer films, relaxation NMR has been combined with high-resolution solid-state NMR (CRAMPS). This provides sufficient resolution to identify functional groups and to separate solvent signals in swelling experiments. Thus it could be shown, that the molecular dynamics in polymer brushes is much faster than in bulk polymers, and they exhibit different swelling behavior. Special experiments permit the selective excitation at the interface between the organic and the inorganic phase. If different isotopes or nuclei are present in the respective phase, trans-interface magnetization transfer generates a short-range magnetization profile at the interface. As an example fluorine-proton cross polarization has been applied on polyelectrolyte multilayers on a thin Nafion film. With the cross polarization only the near-interface polymer is excited. Subsequent spin diffusion can be used to spread the magnetization. Thus the gradient of structure or molecular mobility in the polymer from the surface to the bulk is probed stepwise and compared to the bulk properties.

In other cases selection of the magnetization is based on selective excitation, relaxation or combinations thereof. In nanoparticles from hydroxyapatite the OH signal is selectively excited by a chemical-shift selective spin echo, which benefits from the narrow linewidth. Then the magnetization is spread out by a spin diffusion period. The particles had been coated by polylelectrolyte multilayers from poly(maleic anhydride-co- ethylene) and

poly(diallyldimethylamonium chloride and the outermost layer from poly(styrene sulfonate) (PSS). The aromatic signal from the PSS is identified in the MAS spectrum can thus be used as a ruler to correlate the spin diffusion time with the independently measured layer thickness.

After establishing the techniques on model systems, it has been applied to realistic particle-filled polymer systems and biomimetic hydroxyapatite-gelatine nanoparticles.

O 024
NUCLEAR SPIN CIRCULAR DICHROISM IN FULLERENES

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Novel magneto-optic spectroscopy techniques attract a growing interest of scientific community. The nuclear-spin optical rotation (NSOR) was observed experimentally for Xe and H_2O.[1] Recent theoretical developments have enabled the predictions of the nuclear spin-induced circular dichroism (NSCD),[2] a spectroscopy analogous to magnetic circular dichroism where the CD signal is induced by nuclear spin magnetisation instead of external magnetic field. While awaiting for the construction of efficient experimental devices we predict and analyse the first NSCD spectra of fullerenes C_{60} and C_{70}.[3] We show that NSCD signal is site specific like in traditional NMR.

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O 025
CHARACTERIZATION OF CATALYTIC MATERIALS BY CONVENTIONAL AND DNP-ENHANCED SOLID-STATE NMR METHODS

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Intrinsically low sensitivity is the fundamental challenge in solid-state (SS)NMR, especially in the studies of insensitive nuclei, structural elucidations of minute concentrations of molecules, fast screening of new materials and identification of short-lived intermediates. Recent developments in dynamic nuclear polarization (DNP) afforded remarkable signal enhancements, enabling measurements that are off-limits to traditional NMR. Nonincremental sensitivity gains can be also achieved by using conventional SSNMR schemes utilizing fast magic angle spinning (MAS). We will present several recent examples of applications of these methods to the studies of materials in our laboratory.

The DNP-driven hyperpolarization opens an unprecedented range of opportunities in surface science by enabling studies of much smaller surface areas than previously possible and/or examination of highly dispersed surface species. Here, new approaches to ^{1}H-X heteronuclear correlation (HETCOR) spectroscopy will be presented (for X = ^{15}N, ^{13}C, ^{17}O or ^{29}Si), and applied to functionalized mesoporous silica materials. The results will include the ^{1}H-^{15}N and ^{1}H-^{13}C HETCOR spectra of natural abundance catalytic moieties, which will be compared with the state-of-the-art HETCOR measurements utilizing MAS at rates up to 100 kHz and indirect detection of X nuclei. The first natural abundance ^{17}O DNP-SENS NMR spectrum of surface silanol groups will be presented, in which the hyperpolarized ^{1}H magnetization was efficiently transferred to ^{17}O using the PRESTO technique. The PRESTO approach can be easily altered to measure ^{1}H-^{17}O HETCOR spectra and ^{1}H-^{17}O internuclear distances. This has enabled the detection of hydrogen-bonded and non-hydrogen-bonded silanol groups at the surface of silica gel, which cannot be distinguished otherwise. We will also present the DNP-

enhanced spectra of other challenging systems, including $^{27}Al\{^{1}H\}$ CPMAS spectra small surface Al_xO_y films deposited on silicon wafers, and ^{13}C-^{13}C spectra of molecules reacting on surfaces of metal nanoparticles.

S 06 - Protein Relaxation and Dynamics

O 026
KINETIC COOPERATIVITY, LOOP DYNAMICS, AND ALLOSTERY

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The hallmark of glucokinase (GCK), which catalyzes the phosphorylation of glucose during glycolysis, is its kinetic cooperativity whose understanding at atomic detail has remained open since its discovery over 40 years ago. I will discuss how the origin of kinetic cooperativity is rooted in intramolecular protein dynamics using kinetic CPMG-NMR data of 17 isoleucine side-chains distributed over all parts of GCK. Residues of glucose-free GCK located in the small domain display a distinct exchange behavior involving multiple conformers that are substantially populated ($p > 17\%$) with a $k_{ex} = 509$ s^{-1}, whereas in the glucose-bound form these exchange processes are quenched. This exchange process directly competes with the enzymatic turnover rate at physiological glucose concentrations, thereby generating the sigmoidal rate dependence that defines kinetic cooperativity.

The flexible nature of protein loops and the timescales of their dynamics are critical for many biologically important events at the molecular level, such as protein interaction and recognition processes. Based on a library of 38 proteins, rules about loop dynamics

in terms of amplitude and timescales can be derived using molecular dynamics (MD) simulations and NMR data. These rules have been implemented in the new web server ToeLoop (for Timescales Of Every Loop) that permits the prediction of loop dynamics based on an average 3D protein structure: (http://spin.ccic.ohiostate.edu/index.php/loop/index).

O 027

INCREASING THE SENSITIVITY AND SPEED OF RELAXATION-DISPERSION NMR IN PROTEINS AND NUCLEIC ACIDS

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Biological macromolecules (proteins, nucleic acids) transiently populate conformations that are in continuous exchange with the ground state. These high-energy conformations often play crucial roles for the function, as well as for disease-causing misfolding of these molecules. NMR spectroscopy, and especially Carr-Purcell Meiboom-Gill (CPMG) relaxation dispersion (RD) NMR, is a powerful technique to obtain kinetic, thermodynamic and structural information on conformational exchange processes occurring on the ms time scale (1). CPMG RD experiments are typically based on ^{1}H-^{15}N or ^{1}H-^{13}C correlation experiments comprising a fixed transverse relaxation delay T with a variable number of refocusing pulses. Dispersion profiles for individual nuclear sites are obtained by repeating these experiments for various CPMG frequencies (number of refocusing pulses applied during the relaxation period).

Here we introduce BEST-TROSY (BT) CPMG-RD experiments to study conformational dynamics of amide, imino, and aromatic moieties in proteins and nucleic acids, that combine the features of longitudinal- and transverse-relaxation optimization (2). These new pulse sequences can be successfully applied on modern NMR spectrometers using recycle delays of a few hundred milliseconds, and without the need for RF heating compensation. This results in significant gains in experimental sensitivity, and a reduction of the minimal experimental time requirements to less than one hour, as will be demonstrated for proteins, RNA, and RNA-protein complexes. Most interestingly, BT-CPMG-RD makes short-lived molecular systems, such as folding intermediates or proteins in living cellular environments, amenable to experimental investigation by RD NMR. This is exemplified

here for the folding intermediate of the amyloidogenic protein B2-microglobulin that has been studied by real-time ^{15}N BT-CPMG-RD.

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O 028
CONFORMATION NETWORKS FROM EXACT NOE ANALYSIS

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A multitude of structural states is inherent to biomolecules. One of the major challenges in structural biology is a comprehensive description of the entire structural landscape and the exchange dynamics between structural states at atomic resolution. Whereas NMR relaxation provides important aspects of local dynamics, exciting progress is currently being achieved in formulating more comprehensive descriptions of the structural landscape and the dynamics of a protein. In particular, direct methods to infer atom coordinates of slow-scale motions and to detect concerted motion are much sought-after. We have replaced the standard procedure for structure determination by an approach that generates multi-state ensembles using tight averaged distance restraints derived from exact NOEs (eNOEs) [Vögeli et al. 2012, Vögeli 2014].

We have demonstrated that exact measurements of 1H-1H nuclear Overhauser enhancements (eNOEs) can be converted into distances with an experimental random error of only 0.1 Å. Such eNOEs retrieve a wealth of information, which is sacrificed in routine structure determination. The collection of a dense net of eNOEs traversing a macromolecule serves as an excellent probe towards a more complete representation of structure and dynamics as demonstrated here for three proteins GB3, cyclophilin A and a WW domain.

The ensemble description of the prototypical enzyme cyclophilin reveals the presence of an open and a closed state, which are indicative of large scale correlated motion. In the open state, the catalytic site is preorganized for catalysis. This suggests that the mechanism of action is conformational sampling, while the ligand-binding loop appears to act through an induced fit mechanism. Overall, more than 60-70% of the side chain conformations appear to be correlated.
By applying the multi-state ensemble approach to the WW domain of Pin1, we relate the structural correlations to previously determined networks of residues relevant for folding.
Finally, we cross-validated our method by analyzing states and correlations in GB3 ensembles with large eNOE, RDC and J coupling data sets.

Vögeli, 2014, Prog Nucl Magn Reson Spectrosc 78, 1-46
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O 029

WHAT CAN AND CANNOT BE SAID ABOUT PROTEIN DYNAMICS USING NMR: COMBINING PERSPECTIVES AND TIME SCALES

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In the domain of dynamics studies of proteins, NMR spectroscopy has reached a mature state. Instrumentation and methodology are extremely efficient in selectively measuring various spin relaxation processes and therefore represent a unique tool to probe molecular motions, with site specificity. Indeed, NMR relaxation theory establishes a relationship between molecular motions and spin relaxation. The connection between both is embodied in the spectral density function of the relevant magnetic interactions, which itself is to be related to molecular motions through a dynamical model. Eventually, the practical problem at hand amounts to fitting model parameters from measured data.
One of the issues inherent to the method is to the paucity of data. Therefore, models with few parameters are required, which may be a strong limitation to the model complexity. On the other hand, the models used to describe the dynamics of the molecule should exhibit physical relevance. At this point, a microscopic approach to dynamics, such as provided by MD simulations clearly represents a most useful tool that can be used to test physical models of internal dynamics stochasticity.

In this perspective, we recently investigated the use of fractional Brownian dynamics, and thus this kind of processes with long memory, to account for spectral density functions. [1,2] One of the particularly interesting points is the ability of this approach to account for distributions of time scales, with a fixed number of parameters. This was demonstrated not only for fast, but also slow dynamics (exchange), where a transiently fractional diffusion model was introduced and

where our analyses strongly suggest that short and long time scales obey the same stochastic process. [3]

Moreover, in another perspective, we investigated limitations to the small time scales that NMR relaxation measurements can access, independent of the model used. We give indication that not internal scale below several tens of ps can be identified, and that overall and internal mobility parameters are correlated, which makes the extraction of both questionable. In this context, it is not clear whether the use of multiple fields can circumvent this problem. [4]

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O 030

A CONSISTENT PERSPECTIVE OF PROTEIN DYNAMICS IN THE SOLID-STATE FROM NMR LINESHAPES: THE ROLE OF SYMMETRY

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Deuterium lineshape analysis of CD3 groups has emerged as a particularly useful tool for studying microsecond - millisecond protein motions in the solid-state. The models devised so far consist of several simple jump-type motions combined. The various physical quantities are encoded in their simplest form; hence improvements are only possible by adding yet another simple motion, thereby changing the model. The various treatments developed are case-specific; hence comparison amongst the different systems is not possible. We have developed a new methodology for 2H NMR lineshape analysis free of these limitations. It is based on the microscopic-order-macroscopic-disorder (MOMD) approach. In MOMD motions are described by diffusion tensors, spatial restrictions by potentials/ordering tensors, and geometric features by relative tensor orientations. Barrier-crossing/jump-type scenarios are recovered by appropriate forms of the orientational potential. Model-improvement is accomplished by monitoring the magnitude, symmetry and orientation of the various tensors. The generality of MOMD makes possible comparison amongst different systems. Experimental CD3 spectra from polycrystalline Chicken Villin Headpiece Subdomain are used as examples. All of these lineshapes are reproduced with local potentials comprising the L = 2 and K = 0, 2 spherical harmonics, and with axial local diffusion tensors. Potential strength and rhombicity are found to be ca. 2 –- 3 kBT. The local diffusion tensor is tilted at 120o from the C–-CD3 axis. The perpendicular (parallel) correlation times for local motion are 0.1 - 1.0 ms (3.3 –- 30 microsecond). Corresponding activation energies of 1.1 –- 8.0 kcal/mol are estimated. In virtually all of the cases studied we

find that the symmetry of the local potential changes with temperature. Potential forms exhibiting barriers and wells (materializing in general at local sites in proteins) are illustrated. The local spatial restrictions in internally mobile proteins have been described so far predominantly by second-rank ordering tensors. Even-rank ordering implies apolar (equally probable parallel and antiparallel) arrangement in space, often referred to as alignment. Odd-rank ordering implies polar arrangement in space, often referred to as orientation. Mobile protein moieties are attached physically to the protein; hence their local ordering is largely polar in nature. We investigate the role of symmetry in this context by considering potentials, u, which comprise spherical harmonics of rank 1, 2 and 3. The distinction between orientation and alignment is substantiated particularly well by the relative probability density, exp(–u). We correlate polar ordering (at NH sites) with binding of the third immunoglobulin-binding domain of streptococcal protein G to its cognate FAB fragment. Future prospects, featuring combined mesoscopic/MOMD and atomistic/molecular dynamics approaches, are delineated. An illustration associated with local ordering at NH sites of plexin-B1 is provided.

Plenary Session 2

PL 03
THE EVOLUTION OF KINASE DYNAMICS OVER 1 BILLION YEARS REVEALS A MODERN CANCER DRUG'S MECHANISM

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Protein kinases are attractive drug targets against cancer due to their central role in cellular regulation, but their active site similarity has hampered the development of specific drugs. One of the major success stories is Gleevec, a highly selective inhibitor of Abl kinase. The molecular mechanism underlying Gleevec's selectivity for Abl has not been solved since the discovery of the drug 20 years ago. Gleevec's selectivity is puzzling because the drug-binding pocket with Gleevec bound is nearly identical between Abl and the weak binder Src.

I will present how the characterization of the evolution of kinase dynamics over 1 billion years reveals the mechanism responsible Gleevec's selectivity. We recreated the evolutionary pathway between Src and Abl by resurrecting their last common ancestor. Using a combination of NMR, fast kinetics and x-ray crystallography we characterized the evolution of the energy landscape. In contrast to all other proposed models we show that the origin of Abl's high affinity lies in a conformational change after binding. Such an energy landscape that delivers tight affinity and selectivity via an induced fit mechanism may be general for many inhibitors, highlighting the key role of protein dynamics for drug design. Interestingly, Gleevec resistance is also rooted in protein dynamics.

The results simultaneously shed light on the longstanding paradox of Gleevec specificity while offering insights into how energy landscapes evolve for catalysis. The evolution of energy landscapes may offer a larger variety of signaling mechanisms compared to evolution of structure alone. Interestingly, Gleevec takes advantage of these evolutionary differences "by accident" thereby leading to one of the

most potent and specific cancer drugs to date, and adding an unusual twist to a naturally occurring evolution likely linked to cellular regulation.

R.V. Agafonov et al. **Nat Struct Mol Biol.** (2014) 10, 848-853.
C. Wilson et al. **Science.** (2015) 347, 882-6.

Plenary Session 3

PL 04
SOLID-STATE NMR STUDIES OF MEMBRANE PROTEINS IN SYNTHETIC LIPIDS AND CELL MEMBRANES

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Membrane proteins play critical roles in cells and are involved in many important physiological processes. Many important aspects of their structural and dynamic organization have been revealed by X-ray crystallography and solution NMR. More challenging for these methods are studies of membrane proteins under nearly physiological conditions of a lipid bilayer or in cell membranes. Recent technological and methodological developments in solid-state NMR have led to the possibility of conducting structural analysis of large polytopic membrane proteins. I will describe our recent progress in this area, with particular emphasis on structural insights derived from our studies of a 27 kDa seven-helical photoreceptor Anabaena Sensory Rhodopsin (ASR). My primary focus will be on the determination of structure and oligomeric state of ASR, and its dynamics in the model environment composed of synthetic lipids as well as structural elucidation of ASR in E.coli membranes. Additionally, recent advances in our lab for obtaining DNP enhancements will be presented.

PL 05
A STEPPING STONE TO NEW EXPERIMENTS: FAILURE AND NEW TECHNOLOGIES

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In scientific papers or presentations, it seems the authors had a concrete aim and a plan to achieve something new from the very beginning and could jump into the results straightforwardly. But from my experience, I know it should not be like that in many (most of?) cases. In some cases, failure is a stepping stone to success. Particularly for young people, I believe it is worthy to show unashamedly some of my unsuccessful ideas/experiments/attempts that led something new finally. These unsuccessful attempts involve characterization of "noise", which is a common interest (trouble?) for all of us, and an attempt to affect relaxation. In fact, most of these attempts were prompted by recent development of new technologies, for a few examples, a versatile PC and digital devices that enable us to control amplitude/phase/frequency of RF irradiation precisely. The former is, of course, useful in many ways and I used it for analysis of noise. The latter does, however, make my life as a pulse-sequence maker difficult because there are too many free parameters for a human. It is true that the various kinds of PC-programs for spin-dynamics calculation can help us, but I rather prefer an intuitive way of developing a sequence. I show one example of deducing strange-looking wave forms that realize what we want to do by irradiation. Further, I will show my unsuccessful attempt to affect relaxation, which is not covered by the simulation programs; I wanted to do something that is beyond the current PC programs. I hope the basic ideas behind these attempts can stimulate young people to do something funny but new by themselves.

S 07 - Large biomolecular complexes

O 031
NMR ILLUMINATES TERNARY COMPLEX FORMATION AS IkBa STRIPS NFkB FROM DNA

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The transcription factor NFkB is rapidly activated by a number of extracellular signals. Rapid activation occurs as a result of the presence in resting cells of NFkB in the cytoplasm, in complex with its inhibitor, IkBa. Upon receipt of a signal at the cell surface, IkBa is phosphorylated and degraded by the proteasome in a ubiquitin-dependent manner. In the absence of IkBa, the nuclear localization signal (NLS) of the NFkB is freed, and the transcription factor is translocated to the nucleus, where signal-responsive gene transcription occurs. In many cases, one of the downstream genes is that for IkBa, which is newly transcribed and translated, enters the nucleus and strips the NFkB from the DNA, reversing the signaling switch and returning the NFkB to the cytoplasm as the IkBa complex.

We have previously shown that the presence of IkBa increases the rate of dissociation of NFkB from DNA, and that a ternary complex is implicated in the mechanism. The present research uses differential labeling of NFkB and TROSY NMR spectroscopy to determine whether a ternary complex between NFkB, DNA and IkBa is present in solution and to give insights into its structure. Backbone resonance assignments were made for smaller domains of NFkB in isolation, and the assignments for the p65 monomer in full-length (72 kDa) NFkB dimer were derived from these. Changes in the TROSY-HSQC spectrum upon addition of IkBa to the p65-labeled heterodimer mapped directly to the contact regions identified in the X-ray crystal structures, and the addition of DNA also showed plausible changes that could be rationalized from the NFkB-DNA crystal structure. Even using differential labeling and extensive deuteration, firm conclusions

could not be reached about the contribution of a ternary complex to the stripping mechanism: because of the large number of resonances, resonance overlap was severe. Residue-specific labeling was used to make definitive observations on spectral changes in solution that could be interpreted as evidence for contact between NFkB and DNA and/or IkBa. There are a total of 10 tyrosine residues in NFkB p65, of which 7 have backbone assignments obtained from the uniformly-labeled p65 in the NFkB heterodimer. Tyr36 makes close contacts with DNA in the NFkB-DNA complex, while Tyr306 is present in the p65 NLS, and shows large chemical shift changes between the free NFkB (NLS is disordered) and the complex with IkBa (NLS is folded). The chemical shifts of these residues thus provide diagnostic markers for the contacts between NFkB and DNA, and NFkB and IkBa, and enable us to distinguish whether the NFkB in the presence of both DNA and IkBa is bound to one or both of them. We anticipate using samples specifically labeled with other amino acids, as well as mutant forms of the proteins that show greater propensity for the formation of ternary interactions, to definitively characterize the mechanism of stripping in solution by NMR.

O 032

PROBING THE SUPRAMOLECULAR STRUCTURE OF THE 200KDA β-BARREL ASSEMBLY MACHINERY COMPLEX BY SOLID-STATE NMR

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Protein folding and insertion into cellular membranes, which is essential for physiological, pathogenic and drug resistance functions[1], requires complex molecular protein machines. In E. coli the precursors of outer-membrane beta-barrel proteins (OMP) are synthesized in the bacterial cytoplasm. The unfolded precursor proteins are recognized and translocated across the inner membrane by the Sec translocase. Insertion into the outer membrane is subsequently coordinated by the beta-barrel assembly machinery (BAM[2]) consisting of the core component BamA and accessory lipoproteins (BamB, BamC, BamD, and BamE). Recently, structures were made available of BamA and individual accessory lipoproteins of the BAM complex[3]. In spite of these advancements, atomic information on how BAM dynamically assembles in membranes, the individual roles of the accessory lipoproteins and how substrate insertion takes place is still elusive.

OMP folding and insertion is likely driven by intrinsic dynamics and conformational changes which occur within the BAM complex. We have studied the structure and dynamics of the central component, BamA, and in particular the POTRA domains, in lipid bilayers[4-7]. Most recently, we detected molecular plasticity in POTRA 5 residues known to affect correct beta-barrel assembly in vivo, suggesting that conformational dynamics are related to the interaction of BamA with BamD and possibly to substrate binding.

These results set the stage to study the interactions of essential BAM components in a membrane environment. We have developed a solid-state NMR based approach to address the inherent difficulties encountered when dealing with such large, complex samples. In our

contribution, we report on the latest progress in applying this method to gain insight into interactions which occur upon (sub)complex formation and to elucidate their possible role in substrate insertion.

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O 033

HIV-1 ENVELOPE MEMBRANE PROTEIN GP41: AN NMR STUDY OF DODECYL PHOSPHOCHOLINE EMBEDDED GP41 REVEALS A DYNAMIC PRE-FUSION INTERMEDIATE CONFORMATION

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Human immunodeficiency viral (HIV-1) fusion is mediated by the viral envelope gp120/gp41 complex (ENVelope glycoprotein). After gp120 shedding, gp41 is exposed and elicits membrane fusion via a cascade of conformational changes. In contrast to pre-fusion and post-fusion conformation, little is known about any intermediate conformation. We report on a solution NMR investigation of homotrimeric HIV-1 gp41(1-194) and gp41(27-194), comprising the fusion peptide, N-terminal and C-terminal heptad repeat (NHR and CHR) and transmembrane region and reconstituted in dodecyl phosphocholine (DPC) micelles (1,2).

The protein is mainly a-helical but experiences inter-domain dynamics on the nanosecond and micro- to millisecond time scale and transient a-helical behaviour for certain residues in the N-terminal heptad repeat (NHR), as investigated by a recently proposed set of ^{15}N relaxation experiments that allows Hahn-echo based R_{ex} measurements and ^{15}N R_1, R_{1rho} relaxation and ^{15}N-{^1H} NOE measurements for high molecular weight systems, using a TROSY-detection scheme (3). The optimized set of experiments alleviates systematic errors due to water-saturation and cross-correlated relaxation effects, particularly acute in per-deuterated systems.

For gp41 (27-194) strong lipid interactions are observed, in particular for C-terminal residues of the NHR and immunodominant loop region connecting NHR and C-terminal heptad repeat (CHR). Our data indicate an extended conformation with features anticipated for a

pre-fusion intermediate, presumably in exchange with a lowly populated post-fusion six-helical bundle conformation.

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O 034

AUTOMATED NMR RESONANCE ASSIGNMENT STRATEGIES FOR NUCLEIC ACIDS USING THROUGH-BOND AND THROUGH-SPACE HIGH-DIMENSIONAL EXPERIMENTS

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Sequence specific resonance assignments are the basis for any detailed NMR study of biological macromolecules. In this context projection spectroscopy has proven to be a very versatile tool due to the resolving power of the underlying high dimensional NMR spectra. One application of projection spectroscopy is APSY (automated projection spectroscopy). With APSY robust and fully automated assignments can be obtained in NMR spectra of proteins [1]. We now extended the application of APSY to the automated assignment of NMR spectra of nucleic acids which is quite a challenge due to the narrow chemical shift range of the resonances. We will present two strategies: one for smaller and one for larger [13C,15N]-labeled RNAs [2, 3]. The experiments can be used in the context of sparse sampling or of projection spectroscopy. We used APSY (automated projection spectroscopy) and the FLYA algorithm for the analysis of the spectra.
For small and medium sized nucleic acids we propose a fast, robust and reliable strategy for automated sequential resonance assignment via their phosphodiester backbone based on a suite of 4D and 5D through-bond APSY experiments. The utilized pulse sequences are partially novel, and optimized to enable long evolution times in all dimensions. The highly precise APSY peak lists derived with these experiments could be used directly for reliable automated resonance assignment with the FLYA algorithm [4]. This approach resulted in 98 % assignment completeness for all 13C–1H, 15N1/9 and 31P resonances of a stem-loop with 14 nucleotides.
For medium to larger sized nucleic acids we propose a suite of four through-bond and two through-space high-dimensional APSY experiments. The approach is exemplified with a 0.3 mM sample of

an RNA stem-loop with 48 nucleotides. The highly accurate and precise 3- to 4-dimensional APSY peak lists provided a reliable basis for the subsequent sequence-specific resonance assignment with the algorithm FLYA and resulted in the fully automated resonance assignment of more than 80 % of the resonances of the 13C–1H moieties at the 1', 2', 5, 6, and 8 positions in the nucleotides. The procedure was robust with respect to impurity peaks, low concentration of this for NMR comparably large RNA, and structural features such as a loop, single-nucleotide bulges and a non-Watson–Crick wobble base pair.

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O 035
PHYSIOLOGY AND PATHOLOGY OF TAU BY NMR

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The tubulin associated unit (TAU) was discovered nearly fourty years ago as an unstructred protein that protmotes tubulin assembly into microtubules (Weingarten & Kirschner, 1975), but how it exerts this function remains largely unknown. We will show our recent data with soluble tubulin constructs that give a first hint of the mechanism of this process (Gigant et al., 2014).

After the discovery that Tau composes the paired helical filaments that characterize the neurons of Alzheimer's diseased patients (Kosik et al., 1986; Grundke-Iqbal et al., 1986), intense research has aimed at discovering the molecular mechanisms of this transition. Clinically, the extent of the disease is staged by immunochemistry with the AT8 antibody that recognizes phosphorylated Tau (Braak et al., 2006). Using a combined approach of Molecular Dynamics and NMR, we have characterized this epitope at the molecular level (Gandhi et al., 2015).

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S 08 - NMR Physics

O 036

NMR AS A LOCAL PROBE IN NANOSTRUCTURED STRONGLY CORRELATED MATERIALS.

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Nanostructures are either man made or tend to self organize in materials with strong electron correlations. In these conditions neutron and X ray scattering, normally the technique of choice, may not suffice in unraveling structure from low energy excitations. This is a field in which NMR and other magnetic resonances can still provide very powerful insight. Several examples will be briefly illustrated, ranging from iron superconductors, to charge and spin stripe materials, to single molecule magnets.

O 037

DEVELOPMENT AND APPLICATION OF A NOVEL THZ MAGNETIC RESONANCE SPECTROMETER WITH FIELD- AND FREQUENCY-SWEEP CAPABILITIES

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High Frequency Electron Paramagnetic Resonance (HFEPR) is a powerful method to investigate samples ranging from biomolecules to magnetic materials. It delivers high g-value resolution and access to large zero-field splittings. However, the field domain HFEPR method is very slow in recording data, because it is limited by the sweep rate of the used magnet. Nowadays, with the progress in high frequency microwave technology, a magnetic resonance spectrum can be recorded much faster by sweeping the microwave frequency at a constant magnetic field. Frequency Domain Magnetic Resonance (FDMR) is very convenient as the energy splittings of the system are directly obtained even in absence of a magnetic field. Further, recording the spectra at a constant magnetic field prevents potential changes in physical properties of the sample caused by the changing magnetic field. Hence we decided to take the advantages of both techniques, which is the sensitivity of HFEPR spectroscopy and the convenience of FDMR, and set up a combined HFEPR and FDMR spectrometer at the University in Stuttgart. The combined HFEPR and FDMR spectrometer operates at high magnetic fields up to 17 T and temperatures from 300 K down to 1.8 K. For the microwave radiation an amplifier multiplier chain is used which gaplessly covers a very broad frequency range from 85 GHz to 1100 GHz. A quasi-optical bridge in combination with corrugated waveguides guarantees the propagation of the microwave with only very low losses. We will demonstrate a significant improvement of the FDMR sensitivity by a factor of three orders of magnitude on organic radicals as well as on single molecule magnets in absence of external magnetic field. This large increase of sensitivity was achieved by the implementation of a field modulation. Recently a Fabry-Pérot resonator was developed, which set the current sensitivity of the spectrometer to 10^7 spins/Gauss,

at 370 GHz and at a source output power of approximately 1 mW. This makes the combined HFEPR and FDMR spectrometer an outstandingly powerful tool which recently helped us to resolve several scientific questions concerning Dy…Dy interactions [Nat. Commun., **5**, 5243 (2014)], spin state switching in dicobalt complexes [Chem. Eur. J., **20**, 3475 – 3486 (2014)] and solvent effects in Fe_4 complexes [Chem. Commun., **50**, 15090-15093 (2014)]. We are now improving the combined HFEPR and FDMR spectrometer by implementing a single crystal rotator for orientational studies. In addition, we are developing high frequency rapid scan EPR. By using high frequency rapid scans (5 THz/s) we can gain several additional orders of magnitude in sensitivity and access relaxation rates in one spectrometer at several frequencies.

O 038

FOLLOWING LITHIATION FRONTS IN PARAMAGNETIC BATTERY ELECTRODES WITH IN SITU MAGNETIC RESONANCE SPECTROSCOPIC IMAGING

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Lithium-ion batteries have become the premier energy storage technology for portable electronics and vehicle electrification. A better knowledge of compositional variations within the electrodes during battery operation is however still needed for improving their overall performance.
Ex situ NMR of paramagnetic electrode materials is now considerably easier thanks to ultra-fast MAS, isotopic substitution or "low" magnetic fields. In favorable cases, dipolar recoupling can be used to probe atomic proximities in 2D or site-selective experiments. On the other hand, electrolytes such as polyethylene oxide based block copolymers or sulfides can now be characterized in depth by combining PFG-diffusion measurements and relaxation studies.
In situ NMR spectroscopy may be able to capture the true state of electrodes, however, the electrodes, the separator and free electrolyte can give rise to overlapping signals and complex spectra which may be impossible to deconvolute.
Nowadays, magnetic resonance imaging is essential in the medical field and appears as an elegant tool to unravel changes in batteries. Its implementation for solid paramagnetic materials, however, has until now been a challenge. Implementing imaging techniques that minimize signal loss resulting from fast relaxation allowed us to demonstrate the first 7Li 1D magnetic resonance spectroscopic imaging of a complete 5 mm-diameter, 1 mm-thick operating battery with an unprecedented resolution of 100 micrometers, obtained using strong magnetic field gradients (30 T/m). We provide, through the image-spectrum correlation, information about the chemical

environment of lithium-7 atoms in each 100 microns-thick slice of the battery. Data recording is fast enough to enable the visualization in situ of the displacement of lithiation fronts inside the paramagnetic electrodes during battery operation, showing distinct behaviors depending upon the morphology of the electrode composite systems. This strategy can be applied to other systems suffering from fast relaxation, such as fuel cells, catalysts or paramagnetic rocks or glasses, and may be eventually be combined with more complex relaxation or diffusion measurements.

O 039
Co METAL BASED CATALYSTS PROBED BY FERROMAGNETIC 59Co NMR

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An interest of Co metal based catalysts characterization has risen simultaneously to Fischer-Tropsch synthesis (FTS) due to new opportunities of using biomass for syngas production. New perspectives require novel catalysts to overcome existing limitations of industrially utilized FTS catalysts. Co FTS catalysts are known to produce not only diesel or lubrication oil, but also more valuable products such as alcohols, ethers and olefins. Despite many techniques have been implemented [1] to probe the structure of Co FTS catalysts, the application of ferromagnetic ^{59}Co NMR (f-NMR) is still challenging [2]. The main advantage of f-NMR consists in the type of information providing by the method. First, it gives structural insight into studied active component of FTS catalysts reflecting different Co metal stacking as hcp, fcc or stacking faults (sfs). Second, f-NMR is sensitive to different magnetically ordered parts of sample as magnetic domains, domain walls and single-domain particles. And the last but not least, it is highly sensitive to various substitutions in the Co coronation sphere, which causes a substantial shift of resonance lines due to increase/decrease of magnetic moments on Co nuclei.

A set of different types of Co FTS catalysts has been examined both ex situ and in situ. Co supported on γ-Al_2O_3 has been studied ex situ after activation (fresh catalyst) and after the redox treatment represented by reduction-oxidation-reduction procedure to follow up the catalyst stability to partial uncontrolled oxidation during FTS synthesis. The obtained results on a set of Co/γ-Al_2O_3 catalysts activated in a different way reveal the drastic particle size changes because of the redox treatment. On the other hand, in situ

examination of Co/SiC catalyst performed by sealed glass ampoule heating inside the NMR probe in the range of 300-850 K reveals wide temperature range of sample stability with increasing temperature. The fact is important in terms of legitimation of room and low temperature spectra acquisition of catalytic species, which are actually exposed to reagent mixture at heightened temperature.

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O 040
HETERO-NUCLEAR CROSS EFFECTS DURING DNP ON SOLIDS

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Examples of hetero-nuclear polarization transfer during Dynamic Nuclear Polarization (DNP) experiments have been reported in the literature[1,2]. This transfer mechanism has been described using the Thermal Mixing mechanism for DNP in solids. The spin temperature equilibration process between the electron and nuclear spin baths provides a possible explanation for this transfer process as well as for the equivalence of the hetero-nuclear enhancements.
In the present talk additional experimental evidence showing this type of polarization transfer and enhancement equilibration will be presented and discussed. In particular, ^{1}H-and ^{2}H-DNP spectra and time domain experimental results from glass forming samples, composed of H_2O/DMSO-d_6 and D_2O/DMSO-$h_6$mixtures and containing 40 mM TEMPOL, will be presented and analyzed. This analysis relies on the understanding that during the MW irradiation the electron polarization distribution depends on the spectral diffusion process and that even without MW irradiation the cross-talk between the proton and deuteron polarizations in the samples is possible via the hetero-nuclear cross effect (hn-CE)[3]. The nature of this hn-CE will be characterized and its experimental manifestations will be explained. A model formulating the complex flow of polarization between the electrons and nuclei during DNP on hetero-nuclear spin systems which is compatible with the experimental results will be suggested.

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S 09 - In-vivo and In-cell NMR

O 041
IN-CELL NMR OF LARGER PROTEINS AND COMPLEXES

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The non-invasive character of NMR spectroscopy allows researchers to investigate the conformation and dynamics of biological macromolecules in their natural environment, for example the cytoplasm of cells. We have used in-cell NMR spectroscopy to study both the specific as well as non-specific interactions of biological macromolecules in different cellular systems. The telomeric G-overhang is the 3' single stranded protrusion of double stranded telomeres and consists of repeating d(TTAGGG)n elements. These elements form G-quadruplexes, which however under different in vitro conditions can adopt several different conformations. In order to investigate which of these conformations is the biologically relevant conformation we have injected quadruplexes of different length into Xenopus oocytes or investigated them in oocytes extracts. These investigations revealed that G4 units coexist in two conformations, the hybrid-2 and the 2-tetrad antiparallel basket. In addition, we have used in-cell NMR to investigate the behavior of Pin-1, a peptidyl-prolyl isomerase and show that the protein uses its WW domain to nonspecifically investigate other proteins as potential substrates. This non-specific interaction can be blocked by phosphorylation in the WW domain as welll as by interaction with a specific peptide.

O 042
MAGNETIC RESONANCE DETECTION OF LYMPHATIC BREAST CANCER METASTASIS IN A XENOGRAFT MODEL BY HYPERPOLARIZED 13C-PYRUVATE

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Breast cancer is the most commonly diagnosed cancer among women. Besides significant progress in therapeutic strategies, the prognosis of metastatic breast cancer (MBC) is poor with a five years survival rate around 25%. An early event of MBC is the dissemination of tumor cells in the lymphatic system to form metastasis in lymph nodes (LN). Thus, a key challenge is the ability to non-invasively detect and characterize the metastatic LN in order to improve patient management.
The emergence of dissolution dynamic nuclear polarization (DNP) technique in combination with magnetic resonance imaging and/or spectroscopy (MRI/MRS) has opened new avenues for real-time imaging of the metabolism. The DNP of a ^{13}C-labelled metabolite enables its hyperpolarization leading to an increase in signal-to-noise ratio around 10^4 allowing then for the fast MRI/MRS detection of not only the substrate but also its metabolic products. Taking advantage of the fast anaerobic glycolytic pathway (Warburg effect) in cancer cells, like positron Emission Tomography, this cutting-edge method has already shown its potential for the detection of human prostate tumors. The interest for its clinical application is even larger for the detection and the monitoring of the metastatic process. While the metabolic reprogramming of cancer cells is well identified, little is known about the metabolic profile of the disseminated tumor cells leading to metastasis.
In this study we have used DNP-enhanced ^{13}C MRS method to probe the metabolism of the primary tumors and metastatic LN in a breast cancer xenograft model using 1-^{13}C hyperpolarized pyruvate. MDA-MB-231 human breast cancer cells were injected into the mammary fat pad of seven immunodeficient nude mice. Three to six weeks after

cells implantation, the primary tumor growth and the contralateral inguinal LN were monitored by ^{1}H MRI at 4.7T. Arrays of ^{13}C MRS spectra were recorded on both sites by a 3mm ^{13}C surface coil following the intravenous injection of hyperpolarized ^{13}C-pyruvate. The animals were later sacrificed and the presence of metastasis at the inguinal LN was confirmed or denied by histopathology.
Lactate production from injected pyruvate was observed in both tissues. The highest lactate/pyruvate signal was observed for a metastatic LN (1.14; n=1) while the mean normalized lactate signal from the primary tumors and the non-metastatic LN were 0.50 (± 0.2, n=7) and 0.62 (± 0.2; n=6) respectively. The dynamic data also enabled the extraction of pyruvate-to-lactate rate constant (k-rate). The highest k-rate was observed for the metastatic LN (0.06 s^{-1}), which was twice the mean of the ones calculated from primary tumor spectra (0.03 s^{-1}; ± 0.008). The mean k-rate obtained from the non-metastatic LN was 0.05 s^{-1} (± 0.01). Further studies of metastatic LN are required in order to define the metabolic changes occurring during the infiltration of cancer cells into the lymphatic system. This work, still under progress, presents an unprecedented effort to characterize the metabolism of both the primary tumor cells and their metastatic spread to regional LN, that once successful might be of valuable interest for clinical application.

O 043
ONLINE SPECTROSCOPY OF 13C-LABELLED METABOLITES IN MICRODIALYSATE UTILIZING A MICROCOIL

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^{13}C NMR spectroscopy remains challenging in vivo due to the low gyromagnetic ratio of ^{13}C spins and low natural abundance. To increase the signal-to-noise (SNR) typically high magnetic fields in combination with ^{13}C-labeled substrates are utilized. When samples on the order of one microliter or below are investigated additional solutions are required to increase the limit of detection. Hyperpolarization techniques such as dissolution Dynamic Nuclear Polarization (DNP) or Para-Hydrogen Induced Polarization (PHIP) are very effective approaches for creating spin-polarization.[1-3] However, the in vivo investigation of metabolites in humans has only recently been demonstrated for DNP and is currently only applicable in few research institutions.[4] Another approach to increase the SNR for small sample volumes is the use of microcoils that can be brought in close proximity of the sample.[5] We have constructed a microcoil for NMR spectroscopy that can be mounted inside magnetic resonance imaging systems around the outlet of microdialysis devices. It consists of a double parallel copper coil with a diameter of 780 µm, 2.4 mm length, a wire diameter of 30 µm and a filling factor of 92%. The total volume enclosed by the coil is 1 µL. For improved line narrowing and increased sensitivity the microsolenoid coil is placed inside a fluorinated fluid (FC-63) with a comparable magnetic susceptibility of the used copper wire. Utilizing a microdialysis probe, 3-^{13}C-lactate was injected into a solution containing glioma tumor cells under a continuous flow of 0.5 µL/minute. Having the microcoil positioned around the microdialysis outlet allows for online monitoring of the metabolites exchanged across the microdialysis membrane.

In the future we are planning to expand the investigations to monitor in vivo intracerebral microdialysates which will represent a step

towards in vivo NMR investigation of brain tumor metabolism at submillimetric scale.

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O 044
PROBING PROTEIN QUINARY STRUCTURES BY IN-CELL NMR

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Historically introduced by McConkey to explain the slow mutation rate of highly abundant proteins, weak protein (quinary) interactions are an emergent property of living cells. The protein complexes that result from quinary interactions are transient and thus difficult to study biochemically in vitro. In-cell NMR of proteins and protein complexes can potentially provide atomic resolution information about quinary structures, thus opening the door to study a new level of structural complexity present in intact cells. At present, in-cell NMR is seriously limited by the molecular size of cellular complexes and the intrinsic cytosolic viscosity. Cross-correlated relaxation induced polarization transfer (CRIPT) based in-cell NMR allows us to largely circumvent the limitations of molecular size and characterize protein quinary structures with atomic resolution inside live prokaryotic and eukaryotic cells. We show that RNAs are an important component of protein quinary structures. Our observations provide compelling evidence that quinary interactions are linked to RNA biology and thus are important for regulating protein function and activity. Protein quinary structures are unique to the target protein and may affect physicochemical properties, protein activity, and interactions with drugs.

O 045
A BIOREACTOR FOR NMR OVSERVATION OF BIOLOGICAL EVENTS INSIDE LIVING CELLS

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In-cell NMR spectroscopy is a method used to observe isotopically labeled molecules within living cells. The first in-cell NMR experiment was performed with an E. coli overexpressing a 15N-labeled protein. For the first application of the in-cell NMR method with eukaryotic cells, isotopically labeled target proteins were introduced, by microinjection, into Xenopus laevis oocytes, and a cell-penetrating tag was also utilized. Our group reported an in-cell NMR method for mammalian cells; we used a pore-forming toxin, streptolysin O (SLO), to introduce target proteins by diffusion. By using these methods, protein–drug interactions and intracellular post-translational modifications, such as phosphorylation and acetylation, were successfully detected in vivo. However, the major limitation of the in-cell NMR experiments is the occurrence of cell death during the NMR measurement. As the suspension contains a high density of cells, nutrient depletion occurs rapidly in the anaerobic environment within the NMR tube, thus causing the deterioration of conditions and resulting in cell death during NMR measurements. Therefore, in-cell NMR experiments for eukaryotic cells currently have limited applications, such as for obtaining a single NMR spectrum measured within a very short time. Although sparse sampling methods have been utilized to shorten the time required to acquire multidimensional NMR spectra, many existing in vitro NMR experiments that are used to provide information regarding dynamics and protein interactions take several hours to perform. In this study, we will show a bioreactor system for in-cell NMR, which we developed, to suppress the cell death during NMR measurements.

S 10 - Biomacromolecular Folding and Dynamics

O 046

NEW NMR METHODS TO STUDY RNA FOLDING AND DYNAMICS

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In this contribution, new technologies will be discussed to study RNA and DNA structure and folding by NMR spectroscopy.

Topics covered include:
1.) Development of novel methods to rapidly screen sequence-dependent folding space by PCR-based amplification of RNAs.
2.) Development of T-jump NMR technologies to study temperature-induced folding transitions of RNA and DNA.
3.) Study complex folding pathways of RNAs and DNAs including G-quadruplexes and i-motifs.

O 047
NMR STUDIES OF GUANINE-RICH TETRAHELICAL DNA STRUCTURES

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Canonical model of double helix of DNA can account for information storage and retrieval of genetic information. In addition to double helical structure DNA can fold into a wide range of structures that are associated with its unique biological roles and functions. Guanine-rich repeats can form four-stranded structures termed G-quadruplexes. A wide distribution of G-rich repetitive sequences throughout genome and transcriptome has raised a particular interest.

Nuclear magnetic resonance (NMR) spectroscopy is currently the most versatile spectroscopic techniques for the characterization of molecular structure and dynamics of G-quadruplexes in solution. LNA modified VEGF aptamer RNV66 adopts a single unprecedented parallel-stranded monomeric G-quadruplex with three G-quartets.

Assessment of hydrodynamic properties on d(G4T4G4) originating from 1.5 telomeric repeats of Oxytricha nova has shown that unfolded oligonucleotides do not adopt an extended, but rather some type of random coiled structures. The pre-organized structure held together by transient hydrogen bonds with distinctive fingerprint features represents an intermediate on pathway to G-quadruplex formation.

An oligonucleotide with repetitive G-rich segments found in the regulatory region of the PLEKHG3 gene in the 14th human chromosome that represents a potential candidate contributing to the risk of autism does fold into a G-quadruplex.Nevertheless, it adopts a well-defined tetrahelical structure. No G-quartets or Hoogsteen-type H-bonded guanine residues are present and the overall topology

is conserved in the presence of Li+, Na+, K+ and NH4+ ions. Its four-stranded architecture is stabilized by four G-C base pairs in Watson-Crick geometry, four G·A base pairs in N7-N1 amino carbonyl and six G·G base pairs in N1-carbonyl symmetric geometry.

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O 048
THE WWPDB NMR VALIDATION REPORTS AND UNIFIED NMR DATA REPRESENTATION

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The Worldwide Protein Data Bank (wwPDB) organisation [1], consisting of Protein Data Bank in Europe (PDBe) [2], Protein Data Bank Japan (PDBj) [3], Research Collaboratory for Structural Bioinformatics (RCSB PDB) [4] and Biological Magnetic Resonance Databank (BMRB) [5], jointly manages the Protein Data Bank (PDB) [6], the single global archive of biomacromolecular structure data. In an effort to improve the quality and consistency of the PDB archive [7], the wwPDB convened expert Validation Task Forces (VTFs) for X-ray crystallography [8,9], NMR [10], 3D CryoEM [11] and Small Angle Scattering [12]. PDBe was tasked to implement the corresponding recommendations in software pipelines to be invoked at the time of data deposition to the PDB, annotation, public release and though an anonymous upload server. The validation pipelines are integrated with the new common Deposition and Annotation developed by the wwPDB partners. The X-ray pipeline [8] has been operational since 2013 and the validation reports for all publicly available PDB X-ray-derived entries were made publicly accessible since 2014. The NMR pipeline is expected to become operational in the Summer of 2015 making the validation reports public via the file distribution and wwPDB partner websites.

The principle recommendations of the NMR VTF [10] include the following: 1) identification of well- and ill-defined regions of proteins, 2) application of all relevant routines from the X-ray pipeline for knowledge-based geometric validation, 3) chemical shift referencing corrections, a measure of resonance assignment completeness, identification of statistically unusual chemical shifts and a Random Coil Index (RCI) plot, and 4) simple analysis of the restraints and their violations. Version 1 of the pipeline includes points 1-3 from the above

list. In order to handle the NMR restraints data in a consistent fashion, the issue of format diversity needs to be solved first. The wwPDB and CCPN teamed together to convene two workshops with developers of leading NMR software for structure determination, refinement and validation, who have agreed to develop, comply with and maintain a common format: the NMR Exchange Format (NEF) [13]. The first versions of NMR software supporting data export to and import from NEF files will be released in the Autumn of 2015 with the intention that the wwPDB will require NMR data deposition via NEF files after a transitional period and make restraints validation available in the validation reports.

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O 049

CONFORMATIONAL DYNAMICS AS A KEY FACTOR OF ACTIVATION OF THE RECEIVER DOMAIN OF SENSOR HISTIDINE KINASE CKI1 FROM ARABIDOPSIS THALIANA

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Multistep phosphorelay (MSP) involved in plant hormone signaling represents an interesting evolutionary adaptation of the two-component systems widespread in bacteria. Receiver domains (RDs) of MSP sensors are important components of the system, able to transfer phosphate to downstream interacting partners. While the structure and dynamics bacterial RDs have been studied in details by NMR in bacteria, plant RDs are much less characterized. In this study, we investigated RD of sensor histidine kinase CKI1 of a model plant Arabidopsis thaliana. We studied three forms of CKI1 RD both in solution and in crystal: free protein, magnesium-bound form, and a stable analog of the active, phosphorylated receiver domain. While significant structural changes have been observed in activated bacterial RDs, crystal structures of all three CKI1 RD forms are identical, with the exception of a necessary adjustment of the side chain orientation of residues in a direct contact with the phosphorylation site. In contrast, NMR spectra revealed significant chemical shift changes already upon binding Mg(2+) ions, needed for the active site phosphorylation. An even more striking effect is an alteration of conformational dynamics of a loop close to the phosphorylation site. While signals of the loop residues are broadened beyond the detection limit in a free form, conformational freedom of amino acids in a vicinity of the phosphorylation site is greatly reduced by magnesium binding. With a help of amino-acid selective labeling, we were able to assign the loop resonances and study the loop dynamics by NMR relaxation and relaxation-dispersion experiments. Relaxation data of the active form analog (with phosphorylation of the active-site aspartate mimicked by beryllofluoridation) showed that the conformational exchange in the loop is almost completely eliminated

in the active form. Remarkably, electron density of the loop residues is well defined in crystals of all three forms of CKI1 RD, with a variation of B-factors similar to other loops, which do not exhibit any slow exchange in NMR relaxation experiments. It documents that dynamics of the receiver domain is a key factor of the activation process. Combination of X-ray crystallography and NMR spectroscopy thus shows that magnesium binding and phosphorylation do not induce formation of a new, active conformation of the receiver domain, but shift the equilibrium of conformations in favor of the active state, already present in the ensemble of conformers existing in solution. The identical X-ray structures can be interpreted as trapping the same thermodynamically favored active state conformations of the chemically different CKI1 RD forms. On the other hand, the NMR experiments provided a statistical picture of the complete conformational ensembles in solution, close to physiological conditions.

O 050

THE HETEROGENEOUS STRUCTURAL BEHAVIOUR OF VIRAL INTRINSICALLY DISORDERED PROTEINS REVEALED BY NMR SPECTROSCOPY

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The importance of local flexibility in determining the function of proteins has been recognized long ago and also widely scrutinized. If the extent of local flexibility is taken to its extreme conditions it leads to completely random coil behaviour of a polypeptide chain, indicated as intrinsic disorder, through a wide variety of intermediate cases both in terms of extent of mobility or in terms of protein stretches involved. Many examples of intrinsically disordered proteins (IDPs) appeared in the literature showing how their structural plasticity and intrinsic flexibility can be key features to enable them to interact with a variety of different partners and to adapt to different conditions. These properties provide functional advantages to IDPs enabling them to play key roles in many regulatory processes and their function has also been related to several diseases. IDPs are extensively used by viruses to infect healthy cells since, in virtue of their small genomes able to code only a limited number of proteins, they need economic ways to interfere with the host. This is the case of human papilloma virus (HPV) and of human adenovirus (HAdV). We present here the NMR characterization of E7 from HPV16, one of the most dangerous variants of the virus, and of E1A from HAdV. Their characterization opens the way to study at the molecular level interactions and post-translational modifications of these proteins to reveal functional details that may be linked to their highly oncogenic potential.

S 11 - New Approaches to the MR Measurement

O 051
MULTI-FREQUENCY PROTON NMR RELAXATION FOR THE STUDY OF PROTEIN ROTATIONAL DIFFUSION: APPLICATION TO CROWDED SOLUTIONS

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The rotational diffusion coefficient of proteins, inversely related to the rotational tumbling time, is commonly obtained as a by-product of the high-resolution NMR assessment of internal protein dynamics in terms of the order parameter [1], relying on the Lipari/Szabo "model-free" approach [2]. It crucially relies on the assumption of a single-exponential tumbling correlation function. This assumption, however, can be violated already at moderate concentrations [3], and this violation cannot be inferred from high-resolution data alone due to the limitation of evaluating the spectral density J(w) only at 0 frequency (T_2) and linear combinations of the Larmor frequencies (T_{1r}, NOE). Extending the frequency range, taking advantage of recent developments in ^{1}H fast field cycling relaxometry [4] in combination with rotating-frame (T_{1r}) and transverse (T_2) relaxation [3,5], we can reliably probe the low-frequency spectral density and thus identify "slow tails" of the tumbling correlation function, arising from protein-protein interactions.

In this presentation, I address methodological issues of integral proton relaxometry as opposed to site-resolved observations, requiring the consideration of second-moment distributions, leading to non-exponential relaxation at low frequencies. A reliable analysis is enabled by combination of field-dependent T_1 as well as T_{1r} and T_2 relaxation times [5]. As an application, we compare the slowdown effect observed for rotational as well as translational diffusion of various proteins (aB crystallin [5], bovine serum albumin and lysozyme)

at high concentrations. We find that the reduction in translational diffusion, measured by PFG NMR, follows the increase of the macroscopic viscosity, as predicted by Stokes-Einstein theory. The rotational diffusion, however, decouples from the macroscopic viscosity to a variable extent, which is related to protein shape and interactions [3,5]. Our finding of a less significant rotational slowdown is in line with established principles from colloid science [6], but stands contrast to previous NMR studies [7], highlighting the importance of "slow tails" of the rotational correlation function under protein crowding.

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O 052
DYNAMIC NON-UNIFORM SAMPLING

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Non-uniform sampling (NUS) has become a widely applied way of accelerating multidimensional NMR experiments. Most of the standard signal acquisition software allows to run experiments in the NUS mode. There are also numerous NUS processing methods, among which sparsity-based reconstructions, known also as compressed sensing (CS), have recently become popular [1].

Interestingly, NUS can be also applied in a way slightly different from the usual undersampling of the full Nyquist grid, i.e. it can be used to implement time-resolved experiments with extraordinary temporal resolution. The original idea of Mayzel et al. [2] assumed, that the random sampling of the indirect dimensions is performed in parallel to some chemical or physical process occurring in the sample. Then, overlapping subsets of the acquired dataset are used for the reconstruction of the series of multidimensional spectra corresponding to various moments of the process.

Our group developed CS methods dedicated for the acquisition and processing of the time-resolved data. The examples of both artificially induced processes [3] and uncontrolled reactions [4] have been given. Currently, the idea is being extended to the experiments where the observed change in the spectrum is induced not by the changes in the molecular structure, but by varying the coherence transfer. The examples of applications, as well as the main principles of the method, referred to as dynamic NUS, will be mentioned during the talk.

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O 053
A NEW APPROACH TO ESTIMATE TISSUE ELECTRICAL PROPERTIES BY VARYING THE RF DISTRIBUTION USING HIGH PERMITTIVITY MATERIALS

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Electrical properties tomography (EPT) using MRI is a new technique that has been developed to provide a new image contrast mechanism. Studies have shown that electrical tissue properties - both permittivity and conductivity – contains important physiological information which can be useful for distinguishing, for example, between malignant versus normal tissue. Currently the main imaging method relies on the solution of the homogeneous Helmholtz equation for the electromagnetic field in space, involving a second order derivative of the estimated B_1^+ map. This technique has potential drawbacks at tissue interfaces as well as a high sensitivity to noise. A second method, known as MREIT (Magnetic Resonance Electrical Impedance Tomography) relies on applying currents through tissue via electrodes, but limits on the magnitude of the in-vivo current injection reduces the sensitivity of the method. Different approaches have been introduced to increase the sensitivity of the EPT method, including utilizing a multi-channel transmit coil for a more stable solution. One such work also shows a new approach using Maxwell equations in their integral representations and a multi-channel transmit coil for several B_1^+ phase conditions. Combining ideas from several methods and aiming to design a simple setup for practical implementation, we propose another approach to generate imaging conditions under which the spatial distribution of the B_1^+ can be altered. In this current study we explore an approach that provides an estimation of the tissue electrical properties utilizing the B_1^+ in the presence of a high permittivity dielectric material that is located in the vicinity of the subject in the region of interest, and a second condition in which the material is simply removed.

This method relies on the use of an integral form of the Maxwell equations and on the displacement current that is generated in the presence of a high permittivity dielectric material. In previous studies,

high permittivity dielectric materials have been shown to improve the RF shimming of the B_1^+ in the region of interest. In the vicinity of the dielectric material, the generated displacement current increases the magnetic flux, introducing a specific spatial distribution. In our method, we suggest that measured two B_1^+ maps with and without the dielectric pad in the region of interest and a-priori knowledge of the electrical properties of the dielectric pad, can be utilized to solve the inverse problem and to estimate the electrical tissue properties.

For validation, electromagnetic simulations of the brain region of interest at 3 T were performed to generate the B_1^+ field maps. This was performed using FIT (finite integration technique) software (CST Microwave Studio). The setup included a birdcage coil loaded with the Bio model "Duke" from the Virtual Family with and without high permittivity dielectric pad. Then, the generated slices of the B_1^+ maps in the brain were used as an input for the inverse problem to estimate the complex permittivity maps. The assessments show promising results for phantoms and in-vivo measurements.

O 054
MAGNETIC RESONANCE SPECTROSCOPY AND IMAGING USING A CMOS FREQUENCY DIVISION MULTIPLEXER

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In magnetic resonance imaging (MRI), the use of phased arrays (multiple decoupled coils instead of a single coil) to detect the MR signals offers many advantages. These advantages comprise: increased signal-to-noise ratio (SNR), less scan time, and larger field of view (FoV) of the sample under test. However, increasing the number of receiving coils necessarily leads to increased size, complexity, and cost of the MRI system since each coil needs an individual receiver chain. These drawbacks of increased size and complexity become the major limiting factor to having phased arrays of massive number of coils (≥128-coil array for example).

In this contribution we introduce a novel solution to overcome the challenges associated with MRI phased arrays. It is based on the design of a CMOS frequency division multiplexer (FDM) fabricated in 0.35 µm technology. It is capable of merging the signals from an 8-coil phased array, and transmitting the output on a single coaxial cable. Each channel of the FDM features a wideband low-noise amplifier, a wideband frequency mixer, and a bandpass filter. Utilizing wideband components allows the chip to operate successfully in different MR scanners. Inside the chip, the MR signals are down-converted from the Larmor frequency to eight different bands (10,15,20,25,30,35,40,45) MHz. Characterizing the chip's performance under a high magnetic field (11.7 T) reported a negligible effect of the magnetic field on the functionality. The chip was tested in a real MR spectroscopy experiment in an 11.7 T Bruker scanner. The UHFLI lock-in amplifier from Zurich instruments was employed to digitize and record the MR signals

from the chip. A 5 ml pure water sample and a sample with 100 mmol of sugar were investigated. The results obtained from the chip displayed a very good matching with their counterparts obtained from the scanner.

Furthermore, the chip was examined in an MR imaging experiment in a 9.4 T Bruker scanner (400 MHz Larmor frequency for 1H protons). The MR echo signals from the chip, digitized and recorded by the UHFLI, were processed in Matlab to reconstruct the MR image. The image from the CMOS chip was successfully obtained and showed good resemblance to the original image (from the scanner) but with a decrease in resolution and SNR. This decrease can be compensated by applying techniques such as averaging, oversampling, and phase synchronization.

The CMOS multiplexer is a promising, size-efficient, and power-efficient solution with which to multiplex signals from MR coil arrays and to replace the conventional bulky electronics, also to reduce the number of cables. Moreover, it is expected to contribute to the conception of future MR phased array functional blocks, especially for arrays with a large number of reception coils.

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O 055
LOW-FIELD NMR STUDY OF HEALTHY AND DEGRADED ARTICULAR CARTILAGE AND THE INFLUENCE OF MECHANICAL LOADING

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The layered structure of mammalian articular cartilage is reflected by a pronounced depth dependence of T_2, which is a consequence of different degrees of order of the collagen fibers but also of a gradient of water and glycosaminoglycan (GAG) concentration, respectively. The orientational order results in an angular dependence of T_2 that becomes less pronounced at greater distance from the joint surface [1]. T_1, however, at conventional laboratory field strengths shows little variation in comparison.

In this study, the dependence of magnetic resonance relaxation times in bovine and human articular cartilage is investigated by portable, single-sided scanners at magnetic field strengths of 0.27 T and 0.44 T, respectively. One-dimensional, depth-dependent scans were carried out with spatial resolutions between 20 and 50 µm. While a systematic variation of T_2 is found that is in agreement to similar mammalian cartilage observed at high fields, T_1 also shows a strong depth dependence that correlates with the separation of the tissue into three distinct zones. This pronounced effect is explained by the increased T_1 contrast commonly found towards smaller magnetic field strengths, a consequence of slow and anisotropic molecular reorientations that dominate the relaxation dispersion at low magnetic resonance frequencies [2].

At even lower Larmor frequencies, the so-called quadrupolar dips are observed which indicate cross-relaxation of protons with the partially immobilized ^{14}N nuclei in amino acids in collagen and GAGs. Varying the composition, water content or structural integrity of cartilage affects both the general frequency dependence of T_1 and the shape of the quadrupolar dips, providing a possible diagnostic access to arthropathies such as osteoarthritis (OA) [3].

The effect of enzymes onto cartilage and its constituents is investigated as a model for OA, and results are compared to measurements carried out at bovine and human healthy and osteoarthritic cartilage tissues. While trypsin is known to separate GAGs from the proteoglycan backbone – the distribution of residual GAG having been investigated at low field by the use of the ionic contrast agent Gd-DTPA –, collagenase attacks the collagen structure exclusively. Nitrogen nuclei in both substances are shown to contribute to the quadrupolar dips in a similar way. Experiments for both constituents as well as fresh and enzyme-treated bovine articular cartilage were carried out and the relaxivity in the region of the quadrupolar dips were quantified. The observed strong dependence on water concentration is interpreted by a fast-exchange model and is discussed in conjunction with the low-field imaging results [4]. Loading of extracted cartilage tissue in a pressure cell up to the maximum physiological compression is reflected in a layer-dependent change of relaxation times, whereas the tissue's integrity is artificially affected by enzymatic treatment prior to the loading process.

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S 12 - Exotica

O 056

NUCLEAR SPIN NOISE AND RADIATION DAMPING – NEW INSIGHTS AND APPLICATIONS

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The advent of cryo-probes not only boosted the sensitivity of NMR mostly for the benefit of biomolecular research but also caused renewed interest in fundamental aspects of NMR and facilitated experimental access to spin noise phenomena. Our research on spin noise related phenomena started about 10 years ago [1] and has since then developed into two directions. First, one observes interference effects with radiation damping (RD), as the conditions favoring the detection of spin noise, ultimately also enhance the effects of RD. The interplay of spin noise and RD forms the underpinning of the application of spin noise spectroscopy to analyze and optimize the properties of probes. [2, 3, 4] Second, spin noise detection promises superior sensitivity at very low spin numbers ($<10^8$). This prospect led us to explore the potential of indirect detection via spin noise for spectroscopic experiments, even with standard hardware, paving the way to future nano-scale NMR spectroscopy. [5]

Most recently we have been involved in collaborative research efforts exploring ^{1}H spin noise and RD in highly polarised spin systems by DNP and at very low (down to 18 mK) temperatures. New spin noise gradient echo (SNGE) experiments offer an application potential for rf-pulse free experiments to measure transverse relaxation rates and diffusion. Novel RD interference effects observed in this context enable new approaches to optimise solvent suppression and to enhance detection of secondary isotopomer satellites.

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O 057
ELECTRICAL DETECTION OF ORTHO-PARA CONVERSION IN ENCAPSULATED WATER

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Water exists in two spin isomers, ortho and para that have different nuclear spin states. In bulk water, rapid proton exchange and hindered molecular rotation obscur the direct observation of two spin isomers. The supramolecular endofullerene H2O@C60 provides freely rotating, isolated water molecules even at cryogenic temperatures. We show that the bulk dielectric constant of this substance depends upon the ortho/para ratio, and changes slowly in time after a sudden temperature jump, due to nuclear spin conversion. The attribution of the effect to ortho-para conversion is validated by comparison with nuclear magnetic resonance and quantum theory. The change in dielectric constant is consistent with an electric dipole moment of 0.51+/-0.05 Debye for an encapsulated water molecule, indicating the partial shielding of the water dipole by the encapsulating cage. The phenomenon opens up the prospect of using Kelvin probe force microscopy to study water spin isomers on a single-molecule level. We further explore whether the bulk dielectric constant's dependence on spin isomer composition is a universal phenomenon of polar spin isomers.

O 058
SPIN NOISE GRADIENT ECHO IN STUDYING RELAXATION AND DYNAMICS OF PURE LIQUIDS AND BULK MIXTURES

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This work reports new spin noise (SN) experiments with pulsed field gradient sequences (without RF-pulses) based on a "spin noise gradient echo" (SNGE) and enabling alternative approaches for studying transverse relaxation and diffusion. The experimental details of the SNGE phenomenon were systematically investigated and the application potential of spin noise in echo-attenuation studies of pure liquids and mixtures, where radiation damping (RD) is an important issue, have been explored. To observe a SNGE the difference between SN power spectra integrals acquired with a gradient sequence with equal sign gradients I(+) and one, where the gradient applied during acquisition is compensating any defocusing I(–).

Two and three gradient pulses sequences were developed on the basis of imaging profile sequences. The three gradient sequence compensates for switching artefacts and makes sure the echo is positioned correctly with respect to the acquisition period δ_3 by setting $G_2 \cdot \delta_2 = -(G_1 \cdot \delta_1 + G_3 \cdot \delta_3)$ for the I(–) experiment and inverting G_1 for I(+), with G_n denoting the gradient strengths and δ_n the respective durations in chronological order.

Since the SN experiments may take a long time unless optimised, a small flip angle pulse was prepended to the sequence for rapid parameter adjustment (e.g. positioning of the echo, gradient shapes and pre-emphasis). The repetition delay in these pulse experiments is heavily dependent on longitudinal relaxation T_1 while the SN experiments can be repeated quickly independent of T_1.

An additional complication arises from interference of weak gradients and RD. One observes a "hole burning effect" when a weak gradient

is applied during the acquisition of a radiation damped signal. Simulated SN spectra in the presence of weak gradients showed the results corresponded closely to the experimental SN spectra obtained in pure liquids in presence of a gradient <0.2 G/cm.

SNGE spectra at increasing gradient amplitudes performed on pure liquids and mixtures. While self-diffusion coefficients could be determined easily from the three gradient pulse sequences with a small RF pulse, in pure SNGE experiment the required fast repetition rates could not be attained for gradient amplitudes above 7 G/cm due to limitations of the hardware used (Bruker TCI cryo-probe). At low gradient strengths the dependence of the SNGE integral deviates from the Stejskal-Tanner equation, due to RD interference.

Transverse relaxation rates R_2^* at very high spin concentrations, with reduced interference from RD, was measured by variation of the inter-gradient delays in two and three gradient SNGE sequences. We show first examples on pure solvents and mixtures. Using gradient strengths lower than the chemical shift separation one obtains separate profile images for different spin isochromates, which allows extraction of R_2^* for components in SNGE experiments largely independent of radiation damping.

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O 059
MEASUREMENT OF UNTRUNCATED NUCLEAR SPIN INTERACTIONS VIA ZERO- TO ULTRA-LOW-FIELD NMR

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Nuclear spin interactions are of substantial importance for many fields, including chemistry, quantum information processing, and precision measurement of fundamental symmetries. The most common technique for measuring such interactions is nuclear magnetic resonance (NMR), which is typically performed in large magnetic fields in order to maximize signal via higher nuclear spin polarization and sensitivity of inductive detection. However, the only terms of the spin-coupling Hamiltonians that may be observed in high-field NMR are those that commute with the high-field Zeeman Hamiltonian, which effectively truncates any interaction Hamiltonians that possess different symmetry. Recently, however, NMR experiments have been carried out in the opposite regime of very small magnetic fields [1-4], taking advantage of advances in hyperpolarization [5, 6] and new detection modalities [7-10]. In zero- to ultra-low-field NMR (ZULF-NMR), the strongest interactions are the local spin-spin couplings, which involve coupling tensors that are of different symmetry from the high-field Zeeman Hamiltonian and are many orders of magnitude smaller in amplitude, thus permitting the direct observation of nuclear spin interactions that vanish at high magnetic fields.

We will discuss two notable examples of nuclear spin interactions that are fully observable at zero field, but truncated at high field: the direct dipole-dipole coupling, and the antisymmetric J-coupling. For the first example, we will relate our recent results on the measurement of untruncated residual dipole-dipole couplings in stretched gels at zero field [11]. For the second example, we will describe proposed experiments that make use of the antisymmetric J-coupling for a direct magnetic resonance probe of chirality, and for a new search for molecular parity violation.

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O 060
DYNAMIC NUCLEAR POLARIZATION IN SILICON

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Dynamic nuclear polarization (DNP) has been used to enhance the spin polarization of both silicon nuclei as well as the nuclear spins of donors such as phosphorus. In this talk I will discuss how recent microwave and optical DNP experiments have enhanced our understanding of the spin physics underlying the different DNP mechanisms in both silicon nanoparticles and phosphorus-doped single-crystal silicon wafers. Finally, I will describe our efforts to improve the efficiency of the hyperpolarization process in DNP experiments.

Plenary Session 4

PL 06
DEVELOPMENT OF 13C-BASED METABOLOMICS

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We have been developing several new approaches to metabolomics based on ^{13}C, which offers several advantages both for NMR and LC-MS studies. The NMR methods are greatly facilitated by high sensitivity ^{13}C-optimized superconducting cryogenic probes, which we have made in partnership with the NHMFL. The two NMR approaches are based whether or not isotopic labeling can be employed. At natural abundance ^{13}C, we have shown that it is possible to record 1D ^{13}C and ^{1}H data and statistically correlate resonances, which can be matched to databases. With ^{13}C isotopic enrichment, we can utilize 2D INADEQUATE measurements, which provide a wealth of information about carbon networks in the sample. Both NMR approaches benefit greatly from the large chemical shift dispersion of ^{13}C, which allows for improved multivariate analysis and database matching. In addition to the NMR approaches, we also have been using an LC-MS method called Isotopic Ratio Outlier Analysis (IROA), which was developed by Dr. Chris Beecher. IROA utilizes specific patterns of ^{13}C enrichment and can provide a great deal of information, including the exact number of carbon atoms in each molecule and the relative concentrations of a test vs. reference sample. We are developing new methods to combine ^{13}C NMR with IROA to improve the identification of unknown metabolites.

Plenary Session 5

PL 07
A HYBRID METHODS APPROACH TO DETERMINE THE STRUCTURE OF TETRAHYMENA TELOMERASE HOLOENZYME

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Telomerase is an RNA-protein complex that extends the ends of linear chromosomes, and is a highly regulated determinant of cellular aging, stem cell renewal, and tumorigenesis. We are using a combination of NMR spectroscopy, X-ray crystallography, and electron microscopy to study the structure and function of telomerase. We determined the 3D structure of endogenously assembled Tetrahymena thermophila telomerase holoenzyme at 25 Å resolution using negative stain electron microscopy (EM). Six of the 7 protein subunits and the stem-loop 2 region of TER were localized in the 3D structure by affinity labeling. Fitting with the available high-resolution structures, including RNA structures determined by NMR and a p65-TER complex determined by combining NMR and X-ray crystallography, revealed the organization of TERT, TER, and p65 in the RNP catalytic core. Among the other holoenzyme proteins, p50 has an unanticipated role as a hub between the RNP catalytic core, p75-p19-p45 subcomplex, and the DNA-binding Teb1. A complete in vitro holoenzyme reconstitution correlates activity with structure. This first physical and functional network architecture of a telomerase holoenzyme provided the first view into the structure of the RNP catalytic core and revealed the organization of holoenzyme subunits that confer processivity and bridge telomerase to telomeres. Progress toward obtaining an atomic resolution cryoelectron microscopy structure and detailed protein and RNA domain structures and interactions using NMR, crystallography, and modeling will be discussed.

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PL 08
MICRO-MANUFACTURING TECHNOLOGIES FOR NMR MICRO-DETECTION SYSTEMS

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Numerous ideas motivate the development of miniaturized NMR hardware, yet a common thread is the need to consider the application very carefully, in order to render the measuring instrument practicable, and of course to meet the need of extracting the desired signal from the sample. Additional constraints are imposed by available miniaturization technologies, since only a subset of these lead to assemblies that are suitable for insertion into an NMR magnet.

In my group we are dedicating extensive effort to build very small bespoke NMR detectors [4,5], both for applications in spectroscopy, as well as for imaging. On this adventurous road we are discovering many of the limitations of microfabrication technologies, and also some of the misconceptions about the appropriate scaling laws, such as the way detectibility scales with dimension. In the same process, my grad students and postdocs are inventing new manufacturing technologies and procedures that are gradually overcoming the technical limitations that we encounter in miniaturization. This is simplifying the application of small detectors, mainly because we are focusing on methods of mass fabrication, which brings down the per sensor cost, and encourages experimentation.

Our approach is holistic, in the sense that we are co-developing design software, manufacturing processes, and experimental protocols.

In the talk I will describe a range of our microscale NMR subsystems, including electronics [2], hyperpolarisation with SABRE and DNP, microcoils for MAS, MACS and MRFM [4, 5], in-field electronics for phased microarrays, and numerical design tools for transport modelling, resonator modelling, and coil field tuning [1,3,7]. I will also

cover our ongoing work to simplify micromanufacturing, towards achieving reliable mass fabrication.

The focus of my talk will be on the micro-engineering of these small systems, where I will show results from our design-build-test cycles. I will also illustrate my talk briefly with a few results from the applications, which include C. elegans metabolomics, and brain slice and organ microimaging [6], and which require the hyphenation of other techniques, such as sample nurturing, or organism detection, and optical imaging.

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S 13 - Solid State NMR Techniques

O 061
PULSED ELECTRICALLY DETECTED MAGNETIC RESONANCE: METHODOLOGICIAL ADVANCES AND TOPICAL APPLICATIONS

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Electrical detection methods in combination with the pulsed application of microwaves and/or radiofrequencies allow to very sensitively monitor the coherent manipulation of electron and nuclear spins in semiconductors and to study paramagnetic defects and spin-dependent recombination processes also in actual microelectronic devices. The presentation will summarize the transfer of advanced EPR techniques such as ESEEM and DEER to electrically detected magnetic resonance (EDMR) providing new structural information e.g. on Si interface defects and first quantitative data on the coupling between recombination partners. To fully understand the recombination dynamics involved, we add time-programmed illumination to the toolbox of pulsed EDMR and will summarize the fundamentals of such experiments using the example of P3HT/PCBM organic solar cells. Furthermore, we will show how pulsed illumination improves the sensitivity of electrically detected ENDOR providing access to positively charged donors in Si with superior coherence properties. Using heavier donors such as As, these experiments also give a unique access to crystal fields in this material via the quadrupole interaction, which cannot be studied via nuclear acoustic resonance due to the lack of stable Si isotopes with a nuclear spin greater 1/2. Finally, we will demonstrate that the high sensitivity of EDMR allows to perform broad-band excitation, facilitating the application of shaped microwave and radiofrequency pulses.

This work is performed in collaboration with Konstantin Behringer, Georg Braunbeck, Lukas Dreher, David Franke, Felix Hoehne, Natalie Galfe, Florian Hrubesch, Alexander Kupijai, Yuki Nojiri, Lukas Stelzer,

Martin Stutzmann, Max Suckert and Michael Szech and is funded by DFG via the Priority Program on New Frontiers in Sensitivity for EPR Spectroscopy.

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O 062
1H/1H HOMONUCLEAR MIXING AT ULTRAFAST MAS > 120 KHZ: 1H/1H, 1H CSA/CSA, 15N/15N, 14N/14N CORRELATIONS

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Recent advances in magic angle spinning (MAS) technology have enabled ultrafast MAS above 120 kHz using 0.75 mm rotors. Such a fast MAS allows us to add ^{1}H high-resolution dimension in NMR measurements of rigid solids with enhanced sensitivity. The main advantage of ultrafast MAS is longer ^{1}H T_2 relaxation leading to narrow line width and longer coherence lifetime due to better suppression of ^{1}H-^{1}H dipolar interactions. Nevertheless, ultrafast MAS simultaneously suppresses spin diffusion among ^{1}Hs which hampers the measurement of ^{1}H/^{1}H homonuclear correlations.

In order to correlate several isotropic and anisotropic nuclear interactions, here we demonstrate the utility of ^{1}H/^{1}H recoupling through enhanced ^{1}H/^{1}H spin diffusion process during RFDR mixing under ultrafast MAS. ^{1}H/^{1}H dipolar interactions can be restored by applying pulses synchronous to sample spinning. We have experimentally and theoretically investigated the role of phase cycling in radio frequency-driven recoupling (RFDR) for ^{1}H spin networks. The XY4$^1_4$(XYXY Y-XY-X -X-Y-X-Y -YX-YX) with stronger rf field strength gives the best performance in terms of better preservation of ^{1}H longitudinal magnetization and quicker magnetization transfer between ^{1}Hs because of better tolerance to rf-inhomogeneities and

chemical shift offsets [1, 2]. The use of proper phase cycling gives stronger $^{1}H/^{1}H$ correlations in 2D RFDR exchange spectra.

One can utilize $^{1}H/^{1}H$ spin diffusion to correlate not only ^{1}H isotropic chemical shifts but also other nuclear interactions. (1) $^{15}N/^{15}N$ chemical shift correlation can be obtained by relayed transfer via $^{1}H/^{1}H$ spin diffusion from RFDR at ultrafast MAS. This correlation is extremely challenging to achieve otherwise due to negligible ^{15}N-^{15}N dipolar interactions [3]. The ^{1}H magnetization is first transferred to ^{15}N (first CP) to record ^{15}N chemical shifts and then back transferred to amide ^{1}H nuclei (second CP) followed by RFDR mixing to transfer the magnetization to the other amide ^{1}Hs. The ^{15}N chemical shifts are recorded after magnetization transfer from ^{15}N to ^{1}H (third CP), and the signal is observed in a 3D way, i.e. $^{15}N/^{15}N/^{1}H$, via ^{1}H detection after the fourth CP from ^{1}H to ^{15}N. (2) We successfully obtained the first $^{14}N/^{14}N$ correlation by adopting a similar approach. The only difference here is the replacement of two two-way transfers between^{1}H and ^{15}N achieved by four CPs to two HMQC magnetization transfer between ^{1}H and ^{14}N [4, 5]. (3) Two ^{1}H CSA tensors can successfully be correlated by RFDR mixing, that results in relative orientation among them [6]. Herein, ^{1}H CSA tensors are recoupled by symmetry based sequence [7] and correlated by $^{1}H/^{1}H$ mixing. This clearly helps in the better interpretation of molecular structure in the 3D space.

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O 063
NEW METHODS FOR THE DETECTION OF 14N NUCLEI IN SOLID STATE NMR

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In a 1st step, we demonstrate how frequency-selective pulses allow to achieve an efficient excitation of nuclei experiencing large anisotropic interactions. These pulses can be applied on the indirect channel of HMQC sequences, which facilitate the detection of nuclei exhibiting wide spectra via spin-1/2 isotopes. Selective excitation is achieved using long pulses as well as DANTE trains of long pulses. This indirect method is applicable either to spin-1/2 nuclei with large CSA, or to ^{14}N spin-1 nuclei. The efficiency of selective excitation is larger than that of broadband excitation using the rf field delivered by common probes. Furthermore, it (i) requires moderate rf field, (ii) can be easily optimized, and (iii) displays high robustness to offset, field inhomogeneities, and fluctuations in MAS frequencies.

In a 2nd step, we compare three different categories of ^{1}H-{^{14}N} D-HMQC correlations with indirect evolution of single- (SQ) or double-quantum (DQ) coherences.

DQ sequences require large rf fields, especially the one with a 'classical' DQ observation, which is also the less sensitive method. That with observation of overtone coherences with single pulse excitation is efficient 'on-resonance' but very sensitive to offsets. Its version with WURST excitation is more broadband but at the cost of reduced sensitivity.

SQ sequence with selective excitation is very efficient and easy to optimize and it requires a moderate rf field. Its broadband version with single hard pulse is not efficient most of the time, due to limited rf field. Its version with DANTE excitation scales up the effective rf-field, hence leading to a high efficiency, albeit to the expanse of the offset bandwidth.

As a conclusion, four ^{1}H-{^{14}N} D-HMQC correlation experiments can be used with reasonable efficiency and robustness. These methods correspond to the observation of SQ or overtone coherences with excitation done respectively with DANTE or single pulses if the ^{14}N frequency range is limited or with long selective or WURST pulses otherwise. In both cases, SQ and overtone methods are complementary, in the sense that the first is more efficient than the second, but shows a lower resolution.

In a 3rd step, we propose an improvement of the overtone scheme to increase the efficiency and robustness to offset.

O 064
CRYSTAL STRUCTURE DETERMINATION USING POWDER NMR CRYSTALLOGRAPHY

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Structural characterization is one of the key challenges for modern chemistry. For solids, single crystal diffraction methods are capable of characterizing systems as diverse as membrane proteins, whole virus particles, complex inorganic materials, or supramolecular nanostructures. In contrast, if the sample is a powder, structural characterization represents an enormous challenge.

We established a protocol for natural abundance NMR crystallography for crystal structure elucidation of powdered solids, particularly of pharmaceutical relevance. Towards this end we explore the possibility of complete ab initio structure determination in molecular crystals using combined NMR and computationally based structure prediction techniques. We combine molecular modeling and density functional theory (DFT) calculations of NMR parameters with high-resolution solid-state NMR experiments and powder X-ray diffraction. We illustrate the feasibility of this method in several examples including cocaine and several pharmaceutical drugs.

This method opens the door to structure determination of microcrystalline powdered compounds when the powder under study is not suitable for other structure determination, or can undergo changes between polymorphs during sample preparation. It should be of wide spread interest in many areas, and particularly in pharmaceutical chemistry.

Combining ^{1}H solid-state NMR spectroscopy with DFT calculations can also be applied to the crystal structure determination of metal organic frameworks (MOF). We demonstrate this by reporting the discovery of the previously unknown crystal structure of a novel porous imidazolate substituted metal organic framework with possible applications as a gas storage material. ^{1}H NMR experiments provided a description of the proton environments within the MOF, which in combination with DFT chemical shift calculations and powder X-ray diffraction led to the elucidation of the complete crystal structure.

O 065

DISTANCE MEASUREMENTS IN MAS NMR USING PHASE MODULATED PULSES: THEORETICAL INSIGHTS, DIFFICULT SPINS AND A SMALL TRICK FOR A SPIN-1

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The distance between two spins, when one has a large quadrupolar (or CSA) interaction, can be measured by a variety of magic-angle spinning solid state NMR methods that utilize a long pulse irradiation on the quadrupolar spin (e.g. REAPDOR, TRAPDOR, RESPDOR, LA-REDOR). Current sequences are limited by various properties such as the size of the quadrupolar interaction and rf power, the spinning speed, the sensitivity to off-resonance and rf inhomogeneity and the degree of sensitivity to orientation.

We will show that the incorporation of a phase modulation into the extended recoupling pulse applied to the coupled spin increases significantly the range of the values of the quadrupole moment (or CSA) that can be accessed by a REDOR-based distance measurement experiment. This phase modulated experiment (mod. LA-REDOR#) is not only robust with respect to the spin number and the actual value of the anisotropic interaction, but also very weakly dependent on the actual value of the radio-frequency field. Moreover, experimental results can be fitted by a universal formula corresponding to an equal-transition-probability model.

Theoretical insights reveal that during this pulse, true randomization of the powder crystallites occurs leading to dephasing of spin magnetization that is parallel to the Z-axis.

Based on our theoretical developments we were able to derive a better REDOR dephasing for a spin-1 with a small quadrupole moment, thereby suitable for spins such as 2H and 6Li.

We will show examples of distance measurements to boron-11 and vanadium-51 and two cases, in which the distance to nuclear spins such as Bromine and Bismuth could be measured even when the rf power and exact resonance frequency could not be determined due to their very large quadrupole moments.

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S 14 - Biomolecular Polarization and Relaxation

O 066
CHARACTERIZATION OF PROTEIN DYNAMICS BY NMR RELAXATION

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Molecular motions are central to the biological functions of proteins. Proteins utilize a continuum of conformational space, spanning the entire energy landscape, in performing their biological functions. NMR provides unique information on dynamic processes, advancing our understanding beyond the level of three-dimensional protein structure to reveal the conformational flexibility and motions that are directly relevant to biological function. NMR relaxation measurements provide a powerful approach for direct experimental characterization of protein dynamics and protein folding processes on a broad range of time scales. A combination of room temperature x-ray crystallography and NMR relaxation provides an atomic resolution description of alternate conformational substates that are sampled through ps to ns timescale fluctuations of the protein structure. Relaxation dispersion experiments permit quantitative analysis of the dynamics and thermodynamics of slow, ms timescale conformational fluctuations in proteins and allow structural characterization of weakly populated conformational substates ("excited states"). Such experiments support a view of proteins as dynamic conformational ensembles, providing new mechanistic understanding of allostery, molecular recognition, and enzyme catalysis, and new insights into mechanisms of protein folding and misfolding in disease. Applications of NMR relaxation dispersion experiments to characterize the dynamic conformational ensemble of the enzyme dihydrofolate reductase, a target for anticancer agents, anti-infectives, and anti-malarial drugs, will be described.

O 067
HYPERPOLARIZED PARA-ETHANOL

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Long-Lived States (LLS)[1] are spin states with lifetimes T_{LLS} that can be much longer than the longitudinal relaxation time constant T_1. In a two-spin system, they correspond to a "Triplet/Singlet Imbalance" (TSI)[2], induced by perturbing the equilibrium between the average of the populations of the three triplet states and the population of the singlet state. In para-hydrogen, the TSI can be made observable by using chemical reactions such as additions onto double bonds that break the symmetry of the two hydrogen nuclei[3].
We have shown[4] that it is possible to create a TSI by Dissolution Dynamic Nuclear Polarization (D-DNP) in a pair of magnetically equivalent protons in partly deuterated ethanol $CD_3{}^{13}CH_2OD$ because the population of the singlet state is depleted at very low spin temperatures on the order of 10 mK at 6.7 T. No further preparation methods are required. The TSI can be observed because cross-relaxation leads to population differences across observable transitions of either ^1H or ^{13}C spins, in natural abundance.
In our experiments we used DNP at T = 1.2 K and transferred the sample through a magnetic tunnel (B > 0.9 T) to a 500 MHz NMR spectrometer at room temperature. We observed non-binomial ^{13}C triplets and anti-phase ^1H doublets enhanced by up to two orders of magnitude with respect to Boltzmann's polarization in thermal equilibrium. The lifetime of these signals is somewhat longer than T_1. Simulations using SpinDynamica confirmed that these enhanced and long-lived non-binomial multiplets indeed result from TSI.
Our experimental scheme is applicable to virtually any molecule comprising a $^{13}CH_2$ group in natural abundance, without requiring any chemical reaction prior to detection. This technique could be used as a tracer to obtain biochemical and biophysical information in NMR or

MRI when the molecules of interest have short-lived signals that are too weak to be studied by conventional methods.

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O 068
NMR WITH NANOPARTICLES: MOLECULAR SENSING AND CHROMATOGRAPHY

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Gold nanoparticles (NPs) provide a pincushion-like scaffold onto which molecular receptors can be grafted to form a protecting monolayer. [1] By exploiting different kinds of non-covalent interactions (namely hydrophobic, ion pairing, and metal–ligand coordination) such receptors can in turn provide tailored binding sites for virtually any class of substrates. Remarkably, the variety of monolayers that can be potentially assembled endow a fine-tuning of these interactions not only in terms of selectivity, but also in terms of their strength.
The reduced translational and rotational diffusion rates resulting from the bulkiness of NPs offer a route to manipulate the magnetization of the receptor spins within the monolayer. We show here how relaxation- and diffusion-based NMR techniques can be exploited to label and detect some interacting analytes either by magnetization transfer or by perturbation of their apparent diffusion coefficient. Namely, when the interaction is weak, the spins located on the NPs monolayer can be used as a source of magnetization that is transferred selectivley to the interacting analytes via NOE. As opposite, when the interaction is strong enough to unbalance the analytes populations towards their bound state, a reduction of the diffusion coefficients is observed, which leads to a better separation in DOSY maps. Possible residual signals stemming from the monolayer and interfering with the analytes detection are filtered out by modified CPMG echo-trains. [2]
We have tested this method with several combinations of monolayer-protected nanoparticles and different mixtures of analytes such as benzene derivatives, aromatic anions, and primary ammonium salts [3]. Eventually, the emerging picture is one wherein the interactions of

the analytes with NP monolayers can be tuned in many ways, and even lead to unexpected selectivities.
Among the efforts to turn supramolecular receptors into molecular sensors, we also put monolayer protected NPs at test in a situation as challenging as the analysis of drug metabolites in urines [4]. In this respect, the encouraging results obtained seem to address the classic but still actual problem of realizing systems that can detect organic molecules in water, where most receptors fail in recognizing their targets.

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O 069
KINETIC ISOTOPE EFFECTS ON EXCHANGE RATES OF HN AND DN IN TRYPTOPHAN

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We have quantified the exchange rates between indole protons in tryptophan with the protons of the aqueous solvent, and compared the exchange rate of the analogous process when hydrogen is replaced by deuterium, so that we could determine the H/D kinetic isotope effect (KIE).

$H^N + H' \rightarrow H'^N + H$

$D^N + D' \rightarrow D'^N + D$

The measurement of H/H and D/D exchange rate constants of H^N and D^N on the indole ring in tryptophan is of obvious interest. The isotope effect is considered to be one of the most sensitive tools for the study of reaction mechanisms as it can give insight into free energies and other thermodynamic parameters that describe the stability of hydrogen-bonded structures in different environments.

We have extended our method originally designed for measuring very fast proton exchange rates[1,2,3] to deuterium exchange rates. The effects of scalar relaxation caused by the exchanging H^N or D^N nuclei is determined by detecting the decay of the coherence of a ^{15}N nucleus that has a scalar coupling $^1J(^{15}N, ^1H)$ or $^1J(^{15}N, ^2H)$ to the exchanging nuclei under a multiple-refocusing CPMG pulse train in the presence or absence of proton or deuterium decoupling.

The exchange rates were found to lie in the range $17 < k_D < 10\,000\ s^{-1}$ for $2.2 < pH < 11.8$ and $290 < T < 320$ K. The $\log k_D$ vs. pH plot indicates a combination of specific base catalysis at high pH, un-catalyzed exchange at intermediate pH, and specific acid catalysis at low pH,

which becomes more important at higher temperatures. The kinetic isotope effect k_H/k_D was found to lie in the range $0.22 < k_H/k_D < 14.99$ for $6.3 < pH < 10.5$ and $300 < T < 320$ K. The kinetic isotope effect increases with increasing pH and temperature.

Altogether we can say that in the base-catalyzed mechanism the rate-limiting step of the exchange is the removal of the proton or deuteron from the donor N atom of tryptophan, while for the acid-catalyzed mechanism it is the donation of a proton or deuteron by H_3O^+ , respectively D_3O^+. From the small values of the kinetic isotope effect we can conclude that the H^N/D^N bonds are elongated and not totally broken in the transition state, the H/D atom being more tightly bound to the donor than to the acceptor D_2O or OD^-.

O 070

TRANSIENT COMPLEXES OBSERVED BY PARAMAGNETIC RELAXATION ENHANCEMENT BETWEEN ENZYME 1NTR AND NPR GOVERN SPECIFICITY AND PREVENT CROSS-OVER BETWEEN PHOSPHORYLATION PATHWAYS

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Enzyme I^{Ntr} (EIN^{Ntr})and NPr are the first two enzymes in a newly discovered nitrogen-based phosphorylationtransfer pathway in E. coli. There is a paralogous EIN^{sugar} and HPR involved in thephosphorylation pathway that transfers sugars across bacterial membranes. Thecrystal structure of the EIN^{Ntr} and the NMR structure of NPr showthat the structures of the protein components in these two distinct pathwayslook alike. Therefore we propose that the substrate specificity in these twopathways depends on the detail of their respective molecular interactions.Three specific aspects of the molecular interactions: forces that drive the EIN^{Ntr}:NPrcomplex formation, changes in protein dynamic upon formation of the complex,and transient encounter complex population in the EIN^{Ntr}:NPr systemare being studied.

Using the crystal structure of freeEIN^{Ntr} and the NMR structure of free NPr, we determinedthe complex structure using residual dipolar couplings (RDCs) and chemicalshift perturbations (CSPs) as ambiguous distance restraints between the twointerfaces. RDCs were also used to validate the individual starting structuresof EIN^{Ntr} and NPr. For theparalogous EIN^{sugar}:HPR complex, there is little change in structureupon binding. If this was also the case for EIN^{Ntr} andNPr, RDCs for the complex should also fit the structures in the free form. This was indeed true for NPr, whereas thecrystal structure of EIN^{Ntr} required a rotation of the a and a-βdomains with respect to each other in order to fit the RDCs.The EIN^{Ntr}:NPrcomplex provides clear evidence for specific molecular interactions.

It is known that EIN^{sugar}and HPR sample transient encounter complexes as well as a morestable minor complex prior to productive

binding. EIN^{Ntr} has a higher bindingaffinity to NPr than the EIN^{sugar}:HPR complex and wastherefore not expected to form these encounter complexes. PRE experiments are very sensitive totransient nonspecific complexes whereas RDCs represent an average so describeonly the major, productive complex. Wemeasured paramagnetic relaxation enhancement (PRE) data for our EIN^{Ntr}:NPrcomplex. PRE experiments were collected by mutation of three sites on NPr (T6C,E45C and E74C) and disulfide addition of a nitroxide spin-label MTSL. Our RDC and PRE data did not agree with eachother, confirming the presence of encounter complexes. We therefore use the PREdata to describe the initial stages of complex formation by calculating anensemble of EIN^{Ntr}:NPr encounter complexes.

In addition, we also collectedbackbone relaxation R_1 and R_2 rates, as well as R_2dispersion for the free EIN^{Ntr} as well as the EIN^{Ntr}:NPrcomplex. We noticed that some NPr residues in the interface are broadened andcan not be observed in the complex suggesting that those residues are involvedin an exchange. The relaxation data will be used to characterize additionalexchange processes that occur due to complex formation.

The structure of the EIN^{Ntr}:NPrcomplex, their dynamic behavior, and the ensemble distribution of theirtransient encounter complexes can be compared to the homologous EIN^{sugar}:HPRcomplex to reveal the mechanism that prevents cross-over between the two phosphorylation pathways.

S 15 - NMR Imaging

O 071

POTENTIALS AND CHALLENGES OF IN VIVO 1H NMR SPECTROSCOPY OF BRAIN AT HIGH MAGNETIC FIELDS

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Substantial progress has been made over the past two decades in the technology and methodology of in vivo ^{1}H NMR spectroscopy. High magnetic fields are beneficial also for in vivo applications, but the real gain of an increased sensitivity and chemical shift dispersion is challenged by a number of factors, such as B_0 and B_1 spatial inhomogeneity, the maximum strength of the transmit B_1^+ field, specific absorption rate, increased inherent linewidth or the chemical shift displacement error of a selected volume of interest. The first part of this presentation will review the key methodological aspects of in vivo ^{1}H NMR spectroscopy of the brain. Specifically, current methods of B_0 and B_1 shimming, the volume selection and efficient water suppression will be described. A special attention will be made to the elimination of unwanted coherences. The data processing part will include methods for the frequency and phase correction and elimination of the residual eddy currents. Finally, the metabolite quantification using LCModel analysis and the construction of the basis set with the spectra of brain metabolites will be discussed. The second part of this presentation will demonstrate that this novel advanced acquisition and processing techniques significantly increase the range of NMR detectable metabolites and considerably improve the reliability of their quantification. The examples will be chosen from the studies of neurochemical profiling in mouse models of human diseases. In order to illustrate the current possibilities of in vivo ^{1}H NMR spectroscopy in human brain, the results of functional in vivo ^{1}H NMR spectroscopy will be presented. These results will clearly demonstrate the feasibility of detecting changes in steady-state concentrations of neurochemical in activated human brain by in vivo ^{1}H NMR spectroscopy at 7T.

O 072
RECENT ADVANCES WITH HYPERPOLARIZED 83Kr MRI AND SURFACE QUADRUPOLAR RELAXATION (SQUARE) T1 CONTRAST.

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The noble gas isotope ^{83}Kr has a nuclear spin I = 9/2 with non-vanishing nuclear electric quadrupole moment that is not present in spin I = ½ isotopes such as ^{3}He and ^{129}Xe. Interactions of the quadrupole moment with fluctuating electric field gradients (EFGs) generated by brief interactions of the noble gas atoms with the surrounding surfaces are the dominating cause for the ^{83}Kr longitudinal (T_1) relaxation measured in the gas phase. The ^{83}Kr T_1 relaxation weighted MRI contrast can therefore serve as a probe for surfaces and was previously shown to be indicative of the specific surface treatment in a porous model system [1-3]. The T_1 times in rat lungs [4] are sufficiently long for pulmonary MRI and the relaxation can readily be measured through depolarization of hyperpolarized (hp) ^{83}Kr that enables surface quadrupole relaxation (SQUARE) T_1 contrast [5]. The SQUARE T_1 MRI contrast is dependent on the surface to volume ratio (S/V) and can therefore distinguish between airways and alveolar regions. Work with models surfaces suggest that it is also strongly affected by surface composition, surface temperature, and competitive co-adsorption of other molecules [1-3].
To explore the usage of SQUARE T_1 contrast for pulmonary pathophysiology, hp ^{83}Kr MRI were acquired in excised rat lungs obtained from an elastase induced model of emphysema. A significant ^{83}Kr T_1 relaxation time increase in the SQUARE contrast was found in the elastase treated lungs compared to the baseline data from control lungs. In particular, it was demonstrated that two characteristic T_1^{EV} times, obtained from bimodal fitting of the (T_1) histograms, enable statistically significant distinction between emphysema model and control lung. Beyond statistics, the difference between control group and emphysema model can also readily be identified from visual inspection of the ^{83}Kr SQUARE MR images.

The SQUARE contrast suggests a reduction in pulmonary surface to volume ratio in the emphysema model that was validated by histology. The finding supports usage of ^{83}Kr SQUARE as new biomarker for pulmonary disease related surface-to-volume ratio changes in lungs. It is important to note that molecular oxygen does not dramatically accelerate ^{83}Kr T_1 relaxation and SQUARE measurements in rodent lungs [4] were unaffected by the presence of up to 40% O_2. Because neither the application of magnetic field gradients nor the detection within high magnetic fields is required for T_1 SQUARE measurements, this new biomarker may also be of potential interest for (spatially non-resolved) pulmonary mass screening using small bench-top devices.

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O 073
TRANSPORT IN PHLOEM TISSUE ASSESSED WITH MRI

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Phloem vascular tissue transports phloem sap from source leaves to sinks, i.e., areas of growth and/or storage. The phloem sap consists mainly of water and sucrose, transported in very small amounts (uL) and at very slow velocities (around 200 um s^{-1}). Therefore, phloem flow is difficult to measure. Only MRI is the truly non-invasive technique to measure phloem flow. Windt et al. [1] showed that Pulse Field Gradient Turbo Spin Echo (PFG-TSE) [2] pulse sequence is suitable for phloem flow measurement. PFG-TSE combines MR-imaging with q-space imaging which results in a probability of displacement, a propagator, for every pixel. The propagator contains information about the velocity of phloem sap flow, the area of actual flow, i.e., the flow conducting area (FCA), and the area with stationary, only diffusing water. From velocity and FCA the volume flow of phloem sap is obtained. Windt demonstrated MRI phloem flow measurement over a time-course of 35 hours. However, the behaviour of phloem flow over longer periods of time during an adaptation to different photosynthetic active radiation (PAR) intensity and day-length is unknown. Furthermore, the impact of PAR length on sucrose concentration in the phloem sap in vivo has also yet to be demonstrated. We consecutively measured phloem and xylem flow with the PFG-TSE pulse sequence and T_2 with the Multi Spin Echo (MSE) [3] pulse sequence, and we optimized and compared analysis procedures. In our study we show that T_2 of water in the region of phloem flow is most likely correlated with sucrose concentration in the phloem sap. Sucrose concentration appears to reflect the phloem flow diurnal behaviour, i.e., higher concentration of sucrose during the day and lower concentration of sucrose during the night. Our results disprove a hypothesis about constant phloem sap velocity and phloem sap sucrose concentration over day/night period.

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O 074
RF EFFECTS IN THE STUDY OF METAL SURFACES: INSIGHTS INTO THE MECHANISMS OF BATTERY FAILURE

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When detecting NMR signals of metals, the complex phase of the effective wave vector becomes important because it is modified by the exponential attenuation of the field upon penetrating the object (the "skin depth effect"). A surprising consequence of this effect is the appearance of additional rf phases. These effects are particularly striking when examining the nutation curve of a conductor.

Metal nutation effects are of particular interest to the study of failure mechanisms in rechargeable Li metal batteries. Li microstructures form on the surface of Li metal during electrochemical cycling and can pose a serious safety risk (i.e., short-circuiting, fire). Microstructures are much thinner (< 2 μm) than the bulk metal electrode and are not affected by the skin depth effect. This is apparent in the nutation spectrum of a cycled Li metal cell, with the microstructure resonance nutating differently than the bulk metal resonance. Differences in the phase of the separate signals (microstructure and bulk metal) make quantification difficult. We show that by optimizing the flip angle, the two resonances are acquired in phase allowing for accurate quantification of the amount of microstructures formed. The ability to quantitatively measure Li metal *in situ* gives NMR and MRI an advantage over other techniques to study Li microstructure formation and the conditions under which they form.

We take advantage of the ability to quantify Li microstructures utilizing both *in situ* NMR and MRI experiments. Using MRI, we correlate the growth of Li metal microstructures to changes in the electrolyte concentration gradient across the cell. This allowed us to develop a method to detect the onset of dendritic growth. The properties of the

electrolyte (composition, ionic mobility), along with the rate of charge, play a key role in the amount and type of microstructures formed on a metal electrode. To further understand the mechanism of battery failure and aid to the development of rechargeable Li metal battery technologies, the application of this methodology to the study of novel electrolytes is envisaged.

O 075
IMAGING TREATMENT RESPONSE AND THE TUMOUR MICROENVIRONMENT USING METABOLIC IMAGING WITH HYPERPOLARIZED C-13-LABELLED CELL SUBSTRATES

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A better understanding of tumour biology has led to the development of targeted therapies, in which a drug is designed to disrupt a specific biochemical pathway important for tumour cell survival or proliferation. The introduction of these drugs into the clinic has shown that patients can vary widely in their responses. Molecular imaging is likely to play an increasingly important role in predicting and detecting these responses and thus in guiding treatment in individual patients. We have been developing methods for detecting the early responses of tumours to therapy in mouse models of disease. This has included metabolic imaging with hyperpolarized ^{13}C-labelled substrates, which we have used both to detect treatment response and to investigate the tumour microenvironment. Exchange of hyperpolarized ^{13}C label between lactate and pyruvate and net flux of label between glucose and lactate have been shown to decrease post-treatment and hyperpolarized [1,4-^{13}C]fumarate has been shown to detect subsequent cell necrosis. Tumour pH can be imaged using hyperpolarized $H^{13}CO_3^-$ and redox state can be determined by monitoring the oxidation and reduction of [1-^{13}C]ascorbate and [1-^{13}C]dehydroascorbate respectively. More recently we have shown that we can follow, using hyperpolarized [1-^{13}C]pyruvate, the progression of pancreatic precursor lesions in a mouse model of the disease that recapitulates many of the clinical, histopathological, genetic and metabolic aspects of the human disease. This potentially could be used clinically to guide earlier intervention. Metabolic imaging with hyperpolarized ^{13}C-labelled cell substrates has recently translated to the clinic with a study in prostate cancer and we expect to conduct our first clinical studies using this technique later this year.

S 16 - Biomacromolecules

O 076
TRANSMEMBRANE SIGNALING THROUGH A BACTERIAL HEME TRANSPORTER

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Bacteria use diverse signaling pathways to control gene expression in response to external stimuli. In Gram-negative bacteria, the binding of some nutrients is sensed by their specific outer membrane transporter. A cascade of molecular interactions between several proteins, located in three subcellular compartments, is then used to send this signal from outside to inside the bacteria and upregulate the expression of genes related to the acquisition of these nutrients. We study a heme acquisition system (Has) developed by several commensal and pathogenic bacteria to acquire heme as iron source. Using multidisciplinary approach (NMR, Xray, SAXS and Electron Microscopy) we have determined the structure of multiprotein complexes involved in the Has signaling pathway. Furthermore, we have recently shown, for the first time, that a partially folded protein is involved in this process [1,2,3,4,5]. Our current data represent the first detailed characterization of this type of bacterial signaling.

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O 077

STRUCTURAL INSIGHTS INTO THE DYNAMIC PROCESS OF β2-ADRENERGIC RECEPTOR SIGNALING

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G protein-coupled receptors transduce signals from the extracellular environment to intracellular proteins. Despite structural insights into activation of the β_2-adrenergic receptor (β_2AR), a prototypical GPCR, regulation of cytoplasmic conformations by extracellular ligands remains poorly understood. Here, we use ^{19}F NMR spectroscopy of fluorine labeled β_2AR to identify representative states and exchange rates between these states as a function of ligand efficacy. To provide a structural framework for this conformational heterogeneity, we utilize pulsed electron paramagnetic resonance spectroscopy (double electron-electron resonance or DEER) of nitroxide spin labeled β_2AR.

Recent X-ray crystal structures of the β_2AR show that there is a 14 Å outward displacement of transmembrane 6 (TM6) in the cytoplasmic domain of the receptor. For ^{19}F NMR and DEER spectroscopy, we site-specifically labeled a minimal cysteine version of β_2AR with a trifluoromethyl probe or nitroxide probes at residues located at the cytoplasmic end of TM6 (and TM4 in case of DEER). These studies show that unliganded and inverse-agonist-bound β_2AR exists predominantly in two inactive (the ionic lock intact and broken) conformations that exchange within hundreds of microseconds. Moreover, ^{19}F CPMG experiments show that the inverse agonists regulate the receptor kinetically by shortening lifetimes of the two inactive states, rather than changing populations of the two inactive states.

Presence of agonists shift the equilibrium towards a conformation capable of engaging G proteins, but they do so incompletely, resulting in increased conformational heterogeneity and the coexistence of inactive, intermediate and active states.^{19}F NMR saturation transfer experiment shows that the lifetime of activation intermediate state is on hundreds of milliseconds because of the high energy barrier between the inactive state and the activation intermediate state. Complete transition to the active conformation requires an intracellular-binding G protein-mimetic nanobody, Nb80. Overall, these studies demonstrate a loose allosteric coupling of the agonist-binding site and G protein-coupling interface that may generally be responsible for the complex signaling behavior observed for many GPCRs.

O 078
TOWARDS A MECHANISTIC UNDERSTANDING OF THE OPIOID MY RECEPTOR ACTIVATION BY LIQUID-STATE NMR SPECTROSCOPY

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Opioid receptors (OR), members of the G protein-coupled receptor (GPCR) superfamily, constitute the major and the most effective target for the treatment of pain[1]. The use of opioid drugs acting at these receptors is however a leading cause of death by overdose in Europe and North America. Both beneficial and adverse effects of illicit opioid drugs (opium, heroin) as well as approved therapeutics (morphine and codeine) are mediated by the activation of the mu-opioid receptor (μOR).

We recently described the structure of an inactive conformation of the μOR[2]. It provided important information regarding the binding site of small morphinan antagonists, revealed a largely exposed binding pocket, and demonstrated key molecular determinants for antagonist binding preferences for OR. However, much remains to be learned about the mechanisms by which different agonists can induce distinct levels of Gi protein activation and/or arrestin recruitment upon activation of μOR. Pharmacological and biophysical studies suggest that this versatility can be achieved through the structural plasticity of GPCRs[3].

In this study, we propose to analyse the conformational landscape of the μOR in distinct pharmacological conditions using liquid-state NMR spectroscopy by monitoring signals from methyl-labelled lysines. Assignment of resonances is achieved by a mutagenesis approach. We thus could analyze the structure and dynamics changes upon binding to different ligands ranging from agonist to antagonists, as well as upon binding the effector Gs protein and a mimetic nanobody

thereof. Our results show that there is very weak allosteric coupling between the agonist binding pocket and G protein coupling interface (transmembrane TM 5 and 6). Furthermore, the analysis provides clues on the successive structural events leading to the full active conformation of moR [4]. A better knowledge of the structural basis for opioid drug efficacy may lead to new therapeutic approaches with limited side effects.

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O 079
FROM SEQUENCE TO PROTEIN STRUCTURE USING THREE 4D NMR SPECTRA: OLD TRICKS WITH NEW BRICKS

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NMR spectroscopy has proven to be a powerful technique for 3D structure determination of small to medium size proteins and their complexes. Yet, NMR structure determination still remains very tedious and demanding on both man power and machine time, frequently lacking automation steps which would simplify the protocol. These issues mainly stem from (a) large number of conventional (3D) NMR experiments required for unambiguous assignment of the individual resonance frequencies and (b) extensive signal crowding in the spectra rich in structural information. Recent advances in development of methods for recovering spectra from sparsely sampled NMR data in concert with increased computational power of state-of-the-art computers have opened new possibilities for exploitation of the full potential of some NMR experiments developed already some 20 years ago. Here, we present a novel, highly automated, strategy for de novo determination of protein structures from only three four-dimensional NMR experiments which can be acquired within ~14 days of spectrometer time. We show that combination of the 4D HC(CC-TOCSY)CONH experiment with the 4D HCNH NOESY is sufficient for (a) unambiguous assignment of most (more than 90%) of the resonance frequencies and (b) determination of the protein fold. For the former task, we have adopted the basic ideas used in whole-genome shotgun assembly from next-generation sequencing data to obtain chemical shift assignments automatically in one step. For the latter task, we have tested the ability of autoNOE-ROSETTA to produce a preliminary protein fold using the HCNH NOESY spectrum only. For determination of a high-quality protein structural model we also incorporate restraints from the 4D HCCH NOESY experiment to the structure calculation protocol. The novel strategy

has been successfully employed to determine two protein structures (8 and 20 kDa in size) for which other high-resolution data is available, and for de novo structure determination of an 18 kDa protein. The present approach combines the latest NMR advances with fragment-based assembly tools for high automation in the assignment step, and proves to be very effective for structure determination of proteins as large as 20 kDa. Our strategy outperforms conventional approaches in terms of overall time required for data collection and analysis, improves the fidelity of assignments, and minimizes the level of user intervention. Our data demonstrate that 4D experiments are time-efficient, yielding high resolution spectra void of signal overlaps Therefore we foresee that 4D NOESY spectra provide a viable path for the structure determination of large proteins, because they resolve ambiguities of degenerated proton chemical shifts and facilitate the NOE assignment task for automated structure calculation programs.

O 080

ADVANCING STRUCTURE DETERMINATION OF MEMBRANE PROTEINS IN LIPID BILAYER MEMBRANES

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The biological functions and molecular structures of proteins are highly dependent on the physical and chemical properties of the surrounding environment. Just as water is essential for supporting the native states of soluble proteins, the lipid bilayer is critical for preserving the functional and structural integrity of membrane proteins.

The principal advantage of NMR spectroscopy as a method for structure determination is its ability to examine proteins in samples that are very close to their functional environments. NMR is well suited for studying soluble proteins in water and membrane proteins in lipid bilayer environments.

Here we describe recent progress on NMR structure determination of membrane proteins in lipid bilayers, with examples that include a bacterial virulence factor and a human cytoprotective oncoprotein. We show that samples can be optimized for parallel analysis by solid-state NMR, solution NMR and functional activity assays. Furthermore, we present new computational methods designed specifically to calculate membrane protein structures in a physically realistic membrane environment.

This research is supported by grants from the National Institutes of Health (GM100265; GM110658, CA179087). It utilized the NIH-supported NMR Facilities at the Sanford-Burnham Medical Research Institute (CA030199) and at the University of California San Diego (EB002031).

S 17 - Sensitivity Enhancement I

O 081
STORING 13C SPIN ORDER FOR MORE THAN 1 HOUR IN A ROOM TEMPERATURE LIQUID

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Long-lived states are configurations of nuclear spins that display long relaxation times, relative to ordinary nuclear magnetization. A primary example is the state of singlet order in a pair of spins-1/2, defined as the mean population difference between the spin-0 singlet state and the spin-1 triplet state. In favourable circumstances the lifetime of nuclear singlet order may exceed that of longitudinal magnetization by a factor of 50 or more. Singlet order does not itself provide an NMR signal but it is possible to convert magnetization into singlet spin order, and back again, providing that a small symmetry-breaking mechanism is provided (rather like the keyhole in a safe).

We have used molecular dynamics, quantum chemistry and spin dynamical theory to guide the design of molecular systems providing exceptionally long-lived singlet order. Target systems could then be synthesised through a collaboration with synthetic organic chemists. An example will be given of a molecular system that exhibits a nuclear singlet lifetime of more than 1 hour in a room-temperature liquid.

These systems are expected to be useful as transport agents for nuclear hyperpolarization and for molecular sensing and imaging applications.

Long-lived states are not restricted to two-spin systems. If time permits, some experiments on long-lived states in the three-proton systems of methyl groups will also be presented.

O 082

ENHANCING SABRE WITH MICROTESLA FIELDS: BROADLY APPLICABLE, >10,000 FOLD DIRECT HETERONUCLEAR SIGNAL ENHANCEMENT WITH >20 MINUTE SIGNAL LIFETIMES

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Hyperpolarization, particularly with dissolution dynamic nuclear polarization (d-DNP) (Ardenkjaer-Larsen, PNAS 2003) is opening new applications in NMR and MRI. However, d-DNP has high costs ($3M for the commercial, GE SpinLab™), lengthy hyperpolarization times (~20 min – 1h), and serious scalability challenges (because the mm wave irradiation barely penetrates large samples). An alternative, cost-effective and fast (or even continuous) source of hyperpolarization is "Signal Amplification By Reversible Exchange" (SABRE; Duckett Science 2009). SABRE uses an organometallic Ir-catalyst, which transiently binds both parahydrogen and targeted molecules, permitting transfer of spin order from parahydrogen to the target. Until now, the main limitations of SABRE have been that it only hyperpolarized protons efficiently (associated with few-second T_1 lifetimes), and that it was limited to a relatively small class of molecules. Here we show that both of these limitations are overcome by conducting SABRE in a magnetic shield directly targeting heteronuclei.

We introduced this method as SABRE-SHEATH (SABRE in Shield Enables Alignment Transfer To Heteronuclei). (Theis, JACS 2015; Truong, JPCC 2015). With this method we can directly target spin systems with long signal lifetimes and hyperpolarize much wider classes of molecules. In our first SABRE-SHEATH paper (Theis, JACS 2015) we hyperpolarized ^{15}N-pyridine and ^{15}N-nicotineamide (Vitamin B3 amide) with enhancements of 30,000 fold and 20,000 fold respectively over thermal signals at 9.4 T, and with ^{15}N T_1 of above 1 min.

In our more recent work, the true advance from SABRE-SHEATH is becoming even clearer. Traditional ^{1}H SABRE requires strong J-coupling between the parahydrogen derived hydrides on the catalyst and the protons on the substrate, which has limited effective ^{1}H-SABRE to pyridine like substrates. However, by directly targeting heteronuclei this restriction is removed and many more classes of molecules become amenable. For example, we demonstrate >15,000 fold enhancements on ^{15}N-acetonitrile (with T_1 > 1 min), which is a very poor substrate for traditional ^{1}H SABRE. More importantly, we have discovered that heteronuclear SABRE can directly hyperpolarize long-lived singlet states with very long lifetimes (T_S > 20 min) on ^{15}N spin pairs in diazirine (N=N bound to one C, forming a three membered ring). We report >10,000 fold signal enhancements on the ^{15}N sites even though the molecule displays no proton signal enhancement via conventional ^{1}H-SABRE. Diazirines have not even been reported as Ir-ligands before, yet they hyperpolarize via reversible exchange on the SABRE catalyst. In addition, diazirines can be incorporated as small tags into a large range of biomolecules. The >20 min lifetimes, paired with the significant signal enhancements, set the stage for hour long molecular imaging.

It is our prediction, based on accompanying theoretical models, that these groundbreaking results will enable heteronuclear hyperpolarization of many other substrates that exhibit weak interactions with the reversibly exchanging Iridium complex, be it in a solution or even as 'neat' liquids. (Shchepin, 2015, submitted). Over the last decade d-DNP has changed magnetic resonance research; we believe that SABRE-SHEATH will do so once again, because it puts a generalized hyperpolarization technique into the hands of any interested researcher at a modest budget.

O 083
RF-SABRE MAKES FEASIBLE CONTINUOUS HYPERPOLARIZATION AT HIGH MAGNETIC FIELD

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We present a method of hyper-polarizing protons (^{1}H) and "insensitive" NMR nuclei (here, ^{15}N) by exploiting the SABRE (Signal Amplification By Reversible Exchange) technique and transferring spin order from protons originating from parahydrogen. We demonstrate that hyperpolarization transfer is due to a coherent mechanism operative not only at low (1 mT) and ultralow (~1 μT) magnetic field but also at a high field of an NMR spectrometer in the presence of a suitable RF-excitation scheme, which enables spin order transfer at avoided level crossings. This hyperpolarization technique we term "RF-SABRE".
The achievable ^{1}H-NMR enhancements in the RF-SABRE experiment are around several hundred as compared to NMR signals at thermal equilibrium conditions at 4.7 Tesla; the method is operative for a variety of substrates, which we have studied. The NMR enhancements achieved for ^{15}N-nuclei of pyridine in the ^{15}N-RF-SABRE experiments are more than 1,000 for free pyridine in solution and more than 20,000 for pyridine bound to the SABRE complex in comparison with their thermal signals at 9.4 T.

RF-SABRE experiments are particularly important for solving the sensitivity issue in NMR: they dramatically enhance NMR signals and avoid technically demanding field-cycling. Thus, the proposed method can serve as a new robust and sensitive tool in NMR. Furthermore, the reported RF-SABRE experiments require low power for NMR excitation and make feasible continuous re-hyperpolarization of substrates in high-field experiments. Polarization can be quickly restored to the maximal level within only a few seconds with the polarization levels staying constant over at least several hundred

experiments.

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O 084

EFFICIENT DYNAMIC NUCLEAR POLARIZATION AT 800 MHZ WITH TRITYL-NITROXIDE BIRADICALS

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Dynamic nuclear polarization via the cross-effect (CE DNP) requires two interacting electrons, one of which interacts with a nearby proton. For the cross-effect to take place, the difference between the electron Larmor frequencies of these two electrons must be equal to the proton Larmor frequency, i.e. the cross-effect matching condition, $\omega_{0e1} - \omega_{0e2} = \pm\omega_{01H}$, must be fulfilled. Biradicals, consisting of two, chemically linked nitroxides, were found to be efficient polarizing agents. In recent years, optimization of the chemical structure of these bis-nitroxides has, together with development of instrumentation, contributed tremendously to the increase of the NMR signal enhancement by CE DNP. The most recent milestone is AMUPol, [1] which gives enhancements of 250 at 211 MHz, 235 at 400 MHz, 128 at 600 MHz, and 30 at 800 MHz.

We have tested a series of biradicals consisting of a trityl and a nitroxide [2] for CE DNP at 211, 600 and 800 MHz. The best performing biradical, named CT02-GT, [2] gives an enhancement of 65 at 800 MHz. Thus, at 800 MHz, the enhancement by this trityl-nitroxide biradical is more than a factor of two higher than by AMUPol. In addition, the polarization build-up time is a factor of about 1.3 shorter and is only 3.7 s at 800 MHz.

The enhancement that can be obtained with AMUPol drops when DNP experiments are performed at higher magnetic field. This same

behavior is observed for all bis-nitroxides developed for CE DNP. For the trityl-nitroxide CT02-GT the signal enhancement is only 50 at 211 MHz, but increases to 87 at 600 MHz. We attribute this remarkable field dependence to a relatively strong exchange interaction between the nitroxide and the trityl radicals. The unpaired spin density of trityl is highly delocalized and extends into the chemical linker to the nitroxide. For CT02-GT the exchange interaction between the trityl and the nitroxide radicals was estimated from solution EPR to be around 250 MHz. [2]

A second factor contributing to the good performance of the trityl-nitroxide biradicals is the difference in electronic relaxation rates between trityl and nitroxide radicals. At 140 GHz the T_1 of trityl is roughly four times longer than of TEMPOL. The slow relaxation of trityl has a favorable effect on the populations of the electronic energy levels under microwave irradiation and is predicted to lead to a higher steady-state nuclear polarization. [3] In this case a characteristic asymmetric enhancement field profile is expected, which we indeed observe for CT02-GT.

Our DNP experiments with trityl-nitroxide biradicals show that the development of polarizing agents for CE DNP is not complete and an even further gain in enhancement at high fields seems within reach. They hereby underline the power of CE DNP and how it can open up new possibilities to investigate larger and larger molecular structures by solid-state NMR.

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O 085
PHOTO-CIDNP MAS NMR

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Photo-CIDNP MAS NMR has been developed to a standard analytical method for the analysis of spin-correlated radical pairs [1-3]. The understanding of the mechanism of photo-CIDNP production allows for detailed studies on various reaction centers (RCs) [4-8], and a blue-light photoreceptor [9,10]. Possible functional mechanisms are discussed [2].

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O 086
RELATIVISTIC QUANTUM CHEMISTRY APPLIED TO NMR SHIFTS OF DIA- AND PARAMAGNETIC SYSTEMS

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Examples for the application of relativistic quantum-chemical methods for calculating NMR parameters to various chemical questions will be presented. One topic will be spin-orbit effects on NMR chemical shifts, which can lead to various new and interesting phenomena, particularly for compounds of the heavier elements.[1] Examples will be predictions of giant spin-orbit-induced ^{1}H and ^{13}C shifts in uranium(VI) organometallics (and related transition-metal species), where unprecedented shift ranges have been discovered.[2] For example, detailed analyses provide deeper understanding of ligand effects on the hydride proton shifts in platinum hydride complexes.[3] When one moves to paramagnetic systems, the theoretical and computational background becomes even more challenging.[4] Using methodology related to the Pennanen/Vaara formalism,[5] we report recent applications of first-principles quantum-chemical methods to the computation of pseudo-contact shifts in a paramagnetic Co-substituted metalloenzyme.

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O 087

TWO-DIMENSIONAL LINESHAPE ANALYSIS: METHOD DEVELOPMENT AND APPLICATIONS TO MULTISTATE PROTEIN–LIGAND INTERACTIONS AND ULTRAFAST CO-TRANSLATIONAL PROTEIN FOLDING

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NMR chemical shift changes and lineshapes are a rich source of structural, thermodynamic and kinetic information on macromolecular interactions and conformational exchange processes, which can be extracted by the analysis and fitting of resonance cross-sections. While usefully applicable to most titration measurements, NMR lineshape analysis can be a particularly powerful probe of complex multistate reaction mechanisms that are not readily studied by other methods. However, the more widespread adoption of these methods has been limited by the inability to analyse overlapping cross-peaks common in spectra of biomacromolecules.

Here we describe a method for the simulation and fitting of exchange processes in two-dimensional spectra, based on the direct quantum mechanical simulation of pulse sequences in Liouville space. We show that this method can be used to fully resolve even highly overlapped clusters of residues, greatly extending the general applicability of lineshape analysis. Moreover, as we fully calculate the effects of differential relaxation during the execution of the pulse sequence, normalisation of individual spectra is not required and resonance intensities can therefore be powerful additional constraints on exchange behaviour, resulting in more accurate and precise results than can be achieved by one-dimensional methods.

We illustrate our approach with a range of examples. Analysis of literature data for the interaction of two FBP Nbox peptides with FIR RRM1-RRM2 [1] increased the precision of the measured dissociation constants, while the newly determined kinetic information revealed that the observed variation in affinity are due to changing lifetimes of

the bound state, rather than to differing association rates. As a more complex test case, we have also studied the interaction of Ca2+-calmodulin with the drug TFP [2]. Calmodulin can bind up to four equivalents of TFP, and we show here that this can be fully described by a five-state sequential binding mechanism. We have determined a heirarchy of dissociation constants for the four sites, together with the binding kinetics and chemical shift changes associated with each step, allowing a detailed structural description of each stage of this interaction.

Finally, we describe the analysis of the ultrafast folding of the HP36 villin headpiece [3]. By fitting of HSQC spectra recorded across a range of urea concentrations, we can determine a folding time of ca. 20 μs in water. The fast exchange exhibited by this system can be exploited to provide a chemical shift-based measurement of protein stability, and we describe our development of a villin ribosome-nascent chain complex [4] to use this as a sensitive probe of the effect of ribosomal tethering on protein stability during co-translational protein folding.

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O 088

NESTA-NMR: EFFICIENT AND GENERALIZED PROCESSING OF MULTI-DIMENSIONAL NUS NMR DATA

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NESTA-NMR is a powerful and efficient program for the reconstruction of non-uniformly sampled (NUS) NMR data that incorporates multiple regularization methods, including a novel first-order gradient descent algorithm (NESTA), which enables convergence in significantly fewer steps than existing state-of-the-art methods (JBioNMR, online 2015). NESTA-NMR is able to reconstruct both high dynamic range (NOESY) and standard J-coupled correlation experiments with a high degree of fidelity. Reconstruction times for high-resolution 4D NMR spectra with NESTA-NMR are only a few hours on standard desktop hardware. The program features built-in parallelization and tight incorporation with NMRPipe to optimize both computational speed and ease of use. The software runs on a range of hardware from laptops to computer clusters. New features for artifact suppression will be described that provide confident application to a range of data processing challenges, including IDPs and quantitative relaxation experiments.

O 089
GRADIENT-ENCODED NMR: FROM THEORY TO PRACTICE

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In the last years, a new generation of pulse sequences based on the concept of gradient-encoded NMR has emerged in the field of ultra-high resolution NMR. On the one hand, a considerable effort has been devoted to the implementation of pure shift sequences to acquire spectra along which ^{1}H signals appear as singlets.[1,2] On the other hand, it has been shown that it is also possible to generate along the sample a series of selective spin echoes allowing for the complete edition, within a single acquisition, of the scalar couplings involved around a selected proton site.[3]

Despite the considerable breakthroughs accomplished in this field, the whole experimental and analytical process that leads to the extraction of proton-proton couplings can however remain a hard and time-consuming task for chemists. One reason is the complexity and the amount of information that is made available in state-of-the-art experiments, even for small or medium sized molecules.

We will present our recent theoretical and experimental developments in this field, that aim at controlling spin dynamics in a fully tailored manner in separate regions of the sample, and combining them into high resolution spectra whose analytical content is easily extractible.

We will first introduce a general formalism to simulate gradient-encoded NMR experiments. The action of a selective radiofrequency pulse in presence of a magnetic field gradient is modeled in order to obtain a detailed analysis of NMR signals that are locally created throughout the sample during encoded pulse sequences, involving especially pure shift and J-edited spin evolutions. This theoretical study is intended to describe key features of this spectroscopy such as the spatial resolution and the sensitivity of the encoding process.[4]

We will also present a new correlation experiment cited as "push-G-SERF" that meets the challenge of combining the analytical potential of multi-dimensional correlation spectra with the resolution enhancements provided by pure shift and J-edited spectroscopies. In the resulting phased 2D spectrum, the chemical shift information is selected along the direct dimension through a pure shift real time acquisition scheme, while scalar couplings involving a selected proton nucleus are edited in the indirect domain using a J-edited method. This experiment allows for fully resolving both dimensions of the spectrum that yields a straightforward assignment and measurement of the coupling network around a given proton in the molecule. The robustness of this pulse sequence will be demonstrated on model compounds with increasing structural and spectral complexity, notably on an oligomeric saccharide showing a highly crowded ^{1}H spectrum.[5]

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O 090
MOLECULAR DYNAMICS – NMR EXPERIMENTS AND SIMULATIONS

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NMR experiments enable studying molecular dynamics that span a broad range of time scales from picoseconds, where fast vibrational and librational motions occur, up to seconds, where global refolding of macromolecules takes place. The information about dynamics is, however, difficult to extract from NMR data without a suitable motional model. Molecular dynamics (MD) simulations coupled with calculations of NMR parameters provide direct links between observable NMR data and motional processes. Various MD simulation techniques (classical, DFT, path integral, metadynamics, solutions, solids) will be discussed in the paper and demonstrated on recent examples with the emphasis on fast vibrational motions and solvation shell dynamics.

A combination of DFT molecular dynamics and calculations of shielding and electric field gradient tensors has revealed the impact of vibrational motions on isotropic chemical shifts, chemical shift anisotropies and quadrupolar interactions. Moreover, molecular motion has a significant effect on average molecular structures, and neglecting the effects of motion on crystal structures derived by diffraction methods may lead to significant errors of calculated chemical shifts.[1]

The influence of nuclear quantum effects (NQE – zero-point vibration, delocalisation of the positions of light nuclei) on chemical shifts and quadrupolar couplings has been explored by a combination of path integral molecular dynamics (PIMD) and NMR calculations. NQE were shown to explain previously observed systematic deviations in correlations between calculated and experimental chemical shifts.

The PIMD approach also enables isotope substitution and temperature effects on NMR parameters to be predicted in excellent agreement with experiment.[2]

The structure and dynamics of hydration shells of model amino acids, peptides and nucleotides has been modelled by DFT molecular dynamics.[3,4] In comparison with classical molecular dynamics, the quantum mechanical approach revealed a more structured solvent and significant differences in the radial and angular distributions of the water molecules around the solute. For snapshot MD configurations, the NMR parameters were computed and averaged. Obtained values were significantly closer to experimental parameters than those calculated by the conventional implicit dielectric solvent model or by classical MD simulations.

Chemical shifts of chiral molecules placed into a chiral solvent have been also investigated.[5] Simple alcohols and amines were used as model systems, and differences in NMR chemical shifts dependent on the solute−solvent chirality combination were experimentally detected. Combined quantum mechanic/molecular mechanic (QM/MM) computations were applied to reveal the underlying solute−solvent interactions. The MD results predicted differences in conformer ratios and slight changes in the solvent structure dependent on the solvent/solute chirality combination. The results thus indicate a large potential of NMR spectroscopy and simulations to probe not only the structure of molecules but also their interactions with the environment.

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Plenary Session 6

PL 09
ULTRAHIGH FIELD MRI – ALZHEIMER'S DISEASE, EYE TUMOURS, DIELECTRICS AND PLASMAS

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With the rapid spread of 7 Tesla whole body MRI systems throughout the world there has been significant recent progress in both clinical and clinical research applications. Although predominantly in the neurological area, there have also been many developments in the areas of musculoskeletal, cardiac and ocular imaging. Increased magnetic susceptibility contrast, enhanced magnetic resonance angiography, and much higher signal-to-noise in spectroscopy and heteronuclear imaging/spectroscopy have been the driving forces for much of this progress. The major challenges have been, and continue to be, increased image inhomogeneity, power deposition, and motion-induced artifacts. Many hardware advances have already been necessary to deal with these problems, and many future advances are required to keep the field moving forward.

Examples which will be presented include: (i) the use of navigator echoes and phase imaging for high resolution MRI in Alzheimers patients, (ii) the use of high dielectric materials to improve neuroimaging and spectroscopy at high field, and (iii) the design of new types of RF coil specifically for high field, including dielectric resonators and plasma mediated magnetic resonance.

Plenary Session 7

PL 10
APPLICATION OF DISSOLUTION DYNAMIC NUCLEAR POLARIZATION IN CHEMISTRY

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Dissolution dynamic nuclear polarization (D-DNP) provides transient signal enhancements of several orders of magnitude in liquid state nuclear magnetic resonance. In this presentation, strategies are discussed to use such signal enhancements to extend upon the powerful traditional applications of NMR spectroscopy in chemistry. Apart from improvements in sensitivity, primary benefits include the ability to determine the evolution of signals on a short time scale, and the intrinsic contrast provided by the non-equilibrium spin polarization. Compounds that are involved in chemical reactions or intermolecular interactions are readily and directly hyperpolarized on ^{13}C, ^{1}H, ^{19}F or other nuclei. After rapid mixing with other sample components, high-resolution spectra characterizing the evolution of these species and signal intensities are observed. As an example, the initial time regime of polymerization reactions catalyzed by metallocenes are explored. NMR spectra acquired in real-time permit the identification of intermediate reaction products, and of catalyst-reactant complexes. Further, if the signal intensities are modeled with rate equations, differences in the kinetic behavior among several catalysts become apparent. Since transient changes in NMR signal intensity also result simply from relaxation processes, time-dependent measurements can further provide valuable information even if the involved species are under chemical equilibrium. Firstly, polarization transfer from a hyperpolarized ligand occurs selectively to binding sites on macromolecules. Proximity and molecular dynamics can be derived based on measurement of cross-relaxation rate constants. For working with macromolecules, coherence selection schemes are proposed for the identification of interaction sites in the presence of signal

overlap in one-dimensional spectra. Secondly, spin-relaxation rates of hyperpolarized ligands themselves can be used to report on binding. In an application of potential interest for drug discovery, R_2 relaxation rates are obtained from ^{19}F-hyperpolarized reporter ligands. The changes observed in these relaxation rates upon displacement of the reporter ligand from a binding site directly permit the calculation of the dissociation constant of an inhibitor of interest. Regardless of whether a sample is under chemical equilibrium, however, the non-equilibrium spin populations used inherently limit the number of NMR transients that can be acquired from a D-DNP experiment. It becomes therefore necessary to adapt many of the NMR experiments intended for reporting on spin correlations to these conditions, often involving extensive use of pulsed field gradients (PFG). In a final part, a method for rapid injection of the hyperpolarized sample using a high pressure liquid as a driving force will be introduced. This flow-NMR technique is designed to be compatible with PFG based experiments by substantially reducing the residual fluid motions during the time period encompassing the first several hundred milliseconds after transfer of the hyperpolarized sample into the NMR spectrometer, and allows the application of these NMR experiments for the study of early events after mixing.

PL 11

STRUCTURE AND DYNAMICS OF MICROTUBULE-ASSOCIATED PROTEIN ASSEMBLIES: INSIGHTS FROM MAS NMR AND MD SIMULATIONS

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Microtubules (MTs) and their associated proteins (MAPs) play important roles in vesicle and organelle transport, cell motility and cell division. Dynactin multisubunit assembly is the activator of the cytoplasmic microtubule-based dynein retrograde motor complex. CAP-Gly microtubule binding domain of dynactin's p150Glued subunit is critical for the regulation of dynein's motility. Mutations in the CAP-Gly domain are associated with neurological disorders, but the mechanism by which the CAP-Gly domain recognizes microtubules remains largely unknown, particularly at the atomic level. I will present atomic-resolution 3D structures of CAP-Gly free and assembled on microtubules, determined by MAS NMR spectroscopy. I will discuss the insights gained into structural and dynamic basis of CAP-Gly's biological function and interaction with its binding partners and microtubules, using a hybrid MAS NMR/MD approach. I will present recent results from our investigations into structures and dynamics of other microtubule-associated proteins, p150Glued(1-191) and kinesin motor domain. MAS NMR studies of MAP/MT assemblies required methodological developments, and I will discuss the recent methods from our laboratory for i) determination of intermolecular interfaces in MAP/MT assemblies; ii) correlation spectroscopy and distance measurements under fast MAS; iii) nonuniform sampling for time-domain sensitivity enhancements in multidimensional experiments.

S 19 - Emerging Techniques

O 091
TRANSIENT-COMPENSATED SOLID-STATE NMR

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Pulse sequences in NMR spectroscopy are usually designed under the assumption that amplitude and phase of the radio frequency are identical in experiments, analytical calculations and numerical simulations. In reality, however, the experimental radio-frequency field the nuclear spins experience in the sample can deviate significantly from this ideal behavior [1,2]. Depending on various properties of the rf circuit – e.g., Q-factor, tuning and matching of the probe as well as amplifiers, cables, and filters – an exponential build-up or decay of the in-phase rf-field amplitude as well as quadrature components are observed at any discontinuity during the pulse sequence. The resulting differences from the intended ideal spin trajectories can lead to a significant decrease in the performance of multiple-pulse sequences [3,4]. Therefore, one of the selection criteria for successful NMR experiments is their stability against pulse transients.

Linear response theory offers a way to calculate pulse shapes which counteract the formation of pulse transients and generate the desired pulse shape in the NMR coil [5]. However, it is not possible to generate rf fields with arbitrarily short transition times, like ideal rectangular pulses, but only pulses with edges of finite length, where the amplitude varies continuously.

The use of transient-compensated pulses as building blocks in NMR pulse sequences should lead to more reproducible results between different spectrometers. Using the double-quantum (DQ) recoupling sequence POST-C7 [6,7] as an example we demonstrate that such transient-compensated pulses can be used in symmetry-based recoupling sequences. However, two major implications caused by

the amplitude-shaped pulses need to be addressed. These are the generation of (i) an additional modulation frequency during the symmetry cycle and (ii) a reduced chemical-shift (CS) suppression. Both are caused by deviations of the C-element from an identity propagator. Based on a Floquet analysis, we propose modifications which are necessary to retain the high DQ scaling factors. On one hand, a modified resonance condition due to the additional modulation frequency can be matched by making the sequence asynchronous with respect to the MAS rotation. On the other hand, the rotor-synchronized protocol can be retained, if the amplitude-shaped POST element is modified such that the effective field, which causes a breakdown of DQ recoupling, vanishes. Properties of the transient-compensated sequences with different modified basic elements are analyzed using numerical simulations and experimental data. We show that the application of transient-compensated pulses, where the rf field acting on the spins in the coil is controlled by the spectrometer operator, lead to a high reproducibility of the experiments, especially if small dipolar couplings shall be determined by symmetry-based recoupling.

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O 092
SINGLE-PROTEIN SPIN RESONANCE SPECTROSCOPY UNDER AMBIENT CONDITIONS

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Single-molecule magnetic resonance spectroscopy and imaging is one of the ultimate goals in magnetic resonance and will has great applications in a broad range of scientific areas, from life science to physics and chemistry. We and co-workers have successfully obtained the first single-protein spin resonance spectroscopy under ambient conditions [1], realized atomic-scale structure analysis of single nuclear-spin clusters in diamond [3], detected nuclear magnetic resonance spectroscopy with single spin sensitivity [4], and succeeded in detection of (5nm)3 hydrogen nuclear spin magnetic resonance spectroscopy [6].

Among these works, I will specially introduce the "single-protein magnetic resonance spectroscopy under ambient conditions" [1]. The spin of a single nitrogen vacancy (NV) center in diamond is a highly sensitive magnetic-field sensor. We used the NV center to detect a nitroxide labeled protein and gained the world's first magnetic resonance spectrum of single protein through electron spin resonance under ambient conditions. We not only revealed the position and orientation of the spin label relative to the NV center, but also elucidate the dynamical motions of the protein on the diamond surface. Specially, "atmosphere with room temperature" provided necessary conditions for the broad applications of this technology in life science et al..

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O 093
ELECTRON SPIN COHERENCE NEAR ROOM TEMPERATURE IN MAGNETIC QUANTUM DOTS

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Coherent manipulation of electron spins in semiconductor quantum dots (QDs) has attracted continuously increasing interest to quantum information processing (QIP) applications. [1-3] QDs are zero-dimensional systems that consist of semiconductor nanocrystals (e.g. ZnO, PbS, CdSe etc…) surrounded by organic capping ligands or inorganic shells acting as dielectric insulating barrier between individual nanostructures.
Our aim is to identify and suppress the sources of electron spin dephasing in QDs incorporating magnetic ions by manipulation of the QD environment in order to achieve control on the spin dynamics and the time available for coherent manipulation of the Mn electron spins. Because of this we have chemically synthesized PbS colloidal QDs incorporating on average a single Mn ion [3] and investigated the spin dynamics and quantum coherence properties of the Mn^{2+} spins by pulsed electron spin resonance (ESR) methods.[4]
Solid state pulsed ESR studies reveal that the phase memory time (T_M) of Mn spins $T_M \sim 1$ µs at T = 5 K and is mainly limited by magnetic dipolar coupling between Mn spins in neighbouring QDs as well as by the hyperfine interaction of the Mn ions with the hydrogen nuclear spins of the capping ligands. [5] The suppression of the Mn–Mn interactions by dispersion of the QDs in a diamagnetic matrix and the minimization of the Mn-proton spin interactions by deuteration of the matrix enable us to increase the phase memory time constant up to $T_M \sim 8$ µs and

the spin–lattice relaxation time constant up to T_1 ~ 10 ms.[6] Notably, these long relaxation times have enabled us to observe Rabi oscillations with a figure of merit Q_M ~ 400 and quantum coherence near room temperature (T_M ~ 1 µs at T = 230 K).[6] These observations demonstrate that colloidal QDs represent suitable systems to control and manipulate the spin dynamics of confined electron spins which may find application as qubits in QIP.

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O 094

UTOPIA NMR: RECOVERING LOST MAGNETIZATION USING INTERLEAVED LOW-GAMMA DETECTION

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A growing number of nuclear magnetic resonance (NMR) spectroscopic studies are impaired by the limited information content provided by the standard set of experiments conventionally recorded. This is particularly true for studies of challenging biological systems including large, unstructured, membrane-embedded and/or paramagnetic proteins. Here we introduce the concept of **u**nified **t**ime-**op**timized **i**nterleaved **a**cquisition NMR (**UTOPIA-NMR**) for the unified acquisition of standard proton-detected and low-gamma-detected experiments using a single receiver. Our aim is to activate the high level of polarization and information content distributed on low gamma nuclei (in particular ^{13}C) without disturbing conventional magnetization transfer pathways.

To date, several techniques have been developed aiming to increase the efficiency of data acquisition by making use of the normally lost magnetization. However, they struggle with sensitivity and/or require commonly non-available hardware. We show that using UTOPIA-NMR we are able to recover nearly all of the normally non-used magnetization without disturbing the standard experiments. In other words, additional spectra, that can significantly increase the NMR insights, are obtained completely for free. While we anticipate a broad range of possible applications we demonstrate for the soluble protein Bcl-x_L (ca. 21 kDa) and OmpX in nanodiscs (ca. 160 kDa) that, unlike other techniques, UTOPIA-NMR is particularly useful for

challenging protein systems including perdeuterated (membrane) proteins. Moreover, we also demonstrate that UTOPIA-NMR setup can be useful for screening and/or structural characterization of small compounds.

O 095

FASTER MAS SPINNING FREQUENCIES AND HIGHER MAGNETIC FIELDS: NEW OPPORTUNITIES IN SOLID-STATE NMR.

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MAS spinning frequencies have recently surpassed 100 kHz making proton-detection no only in deuterated and fully backprotonated proteins, but also in fully protonated systems possible. We will discuss the influence of spinning frequency and magnetic field strength on the spectral resolution and the sensitivity and show examples of de-novo structure determination with less than half a milligram of protein.

S 20 - Metabolomics and Small molecules

O 096
NMR METABOLOMICS IN MICROBIOLOGY AND MOLECULAR EPIDEMIOLOGY

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Investigation of small metabolites by ^{1}H NMR spectroscopy in complex biological matrices (biofluids or tissues) allows the detection and characterization of metabolic fingerprints associated with dynamic biochemical processes and their perturbations linked to diseases, genetic mutations, or xenobiotic exposure, opening up vast ranges of applications in the field of medical biology.

We will present how NMR metabolomics strategies can be applied to microbial clinical diagnosis by rapid identification of bacterial species from culture media. While improving the characterization of bacterial isolates is of key public health interest to adapt suitable antibiotherapies in response to bacterial infections and to avoid the development of multiple drugs resistance, metabolic fingerprints of the bacterial exo-metabolome provide rich information throughout bacterial growth. We will show that general antibiotic resistance traits of specific bacterial strains can be detected from untargeted approaches, regardless of the specific antibiotic or the mechanism implied in the resistance.

We will also discuss applications of high-field NMR metabolomics investigations to cohort studies in the context of molecular epidemiology. Within the framework of the European Prospective Investigation into Cancer and Nutrition (EPIC), we have studied pre-diagnostic serum samples obtained from primary hepatocellular carcinoma (HCC) - the most prevalent form of liver cancer – cases and matched controls. We will show that metabolic patterns associated with HCC risk can be identified from high-field untargeted

NMR approaches, and provide meaningful etiologic insight into HCC development that can potentially be used to detect this cancer in its early stages, several years prior to clinical diagnosis.

O 097

RESIDUAL DIPOLAR COUPLINGS AND RESIDUAL CHEMICAL SHIFT ANISOTROPIES FOR THE STRUCTURAL DISCRIMINATION OF SMALL MOLECULES

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For the conformational and configurational analysis of small molecules, available one-bond Residual Dipolar Couplings (RDCs) may not be always sufficient when the molecule has few C-H bonds or has too many parallel bonds. Complementary long range RDCs provide the needed information to allow this under parameterized problem to be solved. We present a method for extracting long range RDCs and their analysis with a new alignment tensor optimization tool and signless J-couplings. In addition, Residual Chemical Shift Anisotropy (RCSA) provides an orientational sampling in the molecule and, importantly, provides information about non-protonated carbons. The differences in ^{13}C RCSA measured between two alignment states obtained by reversible compression/relaxation of PMMA/$CDCl_3$ gel, are demonstrated for the correct determination of the configurations of strychnine and estrone. Double delta RCSAs and delta RDCs that are obtained as the differences between two alignment states are fit separately to different configurations of strychnine and estrone; and result in lower Q factors for the correct configuration. For strychnine, both RDC and RCSA alignment tensors are similar with an inter-tensor angle of 14°. For the correct configuration, the combined fit of RDCs and RCSAs to a single tensor results in a lowest Q factor.

O 098
DISSOLUTION DNP IN LDH ACTIVITY AND CCL39 MURINE CANCER CELLS METABOLISM NMR MEASUREMENT

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The real-time measurement of enzymatic activities by dissolution-DNP [1] enhanced NMR can help current efforts in cancer diagnosis. The large polarization can be used to follow real-time molecular metabolic changes for a time span as long as a few T_1's of the target nuclei. The combination of dissolution DNP with LLS-based techniques [2] promises to extend the measurement time span for enzymatic reactions to timescales longer than T_1.

The pyruvate-to-lactate transformation by LDH is fast and it has been shown that the hyperpolarized ^{13}C-lactate signal measured in vivo, including in cells, following the injection of ^{13}C-labelled pyruvate is mainly due to a fast label exchange between pyruvate and lactate rather than to lactate production. [3] Using dissolution-DNP NMR, this study follows the real-time flux of pyruvate through LDH, in absence of initial lactate in the sample. Applications of pyruvate-based DNP to prostate cancer diagnosis by MRI are at clinical stage. [4]

Hyperpolarized ^{13}C-labelled pyruvate was prepared by dissolution DNP and transferred to LDH solutions in buffer. No lactate was added to the initial solution. The enzymatic activity of lactate dehydrogenase (LDH) could be assessed measuring the real-time lactate formation speed, at different enzyme concentrations, down to 10^{-3} U.I.. We applied the same technique to the measurement of the ^{13}C-labelled pyruvate metabolism in colon cancer CCL39 cells that over-express mono-carboxylate transporter MCT4 in the plasma membrane. This facilitates the intracellular transport of pyruvate inside the CCL39 cells, where it undergoes reduction into lactate by endogenous LDH.

Further developments in the application of LLS-based techniques to hyperpolarized NMR will be discussed.

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O 099
NMR STUDIES OF AN OXYGEN SENSING OXYGENASE

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Prolyl hydroxylase domain containing protein 2 (PHD2) is a Fe(II) and 2-oxoglutarate (2OG) dependent oxygenase that plays key roles in the regulation of the human hypoxia signalling and response pathways. In hypoxia, the a-subunit of hypoxia inducible factor (HIF) dimerise with its β-subunit to trigger hypoxic responses; In normoxia, however, PHD2 initiates the degradation of HIF-a by catalysing the hydroxylation of two conserved prolyl residues on HIF-a. Given the central role PHD2 plays in oxygen and hypoxia sensing, PHD2 is a current inhibition target for the treatments of many diseases including anaemia, cancers and ischemia.

We are interested in the mechanistic and inhibition studies of PHD2, and in the last five years we have applied NMR spectroscopy extensively to study the interactions between PHD2 and its ligands (e.g. substrates and inhibitors). In this talk I will review four of the methods that we routinely use to study PHD2-ligand interactions, which include both ligand-observed and protein-observed NMR techniques:

1. Solvent relaxation method in which ligand binding to PHD2 is reflected by changes in the bulk water longitudinal relaxation rate. This is made possible by replacing the native Fe(II) of PHD2 with a paramagnetic metal such as Mn(II).

2. Reporter displacement method in which the cosubstrate 2OG was used as a reporter ligand to monitor site-specific ligand binding events using simple 1D NMR techniques.

3. The applications of a combined waterLOGSY, transferred NOE and CPMG-based methods for the screening of PHD2 ligands so that both high- and low-affinity ligands can be captured, and to help avoid false negatives in screens.

4. Protein NMR studies including the applications of both chemical shift perturbation and relaxation studies to study the conformations and dynamics of PHD2 upon substrate binding in order to probe the mechanistic basis of the substrate selectivity of PHD2.

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O 100
PHOSPHOLIPID AND STEROL INTERACTIONS BY SOLID-STATE NMR: ROLES IN BLOOD CLOTTING AND ANTIFUNGAL DRUG MECHANISMS

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Atomistic information about lipids is challenging to obtain by most experimental methods. Magic-angle spinning solid-state NMR enables new insights into the structure, dynamics and functional molecular interactions of phospholipids and sterols. In this talk, first I will present approaches to examine the assembly of phosphatidylserine in the presence of calcium into complexes that accelerate blood coagulation. We have developed semi-synthetic isotopic labeling strategies, combined with assembly in Nanodiscs and NMR measurements to report detailed structural information in this context. Second, I will describe our studies to elucidate the mechanism of action of the antifungal drug amphotericin, which is the gold standard small molecule drug for treatment of life-threatening fungal infections. For over half a century, this powerful but also highly toxic small molecule has evaded development of microbial resistance. Understanding how amphotericin kills yeast is key to guide the development of derivatives with an improved therapeutic index. Through a series of NMR and functional studies, we demonstrate that amphotericin exists primarily as large, extramembranous aggregates that kill yeast by extracting ergosterol from lipid bilayers. This new mechanistic understanding is guiding development of amphotericin B analogs with improved efficacy.

"Amphotericin forms an extramembranous and fungicidal sterol sponge", T.M. Anderson, M.C. Clay, A.G.Cioffi, K.A. Diaz, G.S. Hisao, M.D. Tuttle, A.J. Nieuwkoop, G. Comellas, N. Maryum, S. Wang, B.E. Uno, E.L. Wildeman, T. Gonen, C. M. Rienstra, M.D. Burke. Nat. Chem. Bio. **2014**, 10, 400-406.

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S 21 - Relaxation and Transport Phenomena

O 101
NMR RELAXATION IN SOLIDS

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NMR relaxometry is one of the most powerful tools for studying dynamical processes in condensed matter. Nevertheless, in most cases, it is applied to liquids. The reason for this situation is combined effects of strong anisotropic interactions and slow molecular dynamics (both typical of solids) that has proven to be a great challenge from the theoretical point of view.

Standard NMR relaxation experiments are performed at a single frequency. NMR spectrometers based on Field Cycling (FC) technique [1] allow relaxation experiments in the frequency range of 10 kHz – 40 MHz (for ^{1}H) . This range can be extended towards lower fields by Earth field compensation and towards higher fields (up to 3T) by using external magnets.

Relaxation dispersion profiles for solids show a very rich structure compared to liquids [2,3]. Interplay between dipolar and quadrupolar spin interactions modulated by various motional processes leads to a variety of effects from which unique information about dynamical and structural properties of solid systems can be revealed, provided appropriate theoretical models are available. To meet the challenge a suitable relaxation theory must be valid for an arbitrary magnetic field and spin quantum number of the quadrupolar nuclei, arbitrary interaction strengths and motional conditions. Moreover, the theory has to account for non-exponential relaxation and cross-relaxation effects. Eventually, the theoretical approach has to include advanced models of dynamical processes in solids.

In this lecture principles of relaxation theories fulfilling these requirements are discussed. Specifically, the following topics are addressed:

-Relaxation theory beyond the validity range of perturbation treatments. Slow molecular motion and/or strong spin interactions often turn solid state systems beyond validity regimes of perturbation theory. For solids the perturbation conditions become, with decreasing temperature, sooner or later violated. A way to circumvent these limitations is the Stochastic Liouville Equation (SLE) [4].

-Multi-exponential relaxation. The presence of structurally and dynamically non-equivalent sublattices in solids can lead to a non-exponential relaxation. Analogous effects can be caused by cross-relaxation between different nuclei (for example ^{1}H- ^{19}F).

-Quadrupolar relaxation enhancement. This effect is often referred to as "quadrupolar peaks" ("dips"). They are observed when the magnetic field is set to a value for which the Zeeman splitting of the spin- 1/2 nucleus matches the energy splitting of the quadrupolar nucleus (determined by its residual quadrupolar coupling and Zeeman interaction).

-Motional models. It will be shown how different motional models can be incorporated into the SLE-based relaxation theory.

- Translational diffusion. It will be shown how "apparent" translational diffusion coefficients in solids can be extracted from spin-lattice relaxation dispersion data.

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O 102
SOLID-STATE NMR STUDIES OF A SUPER IONIC CONDUCTOR, Li7P3S11

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Lithium ionic conductors are promising electrolyte of solid-state batteries. Their major advantages are high ionic conductivity, electrochemical stability and inflammability. However, not much is known about high lithium ionic conduction mechanism. The high ionic conductivity (~10^{-3} S/cm) of $70Li_2S$ -$30P_2S_5$ (mol%) glass ceramics has been attributed to the precipitation of a metastable $Li_7P_3S_{11}$ crystal. By analyzing a synchrotron X-ray powder diffraction pattern of the crystal, Yamane et al. showed that Li ions are situated between PS_4 tetrahedra and P_2S_7 ditetrahedra. In this work, we applied $^{6/7}$Li and ^{31}P solid-state NMR to examine structural/dynamical variation of these tetrahedral units in two $70Li_2S$-$30P_2S_5$ samples; one is glass ceramic and the other is glass, whose ion conductivity is low (<10^{-4} S/cm). While $^{6/7}$Li and ^{31}P spin-lattice relaxation times exhibit that ^{31}P relaxation of the glass sample is governed by ^{7}Li motion, those of the glass-ceramics sample indicate ^{31}P motion is appreciable at temperatures higher than ca. 300K. High-resolution ^{31}P MAS NMR spectra of the glass-ceramics sample bear the ^{31}P signals of PS_4, P_2S_7 and P_2S_6. In addition to these reported previously, we found two signals, which have not yet been assigned. Interestingly, these two signals are merged into the P_2S_7 signal at higher temperatures and, further, related with each other by ^{31}P-^{31}P dipolar correlation experiment. We show that the high ion conductivity is supported by dynamical fluctuation of the P_2S_7 unit.

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O 103
ULTRAFAST MULTIDIMENSIONAL LAPLACE NMR FOR A RAPID AND SENSITIVE CHEMICAL ANALYSIS

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Relaxation and diffusion NMR experiments provide versatile information about the dynamics and structure of substances such as proteins, polymers, porous media etc. They may also enable separation of different components in the systems lacking spectral resolution, leading to improved chemical resolution. Since the relaxation and diffusion data consists of exponentially decaying components, the processing requires a Laplace inversion in order to determine diffusion coefficient and relaxation time distributions. Consequently, these methods can be referred to as **Laplace NMR** (LNMR). [1]

Like in traditional NMR spectroscopy, the resolution and information content of LNMR can be increased by the multidimensional approach [1]. However, long experiment time restricts the applicability of the multidimensional methods. As a solution for this problem, we are developing a broad range of ultrafast, **single-scan multidimensional LNMR** experiments [2,3], based on the principles of continuous spatial encoding that have been recently successfully applied in ultrafast multidimensional NMR spectroscopy [4]. The method shortens the experimental time by one to three orders of magnitude as compared to the conventional method, offering unprecedented opportunities to study fast processes such as polymerization, gel formation, phase changes and metabolism, in real-time.

The sensitivity of NMR can be increased by several orders of magnitude by nuclear spin **hyperpolarization** techniques such as dynamic nuclear polarization (DNP), parahydrogen-induced polarization (PHIP) and spin-exchange optical pumping (SEOP), allowing investigations low concentrations of molecules. The

proposed new LNMR techniques enable using hyperpolarized substances in the multidimensional approach [3], which is not feasible in the case of traditional methods requiring extensive repetition of the experiments.

In the presentation, we explain the **principles** of various ultrafast multidimensional LNMR experiments, and with **experimental demonstrations** as well as simulations we show that they provide detailed information about sample components that are not spectrally resolved, with a high resolution which is not usually considered to be achievable by ill-posed Laplace inversion. Furthermore, we show the first application of hyperpolarized (PHIP) substances to significantly boost the sensitivity of ultrafast multidimensional LNMR [3].

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O 104
DIFFUSION AND TRANSPORT VIA SINGLET TAGGING

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Magnetic resonance imaging can be used to study motional processes such as molecular diffusion and flow. However, in conventional experiments the accessible timescales are limited by either transverse (T_2) or longitudinal (T_1) relaxation times. Long-lived nuclear singlet spin states allow spin magnetization to be stored for a considerably extended period of time, typically an order of magnitude longer than the longitudinal relaxation decay constant. Recently, we introduced a technique able to prepare long-lived singlet spin order only in selected regions of space. We call this technique "singlet tagging". Singlet-tagged portions of a sample can be followed for much longer than T_1 thus offering the possibility to use magnetic resonance to study smaller diffusion coefficient and slower flow compared to magnetization tagging experiments run on identical hardware. We demonstrate the use of this technique for monitoring diffusion over a macroscopic scale and tracking a very slow flow.

O 105

NMR RELAXATION AND MOLECULAR DYNAMICS IN HOST-GUEST COMPLEXES: CHLOROMETHANES@CRYPTOPHANES AS AN EXAMPLE

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Cryptophanes [1] are cage-like molecules, composed of two cyclotribenzylene caps bound together by flexible linkers. They are able to bind small organic guests (such as chloroform or dichloromethane) inside their cavity. The host-guest complexes of cryptophanes with chloromethanes are characterized by the occurrence of several dynamic processes: guest exchange between free and bound sites, guest motion inside the cavity, conformational exchange for the host (the three linkers and the methoxy groups are conformationally flexible). The dynamic processes are clearly visible in the proton and carbon-13 NMR spectra, taken at different temperatures. Quantitative analysis of guest exchange processes is possible using line-shape analysis and EXSY, while the guest reorientation can be followed through ^{13}C relaxation. Under advantageous conditions, the dynamics of the conformational exchange processes can be characterized using the CPMG relaxation dispersion experiments [2].

Complementary information on the various dynamic processes can also be obtained through measurements of cross-correlated relaxation rates [3].

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S 22 - Disordered proteins

O 106
DISORDER REGULATION OF AN INSTRINSICALLY DISORDERED PROTEIN: BEYOND THE STRUCTURE-FUNCTION PARADIGM

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About two thirds of the proteins in eukaryotic organisms contains large intrinsically disordered regions, i.e. highly dynamic regions rapidly sampling a very large number of conformations. Many of these proteins are associated with high-level regulation processes and their ill functioning leads to diseases, such as cancer. c-Src, is a paradigmatic example. c-Src is the leading member of the Src family of kinases that share a common domain structure formed by a membrane anchoring SH4 domain, an intrinsically disordered Unique domain, a polyproline binding SH3 domain, a SH2 domain recognizing phosphotyrosine containing peptides and a kinase, or SH1, domain, followed by a short regulatory tail. All members of the family show high homology in the SH4, SH3, SH2 and SH1 domains but diverge strongly at the Unique domain.

Following the insight provided by NMR studies, we could demonstrate that the Unique domain is part of a previously unrecognized regulatory system, confirming the functional relevance of the intrinsically disordered Unique domain. In this talk we shall present recent results suggesting that conformational bias in the Unique domain is regulated by weak interactions involving neighbor SH4 and SH3 domains, as well as transient contacts within the Unique domain itself, and of the Unique, SH4 and SH3 domains with lipids. These interactions are allosterically modulated by polyproline ligands

binding to the SH3 domain as well as by phosphorylation at various sites in the Unique domain.

The results that will be presented are building a much more complex picture of c-Src regulation than the widely accepted current model. This new picture, however is more realistic and is consistent with the role of c-Src as a regulation hub for a large number of signaling pathways.

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O 107
THE CONFORMATIONAL ENSEMBLE OF INTRINSICALLY DISORDERED WIP: BIOLOGICAL AND BIOPHYSICAL INSIGHTS FROM 13C-DETECTED SPECTROSCOPY

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Intrinsically disordered proteins (IDPs) are multi-conformational polypeptides that lack a stable three-dimensional structure. Two facts are becoming increasingly clear, (i) the versatile IDPs play key roles in a multitude of biological processes, and (ii) given their flexible nature, NMR is emerging as a leading method for investigating IDP behavior on the molecular level. Together these considerations have motivated exciting methodological developments, most notably 13C'-detected spectroscopy, enabling NMR to overcome the unique challenges presented by this class of proteins (1-3).

The focus of our structural study is the intrinsically disordered WASp Interacting Protein (WIP) from human T cells, whose C-terminal interaction with WASp and N-terminal interaction with actin are crucial for the cytoskeletal changes accompanying cell activation. The pivotal role of the WASp-WIP complex in the immune response is demonstrated by the fact that WASp mutants unable to bind WIP lead to hereditary immunodeficiencies Wiskott-Aldrich syndrome and X-linked thrombocytopenia, and uncontrolled WASp expression is involved in hematopoietic malignancies (4). A range of 13C'-detected NMR experiments tailored for IDP study was employed to discover transient structural motifs in WIP. Chemical shifts, relaxation rates, solvent exposure, temperature effects and residual dipolar couplings all concurred in identifying WIP segments exhibiting a structural propensity, or a bias towards partial secondary structure, in their conformational ensemble. Remarkably, in both unstructured WIP terminal domains these transient structural elements echo the eventual conformation of WIP in its bound state in complex with actin or WASp, and a previously unrecognized segment in each domain was determined to contribute to the binding affinity (5, 6).

We have also enhanced the NMR toolbox for investigating IDPs by developing a J-modulated experiment designed to detect small changes in backbone one- and two-bond J(15N,13Ca) couplings reporting on shifts in the structural ensemble. This concept was realized using a unidirectional (H)NCO 13C-detected experiment suitable for the poor spectral dispersion characteristic of disordered proteins and optimized for maximum coverage of amino acid types. We demonstrate the utility of this method for the WIP N-terminal domain by following ensemble shifts induced by temperature changes, denaturing environment and crowding conditions. Taken together, our results provide a comprehensive map of WIP structure and dynamics and how these affect its interaction with T cell binding partners, as well as highlight the potential impact of high-resolution NMR upon the field of biologically active unstructured proteins.

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O 108

STRUCTURAL AND ENERGETIC DETAILS OF THE UNFOLDING LANDSCAPE OF STAPHYLOCOCCAL NUCLEASE FROM HIGH-PRESSURE NMR.

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Staphylococcal nuclease (SNase) has long served as a model system for protein folding. It is a globular protein of moderate complexity, consisting of three structural sub-domains. The major N-terminal sub-domain (SubD1) belonging to OB-fold family, sub-domain 2 (SubD2) corresponding to the C-terminal helix, and the interface between the two sub-domains (IntD).

To begin to identify the structural and energetic determinants of its folding free energy landscape we have examined in detail using high pressure NMR the consequences of cavity creating mutations in each of the sub-domains of SNase (1). These latter enhanced the population of the major folding intermediate. Cavity creation in different regions of the reference protein, despite equivalent effects on global stability, had very distinct consequences on the complexity of the folding free energy landscape. The L125A substitution in the C-terminal helix of SNase slightly suppressed the major intermediate and promoted an additional folding intermediate, involving disorder in the N-terminus. The I92A substitution, located in the hydrophobic OB-fold core, had a much more profound effect, resulting in a significant increase in the number of intermediate states and implicating the entire protein structure (2).

To address the effect the effect of cavity creation on SNase folding kinetics, and thus to the Transition State Ensemble (TSE), we also performed pressure-jump relaxation studies on these proteins (3). Real-time ^{1}H-^{15}N 2D correlation peak intensity profiles were collected at

over 100 residues for SNase and several cavity containing variants as a function of pressure. For SNase, the intermediate with a folded SubD1 and a disordered SubD2 is populated not only at equilibrium under certain conditions, but also transiently. In this intermediate, the tertiary interactions in SubD1 are nearly fully in place, whereas in the TSE, SubD1 has collapsed, formed the cavity and excluded solvent, but the tertiary interactions are not in place.

Pressure therefore facilitates the identification and characterization of the multiple conformations on a folding landscape, and has provided crucial information for understanding the sequence and structural determinants of this complex process.

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O 109

VISUALIZING THE MOLECULAR RECOGNITION TRAJECTORY OF AN INTRINSICALLY DISORDERED PROTEIN USING MULTINUCLEAR RELAXATION DISPERSION NMR

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Despite playing important roles throughout biology, molecular recognition mechanisms in intrinsically disordered proteins remain poorly understood. We present a combination of 1H, 13C, and 15N relaxation dispersion NMR, measured at multiple titration points, to map the interaction between the disordered domain of Sendai virus nucleoprotein (NT) and the C-terminal domain of the phosphoprotein (PX). Interaction with PX funnels the free-state equilibrium of NT by stabilizing one of the previously identified helical substates present in the prerecognition ensemble in a nonspecific and dynamic encounter complex on the surface of PX. This helix then locates into the binding site at a rate coincident with intrinsic breathing motions of the helical groove on the surface of PX. The binding kinetics of complex formation are thus regulated by the intrinsic free-state conformational dynamics of both proteins. Our approach provides high-resolution structural and kinetic information about a complex folding and binding interaction trajectory.

O 110
NMR CONTRIBUTIONS TO STRUCTURAL DYNAMICS OF INTRINSICALLY DISORDERED PROTEINS

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Intrinsically disordered proteins (IDPs) are characterized by substantial conformational plasticity. Given their inherent structural flexibility X-Ray crystallography is not applicable to study these proteins. In contrast, NMR spectroscopy offers unique opportunities for structural and dynamic studies of IDPs. The past two decades have witnessed significant development of NMR spectroscopy that couples advances in spin physics and chemistry with a broad range of applications. This presentation will summarize key advances in basic physical-chemistry and NMR methodology and illustrate the approaches with applications to biologically interesting IDPs.

S 23 - Paramagnetic Systems

O 111
PARAMAGNETIC NMR TOOLS TO STUDY PROTEINS AND PROTEIN COMPLEXES

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Unpaired electrons cause paramagnetic effects in NMR spectra. These effects were considered a nuisance because they can lead to broad lines and shifts of the resonances. However, nowadays the electrons have been tamed and used to our advantage. I will describe design and application of two-armed paramagnetic probes, called **Caged Lanthanide NMR Probes** (CLaNPs). These probes are attached to protein surfaces at specific sites and cause either shifts or broadening of resonances in a controlled way, providing long-range restraints -pseudocontact shifts (PCS), paramagnetic relaxation enhancements (PRE) and residual dipolar couplings (RDC)- for structure calculation. I will present examples of protein-protein and protein-ligand complexes that were studied with CLaNPs.

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O 112
RADICALS AND RADICAL PAIR IN LIGHT-ACTIVE FLAVOPROTEINS

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Three different classes of flavin-containing blue-light photoreceptors, namely LOV domains, BLUF domains and cryptochromes, are known to date. Although these proteins share the same flavin chromophore, their primary photoreactions differ significantly. Electron-transfer reactions are supposed to be involved an all three classes of blue-light photoreceptors, therefore electron paramagnetic resonance (EPR) methods in all flavors are particularly suited to identify and characterize (short-lived) paramagnetic intermediates (radicals, radical pairs and triplet states) [1] even in in vivo systems [2].

In this contribution, we describe the identification of key amino acids for efficient electron transfer in cryptochromes (and photolyases), and the finding of a secondary electron transfer if conserved amino acids of the primary electron transfer pathway are blocked [3]. Moreover, we could demonstrate that the kinetics of this photo-induced electron transfer reaction is magnetically sensitive, which argues that cryptochrome is fit for purpose as a magnetoreceptor [4].

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O 113
RIGID, HIGH-AFFINITY LANTHANIDE CHELATING TAGS MONITOR PROTEIN-LIGAND INTERACTIONS BY NMR (PCS, PRE AND RDC), DEER-EPR AND FRET

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Deciphering the countless interactions of biopolymers in a living cell is one of the most challenging tasks for life sciences for the next couple of decades. Understanding these exceedingly complex interplays on an atomic scale structural level is the prerequisite for a quantitative description of signalling and regulation circuits in any biological system. While X-ray crystallography is a powerful tool to determine the solid state structure of individual proteins, its ability to characterise protein – protein complexes, that are often weak or even transient is rather limited.

The solution NMR characterization of protein – protein complexes and protein – ligand complexes by classical, NOE-based methods is a difficult and laborious task due to the short range of the NOE effect. In contrast, synthetic lanthanide chelating tags (lct) that are site-specifically attached via a cysteine thiol to a protein induce pseudo-contact shifts (pcs) in the nuclei of the protein over a distance of up to 70 Å. This long-range effect can be used to determine structure and dynamics of proteins and their complexes by simple and sensitive one- and two-dimensional NMR experiments [1]. We have recently developed [2] an unusually rigid, high affinity lct, DOTA-M8. Here we present applications of this tag to the monomeric 261 residue protein human carbonic anhydrase type II (hCA II). Host guest transition metal complexes bound to hCA II have successfully been applied as synthetic metalloenzymes in homogeneous catalysis [3]. Five different serine to cysteine mutants of hCA II have been designed and the DOTA-M8 tag, loaded with diamagnetic lutetium or with paramagnetic thulium was conjugated to the hCA II. We have thus obtained five linear independent susceptibility tensors that allow the exact positioning in space of any nucleus with an observed pcs in a complex with hCA II. The PCS, PRE and RDC NMR experiments

presented, demonstrate the structural characterization of a complex between hCA II and a fluorine-containing inhibitor by simple one-dimensional ^{19}F-NMR experiments. The methodology can in principle be applied to even much larger protein complexes as long as meaningful (TROSY)-HSQC (^{15}N or ^{13}C) of the protein can be obtained.

In addition to the application of our tags in NMR spectroscopy, we have also carried out electron – electron double resonance experiments between two Gd(3+) tags on hCA II double cystein mutants and between a Gd(3+) and a Nitroxyl labelled inhibitor of hCA II in a collaboration with Maxim Yulikov at the ETH Zurich.

A further versatile implementation of DOTA-M8 based lcts is the exploitation of the long-lived photo excited states of Europium and Terbium derivates of our tags aimed at FRET spectroscopy and we will present preliminary results in these efforts.

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O 114
DYNAMICS OF CHARGE SEPARATION IN POLYMER-FULLERENE BULK-HETEROJUNCTIONS VS PHOTOSYNTHESIS AS REVEALED BY TIME-RESOLVED EPR/ENDOR/DFT STUDY

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At present, fossil fuels are the predominant source of energy driving world technological development. Due to the increasing world-wide demand for energy, new renewable and clean energy sources are needed to supplement, and eventually replace, the environmentally harmful and finite supply of fossil fuels. Solar energy provides one of the most promising sources of energy to meet future energy needs. Two possible pathways convert solar energy to useful forms of energy. The first, a "solar to fuel" approach, stores captured solar energy in high energy chemical bonds of molecules such as hydrogen. This approach is inspired by Nature's photosynthetic solar energy conversion processes. A second approach involves "solar to electricity" conversion. Both approaches include light harvesting and light-induced charge separation (CS) steps. In natural photosynthesis, light-induced long-lived charge separation occurs with a quantum yield that approaches 100%. This efficiency is so far unmatched by any man-made artificial system.

To this end, we use light-induced EPR/ENDOR spectroscopy combined with DFT calculations to study mechanisms of charge separation and charge stabilization in active organic photovoltaic materials (OPV) based on the composites of multiple and fullerene derivatives. Time-resolved EPR spectra show a strong polarization pattern for all polymer-fullerene blends under study, which is caused by non-Boltzmann population of the electron spin energy levels in the radical pairs. Similar polarization patterns were first reported in molecular donor-acceptor systems, such as natural and artificial photosynthetic assemblies, and comparison with these systems allow us to better understand CS processes in OPVs. The spectral analysis presented here, in combination with DFT calculations, shows that charge separation processes in OPV materials are similar to that in natural and

man-made organic photosynthetic systems. This work is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences, under contract number DE-AC02-06CH11357 at Argonne National Laboratory.

O 115
TRIPLET STATE DELOCALISATION IN LINEAR AND CYCLIC PORPHYRIN ARRAYS

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Porphyrin-based supramolecular structures have long been investigated with regard to their applications in molecular engineering, artificial photosynthesis and spintronics. In this paper we investigate photochemically generated triplet states of these supramolecular architectures so as to study the electronic communication between the single porphyrin units. Within the project we are able to combine information obtained by both continuous wave (cw) and pulsed Electron Paramagnetic Resonance (EPR) techniques to form a full picture of the charge distribution in a number of porphyrin structures:

1. linear conjugated polymers with with up to six porphyrin units
2. a cyclic six-membered ring
3. a cyclic six-membered ring arranged around a rigid template

Zero-field splitting parameters, spin polarisations and ^{1}H hyperfine couplings are combined to study the extent of delocalisation of the excited triplet state. Further geometric information on the relative orientation of the principal optical axis with the Z-axis of the zero-field splitting tensor is obtained using magnetophotoselection experiments.

S 24 - Sensitivity enhancement II

O 116

HYPERPOLARIZATION OF NUCLEAR SPINS BY PARAHYDROGEN FOR CATALYTIC AND IMAGING APPLICATIONS

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The utility of parahydrogen for NMR signal enhancement has grown tremendously over the last several years. Biomedical applications of MRI and MRS clearly represent a powerful driving force for the entire hyperpolarization field. However, there are other important applications of NMR and MRI which can benefit greatly from significant NMR signal enhancement.

PHIP has both advantages and disadvantages in the context of such applications. Compared to dissolution DNP, it is much less universal, but it is a relatively cheap technology which in addition can be used for producing hyperpolarized fluids continuously as opposed to the production of small batches of hyperpolarized substances inherent to DNP. Furthermore, as PHIP effects are generated in chemical reactions which involve p-H_2, PHIP is a powerful tool for the mechanistic studies of homogeneous catalytic processes that involve H_2 activation by transition metal complexes in solution. In particular, the mechanisms of homogeneous hydrogenations with p-H_2 can be addressed using PHIP as the signal enhancement of 3-4 orders of magnitude makes the short-lived reaction intermediates and their dynamic transformations readily detectable in many cases. Recent studies show that in addition to transition metal complexes, this also applies to metal-free systems capable of activating H_2. However, most catalytic processes of industrial importance are heterogeneous, and in many cases their mechanisms are poorly understood. Extension of PHIP-based signal enhancement to the studies of heterogeneous gas-solid and liquid-solid hydrogenations can potentially provide useful

mechanistic insights for these very complex systems. To achieve this, in our research we continue to explore the influence of various substrates, catalysts, catalyst supports, and reaction conditions on the PHIP effects observed in hydrogenations catalyzed by heterogeneous catalysts. The technique can be extended to other industrially important catalytic reactions as well. Our recent achievements in the exploration of all these possibilities will be presented.

Combination of PHIP with MRI is a promising approach for boosting MRI sensitivity required for many novel applications. In particular, this combination is of significant interest for catalytic research. Addressing complex processes in operating model reactors is difficult with conventional MRI and MRS approaches because in many cases their sensitivity is insufficient for such studies. We have demonstrated that heterogeneous PHIP is a promising tool for studying relevant processes in operating model catalytic reactions which allows one to detect transport of reactants and catalytic conversion quantitatively and non-invasively in a spatially resolved fashion. Such advanced techniques for operando studies of catalysts and reactors are crucial for modern chemical engineering and catalysis research and practice.

PHIP has been demonstrated also in the context of in vivo MRI/MRS studies. The major challenges that need to be addressed on this way are the production of biocompatible chemicals suitable for such in vivo studies, and the separation of the produced hyperpolarized substances from the hydrogenation catalyst and organic solvents. Suitable new approaches based on the use of p-H_2 in heterogeneous hydrogenations as well as recent progress with SABRE hyperpolarization technique will be discussed.

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O 117

MEASURING ABSOLUTE SPIN POLARIZATION IN DISSOLUTION-DNP BY SPIN POLARIMETRY MAGNETIC RESONANCE (SPY-MR)

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In recent years, Dissolution Dynamic Nuclear Polarization (D-DNP) has become a technique of choice for dramatically increasing the sensitivity of magnetic resonance in solution [1]. The nuclear polarization of a wide range of nuclei (^{1}H, ^{6}Li, ^{13}C, ^{15}N, ^{29}Si, ^{89}Y…) can be increased by saturating the ESR transitions of polarizing agents such as TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl) at low temperatures and moderate magnetic fields (in our laboratory at $1.2 < T < 4.2$ K and $B_0 = 6.7$ T). Thus, the proton polarization can be boosted by DNP to $P(^{1}H) > 90\%$ and by cross-polarization (CP) from ^{1}H to ^{13}C one can achieve $P(^{13}C) > 70\%$ [2]. Thereafter, the DNP samples can be rapidly dissolved and transferred to an NMR or MRI system, while preserving most of the polarization.

One of the practical challenges of D-DNP is the accurate determination of the resulting nuclear spin polarization $P(^{13}C)$. This is usually done by comparing signals obtained with and without DNP at low temperature prior to dissolution, then again in solution at room temperature immediately after dissolution, and finally at thermal equilibrium (TE) after complete relaxation. However, when a hyperpolarized substance is injected into a living organism, or more generally in any experiment where the hyperpolarized substance is diluted or undergoes irreversible biochemical transformations, the measurement of a thermal equilibrium signal can be difficult or even impossible.

After hyperpolarization of [1,2-^{13}C]pyruvate by dissolution-DNP, the strong polarization of spin $^{13}C^1$ is known to lead to an asymmetry A_S of the multiplet of the neighboring scalar-coupled spin $^{13}C^2$ and vice-versa [3-6]. This feature can be used for a broad range of homo- or heteronuclear scalar-coupled spin systems, and provides an elegant way to measure spin polarizations 'on the fly' while obviating the need for laborious measurements of signal intensities at thermal equilibrium.

We demonstrate that this method entitled Spin Polarimetry for Magnetic Resonance (SPY-MR) can be applied, provided (1) that DNP leads to a uniform spin temperature in the ^{13}C nuclear spin reservoir in the frozen solid; (2) that after dissolution the cross-relaxation rates that describe the coupling between Zeeman polarization and two-spin order can be neglected and (3) that effects of second-order coupling on the intensities of the transitions ("roof effect") are properly taken into account.

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O 118
NUCLEAR DEPOLARIZATION AND ABSOLUTE SENSITIVITY IN MAS-DNP: AMUPOL VS TOTAPOL WHO IS WINNING?

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Over the last two decades solid-state Nuclear Magnetic Resonance (NMR) has witnessed a breakthrough in increasing the nuclear polarization with the advent of Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP).[1] This approach requires the use of paramagnetic dopants (polarizing agents) and their efficiency is usually assessed comparing the ratio between the NMR signal intensity in presence and absence of microwave irradiation, $\varepsilon_{on/off}$. However, it has recently become clear that this parameter alone is not sufficient to account for the true sensitivity afforded (or not) by the technique.[2],[3] The presence of dopants in the system has several side-effects such as signal losses and faster nuclear decay times, which need to be taken into account when optimizing the overall sensitivity.

In this contribution we investigate NMR signal losses occurring during MAS-DNP experiments and specifically compare the (spinning-dependent) depolarization effect[4] for two "gold-standard" biradicals currently in use in most MAS-DNP studies. While TOTAPOL[5]typically yields a maximum enhancement of about 60 at 10 T and 100 K using standard samples, the recently introduced AMUPOL biradical[6] yields an enhancement factor 3 to 4 times larger. In this work we demonstrate that significant depolarization losses can be observed in the absence of microwaves at ~110 K and 10 T for both TOTAPOL and AMUPOL biradicals at 10 kHz MAS frequency: up to 20% for TOTAPOL and 60% for AMUPOL.

Using MAS-DNP simulations,[7] we show that these observations can be rationalized and are consistent with the biradical properties. Further insight into the depolarization mechanism (multi-parameters

phenomenon) can be obtained comparing the result for each crystallite orientation with the result obtained on the powder average. Overall, this work demonstrates for the first time that a DNP enhancement factor higher than the "theoretical limit" can be obtained. In light of these new results, the outstanding performance of AMUPOL must be revised and we propose a new and simple method to honestly assess the polarization gain during MAS-DNP experiments.

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O 119
SOLID-STATE DYNAMIC NUCLEAR POLARIZATION AT HIGH-TEMPERATURE, HIGH-FIELD & FAST MAS

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Dynamic Nuclear Polarization (DNP) enhanced solid-state NMR is a powerful technique to boost sensitivity by around 2 orders of magnitude. It has found rapidly growing applications in the characterization of biomolecules, materials and functionalized surfaces. The best DNP enhancements obtained so far for in situ MAS experiments are achieved at temperatures of ~100 K or lower, at fields of 9.4 T or lower. Most DNP experiments are performed in a glassy frozen matrix where the electronic and nuclear properties of the polarizing agent (the electron and nuclear relaxation times, …) make efficient Cross-Effect, Solid-Effect or Overhauser DNP mechanisms possible. On the other hand, low temperatures and low fields pose major bottlenecks for some applications, especially to biological samples, where spectra are significantly less resolved at low temperature and low fields. Increasing the temperature or the field normally leads to a very rapid drop in DNP performance, leading to negligible enhancements above 200 K at 9.4 T.[1,2]

Here we show how the careful choice of the solvent and the polarizing agent make it possible to obtain DNP enhancements of the order of 20 @ 273 K (0˚C) and 9.4 T. We will also present strategies to obtain proton DNP enhancements above 100 at higher fields (18.8 T, 800 MHz) and investigate the effects of fast magic angle spinning on DNP enhancements.

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O 120
TOWARDS SUPER-HIGH FIELD AND ULTRA-COMPACT SIZE NMR MAGNETS OPERATED BEYOND 1 GHZ (REVIEW)

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Super high field NMR spectrometers operated beyond 1GHz are being actively pursued for use in many fields including life sciences, pharmaceutical research, organic chemistry, and materials science. Conventional low temperature superconducting (LTS) NMR magnets cannot exceed 1 GHz, 23.5 T, as the critical current density of a typical LTS conductor decreases steeply beyond 23 T. The use of a high temperature superconducting (HTS) magnet, which is made of (a) a $Bi_2Sr_2Ca_2Cu_3O_x$ (Bi-2223) multi-filamentary HTS conductor or (b) a RE(Rare Earth)$_1Ba_2Cu_3O_x$(REBCO) coated HTS conductor, provides a high current density above 23 T, enabling an NMR magnet to substantially exceed 23.5 T, i.e. 1 GHz. Thus, super-high field NMR magnets producing a field as high as 1.2 GHz, are made possible if we employ low-field LTS outer coils and high-field HTS inner coils, which is called here as "an LTS/HTS NMR magnet". The LTS/HTS configuration was initially proposed by MIT in 2003. Only MIT and Japan have so far developed LTS/HTS NMR magnets. In this presentation, the Japanese project will be reviewed.

Japan firstly conducted basic experiments and made a precise comparison between two 500 MHz-class LTS/HTS NMR spectrometers operated in driven mode; one with a Bi-2223 innermost coil [1,2] and another with an REBCO innermost coil [3]. She evaluated (a) the temporal magnetic field drift due to the screening current induced in the HTS conductor, (b) the diamagnetic effect of the HTS coil which degrades the cryoshim performance, (c) the NMR sensitivity and NMR signal resolution, and (d) the quality of protein NMR spectra obtained. The NHMFL (USA) will fabricate another innermost coil made of a Bi-2212($Bi_2Sr_2Ca_1Cu_2O_x$) multi-filamentary round conductor, which will be similarly evaluated in RIKEN by NMR measurements in this year.

Based on these research results, Japan started to construct the world's first beyond 1 GHz LTS/Bi-2223 NMR spectrometer, which was designed

to operate at 1.03 GHz (24.2 T). The magnet was completed in 2014 and cooled by pressurized superfluid helium at 1.8 K. The charging experiment of the NMR magnet is being made in the National Institute of Materials Science (NIMS). Results of NMR measurements using this machine will be briefly reviewed at the conference.

As a final part of the presentation, a perspective on the potential of super high-field and compact-size NMR spectrometers, such as a 1.2 GHz machine, will be discussed based on the above results; new types of Bi-2223 multi-filamentary HTS conductor and REBCO coated HTS conductor are assumed for optimal coil design.

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Plenary Session 8

PL 12
SOLID-STATE DYNAMIC NUCLEAR POLARIZATION AT 263 TO 527 GHZ: INSTRUMENTATION DESIGN AND POLARIZING AGENTS

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Dynamic Nuclear Polarization (DNP) experiments transfer the high Boltzmann polarization of unpaired electron spins to nuclear spins for large gain in sensitivity and dramatic reduction in signal averaging time. This sensitivity gain opens the door to applications in solid-state NMR that may not have been achievable otherwise, and a range of applications have been demonstrated spanning from small molecules to large biological complexes and materials.[1] The polarization transfer is driven by microwave irradiation of the electron spins near their Larmor frequency. For DNP experiments at ^{1}H frequencies in the range of 400-800 MHz, microwave sources operating in the range of 263-527 GHz are required with high output power, spectral purity, and frequency and power stability.

We report on the development of DNP spectrometers for solid-state NMR applications at 263 GHz/400 MHz[2], 395 GHz/600 MHz, and 527 GHz/800 MHz. Microwaves are generated with continuous-wave gyrotrons and transmitted to the NMR sample via corrugated waveguide. Second-harmonic design and cryogen-free gyrotron magnets were introduced for reduced size and high output beam purity. The microwaves are coupled into a low-temperature (100 K) NMR probe and the sample is then irradiated for DNP-NMR experiments while spinning at the magic angle in a 1.3, 1.9, or 3.2 mm rotor.

The optimization of polarizing agents for DNP experiments at high field has been a critical factor in the extension of DNP experiments beyond 250-263 GHz. Initial DNP experiments at lower field were performed with nitroxide radicals such at 4-amino TEMPO with broad EPR line and

Cross Effect polarization mechanism. The development of biradical polarizing agents (two covalently-linked nitroxides) allowed for increased DNP efficiency and reduced radical concentration.[3]Building up on this work we have introduced a series of water-soluble nitroxide biradicals with higher DNP efficiency and reduced temperature and magic angle spinning dependence, enabling DNP experiments over a wider range of experimental conditions.[4] Nevertheless, these nitroxide biradicals still exhibit a decreased DNP efficiency with increasing frequency. Looking at narrow-line polarizing agents and the Overhauser mechanism offers opportunities for more favorable scaling with increasing frequency and reduced microwave power requirement.[5]

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PL 13

EXPLOITING CHEMICAL SHIFTS AND RDCS IN THE STUDY OF STRUCTURED AND INTRINSICALLY DISORDERED PROTEINS

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Chemical shift and RDC data are rich in structural information and accessible at the early stages of a structural study. An alternate approach to protein structure determination, which is applicable to larger proteins representing a wide variety of folds, utilizes these data and directly exploits the powerful bioinformatics algorithms previously developed for sequence-based homology modeling. For a protein with assigned chemical shifts, the new POMONA program finds structural homologs in the crystallographic database that best agree with experimental values, and subsequently uses chemical shift based Rosetta comparative modeling to yield full atom representations. For disordered proteins, chemical shifts complemented by multiple J couplings and short range NOE data are proving useful to identify Ramachandran distributions of individual residues, showing a close to random coil picture for most a-synuclein residues, but substantial deviations for many residues in the Alzheimer's related Abeta peptide, indicative of distinct structural propensities.

Poster Session 1

P 001

APPLICATIONS OF 19F-NMR TO STUDY PROTEIN-LIGAND INTERACTIONS AND PROTEIN CONFORMATIONAL CHANGES IN SOLUTION

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Nuclear magnetic resonance (NMR) is a powerful biophysical method for studying protein-ligand interactions in solution and elucidating the mechanism of action of potential inhibitors. However, protein NMR can be complicated by the overlap of ^{1}H and other resonances, hence the resolution needed to assign spectra precisely can be hard to achieve[1]. ^{19}F-NMR is increasingly being used to study conformational changes and protein-ligand interactions in solution because ^{19}F is (i) a spin ½ nucleus, (ii) 100% naturally abundant, (iii) 83 % as sensitive to NMR detection as ^{1}H, (iv) not present in most biological systems, and (v) its chemical shift is particularly sensitive to changes in local environment[2]. Recent advancements in NMR instrument and probe design have made ^{19}F-NMR more sensitive and more widely available; consequently, ^{19}F-NMR is finding growing application in research. Here, we report the use of ^{19}F-NMR to study two biomedically important protein systems. Proteins can be fluorine-labelled either by biological incorporation of fluorinated amino acids or by site-specific chemical ligation[3]. 3-bromo-1,1,1-trifluoroacetone (BTFA) has been developed as a useful reagent for importing fluorine into proteins via nucleophilic substitution such as with a cysteinyl-thiol[4]. The São Paulo metallo-β-lactamase-1 (SPM-1), a B1 sub-family metallo-β-lactamase, containing only one cysteine (Cys221) coordinating the second Zn(II) cation in its active site, was ^{19}F-labelled using BTFA. The interactions of SPM-1 with various potential inhibitors were reported by ^{19}F-NMR, which enabled monitoring SPM-1 conformational changes on ligand binding and informed on binding

strength by enabling K_D measurements. In a second study, prolyl hydroxylase 2 (PHD2), an enzyme involved in human oxygen sensing, was ^{19}F-labelled and its interactions with its co-substrate, 2-oxoglutarate (2OG), and peptide substrate (CODD) were monitored by ^{19}F-NMR. Conformational changes of PHD2 on 2OG and CODD binding were consistent with crystallographic analyses. Finally, ^{19}F-NMR was used to study solvent exposure of the complex and its dynamics in solution through relaxation dispersion of the ^{19}F-nucleus at different temperatures. Overall, the results illustrate the power of ^{19}F-NMR for monitoring ligand binding and conformational changes.

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P 004
CURCUMIN BINDING TO AMYLOID-BETA OLIGOMERS

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Curcumin, a dietary polyphenol, is a natural spice, which possesses a spectrum of anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic properties and has preventive and therapeutic potential for neurodegenerative diseases such as Alzheimer´s and Parkinson´s diseases. We have recently reported on the effect of curcumin on lateral diffusion of lipids in saturated and unsaturated bilayers as studied by the 1H NMR diffusometry (Filippov et al., Langmuir, 2014): the lateral diffusion coefficients of lipids in phospholipid bilayers are significantly reduced by addition of curcumin. We suggested that the curcumin molecule binds to the lipids (hydrogen bonding of –OH groups in curcumin with phosphate groups in lipids), thereby increasing the size of the diffusing entity.

Interestingly, a number of groups have reported on binding of curcumin and curcuminoids to amyloid fibrils of Alzheimer's Amyloid-beta peptides, though no studies on binding of curcumin to the most neurotoxic species of Amyloid-beta, oligomers and protofibrils, were yet performed, to the best of our knowledge. One of the most plausible neurotoxicity hypothesis of Amyloid-beta peptides is incorporation of small oligomers of these peptides into phospholipid membranes and formation of channels/pores. Therefore, it is an intriguing topic to be further explored whether and how: (i) curcumin binds to oligomers of Alzheimer's Amyloid-beta peptides, in particular to the Amyloid-beta(1-42) variant and whether and how (ii) curcumin blocks Amyloid-beta channels/pores in phospholipid membranes.

In order to answer the above questions we used solid state NMR on complexes of selectively 13C and 13C/15N labeled curcumin, the wild-type Amyloid-beta(1-42) and a variant of synthetically prepared cross-linked Amyloid-beta(1-42)Cys21-Cys30 previously designed and

thoroughly studied by Härd and co-workers (Lendel et al., PNAS 2010 and Angew. Chem. Int. Ed. 2014).

P 007

IN-SITU SOLID STATE NMR ON UNIFORMLY 13C-15N LABELED C. REINHARDTII THYLAKOID MEMBRANES

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In oxygenic photosynthesis, sophisticated regulation mechanisms have evolved to enable the splitting of water via P680, the strongest oxidizer found in Nature, while preventing the system from photo damage. Under excess light, remodeling of the photosynthetic membrane takes place and molecular switching of light-harvesting antenna proteins into a photoprotective, light-quenching state. Photosynthetic thylakoid membranes are densely packed with proteins (about 70%) and are abundant with proteins from the light-harvesting multigene family. Here we aim to study the structure and dynamics of protein and lipid components inside entire photosynthetic membranes by applying high-resolution Magic-Angle Spinning NMR on uniformly ^{13}C-^{15}N thylakoid membranes of the green alga Chlamydomonas reinhardtii in active and in quenched states, which contain different xanthophylls. Thylakoid membranes of wild-type C. reinhardtii (WT) contain violaxanthin and NPQ2 mutant membranes contain zeaxanthin. 2D ^{13}C-^{13}C MAS-NMR experiments (PDSD, PARIS) were performed and results compared with datasets on isolated C. reinhardtii light-harvesting complexes embedded in detergent micelles. In addition, to study the dynamics of membrane proteins and lipid components, cross-polarization, through-bond spectroscopy and direct polarization transfer experiments were performed at different temperatures. The first results are presented.

P 013

INSIGHTS INTO THE STRUCTURE AND DYNAMICS OF THE N-TERMINAL FRAGMENT OF THE HUNTINGTIN PROTEIN

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Studying proteins at atomic resolution both in vitro and in their native environments, is fundamental to understanding protein folding and aggregation. This work studies a CAG expansion within the huntingtin (Htt) gene, that encodes a polymorphic glutamine tract near the protein N-terminus and that is associated with Huntington's disease. The ensuing polyQ peptide is preceded by a 17 residue region that modulates the glutamine tract's behavior. To help elucidate the molecular basis of Htt aggregation, we investigated wild type Htt's 17 amino acid N-terminal segment with a 17 residue polyQ stretch (HttN17Q17). Studying Htt peptides presents a number of unique challenges: they display a high degree of conformational flexibility leading to averaging of NMR chemical shifts, and a large portion of their backbones are solvent-exposed leading to fast hydrogen exchange and causing extensive line broadening. To proceed with the NMR study, hydrogen exchange was suppressed by dissolving HttN17Q17 in a low pH solution. Resonances in the neutral (pH = 7.4) in vitro samples were then mapped to their low pH counterparts by performing NMR titration experiments. Molecular dynamics simulations starting from CS-ROSETTA derived structures based on the experimental chemical shifts were used to extract order parameters for the Htt peptide at low and neutral pH. All these data confirmed the high flexibility that the N17Q17 residues display in solution at neutral pH.

P 016
THE STRUCTURE OF NANODISCS: IMPLICATIONS FOR HIGH-DENSITY LIPOPROTEIN PARTICLES

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Since several decades, high-density lipoprotein (HDL) particles have not lent themselves to high-resolution structural techniques like NMR and X-ray crystallography due to its heterogeneity in density, size, shape, and protein as well as lipid composition. Irrespective of the different subclasses, the presence of apolipoprotein A-I (apoA-I) is common to every HDL particle. ApoA-I is the major protein component of all HDL particles found in human plasma, accounting for approximately 70% of total HDL protein. On the basis of sequence homology, residues 44-243 of apoA-I contain two 11-residue and eight 22-residue amphipathic a-helical motifs thought to represent the fundamental lipid-binding motifs. The pattern found for hydrophobic residues within the amphipathic a-helices is similar to the heptad repeats (abcdefg)n of a-helical coiled coil proteins. It is speculated that the modified heptad sequence (abc[c']defg)n with the additional residue c' is required for the protein to efficiently orient hydrophobic residues towards the lipid environment.

Discoidal and spherical HDL particles are found in human plasma, whereas discoidal HDL particles are composed of two apoA-I molecules and are transformed to spherical HDLs by the enzyme lecithin/cholesterol acyltransferase. Interestingly, the increase in diameter for HDL particles not containing apolipoprotein A-II (apoA-II) is attributed to an increasing number of apoA-I molecules, whereas for apoA-I/apoA-II HDL particles the number of apoA-I remains constant at two copies per particle irrespective of the diameter. Recent experiments revealed a similar structural organization of apoA-I in discs and spheres of reconstituted and plasma-derived HDL particles, regardless of their diameter. These results suggest an important role for discoidal dimeric apoA-I as a basic building block

in early HDL assembly and that structural features of discoidal apoA-I perpetuate to spherical assemblies. Insights into ApoA-I organization have come from a wealth of low-resolution biochemical and biophysical studies supporting various but also conflicting models. However, the double-belt model is currently the only model that is supported by crystal structures of lipid-free ApoA-I and nuclear magnetic resonance (NMR) data from a lipid-apoA-I complex. We decided to structurally investigate reconstituted discoidal HDL particles, also termed nanodiscs, by using MSPΔH5, a shortened version of apoA-1 lacking the first 54 N-terminal residues and helix 5 (residues 121-142). The use of an extensive selective amino acid depletion approach allowed for a sequential assignment of 95%. Introducing paramagnetic MTSL at 7 different positions equally distributed along the sequence resulted in long-range EPR distances of up to 65 Å. Furthermore, recording NMR spectra of mixed nanodiscs containing 14N-MTSL-MSPΔH5 and 15N-MSPΔH5 resulted in 910 intermolecular PRE distance restraints. A sample containing 15N-MTSL-MSPΔH5 and 14N-MSPΔH5 with MTSL at position 67 resulted in 220 intramolecular PRE restraints and allowed for the proximity of N- and C-terminus to be assessed. Together with intermolecular CH3-CH3 and HN-CH3 NOEs, intramolecular HN-HN NOEs, hydrogen bond restraints, angle restraints and HN-N and N-CO RDCs a high-resolution structure of the supramolecular lipid-containing nanodisc complex could be derived. The structure is stabilized by intermolecular salt bridges and cation-pi interactions and confirms the proposed antiparallel orientation of the two MSP molecules with a fixed 5/5 registry. In addition, the same structure is able to explain the recently proposed 5/2 registry.

P 019

EGFR TRANSMEMBRANE DOMAIN PACKING DIVERSITY SUGGESTS THAT COUPLED PROTEIN-PROTEIN AND PROTEIN-LIPID INTERACTIONS UNDERLIE IN THE SIGNAL TRANSDUCTION ACROSS MEMBRANE

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Signal transduction by receptor tyrosine kinases (RTKs) has been in the spotlight of scientific interest owing to the central role of these single-spanning membrane receptors in the regulation of development, cell motility, proliferation, differentiation, and apoptosis. During signal transduction across plasma membrane, RTKs are activated by proper ligand-induced homo- and heterodimerization or by reorientation of monomers in preformed receptor dimers upon ligand binding. Nowadays, the elucidation of high-resolution structure of full-size RTK having flexible multiple-domain composition is still a challenge.

The human epidermal growth factor receptor (EGFR) family, also known as HER or ErbB, serves as excellent model RTK to illustrate how ligand-induced conformational rearrangements and specific dimerization of extracellular domains lead to the allosteric activation of the cytoplasmic kinase domains, resulting in signal propagation across the membrane. Besides, HER/ErbB relatives are known oncogenic drivers in many cancers, and inhibitors of these receptors have been among the most successful examples of targeted cancer therapies to date. Many essential aspects of the HER/ErbB signal-transduction mechanism at the molecular level have been elucidated lately. Nevertheless, there are several issues yet to be resolved, including the particular role of the single-span helical transmembrane domain and flexible juxtamembrane regions in the receptor activity switching in terms of an apparent loose coupling between structural rearrangements of the extracellular and intracellular RTK regions.

Previously, the number of homo- and heterodimeric conformations of the HER/ErbB transmembrane domains assembled via polar N-terminal double GG4-like motifs in a fashion permitting proper kinase activation was experimentally determined with the aid of solution heteronuclear NMR spectroscopy in lipid bicelles. In the present work we used another micellar membrane mimicking environment to study an alternative self-association of the EGFR transmembrane domain via the weakly polar C-terminal $A^{661}xxxG^{665}$ motif, which is considered to allow inhibition of the receptor kinase activity. Fine adaptation of intermolecular polar and hydrophobic contacts that we found to accompany the dimer formation suggests that certain membrane properties can govern the transmembrane domain helix packing and, thus, their alteration can trigger the receptor state. There is a straightforward correlation between the hydrophobicity of the dimerization interface and the polarity of the lipidic environment, in which an appropriate dimerization mode occurs. This implies that signal transduction through membrane can be mediated by coupled protein-protein and protein-lipid interactions, elucidating paradoxically loose linkage between ligand binding and kinase activation and providing novel insights into RTK signal transduction across membrane.

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P 022

BACTERIAL AND CELL-FREE PRODUCTION OF TRANSMEMBRANE FRAGMENTS OF HER/ERBB RECEPTOR TYROSINE KINASES FOR STRUCTURAL STUDIES OF SIGNAL TRUNSDUCTION MECHANISM

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The epidermal growth factor receptor family, also know as HER or ErbB, of receptor tyrosine kinases mediates a variety of cellular responses in normal biological processes and in pathological states of multicellular organisms. During signal transduction across plasma membrane, four human HER/ErbB members are activated by proper ligand-induced homo- and heterodimerization or by reorientation of monomers in preformed receptor dimers upon ligand binding. Establishing structure-function relationship as well as rational drug design requires precise structural-dynamic information about this class of biologically significant bitopic membrane proteins. Protein-protein and protein-lipid interactions of single-span transmembrane domains and juxtamembrane regions of HER/ErbB receptors are believed to be important for the receptor functioning and signal transduction across membrane. A combination of heteronuclear NMR, optical spectroscopy, protein engineering and molecular modelling made it possible studies of these intra- and intermolecular interactions inside the supramolecular complexes mimicking membrane environment.

We designed high-performance systems of bacterial and cell-free expression and purification for biochemical and biophysical studies of the HER/ErbB TM fragments, containing transmembrane and juxtamembrane regions of different length, as well as the fragments with pathogenic mutations. The systems allow obtaining milligram quantities of the HER/ErbB TM fragments with isotope labeling more than 95%. The $^{13}C/^{15}N$-isotope labeled samples were produced for heteronuclear NMR studies, which subsequently make it possible to characterize in detail spatial structure, dynamics, and kinetic parameters of specific dimerization of the HER/ErbB TM fragments in

different membrane mimetics. Over current stage NMR structural-dynamic data were obtained for several monomeric and dimeric HER/ErbB TM fragments. The fragments were solubilized in detergent micelles and lipid bicelles, 40-60 kDa supramolecular membrane-mimicking complexes, which allows acquiring proper NMR spectra despite low sample stability and aggregation. The already available information about structural-dynamic properties of the transmembrane domains as well as extracellular and cytoplasmic juxtamembrane regions of HER/ErbB receptor tyrosine kinases along with the available biophysical and biochemical data provides useful insights into their functioning in normal and pathological states of human organism.

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P 025

NMR STUDIES ON A MEMBRANE-EMBEDDED DOMAIN OF THE LYSOSOMAL PEPTIDE TRANSPORTER TAPL

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ABC-transporters comprise a huge family of primary active membrane transport proteins, which couple ATB binding and hydrolysis with conformational changes to transport a broad spectrum of substances. They share a common fold of two nucleotide binding domains (NBDs) and two transmembrane domains (TMDs), but also accessory domains are often found. These accessory domains include extra cytoplasmic, cytosolic regulatory, as well as catalytic or membrane-embedded domains. However, the structure of an additional membrane-embedded domain of any ABC-transporter has not been determined so far. Therefore, we are interested in solving the structure of the membrane-embedded TMD0 of the homodimeric lysosomal polypeptide transporter TAPL (transporter associated with antigen processing like, ABCB9). The 17 kDa TMD0 is dispensable for peptide transport and dimerization of the half transporter, but includes the lysosomal targeting signal and mediates the interaction with the lysosomal associated membrane proteins LAMP-1 and LAMP-2, which highly increase the half-life of the transporter.

To elucidate the structure of TMD0 we use cell-free expression and solution NMR. An AT-rich expression tag strongly increased the quantities of cell-free expressed TMD0. Furthermore, conditions were optimized for solution NMR studies in respect of high solubilization efficiency, long-term stability and high quality spectra for TMD0 in 6-DHPC micelles. Correct folding of cell-free expressed TMD0 was proved by interaction with coreTAPL lacking TMD0. With the help of

combinatorial labeling schemes we succeeded in the full backbone and partial side chain chemical shift assignment, which led to a secondary structure model. To obtain spatial distance restraints for 3D structure calculations we established spin probe labeling procedure and collected paramagnetic relaxation enhancement (PRE) restraints. Also nuclear Overhauser effect (NOE) experiments as well as distances within TMD0 by EPR were performed.

P 028

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF NADH BINDING TO THE HUMAN VOLTAGE-DEPENDENT ANION CHANNEL (VDAC)

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The voltage-dependent anion channel (VDAC) is the most abundant protein in the eukaryotic outer mitochondrial membrane. VDAC forms the primary path for diffusion of adenosine triphosphate (ATP), adenosine diphosphate (ADP), other metabolites and ions between the mitochondrial intermembrane space and the cytosol. VDAC-1 is a 31 kDa b-barrel consisting of 19 b-strands (1–3). The N-terminal a-helix that extends into the pore has been implicated for voltage gating, allowing VDAC-1 to switch between a high- and a low-conductance state. In the low-conductance state that can be triggered by NADH, nucleotide flux through the VDAC pore is drastically reduced (4). Here, we describe our experimental progress towards a structural characterization of the VDAC–NADH interaction at the atomic level by solution NMR spectroscopy. NMR titration experiments of VDAC in micelles and bicelles with NADH show that the nicotinamide moiety is the minimal binding unit. Subsequent ^{13}C- and ^{15}N-resolved 3D NOESY experiments recorded on ILV-labeled VDAC reveal specific intermolecular NOEs, enabling the structure determination of the VDAC-NADH complex. The data provide a molecular model for the regulation of cellular metabolism by VDAC and insights into its voltage-gating mechanism.

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P 031

NMR SOLUTION STRUCTURE OF A DNA QUADRUPLEX CONTAINING ALS AND FTD RELATED GGGGCC REPEAT

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Guanine rich nucleic acids can form non-canonical four-stranded structures called G-quadruplexes stabilized by monovalent cations such as K^+ or Na^+. G-quadruplexes are composed of stacked layers of G-quartets, formed by coplanar arrangement of four guanines connected by Hoogsteen-hydrogen bonds. Specific G-quadruplex structure adopted by an oligonucleotide can be influenced by small changes in its sequence and changing solution composition. G-rich sequences are present in critical regions of the human genome including telomere ends, oncogene promoters and 5'-UTR regions. Furthermore, formation of G-quadruplexes has been shown to be implicated in mechanisms of certain diseases. Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are related neurodegenerative diseases that share a common genetic and pathological background. Expansion of GGGGCC repeat tract within the first intron of C9orf72 was identified as the most frequent genetic cause of ALS and FTD. A disease mechanism has been proposed in which G-quadruplex formation within expanded GGGGCC repeat triggers pathological pathways leading to ALS and FTD.

In order to probe G-quadruplex formation in the GGGGCC repeat, we initiated an NMR study of several different DNA oligonucleotides containing four GGGGCC repeats representing the simplest model for unimolecular G-quadruplex. Screening with ^1H NMR revealed that oligonucleotides fold into multiple G-quadruplex structures in the presence of K^+ ions. Oligonucleotide $d[(G_4C_2)_3G_4]$ showed the presence of two predominant G-quadruplex species with antiparallel orientation of strands and syn conformation of some guanines residues. Since 8-bromodeoxyguanosine residue preferentially adopts

a syn glycosidic conformation, substituting guanine with its 8-bromo analogue at a desired position in a sequence can lead to stabilization of anticipated structure. Oligonucleotide d[$(G_4C_2)_3GG^{Br}GG$] with single dG to 8Br-dG substitution at position 21 exhibited favorable spectral characteristics with only two sets of sharp signals in ^1H NMR spectrum. In order to favor a single structure we tested folding of oligonucleotide with different solution compositions. We observed that the ratio between the two structures was very sensitive to pH of the solution. This characteristic was employed to obtain a sample with 70% major and 30% minor species, suitable for structure determination. We determined the structure of the major G-quadruplex species adopted by oligonucleotide d[$(G_4C_2)_3GG^{Br}GG$] under physiologically relevant pH 7.2 and 120 mM KCl. G-quadruplex structure adopted by d[$(G_4C_2)_3GG^{Br}GG$] is unimolecular and composed of four G-quartets. Every strand is antiparallel with respect to adjacent strands with syn-anti progression of glycosidic conformation of guanine residues along the strands. G-quartets are connected by three edgewise loops, made of two cytosine residues each. Two edgewise loops span a narrow groove and one loop spans a wide groove. One of the cytosine residues in every loop is stacked upon the neighboring G-quartet contributing to a very compact and stable structure.

P 034
STRUCTURE OF MICROTUBULE-BOUND(296-321)

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The microtubule-associated protein Tau promotes formation and stabilization of axonal microtubules and thus influences axonal stability and cell morphology. Human Tau protein has six different isoforms, which are expressed in neurons of the central nervous system. The six tau isoforms differ from each other in the number of microtubule binding repeats and in the presence or absence of one or two N-terminal inserts. In fetal neurons only the smallest isoform is expressed and all six isoforms are expressed in adult human brain. Binding of Tau to microtubules is mediated by the C-terminal microtubule binding domain. The hyperphosphorylation of tau leads to the formation of neurofibrillary tangles which is one of the hallmarks in neurodegenerative diseases.

Microtubules are regulated by Tau, which belongs to the class of intrinsically disordered proteins (1). In solution, it does not fold into a well-defined structure but populates a dynamic ensemble of conformations (2). However, little is known about the three-dimensional structure of Tau bound to microtubules, because of the high-molecular weight of microtubules and the dynamic nature of the interaction. Using NMR spectroscopy we here provide insight into the characterization of the interaction of Tau isoforms and conformation of the repeat domain of Tau in complex with microtubules. The microtubule-induced NMR broadening profiles obtained for the smallest and largest isoforms of tau, htau23 and htau40, suggested that distinct short regions of Tau are important for binding to

microtubules (3). To calculate the structure of microtubule-bound Tau(296-321), we recorded two-dimensional Nuclear Overhauser Effect (NOE) spectra in the absence and presence of microtubules to observe transferred NOEs. Tau(296-321) is particularly interesting as it contains the hexapeptide at the beginning repeat three, which is converted into β-structure upon aggregation of Tau into amyloid fibrils. On the basis of the experimentally-derived contacts, structure calculation for Tau(296-321) was performed and the calculated structures converged to a hairpin-like conformation (4). Thus we show that the microtubule-associated protein Tau, which is intrinsically disordered in solution, adopts a stable conformation upon binding to microtubules.

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P 037

FASTER PROBES AND HIGHER PROTON CONTENTS: WHEN RESOLUTION MEETS SENSITIVITY IN BIOMOLECULAR MAS NMR

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Proton dilution by perdeuteration is one of the most efficient ways to achieve narrow ^{1}H linewidths in biomolecular solid-state NMR. This is typically achieved by expressing a protein in a deuterated medium, and then reprotonating the amide groups by exchange in water buffers with controlled H_2O/D_2O ratios during the purification stage. This approach however impacts the sensitivity, prevents the observation of ^{1}H in the side-chains, and is not viable for samples that do not unfold reversibly. Fast MAS at high magnetic field is an additional key tool to narrow ^{1}H linewidths, allowing to record spectra with resolved ^{1}H lines from samples with progressively higher ^{1}H contents. In this work, we report the ^{1}H linewidths on a set of model microcrystalline protein samples spinning at rates from 20 kHz to 111 kHz. By investigating proteins with variable protonation levels, we show that fast MAS rates on small rotors conjugates the availability of resolved ^{1}H-^{15}N (backbone) and ^{1}H-^{13}C (side-chains) correlations with high sensitivity.

P 040

STRUCTURAL BASIS FOR THE CONSERVED BINDING MECHANISM OF MDM2-INHIBITING PEPTIDES AND ANTI-APOPTOTIC BCL-2 FAMILY PROTEINS

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The interaction between tumor suppressor p53 and the anti-apoptotic Bcl-2 family proteins serves a critical role in the transcription-independent apoptosis mechanism of p53. Our previous studies showed that an MDM2-inhibiting motif (residues 15–29) in the p53 transactivation domain (p53TAD) mediates the interaction with anti-apoptotic Bcl-2 family proteins. In this study, we provided structural models of the complexes between the MDM2-inhibiting p53TAD peptide and Mcl-1, Bcl-w, and Kaposi sarcoma-associated herpes virus (KSHV) Bcl-2 using NMR chemical shift perturbation data. The binding mode of the MDM2-inhibiting p53TAD peptide is highly conserved among the anti-apoptotic Bcl-2 family proteins despite their distinct specificities for pro-apoptotic Bcl-2 family proteins. We also identified the binding of a phage-display-derived MDM2-inhibiting peptide 12-1 to anti-apoptotic Bcl-X_L protein by using NMR spectroscopy. The structural model of the Bcl-X_L/12-1 peptide complex revealed that the conserved residues Phe4, Trp8, and Leu11 in the MDM2-inhibiting peptide fit into a hydrophobic cleft of Bcl-X_L in a manner similar to that of pro-apoptotic Bcl-2 homology 3 (BH3) peptides. Our results shed light on the mechanism underlying dual-targeting of the FxxxWxxL-based a-helical motif to MDM2 and anti-apoptotic Bcl-2 family proteins for anticancer therapy.

P 043

GLOBAL FOLD OF THE TRANSMEMBRANE DOMAINS OF HEPATITIS C VIRUS GLYCOPROTEINS E1 AND E2 IN LPPG MICELLES

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E1 and E2 are two hepatitis C viral envelope glycoproteins that assemble into a heterodimer essential for membrane fusion and penetration into the target cell (1, 2). Both extracellular and transmembrane (TM) glycoprotein domains contribute to this interaction, but study of TM-TM interactions has been limited because synthesis and structural characterization of these highly hydrophobic segments present significant challenges. In this NMR study, as a first step towards our long-term goal of understanding the interaction between E1 and E2 in the membrane environment we have successfully expressed and purified the E1- and E2-TM domains as maltose binding protein fusion constructs and determined their global folds when incorporated in lysophospholipid micelles.

Backbone resonance frequencies, relaxation rates and solvent exposure measurements concur that both E1-TM and E2-TM adopt a helical conformation, with two helical segments spanning E1-TM (E2-TM) residues 354-365 (717-726) and 371-379 (732-746) connected by an unstructured linker. The unstructured linkers include charged residues K370, D728 and R730 involved in heterodimer formation. The K370A mutation did not affect the secondary structure of E1-TM but did shift the linker within the micelle (3). Both peptides exhibited similar ^{15}N relaxation behavior, corresponding to a global tumbling time in the 12-13 ns range, consistent with the expected size of the peptide/LPPG mixed micelles. The N-terminal helix of E1-TM exhibits increased motions on the ps timescale which may be attributed to the presence of the GxxxG motif. The positioning of the helix-linker-helix architecture within the mixed E2-TM/LPPG micelle was established by paramagnetic NMR spectroscopy and phospholipid-peptide cross relaxation measurements. These indicate that while the helices traverse the hydrophobic interior of the micelle, the linker lies closer to the micelle perimeter to accommodate its charged residues which

'snorkel' out towards the less hydrophobic phosphoglycerol headgroups (4). These results lay the groundwork for structure determination of the E1/E2 complex and a molecular understanding of glycoprotein heterodimerization.

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P 046

STRUCTURE AND DYNAMICS OF THETA-DEFENSINS; ANTIMICROBIAL CYCLIC PEPTIDES FROM PRIMATES

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The only known backbone cyclic peptides in mammals are theta-defensins (θ-defensins), which are part of the innate immune systems of some primates and comprise 18 residues cross-braced by three disulfide bonds.[1] The pharmaceutical industry has shown interest in θ-defensins because of their antibacterial, antiviral, and immunomodulatory activities and their stability to thermal and enzymatic degradation. In contrast to their antimicrobial properties, which have been widely studied, relatively little is known about the three-dimensional structures and dynamics of θ-defensins. Herein we describe how nuclear magnetic resonance (NMR) spectroscopy techniques have been used to characterise θ-defensins in terms of their structure and dynamics. High-resolution structure determination from homonuclear two-dimensional NMR spectroscopy of native and chemically modified θ-defensins showed that they have a well-defined β-sheet structure constrained by three disulfide bonds: a structural feature termed the 'cyclic cystine ladder'.[2] The three disulfide bonds were found to be important for the structure and stability of θ-defensins; however, they are not essential for antibacterial activity.[3] NMR relaxation experiments gave insights into the overall and internal motion of θ-defensins in aqueous solution, showing that some internal motion might be present.[4] In addition to contributing to our understanding of stable naturally occurring cyclic peptides, the structural characterization of θ-defensins will help to guide the design of peptide drugs based on the θ-defensin scaffold. Moreover, these studies illustrate the value of NMR spectroscopy in providing insights into peptide structure and function.

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P 049

INTERACTION OF A HISTONE CHAPERONE INVOLVED IN DNA REPAIR WITH CORE HISTONES

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Histones are amongst the proteins with the longest and biggest history in science. Their important function is the fundamental process of DNA condensation to form chromatin in the living cell. In this process, two copies of each of the four main histones H2A, H2B, H3, and H4 form the protein core complex. Each histone core is wrapped by 147 base pairs of DNA to form the nucleosome as the basic unit of chromatin. Up to several million nucleosomes line up in a beads-on-a-string like fashion. Cellular growth and differentiation, gene expression, and DNA damage require eviction of the nucleosomes for the replication, transcription, and DNA repair machineries to access the DNA. This requires the sequential removal of the histones from the DNA which is catalysed by histone chaperones. Histone chaperone APLF has been shown to be involved in DNA repair. We have determined its mode and affinity of binding and mapped its interaction interface with histone complexes using biophysical methods like microscale thermophoresis, isothermal titration calorimetry, and high resolution NMR spectroscopy.

P 052

SOLUTION STRUCTURE OF PRIC, A SCAFFOLD PROTEIN FOR DNA, SSB, AND DNAB/DNAC BINDING DURING REPLICATION RESTART

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Our understanding of the mechanisms that drive DNA replication, recombination, and repair (the classically defined genome maintenance pathways of all cells) is highly advanced. In contrast, the mechanisms that drive the fourth core genome biology pathway, **DNA replication restart** (only recently recognized) remain poorly understood. DNA replication restart is an essential process in bacteria that reloads DNA replication complexes (replisomes) ejected prematurely from replication forks due to encounters with impassable DNA damage or protein complexes. In E. coli and related bacterial species, the **Replication Restart Proteins (RRPs)** (PriA, PriB, PriC, DnaT, and Rep) catalyze this activity. The 20-kDa PriC protein is unique among them in that it can mediate in vitro replisome reloading in the absence of other replication restart proteins. PriC forms a direct complex with the single-stranded DNA binding protein (SSB) and this interaction is involved fork recognition and remodeling. Fork recognition is also predicted to rely on an interaction with ssDNA, which PriC binds cooperatively and with high affinity.

To better understand the mechanisms of DNA replication restart, we have solved the full-length solution structure of PriC from Cronobacter sakazakii and have identified and characterized an interaction between PriC and DnaB. PriC is a single domain consisting of a bundle of five alpha helices with an extended loop connecting alpha helices 1 and 2. Additionally, we found that PriC binds to DnaB with a strong preference for DnaB within the DnaB/DnaC complex over DnaB alone. A tryptophan residue within the second alpha helix of PriC may play a role in stabilizing this interaction, which suggests that the binding site for DnaB may not overlap with the binding sites for SSB or DNA. These data support a model in which PriC acts both as a

mediator of SSB/DNA binding and as a scaffold for bringing together all of the elements required for successful DnaB loading.

P 055
NMR STUDY OF GP36-MPER–C8 PEPTIDE INTERACTION

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The feline immunodeficiency virus (FIV) is a lentivirus that resembles the human immunodeficiency virus (HIV). It is studied as a model system for anti-HIV vaccines and anti-HIV drugs development.[1] FIV and HIV have a common mechanism of virus cell fusion mediated by Gp36 and GP41 glycoproteins respectively.

The gp36 ectodomain contains several characteristic functional domains, including the fusion peptide (FP), N-terminal heptad repeat (NHR), C-terminal heptad repeat (CHR) and membrane proximal extracellular region (MPER).

During the virus entry, NHR and CHR, automatically, fold back to form a low energy stable six-helical bundle (6HB) with NHR trimer as the inner core and anti-parallel binding of three CHRs.

an octapeptide derived from gp36 MPER (^{770}WEDWVGWI^{777}), dubbed C8, We previously demonstrated that elicited antiviral activity as result of blocking cell entry.

In the hypothesis that antiviral activity of C8 occurs via the interaction with its counterpart in Gp36, preventing 6HB formation and terminating the FIV cell fusion process, we studied the interaction of C8 with GP36 MPER using chemical shift mapping. Accordingly we solved Gp36 MPER structure on the basis of heteronuclear 3D NMR experiments on double labeled sample GP36-MPER sample. C8-Gp36MPER interaction was analyzed using the chemical shift mapping approach. The comparison of the ^{15}N-NHQC Gp36-MPER spectra recorded after the addition of increasing amounts of C8 peptide, shows changes in several HN backbone chemical shifts, providing evidence that antiviral activity of C8 peptide resides into the structural interaction with gp36 MPER region to modify the properties of virus cell entry.

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P 058
NMR STUDY OF NEW LIGANDS OF FARNESYL PIROPHOSPHATE SYNTHASE

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Farnesyl Pirophosphate Synthase (FPPS) is a key enzyme in the mevalonate, isoprenoid biosynthesis pathway. FPPS is nowadays the target of bisphosphonate drugs used in osteoporosis disease, nonetheless it is studied as target for anti-cancer therapeutics.[1] N6-Isopentenyladenosine (i6A) is a modified nucleoside exhibiting anti-tumor effects on human and murine cells.[2] Growing biochemical evidence demonstrate the involvement of FPPS protein in i6A anti-tumor action.[3]

Here we report an NMR based investigation of i6A-FPPS interaction using saturation transfer difference (STD) and WaterLOGSY NMR experiments. i6A proves to occupy FPPS enzymatic pocket with a calculated K_D of ~1mM. Molecular docking calculations based on NMR data allow for the determination of a binding pose model coherent with a prevalent role of isopentenyl moiety in the interaction with FPPS binding site.

Newly synthesized analogs of i6A, designed on the basis of i6A-FPPS interaction data, and screened with STD NMR experiments confirm the binding mode of i6A, underlining the importance of N6-adenosine substituent in the interaction with FPPS binding site. Introduction in this position of a benzyl moiety results in a significant improvement of the interaction with FPPS target, opening the perspective that appropriate modifications of the benzyl portion on adenosine scaffold, may lead to new interesting FPPS inhibitors.

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P 061

INSIGHTS INTO THE ROLE OF CYSTEINES FOR PROTEIN STRUCTURE AND FUNCTION FROM MD SIMULATIONS, NMR SPECTROSCOPY, AND OTHER BIOPHYSICAL METHODS

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Cysteines cannot only stabilize protein folds by forming disulfide bonds or coordinating metal ions, they play also an important role for the regulation of protein function by enabling redox-sensitive conformational changes. Here, we present three examples of the analysis of the role of cysteines for protein structure and function:

1) MD simulations of two about 25 residue long cysteines rich domains (CRDs) form hydra proteins that share the same cysteine sequence distribution, however adopt different disulfide bond patterns and folds. The MD data nicely complements earlier published NMR residual dipolar coupling (RDC) data. Additional MD simulations may provide information about the redox potentials of the cysteines and the reshuffling of intra- to intermolecular disulfide bonds upon formation of the extremely stable capsule wall.

2) The analysis of the redox-potential and the structure and dynamics of the free oxidized as well as the oxidized and reduced membrane associated states of the FATC domain of the central cell growth regulator 'target of rapamycin' (TOR) that has two conserved cysteines by NMR, fluorescence, and MD simulations.

3) The NMR analysis of the redox-sensitive rubredoxin domain (RD) that is N-terminal of the catalytic domain of the mycobacterial kinase G (PknG). Unfolding upon oxidation and metal release may facilitate substrate access to the kinase active site. Also here, specific MD simulations may well complement the NMR data. The effects of redox changes or deletions on the catalytic activity are derived from kinase assays.

P 064
STRUCTURAL ANALYSIS OF PYROGLUTAMATE AMYLOID-β(3-42) BY SOLUTION STATE NMR SPECTROSCOPY

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive decline of cognitive functions and has become the main cause for dementia in the elderly. A hallmark of AD is the accumulation of extracellular amyloid-β (Aβ) plaques in the brains of patients. N-terminally truncated pyroglutamate-modified Aβ (pEAβ) has been described as a major compound of Aβ species in AD brains. Moreover, pEAβ(3-42) bearing pyroglutamate at its N-terminal position has been described to be a dominant isoform in senile plaques [1]. Compared to non-truncated Aβ species, pEAβ(3-42) is more resistant to degradation, shows higher toxicity and has increased aggregation propensity and β-sheet stabilization. However, there is only little insight about the structural difference [1]. Circular dichroism (CD) spectroscopy data suggest that pEAβ(3-42) shows an increased tendency to form β-sheet-rich structures in solution conditions, where Aβ(1-42) forms α-helices. Based on a previous study [2], we established a protocol for reproducible expression and purification of 15N- and 13C-enriched pEAβ(3-42) for structural analysis by solution state NMR spectroscopy. Three-dimensional triple resonance experiments for backbone and partial side chain assignments were performed. Secondary structure prediction by NMR chemical shift data indicates that soluble pEAβ(3-42) contains two helical regions connected by a linker similar to Aβ(1-42) under these conditions. This is in accordance with previous results comparing pEAβ(3-40) with Aβ(1-40) [3]. However, some peak intensities of pEAβ(3-42) are drastically decreased compared to its non-modified isoform indicating the presence of exchange processes. Density gradient centrifugation and transmission electron microscopy confirm

the data on pEAβ(3-42) in solvent conditions used for the liquid state NMR experiments by showing increased oligomerization, large fibrils and high molecular weight aggregates.

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P 067
UNDERSTANDING CYTOCHROME C TRANSIENT COMPLEXES WITHIN THE ELECTRON TRANSPORT CHAIN

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Electron transport chains demand a high turnover of the soluble electron carriers at the functional sites of the membrane complexes to attain an efficient functionality. Thus, lowering the lifetime of the complexes allows fast replacement of the mobile protein. Additionally, complexes anchored to the membrane may provide secondary binding places to aid the release of the soluble partner from the active site. Further, gathering the membrane complexes into supercomplexes may yield a diffusional restraint or "channeling" that avoids random diffusion across the bulk phase to increase the electron transport throughput.

In mitochondria, cytochrome c (Cc) plays an essential role as an electron carrier between complexes III and IV. Kinetic analyses of the intermolecular electron transfer indicate the presence of secondary binding sites in these complexes. Hence, we have performed titration experiments to characterize the interaction of Cc with the soluble domain of cytochrome c_1 (Cc_1) from complex III, and compared the physiological interaction with cross-complexes using partners from distinct organisms. NMR Chemical Shift Perturbation data from Cc obtained at a low ionic strength clearly indicates the presence of two Cc-binding sites on the surface of Cc_1. Both, NMR and Isothermal Titration Calorimetry analyses revealed that the two sites in Cc_1 differ markedly in their affinities towards the soluble carrier, despite both laying within the micromolar range. Consistently, Ambiguous Restraint

Driven Docking and ab initio Brownian Dynamics computations clearly showed two binding conformations. Both are compatible with the full complex III structure. The first one agrees with the X-Ray structure and is capable for fast electron transfer. In the second one, however, Cc binds too far from the heme moiety of Cc_1 to accept electrons from it. However, it could represent a channel to drive cytochrome c towards the nearby complex IV within the mitochondrial respirasome.

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P 070

MODIFICATION OF THE AGLYCONE JOSAMYCIN WITH USING THE REGIOSELECTIVE NUCLEOPHILIC SUBSTITUTION SN2' TYPE AND DIPOLAR HUISGEN CYCLOADDITION

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Josamycin (**1**) is a macrolide which belongs to the leucomycin group of antibiotics.[1] This antibiotic have 16-membered aglycone ring substituted with a 4-O-isovalerylmycarosylmycaminose sugar moiety. Josamycin is produced by Streptomyces narbonensis and was discovered by Umezawa et al. in 1967.[2] This macrolide has a broad spectrum of antimicrobial activity against Gram-positive and erythromycin-resistant bacteria.[3]

The first stage of the work was to prepare the aglycone Josamycin to introduce of the expected alkyne substituent. New derivatives have been synthesised by elimination of the acetate group with the formation of α,β-unsaturated Josamycin of (2E) configuration (**2**). Nucleophilic substitution of S_N2' type performed at diene system of the aglycone ring yielded new derivative (**3**) as exclusive product being a result of regioselective and stereospecific reaction. Absolute configuration of newly formed stereogenic center as (13S) and the site of the substitution were evidenced by ^1H-^1H NOESY contacts and ^1H-^{13}C HMBC long-range couplings, respectively. 1,3-Dipolar Huisgen cycloaddition of **3** with different azides enable to obtain new triazole leucomycin derivatives of type (**4**) at the aglycone.[4]

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P 073
PROTEIN DIFFUSION FOR FOLDED AND DISORDERED SYSTEMS

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Diffusion ordered spectroscopy (DOSY) is finding increasing use for the analysis of mixtures and for the characterization of protein structure. Not only in pharmaceutical analysis, but also in the structural characterization of proteins, the measurement of the molecular dimension is of great importance. The diffusion coefficient (D) of a certain molecule under given conditions is closely related to its size. Diffusion coefficients can be measured by PFG-NMR methods by various pulse sequences. The results of the PFG-NMR experiments allow estimation of the molecular weight (M), also the monitoring of aggregation.

In our study – based on our previous research – we investigated the diffusion properties of two groups: folded and intrinsically disordered proteins. We performed a careful validation of the method, including gradient and temperature calibration, and basic diffusion coefficient values checked for water, glucose, and lactose. The measured biological systems had sequence diversity and varying lengths (between 15-525-residues). All studied systems behaved as monomers under the experimental conditions. The obtained diffusion coefficients were subject to hydrodynamic radii calculation based on the Stokes-Einstein equation:

$D_t = k_B T/f_t$, where $f_t = 6\pi\eta r_H$

We tested both approaches present in the literature for calculating the r_H value (with the assumption of spherical particles): (a) the relative method using dioxane as reference, and (b) the absolute method, that needs exact viscosity data, which we determined for various conditions. For several of our proteins we have independent data obtained by SAXS or SEC techniques, which we use for comparison, or possible shape determination.

Based on the measured data pool there is a clear difference between the behavior of folded and disordered proteins. For both cases we were able to provide equations describing the correlation between D-M, r_H-M and r_H-N.

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P 076

STUDYING HIGHLY FLEXIBLE INTRINSICALLY DISORDERED PROTEINS NEAR PHYSIOLOGICAL CONDITIONS: THE CONTRIBUTION OF 13C-DETECTED EXCLUSIVELY HETERONUCLEAR NMR EXPERIMENTS

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Intrinsically disordered proteins (IDPs), characterized by highly flexible solvent exposed backbones and by many conformations accessible at physiological pH and temperature, carry out a variety of functions, highly complementary to those carried out by folded proteins.

It is thus important to expand our view of how protein structural and dynamic features affect function, beyond the static picture of a single well defined 3D structure that has influenced so much our way of thinking.

NMR spectroscopy provides a unique tool for the atomic resolution characterization of IDPs. The contribution of exclusively heteronuclear NMR experiments based on ^{13}C direct detection to study highly flexible IDPs near physiological conditions (pH, temperature, in-cell) is discussed here[1-4].

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P 079
STRUCTURAL CHARACTERIZATION OF IRREGULAR TELOMERIC DNA FROM S. CEREVISAE

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Telomeres protect the ends of the linear eukaryotic chromosomes from end-to-end fusions and serve as buffer zones against sequence loss due to incomplete replication. They are maintained by the ribonucleoprotein enzyme telomerase, a cellular reverse transcriptase that uses a specific region of its RNA subunit as template for DNA synthesis. The template region of the RNA is copied repeatedly onto the 3' ends of the chromosomes, thus specifying the telomere repeats. The ability to maintain telomeres is a prerequisite to undergo unlimited rounds of replication, and reactivation of telomerase is seen in more than 80% of human tumors.

Although Saccharomyces cerevisiae contains only one telomerase RNA gene, telomere repeat sequences are degenerate/irregular in this organism (Cohn et al. 1998). Nonetheless, similarly to the telomeric DNA based on regular telomeric repeats, the telomeric DNA from S. cerevisae has the ability to form tetraplex-based structures (Školáková et al. 2015). In other eukaryotes, the tetraplex-based structures that are forming from telomeric DNA defined by regular repeats correspond to canonical G-quadruplex (in the G-rich strand) and DNA i-motif (in the C-rich strand). However, both the sequence composition as well as its irregularity preclude formation of canonical G-quadruplex and DNA i-motif in telomeric DNA from S. cerevisae.

Here we use 1D and 2D proton and heteronuclear NMR spectroscopy to characterize evolutionarily conserved tetraplex-based motifs within telomeric DNA of S. cerevisae. We aim at addressing the role of tetraplex structures formed in 3' G-rich telomeric overhang in diversification of the telomeric repeats in budding yeast.

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P 082
DISTANCE MEASUREMENTS IN A PTB/IRES COMPLEX BY PULSE EPR

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The Polypyrimidine Tract Binding Protein 1 (PTBP1), consisting of four RNA Recognition Motif (RRM) domains, plays an important role in alternative splicing and initiation of 5'-cap independent translation. This 57 kDa RNA-binding protein forms a complex with different stemloops of the Internal Ribosomal Entry Site (IRES) of Encephalomyocarditis Virus (EMCV) [1,2]. In this project we intend to use Electron Paramagnetic Resonance (EPR) in combination with Nuclear Magenetic Resonance (NMR) to determine a 3D model of PTBP1 in an unbound state as well as in a complex with the IRES, by utilizing the approach reported recently [3]. Here we report the design of labeling positions, preparation of mutants for Site-Directed Spin Labeling (SDSL), and comparison of Gd(III)-based and nitroxide-based spin labels, with respect to their performance in such a structural study. Based on the determined 3D structures of the isolated single RRM domains [4], we chose different positions in the alpha-helices for introducing pairwise cysteine mutations for SDSL in full-length PTBP1 as well as in its individual RRMs. Mutants were expressed in Escherichia coli and purified by affinity, cation exchange and size exclusion chromatography. Inter- and intra-domain mutated proteins were labeled with nitroxide radicals and Gd(III)-complexes. We used Continuous-Wave EPR (CW-EPR) at X-band (9.5 GHz) to analyze the labeling efficiency of the attached nitroxide radicals. First inter- and intra-domain distance distributions were investigated by Double Electron-Electron Resoncance (DEER) at Q-band (35 GHz) which gave information about differences between the labeled single domains and the full protein as well as the influence of the binding of native EMCV-IRES RNA. We could show that the simulated distance

distributions for nitroxide radicals and Gd-complexes of the chosen labeling positions within the single domains agree with the DEER measurements. Interestingly, we observed that binding of the native EMCV-IRES does not significantly influence the site-to-site distance between RRM1 and RRM2 of PTBP1.

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P 085

THE STRUCTURE OF AN INTACT BACTERIOPHAGE VIRUS CAPSID FROM MAGIC-ANGLE SPINNING SOLID-STATE NMR AND ROSETTA MODELING

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The M13 bacteriophage is a filamentous virus that infects bacteria. It consists of thousands of identical coat protein subunits that wrap a circular single-stranded DNA genome. Using distance restraints from magic-angle spinning NMR and Rosetta model building we obtained a structural model for the capsid of the intact phage (pdb 2MJZ)[1]. The capsid is composed from pentamers, each representing five identical coat protein subunits, and our data provides the structure of each subunit as well as the distance and angular relation between the pentamers. The model suggests that the coat protein is mostly helical and that the capsid is stabilized by a repeating hydrophobic/aromatic binding epitope that spirals along the entire structure. The overall orientation of the subunits could be validated independently by observing their interactions with the DNA[2] and the orientation of most aromatic residues are in agreement with prior Raman studies. Our structure is closely related, but different, from that of the Y21M mutant of fd phage (M13-N12D-Y21M) obtained from a mutual refinement of fiber diffraction and aligned solid state NMR studies (pdb 2C0X).

In order to probe the dynamics of the capsid, we developed a modified version of the coupling-amplified DIPSHIFT experiment (Hong et al. JMR 129, 85, 1997) and adapted it to pseudo-3D acquisition at moderate spinning speeds (10-30 kHz) suitable for protein studies[3]. The sequence was applied to a carbohydrate binding module protein and its reduced pseudo-2D version validates prior observations that the N-terminus of the M13 capsid subunit is dynamics.

The M13 capsid structure has been obtained with Omry Morag in collaboration with Nik Sgourakis (NIH) and David Baker (Seattle). The

dynamics studies were developed by Hadar Ivanir and Evgeny Nimerovsky in collaboration with PK Madhu (TIFR).

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P 088

EFFECTIVE BACTERIAL AND CELL-FREE PRODUCTION OF MEMBRANE PROTEIN TRANSMEMBRANE FRAGMENTS FOR NMR APPLICATIONS

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Due to hydrophobic nature, membrane proteins remains "terra incognita" of structural biology for long time. This prevents rational investigation of the proteins at molecular level and effective structure-based drug design. Fibroblast growth factor receptor 3 (FGFR3) plays an important role in human development and diseases. There are mutations in the transmembrane region of FGFR3 (tmFGFR3), including point pathogenic mutations G380R and A391E, associating with human pathology states. We propose effective cell-free and bacterial expression systems that allow us to produce the target peptides in sufficient amounts for NMR structural studies. Interesting that for wild-type tmFGFR3 and for tmFGFR3(A391E) best yields were obtained using bacterial expression system whilst tmFGFR3(G380R) was best expressed in cell-free system. Purification procedures were developed for all peptides and isotope-labeled derivatives. Purified proteins were reconstituted into membrane-mimicking environment and characterized using dynamic light scattering, CD and NMR spectroscopy. High-resolution NMR structure of tmFGFR3 dimer was obtained. Some pathogenic mutations fall within the helix-helix interface and the others are within a putative alternative interface. Based on the obtained structure and other known data we propose a mechanism of the FGFR3-mediated signal transduction across the cellular membrane.

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P 091

STRUCTURAL INVESTIGATIONS OF TRANSMEMBRANE DOMAINS OF TOLL-LIKE RECEPTORS IN THE DIMERIC AND TRIMERIC STATES

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Toll-like receptors (TLRs) are one of the key players in both the innate and adaptive immune systems. These proteins recognize conserved products unique to microbial metabolism or viral nucleic acids and induce activation of inflammatory and antimicrobial innate immune responses. TLRs are type I transmembrane proteins. Each of them consists of the extracellular domain (ECD) containing leucine-rich repeats that mediate the recognition of pathogen-associated molecular patterns (PAMPs); single transmembrane a-helix domain; and intracellular Toll–interleukin 1 receptor domain (TIR) required for downstream signal transduction. It was shown that TLRs form homo- or heterodimeric signaling complexes interacting with the single PAMP molecule. The medical and biological significance of TLR signaling is obvious, since the disregulation of the TLR system may cause various autoimmune diseases and septic shock, and some therapeutic strategies targeting TLRs have already emerged. There is a lot of data available on the structural and biological aspects of the TLR signaling. Structural analysis of several TLRs have elucidated the mechanisms of PAMP recognition by TLR. But mainly these data concern to the roles of the ECD and TIR domains. There is only one computer model of the dimeric full-length TLR3 receptor in the active state. But the role of the transmembrane domain (TMD) in TLR signaling is still elusive, while its significance for the TLR activation was demonstrated in recent studies. Isolated TMDs of all TLR receptors were shown to homodimerize in bacterial membranes, with TMDs of TLR2,3,8,9 having the highest propensity to perform homotypic interactions. Taking into account all aforesaid, it is obvious that the structural investigations of TLR TMD dimers or oligomers are necessary, because these domains can serve as targets for emerging therapies. In this study the efficient cell-free

expression system of TLR3 and TLR4 TMDs and purification protocols are described. The ability of these TMDs to form dimers and trimers in detergent micelles was shown by solution NMR spectroscopy. Spatial structures and free energy magnitudes were determined for both TMDs in dimeric and trimeric states. Possible surfaces of TMDs that may be used for the helix–helix interaction by the full-length receptors were presented.

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P 094
NMR INVESTIGATION INTO HEME REGULATORY MOTIFS

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Besides the well-known example as permanently (often covalently) bound cofactor, like e.g. in hemoglobins and cytochromes, heme is now increasingly recognised as an essential effector in biological processes. There is an increasing evidence for regulatory roles in transcription, signal transduction, transmembrane ion transport, cell cycle and translation. Here, heme is frequently only temporarily associated with peptides or proteins. These interactions are mediated by heme-binding motifs (HBM) or heme-regulatory motifs (HRM), the "Cys-Pro motif" being a prominent example. Nevertheless, no distinct classification and characterisation of heme-regulatory motifs is available. Solution NMR spectroscopy is an excellent tool in determining different heme-binding modes and investigation into conformational changes of peptides upon heme-binding.

A literature survey indicated cysteine, histidine and tyrosine as the three most reported iron-coordinating amino acids. A combinatorial peptide library screening was performed by our collaborators lab to identify heme-binding preferences of short peptides (9-mers) using UV/Vis, mass and EPR spectroscopies. Finally solution NMR spectroscopy was employed to determine different heme-binding modes and to investigate into conformational changes of these peptides upon heme-binding. Now we intend to transfer this heme-binding knowledge on to the protein level and investigate their heme regulation.

As an example of cysteine-based peptide, we are currently looking into heme regulation of the N-terminus of the a-subunit of potassium

channel Kv1.4. Voltage-gated potassium channels are responsible for the regulation of several ion conductance pathways and their inactivation is one of the most critical events that shapes the presynaptic potential in the central nervous system. Heme binds to this N-terminus leading to structural changes, impairment of the inactivation process and finally important effects in downstream signalling.

We will present results of our NMR structure determination of heme:peptide/protein complexes as part of our attempt to classify the HBM and HRM based on detailed functional and structural characterisations.

P 097
HOW TO CHOOSE AN OPTIMAL SET OF EXPERIMENTS FOR RESONANCE ASSIGNMENT OF IDPS?

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Intrinsically disordered proteins (IDPs) are a numerous and functionally important class of proteins. As IDPs lack fixed three-dimensional structure, the primary method to obtain information about those proteins is NMR spectroscopy. However, because of small chemical shift dispersion standard NMR techniques usually do not provide sufficient peak resolution for IDPs studies.

A number of high-dimensional (>= 4D) techniques have been introduced to overcome this problem [1-6], they differ in terms of detected nuclei type, dimensionality and obtained resonances. Choosing an optimal set of experiments can be crucial for the success of the assignment. Here we present a comparison of several sets of high-dimensional experiments with non-uniform sampling.

Using chemical shifts data deposited in Biological Magnetic Resonance Data Bank (BMRB) [7] we have simulated results of tested experiments, as if they were performed on various IDPs and processed using SMFT algorithm [8]. Peak lists generated in this way were used for automatic resonance assignment using TSAR program [9].We then investigated how properties of the protein, such as size, number of prolines, number of glycines, presence of repetitive sequences and peak dispersion affect the completeness of the assignment.

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FUNCTIONAL AMYLOIDS FROM THE FUNGAL PATHOGEN ASPERGILLUS FUMIGATUS

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Hydrophobins are fungal proteins characterised by a conserved amphipathic profile and an idiosyncratic pattern of eight cysteine residues involved in four disulphide bridges. Their functions are due to their remarkable physicochemical properties. Hydrophobins are secreted in a soluble form that self-assembles at hydrophobic/hydrophilic or air/water interfaces to form **amphipathic monolayers**. These proteins are used by fungi to breach the air/water barrier and develop aerial hyphae, to prevent water-logging, to cover aerial hyphae and spores rendering them hydrophobic thus facilitating aerial growth, spore dispersal and resistance to desiccation, to participate in the extracellular matrix or to form a protective layer during fruiting body development. Hydrophobins can also participate in host-fungi interactions (1).

Aspergillus fumigatus is the most important airborne fungal pathogen, causing over 200 thousands deaths per year among immunocompromised people. Its spores, which are the infectious form of the mould, are covered by **an amyloid-fibre layer with rodlet morphology** formed by a hydrophobin called RodA. This **rodlet coat renders the spores inert relative to the innate and adaptive human immune systems**, preventing the recognition of the mould pathogen-associated patterns (PAMs) (2).

We aim at describing the self-association of RodA into rodlets, characterising the structure of the amyloid rodlets and shedding light on the possible relationships between structure and the lack of immune response to RodA. We have shown by NMR that **recombinant RodA** expressed in Escherichia coli can be **successfully refolded** in vitro into a native protein with the same structure than the protein extracted from fungal spores. Moreover, recombinant RodA can **auto-associate into rodlet layers in vitro** that show the same morphology as the rodlets at the surface of the spores as assessed by atomic force or electron microscopies. We have studied the **structure** and **dynamics** of RodA by **solution NMR**. The structure displays a **rigid core** with secondary structures **organized around the four S-S bonds** and long **inter-cysteine flexible loops**. The kinetics of rodlet formation of wild type and mutants of RodA have been monitored by the binding of the amyloid fibre marker thioflavin T. The latter **mutational analysis** has highlighted **residues in the flexible inter-cysteine loops** that may be involved in the **cross-β spine of the amyloid fibres** as well as an N-terminal **region** that is important for the **lateral association** of the rodlets to form a monolayer. The **kinetics of rodlet formation of A. fumigatus point mutants in vivo are correlated to the observed kinetics in vitro**, indicating that the mechanistic and structural information obtained in vitro are relevant to rodlet formation on the fungus spores. We have also explored the **relationship** between **structure (unfolded and native soluble forms, rodlets, mutants)** and **immunological inertness** using human dendritic cells, macrophages and T cells.

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P 103
STRUCTURAL CONVERGENCE OF UNSTRUCTURED P53 FAMILY TRANSACTIVATION DOMAINS IN MDM2 RECOGNITION

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The p53, p63, and p73 proteins belong to the p53 family of transcription factors, which play key roles in tumor suppression. Although the transactivation domains (TADs) of the p53 family are intrinsically disordered, these domains are commonly involved in the regulatory interactions with MDM2. In this study, we determined the solution structure of the p73TAD peptide in complex with MDM2 using NMR spectroscopy and biophysically characterized the interactions between the p53 family TAD peptides and MDM2. Upon binding with MDM2, the intrinsically disordered p73TAD and p63TAD peptides adopt an amphipathic a-helical conformation, which is similar to the conformation of p53TAD, although the a-helical content induced by MDM2 binding varies. With ITC and CD data, our biophysical characterization showed that p73TAD resembles p53TAD more closely than p63TAD in terms of helical stability, MDM2 binding affinity, and phosphorylation effects on MDM2 binding. Therefore, our structural information may be useful in establishing alternative anticancer strategies that exploit the activation of the p73 pathway against human tumors bearing p53 mutations.

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FAST PROTEIN BACKBONE ASSIGNMENT BY COMBINATORIAL LAEBLING: APPLICATION TO SMALL MOLECULE BINDING STUDIES

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Structural information of target proteins is crucial for the development of high affinity, low molecular weight ligands. Especially fragment based approaches require the exact binding position of the fragment as well as detection of interactions between the small molecule and the protein of interest. This information is routinely gathered by x-ray crystallography. However in some cases the desired co-crystals do not grow or diffract poorly. This can be due to conformational changes upon ligand binding or if the binding site is not accessible in the crystal lattice. In these cases NMR is a valuable technique to confirm the binding of potential hits and gives information about the binding location, for the measurement is in solution and allows for conformational changes. To achieve a fast backbone assignment, which is necessary for the data analysis, a combinatorial selective labeling scheme in conjunction with cell-free protein expression is used. Cell free expression offers great opportunities for specific labeling, because single amino acid types can be labeled independently with considerably reduced isotope scrambling. This allows the use of 2D H,N-detected triple resonance spectra to identify unique amino acid pairs and therefore a fast and unambiguous assignment even with high signal overlap.

The approach was used to test different molecules that bind to the prolyl-cis-trans isomerase CypD. Some low affinity fragments (K_D in mM range) as well as high affinity ligands (K_D in nM range) were tested, of which some did not yield any co-crystal structure. Even for the low affinity ligands the binding site could be determined and is situated on a site where the protein forms crystal contacts.

One of the inhibitors showed 15N-1H spectra which did not reflect the expectations derived from the crystal structure of the complex. The chemical shift perturbations (CSP) as well as changes in linewidth pointed to a conformational change and an increase in protein dynamics, while the crystal structure showed only minor conformational changes in the involved sidechains with barely any changes in the backbone. These observations were further investigated with the final goal to calculate a structure in complex with the inhibitor to see if a large conformational change takes place.

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TIME-CORRELATED NETWORKS OF MOTIONS IN PROTEINS: A BASIS FOR NMR-RELATED MODELS OF INTERNAL DYNAMICS

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NMR relaxation experiments in isotopically labeled proteins represent unique strategies to probe internal protein dynamics. Relaxation rates obtained from these experiments can be analysed to derive amplitudes and time scales of internal motions. However, it is well known that their interpretation in terms of dynamical parameters is not straightforward and requires, at least plausible, modeling of the motions and their statistics. These reasons are sufficient to justify the use of molecular dynamics (MD) simulations and theoretical models.

We will present a novel approach based on the analysis of different auto- and cross-correlation functions obtained from MD simulations. In the proposed method, the clustering of effective correlation times allows to decompose protein structures in terms of time-scale dependent networks of dynamically correlated local domains. This segmentation of the protein on the basis of motion time scales should provide an adaptive strategy for coarse-graining internal motions, depending on the problem at hand, and could be used in the derivation of stochastic models for flexible macromolecules. This approach may serve as a basis for the development of a unified framework for the derivation of dynamic models that permit to extend the range of time scales accessed by MD simulations. This is of particular interest for the interpretation of magnetic resonance relaxation experiments, where dynamical processes at different time scales can be probed.

Preliminary results obtained for several prototypal proteins will illustrate this approach.

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P 112
INTEGRATED COMPUTATIONAL INTERPRETATION OF SDSL-EPR OBSERVABLES IN BIOSYSTEMS

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Dynamics play a basic role in determining and regulating physical and chemical properties of biomolecules, including their biological functions and spectroscopic signatures. Examples of dynamic-controlled classes of processes are the allosteric effects in enzyme catalysis, the formation of non-specific transient encounter complexes in the protein-protein association and the regulation of molecular recognition.

Internal and global motions in solution affect directly or indirectly most spectroscopic methods aimed at the characterization of biomolecules as in traditional approaches such as nuclear magnetic resonance relaxation, fluorescence anisotropy decay, time resolved X-ray and in single-molecule experiments such as site-direct-spin-labeled electron spin resonance, Förster fluorescence resonance energy transfer, and many other techniques [1-3].

In particular, a wealth of information on relaxation processes can be extracted from Electron Paramagnetic Resonance (EPR) experiments for studying structural and dynamic properties of peptides or proteins in solution. Among different EPR techniques, one of the most used is site directed spin labeling (continuous wave) EPR (SDSL-EPR), in which the paramagnetic probe (e.g. a stable nitroxide radical) is covalently bound to a particular residue of interest (e.g. cysteine or serine) or directly incorporated into the peptide backbone (i.e. TOAC spin probe), giving direct information on diverse properties, like local mobility and solvent accessible surface.

EPR data can be interpreted by stochastic approaches within the Stochastic Liouville Equation formalism, in which motional dynamics is included in terms of stochastic operators in the super Hamiltonian governing the time evolution of the system [4]. Parameterization of the Stochastic Liouville Equation can be successfully pursued within an Integrated Computational Approach (ICA) [5], i.e. using different theoretical and computational methods for evaluating molecular [6] (e.g. rotational and conformation diffusion tensor components) and magnetic (e.g. Zeeman and hyperfine tensors) parameters.

Here we present an ICA interpretation of SDSL-EPR data obtained for three different systems: i) rigid 3_{10}-helical peptides with TOAC spin label (mono and bis-labelled), ii) phospholamban (PLB) in DOPC/DOPE membrane with TOAC spin label, and iii) Domain I of HydF protein labelled with MTSL spin probe in different positions.

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IMPLEMENTATION OF FAST AND EFFCIENT TECHNIQUES FOR SPIN NOISE PROCESSING WITHIN THE TOPSPIN ENVIRONMENT

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A software toolkit was originally developed to accelerate the processing of 2D spin noise experiments.[1] Common operations like the Fourier transformation are already implemented in a very efficient manner within most manufacturers' spectrometer programs. Previously customized and novel processing techniques could be tested only by exporting the data to an external tool (MATLAB or Python) or tedious programming in C within the Topspin AU-environment. The former approach has severe performance issues, which can slow down the development of new experimental techniques significantly. This applies in particular to techniques such as 2D spin noise detected spectroscopy depending on non-standard processing algorithms applied to large amounts of data. For example for 2D spin noise detected HMQC 32GB of raw data are usual, data transfer and reformatting can thus become major bottlenecks to develop iterative improvements, due to the high turnaround times. To achieve a balance between performance and programming complexity a general toolkit was developed within the TopSpin (3.2) environment that allows users with limited programming skills to implement new methods and with a maximum of computational performance. The final solution is an EDSL (embedded domain specific language) written in C++. A simplified syntax is used, which is suitable for persons with focus on NMR and elementary programming skills. The memory requirements are scalable, and generally low, even for very large data sets. The toolkit works on all major operating systems (Linux, Mac OSX and Windows) with support for the x86-64 architecture.

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P 121
PREDICTION OF SINGLET STATE RELAXATION WITH MD AND QM CALCULATIONS

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Under certain conditions systems of two spin-1/2 nuclei support singlet states that are immune to the intra-pair dipolar relaxation superoperator. This enables characteristic decay constants TS as long as an hour and 50 times longer than T1 [1]. These enhanced lifetimes are of particular interest as polarization (and indeed hyperpolarization) can be stored for longer time intervals with important application in MRI, for example. Because of the extended time regime, many other "minor" relaxation mechanism interplay to relax these states. An exact knowledge of these mechanisms can indicate the conditions to maximize and extend even further singlet lifetimes.

However, even relatively small molecules in solution impose a number of challenges in the task of modeling T1 and TS from molecular dynamics simulation (MD) and quantum mechanics calculations QM. Computational cost is one major bottleneck. In addition, in order to accurately model long TS, attention is required to the weak and rather poorly explored relaxation mechanisms such as spin-rotation.

In this work we use novel approaches to address chemical shift anisotropy, spin-rotation and inter-molecular dipole-dipole relaxation mechanisms within the joint framework of MD and QM calculations. We take particular care of molecular flexibility. The CSA contribution, for example, is computed using a timescale-separated spin Hamiltonian, paying particular attention to internal molecular degrees of freedom. In the case of spin-rotation, a generalization of the local spin-internal Hamiltonian [2,3] is proposed. We demonstrate a good quantitative agreement between experiments and calculations without adjustable parameters. We discuss the field-dependence of TS and T1.

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DETECTION OF PEGYLATED SPECIES IN RATS USING QUANTITATIVE NMR SPECTROSCOPY

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The attachment of a protective polymer, poly(ethylene glycol) (PEG), is a very common strategy to improving the pharmacological properties of drugs. PEG is a non-toxic molecule known to reduce antigenicity, immunogenicity, proteolytic degradation and renal clearance, while improving stability, shelf-life and the solubility of entities to which it is attached. As such, it has been used to enhance the efficacies of small molecules, peptides and proteins, while also improving the performance of polymeric nanoparticles, liposomes and micellar drug delivery systems. Numerous pegylated conjugates have been approved for clinical use, while others are in clinical trials. Biodistribution data is needed for clinical approval, and tracking PEG is one method to determine PEG-drug localization. We propose the use of NMR spectroscopy, in combination with ^{13}C-labelling of PEG, as a direct, rapid and sensitive method to detect pegylated species in complex samples with little sample preparation. Signal amplification is achieved by the large number of chemically equivalent protons in PEG, while background signal can be eliminated by filtration of PEG proton signal through an isotopically enriched nucleus. Following proof-of-principle detection of both unlabelled and labelled PEG in blood, we quantify clearance of pegylated species in rats following intravenous injection. Overall, NMR is a valuable addition to the toolbox of techniques used to quantify the growing number of pegylated entities. While not necessarily as sensitive as some of the other techniques, its strength lies in its insensitivity to interfering components to analysis in complex samples, thus, for example, allowing detection of PEG species in unprocessed blood or even if the analyte is encased within a protective barrier.

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SUSCEPTIBILITY ARTIFACT CHARACTERIZATION OF ELECTRODE MATERIALS AND GEOMETRIES FOR NEURONAL IMPLANTS

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Motivation:
MRI is one of the most versatile examination tools for physical investigation of the brain in research and in clinical approaches [1]. Additionally there are more and more patients carrying implants connected to the brain [2] that have to undergo an MRI procedure. Hence, for future development of neuronal implants it is absolutely necessary to determine their interactions with the MR environment, such as susceptibility artifacts, since these can lead to loss of information or misinterpretation of acquired data.

Fundamentals:
One reason for MR image artifacts is the distortion of the static magnetic field (B_0) by the material. The extend of the distortion strongly depends on the materials magnetic property (susceptibility), shape and orientation w.r.t. B_0. Within this research we fabricated electrode-like metal pads deposited on PDMS with varying electrode distances and different materials compositions (Al and Pt/Ir). To enable the repetitive placement of these substrates within various MRI scanner systems (horizontal & vertical bore, 300, 400 and 500 MHz), with different orientations of the sample w.r.t B_0 we developed a sample mount. Additionally a FEM simulation has been conducted to calculate the B_0 deviations for comparison of simulation and MRI data. Based on the simulations further experiments can be prepared.

Fabrication and MR measurement:
The PMMA mount was fabricated with the aid of an infrared laser cutter. The mount enables to clamp the test substrates with two hinges parallel and with five slits horizontal w.r.t. B_0. After mounting the test sample to the PMMA mount, it was placed inside a standard NMR glass tube and the sample will automatically be aligned to the isocentre field. The metal electrode-like substrates are fabricated by laminating different metal foils on a PDMS substrate. With a UV laser cutter the metal pads, with varying distance and shapes, were structured and peeled off. The MRI measurement was conducted with the samples immersed in $CuSO_4$ doped water to reduce T_1 and using a standard FLASH sequence.

Results & Outlook:
Depending on the electrode shapes, distances between the individual electrode-like pads and the material susceptibility, the resulting artifacts are overlapping and thus suggest a larger connected huge object in the MR image. The simulations agree with the experimental results in terms of the least distance without having overlapped distortions of the MR image. Based on the results we can predict with FEM simulations the minimum distances between electrodes in order to avoid overlapping artifacts and thus give a realistic MR image. More investigations have to be made to include the influence of different MRI pulse sequences on the artifacts occurring in the MR image.

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PHOTO-INDUCED PHENOMENA IN MOLECULAR MAGNETS Cu(hfac)2LR : RECENT ACHIEVEMENTS

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Opportunity of using molecular spin states as quantum bits in data storage devices, quantum computers and multifunctional spintronics has caused considerable interest in this area and stimulated active search for appropriate compounds. Among them are the spin-crossover (SCO) complexes based on transition metal ions (d^4 – d^7 electron configuration) and organic ligands that can be switched between low-spin and high-spin states by external stimuli.

Recently a new class of SCO-like compounds was discovered – polymer-chain complexes $Cu(hfac)_2L^R$ based on alternating fragments of copper(II) ions and various nitroxide radicals. In contrast to classical SCO systems, the inherent bistability is provided by variation of exchange interaction in exchange-coupled three-spin clusters nitroxide-copper(II)-nitroxide. The system can be found in two situations: (1) the weakly-coupled spin state (WS) with weak ferromagnetic exchange between copper(II) and nitroxides; (2) the strongly-coupled spin state (SS) with strong antiferromagnetic exchange interaction. Switching between these two states can be triggered by temperature, pressure or light. The effect of photo-switching and light-induced excited spin state trapping (LIESST) is a subject of special interest due to the promising technological applications in the future.

In this work we overview our recent achievements in photo-studies of $Cu(hfac)_2L^R$ compounds using multifrequency EPR and

supplementary techniques (FTIR, UV-Vis spectroscopy). In particular, we showed that chemical tuning of ligand structure has influence on photo-induced state stability: new complex $Cu(hfac)_2L_{tert}^{Me}$ shows significantly higher LIESST observation temperature (60-65 K) compared to typical ones (~20 K) [1]. We also report the first example of photo-generation of thermally-inaccessible state with detailed characterization of its photo-switching and relaxation mechanisms on different timescales [2]. Moreover, we have found and investigated new type of photo-switchable complexes $Cu(hfac)_2L^R$ containing two-spin clusters nitroxide-copper(II); they already demonstrated higher stability of photo-induced metastable states and capability for complete spin-state conversion. Finally, we fully characterized main properties of photo-induced metastable states in $Cu(hfac)_2L^R$ and compared them with thermo-induced stable WS states: electronic structure and relaxation properties by means of EPR spectroscopy and geometrical structure of photo-induced metastable state using variable-temperature FTIR spectroscopy.

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LOW MOLECULAR WEIGHT ORGANIC GELATORS AS A HRDENER FOR GEL ELECTROLYTES

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Gel electrolyte based on low molecular weight organic gelator methyl-4,6-O-(p-nitrobenzylidene)-a-D-glucopyranoside was formed by the self-assembly phenomena in aqueous solution of high temperature ionic liquid tetramethylammonium bromide. The solidification process was based on sol-gel technique with controlled gelation temperature. When the temperature was below the characteristic gel-sol phase transition temperature, T_{gs}, the gel electrolyte was solid-like. The gel electrolytes showed enhanced ionic conductivity to those of the pure electrolyte in liquid state in whole temperature range below T_{gs}. The thermal stability, ionic conductivity and molecular dynamics investigated as a function of temperature and concentration of the gelator, together with the gel microstructure were performed to get some insight in to the origin of the enhanced conductivity properties. Intermolecular interaction between ion complexes and gelator aggregates was implicated by the data obtained and suggested as the origin of the conductivity enhancement effect.

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STRUCTURAL STUDIES OF HIGH PERFORMANCE POLYARAMID FIBRES

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We study poly(p-phenylene terephthalamides) (PPTA) obtained from Teijin Aramid by solid state NMR to get a better understanding of their structure-function relationship. The strong properties of aramids make it difficult to get information on the molecular level using analytical techniques. Also for NMR the spectral resolution is limited due to strong dipolar couplings and a limited chemical shift range due to the fact that protons and carbons belong to aromatic moieties. Combining single-quantum double-quantum correlation experiments, 1H-13C correlations at fast magic angle spinning speeds and first-principles calculations we are able to present a solid state proton chemical shift assignment of PPTA. Based on the 1H-13C correlation experiments we suggest different assignment of the 13C chemical shifts compared to literature.1,2

DFT calculations allow us to compare the chemical shifts to structural models of PPTA giving us insights into the relative packing of the polymer chains in the unit cell. Based on fibre X-ray3 and single crystal X-ray4 diffraction experiments two structural models for PPTA were proposed. The main difference is the packing of the alike aromatic rings in adjacent chains. In literature more structural models based on the direction of the hydrogen-bonded sheets are proposed5, however so far no experimental evidence is found for these structures. In our comparison we considered a whole family of possible structures consisting of different packing of adjacent rings and different direction of hydrogen bonding between the chains. We will discuss the most probable packing of the polymer chains based on the chemical shift comparison of the experiments and the calculations. It should be noted that the 1H shifts provide the best discrimination between the different proposed structures.

To investigate the existence of various polymorphs in differently processed fibers and to understand strength variations in these materials we aim to perform ssNMR spectroscopy of individual fibers which demands a leap in sensitivity and resolution. We have shown that miniaturizing the detection coil increases the mass-sensitivity and allows the use of extremely high decoupling-fields. Based on this we developed a so-called piggy-back μMAS NMR setup (sample volume 50 nL) in which we achieve a resolution that significantly surpasses state-of-the-art ultra-fast MAS (~100kHz) probe heads. "Inverse" detection experiments in combination with homonuclear decoupling enables the structural study of mass-limited samples (with improved sensitivity). First results showing the sensitivity enhancement at low spinning speeds will be shown and possibilities for the application to aramids and related polymeric systems will be discussed.

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P 142
EXPANDING THE NMR PALETTE: INSIGHTS ON ARTIFICIAL CHARGE SEPARATORS

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Spurred by worries over climate change, there is increasing interest in mimicking natural photosynthesis for the conversion of solar energy into fuel. The molecular structure and packing of self-assembled Zinc Salphen/NDI dyad and Perylene-based molecules, which are potential, charge separators were studied in detail in the solid state.

While dynamic scattering, availability of diffraction grade crystal, destruction of crystal due to electron beam were the drawback of TEM, difficulty to index the bragg peaks due to overlap is the limitation of the powder XRD. The combination of MAS NMR, TEM, Powder XRD and molecular modeling provide a powerful methodology that can be of use to investigate molecular geometry (and properties) of larger unlabeled - aggregated supramolecular systems. DFT calculations were performed using the CASTEP module in the material studio with GIPAW wave function. Quantum mechanical calculations allow experimental ^{1}H and ^{13}C solid-state NMR spectra to be assigned in a quantitative manner to a specific molecular packing arrangement, starting from the chemical structure of a moderately sized molecule. The incompleteness of SSNMR data is supplemented by data from TEM and powder XRD.

Here we simulated the distance constraints obtained from the LGCP build up curve using Simpson/Spinevolution for the selected carbon atoms. An electron density map of the proposed structure is generated and its projected down in the right orientation followed by fourier transform using EMAN2 software is employed to simulate the electron diffraction pattern. To confirm the model we simulated the powder XRD pattern using Reflex module in the material studio. We described a methodology in which the computational integration of MicroED, Powder XRD and SSNMR to propose a model for a molecule with high molecular mass, with less ambiguity. One of the biggest challenges with smarter crystallography is that it is limited to small molecules but here we proposed structures for molecules with higher atomic weight, which is around 1000gm/mol. This methodology could be extended to understand the mechanism of battery in the near future.

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SOLID STATE NMR, QUANTUM MECHANIC AND MOLECULAR DYNAMIC SIMULATIONS DELIVER SPATIAL INFORMATION ABOUT THE ORGANIC/INORGANIC INTERFACE IN BIOHYBRIDS

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Interactions between silica and organic molecules are important in various fields. One example is normal phase chromatography where the adsorption and desorption of molecules in a mobile phase with silica-packed column leads to their separation. Another well-known example of silica interacting with organic molecules are biominerals.

Especially diatoms are known for their formation of well-defined silicified cell walls [1]. The interaction of biosilica-associated molecules like polyamines, peptides and polysaccharides with silicic acid / silica was studied in vitro using microscopic techniques like SEM and optical spectroscopy. Solid state NMR is capable of providing information about the composition and spatial arrangement of the molecules at the interface between the organic phase and silica. It also allows to derive models for the predominant interaction mechanisms between the molecules during and after the formation of biosilica.

In our studies, isotopically labeled choline and polyamines (^{13}C, ^{15}N) are used to study the interaction with ^{29}Si-labeled monosilicic acid. The formed nanocomposites were analyzed with respect to interactions between the organic molecules and silica.

The application of CP MAS-based experiments like ^{1}H-^{13}C-{^{29}Si}-REDOR [2] and ^{1}H-^{13}C/^{1}H-^{29}Si-HETCOR [3] allows the determination of

spatial parameters, especially ^{29}Si-^{13}C internuclear distances and molecular orientations in this system. Thus the type of interaction (hydrogen bonding or electrostatic interactions) between the organic molecules and the silica surface can be determined. Furthermore, the different phases resulting from the silica formation are distinguishable. The derived NMR-parameters are in excellent agreement with the results of corresponding QM and MM simulations. QM calculations where made with CP2K which provides density functional theory (DFT) methods as well as classical pair and many-body potentials.

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ELECTRON PARAMAGNETIC RESONANCE STUDY OF EXCHANGE INTERACTIONS BETWEEN CERIUM IONS IN YAIO3 SINGLE CRYSTAL SCINTILLATOR

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Cerium doped yttrium-aluminum perovskite ($YAlO_3$, YAP) was intensively studied in the past decades due to prominent scintillating characteristics which allow its application in the g-rays detection [1-4]. Ce^{3+} ions are used as activation dopants (5d-4f transition) responsible for the broad emission band peaking at approximately 370 nm. Our study is thus focused on the crucial point – the incorporation of the Ce^{3+} ions inside the YAP host.
YAP single crystals have an orthorhombic perovskite structure, space group D-P_{bnm} [5]. They were grown from melt by the Czochralski method in a Mo crucible in reducing atmosphere with 0.5 at.% content of Ce^{3+} ions. Some of the YAP:Ce single crystals were grown with about a thousand times lower concentration of the Ce^{3+} ions.
Electron Paramagnetic Resonance (EPR) spectra of the YAP:Ce were composed of very strong lines originating from the Ce^{3+} ions at two magnetically inequivalent yttrium sites and much weaker doublets of lines. The doublets originate from the exchange interaction between the two coupled Ce^{3+} ions. These pairs of ions could be further classified accordingly to the type of interaction - either next to the nearest (nn, antiferromagnetic direct exchange between the two ions) or next to the next to the nearest (nnn, ferromagnetic superexchange via an oxygen ion). By using the binomial distribution, the probabilities of finding the nn or nnn pair were calculated. Comparing them with the relative intensities of the doublets in spectra, the pairs were classified by the type of interaction. The corresponding g tensors were determined. The coupling constants were assumed to be the sum of magnetic dipolar, isotropic (Heisenberg) and anisotropic exchanges. The z-components of magnetic dipolar constants were roughly estimated for the both types of the pairs. However, the real values of exchange constants cannot be determined from our data directly, since the doublets positions in

the spectra of the coupled Ce^{3+} ions are given by the combination of the dipole and exchange interactions.

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NMR STUDY OF CHEMICAL ORDERING AND ANOMALOUS 207PB HYPERFINE INTERACTION IN MULTIFERROIC PEROVSKITES PB(FE0.5SB0.5)O3

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Multiferroic materials with the coexistence of ferroelectric and ferro- or antiferromagnetic orders have recently attracted significant interest due to their potential for applications as multifunctional materials, for instance, in spintronics and magnetic random access memory. Bulk characteristics of Fe-based double perovskites, which show multiferroicity, are strongly dependent on the structure in micro- and nanoscopic scale: the chemical order is an essential to formation of superspin clusters which are responsible for the observed magnetic relaxor properties [1]. To examine the local structure of these compounds, NMR methods with resolving hyperfine interactions can be successfully applied [2].

In this work, we studied a new multiferroic material $Pb(Fe_{0.5}Sb_{0.5})O_3$, which was synthesized with varying degree of Fe^{3+} and Sb^{5+} ions ordering, by nuclear magnetic resonance. The four ceramic samples with ordering parameter values S= 0.93, 0.67, 0.46, and 0.17 (determined by X-ray diffraction) were studied by means of ^{207}Pb and $^{123,121}Sb$ NMR spectroscopy. The spectra were measured in a field of 9.4 T at various temperatures. ^{207}Pb NMR spectra of the ordered sample feature large paramagnetic shift: ~1.8 MHz at 300 K that increases up to ~3 MHz at temperatures around 100 K. Samples with cationic disorder display two distinct components which are attributed to ordered and disordered regions.

The experiments were supplemented by density functional theory calculations and the experimental and calculated NMR parameters were compared.

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91Zr, 137Ba AND 47,49Ti NMR STUDY OF RELAXATION PROCESSES IN THE LEAD-FREE RELAXOR FERROELECTRIC xBaZrO3-(1-x)BaTiO3

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$xBaZrO_3$-(1-x)$BaTiO_3$ (BZT) solid solutions have become recently extremely popular as one of the possible lead-free piezoelectric and relaxor ferroelectric systems with a potential use for applications as microwave electric-field tunable materials and from the fundamental physics point of view as a prototype isovalent mixed system with relaxor ferroelectric properties [1]. It is assumed that due to a smaller ionic radius of Ti^{4+} (0.605 Å) compared to Zr^{4+} (0.72 Å), the Ti^{4+} ions occupy off-center position within the BO_6 octahedra in contrast to centric Zr^{4+} positions for the whole composition range. As NMR is one of the most suitable methods to prove off-centering (static and dynamic) of ions with quadrupolar nuclei, we have performed detailed studies of the spin-lattice and spin-spin relaxation rates of ^{137}Ba, ^{91}Zr and $^{47,49}Ti$ in the solid solutions $xBaZrO_3$-(1-x)$BaTiO_3$ in the temperature region 10-340 K.

$xBaZrO_3$-(1-x)$BaTiO_3$ powders were prepared using the standard solid state reaction by calcination at 1000°C (4 hours) and the ceramics were processed by cold isostatic pressing (1500 bar) and sintering at 1600°C for 4 hours. The ceramics were of single phase perovskite structure with over 99% theoretical density and grain size ~1 mm. NMR measurements were carried out in 9.4 T magnetic field at various temperatures. The obtained NMR data are compared with the dielectric spectroscopy data measured in the same samples.

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P 157
HOST-GUEST INTERACTIONS IN CONTROLLED DRUG DELIVERY SYSTEMS

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Second-order interactions between active substances and different one- and multicomponent macromolecular systems basically determine the properties of controlled drug delivery systems. These interactions influence not only the release properties but by our basic hypothesis the morphology and size of active pharmaceutical ingredient (API) particles too.

Responsive hydrogels are one of the most frequently proposed vehicles for targeted and controlled drug delivery. Interaction between the transported drug and the three-dimensional polymer network could compromise the kinetics and the efficiency of delivery in thermoresponsive polymers. Poly(N-isopropylacrylamide) (PNIPA) gel was equilibrated with excess aqueous solutions with different concentration of three model drug molecules, phenol, ibuprofen and dopamine. After drying to constant mass of the loaded samples were investigated by solid-state NMR, thermal (STA, DSC), X-ray powder diffraction and transmission electron microscopy methods. Differences were found in the morphology and distribution of active substances in the polymer network.

The crystalline/amorphous structure of API can be determine more accurately from the 13C CP MAS spectra than from the X-ray powder diffraction. Depending on the concentration of the swelling media and the nature of the API, their morphology can be very different in the loaded systems. To better understand the possible host-guest interactions between small molecules and the polymer 1H-1H double-quantum CRAMPS (combined rotation and multiple-pulse sequence) spectra were recorded.

Phenols exert a major influence on PNIPA in solution or swollen gel by reducing its phase transition temperature. The strong interaction between phenol and the polymer that is detected by solution- and solid-state NMR hinders the crystallisation of phenol when the water is gradually evaporated. The similar H-bonded structure was identified on the 1H-1H DQ spectra in dry state than in swollen gel by 1H-1H SQ correlation method. The amino ethyl phenol derivative dopamine has a much more limited effect, but in the opposite direction – the transition temperature increases slightly. The strong interaction observed among the dopamine molecules disables the polymer–dopamine interaction and favours crystallization of the dopamine when water is removed. Additionally the difference of the chemical shift of the aromatic protons in amorphous and crystalline dopamine was also determined by DQ spectroscopy. In case of ibuprofen the strength of the host-guest interactions are lie between phenol and dopamine.

These results reveal that embedding the drugs into polymer matrices for controlled delivery can alter the crystallinity of the stored molecules. As morphology is one of the crucial factors in delivery, this may compromise the rate and the efficiency of release.

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PROBING OF CHAIN CONFORMATIONS IN CONJUGATED NANOPARTICLES BY EPR SPECTROSCOPY

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It is an open question how individual polymer chains adopt their conformation inside nanoparticles. We establish electron paramagnetic resonance spectroscopy as a method allowing for a direct observation.

A knowledge of the conformations of polymer chains in nanoparticles is desirable in many instances. They determine e.g. luminescent and conductive properties of the individual chain, and likewise packing and consequently inter-chain interactions. A particularly relevant and timely example a nanoparticles of fluorescent conjugated polymers. The generally assumed rigid nature of such polymer chains, as reflected by their persistence lengths, appears contradictive to the fact that nanoparticles with sizes lower than the persistence length have frequently been encountered. However, no methods exist to date to probe chain conformations in nanoparticles experimentally.

Here, we show how this problem can be resolved by electron spin resonance (EPR) spectroscopy. Oligo(phenylene ethynylenes) were chosen as a model due to the particular rigidity and high persistence length of this type of compounds. Our studies reveal that these probes adopt a bent conformation when confined in nanoparticles. Considerations of surface energies vs. bending energies agree with this picture.

P 163
NMR INVESTIGATIONS OF THE MOTOR OIL AGING PROCESSES

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In the last years, changes in the ecological mindset can be observed. The interest in oil recycling or at least in a reduction of oil change intervals at simultaneously constant performance increases significantly. To achieve longer oil lifetimes, knowledge about the oil aging processes is indispensable. Common engine oils consist of a variety of additives dispersed in a hydrocarbon oil base in which the specific composition depends on the individual requirements of the engine. During the engine operation, oils underlie an aging process, which lead to a change in chemical composition.

Generally, the oil aging process is an irreversible modification of the physicochemical properties of the oil. Different analytical methods have been applied, like infrared (IR) spectroscopy, mass spectrometry coupled with gas chromatography (GC-MS), inductively coupled plasma optical emission spectrometry (ICP-OES) and nuclear magnetic resonance (NMR) spectroscopy. In contrast to IR-spectroscopy, NMR spectroscopy is not limited by surface effects, depth of penetration and signals overlap.

For better understanding of the oil aging processes, different motor oils have been characterized by diverse NMR spectroscopic methods. Already one-dimensional ^{1}H ^{13}C spectra show specific changes in the chemical composition during their lifetime, like the formation of e.g. acids, alcohols and aromatics. To get deeper insight into the chemical composition of the molecules present in the oil, two-dimensional spectra were measured. Finally, the chemical changes in composition can be quantified. Depending on the motorization of the automotive, a significant difference in motor oil aging can be observed. The different NMR spectra are discussed and interpreted.

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THREE-DIMENSIONAL STRUCTURE DETERMINATION OF SURFACE SPECIES BY DNP ENHANCED SOLID-STATE NMR

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NMR spectroscopy (often in conjunction with diffraction methods) is the method of choice for characterizing surfaces whenever possible, but the detection limit of NMR is far too low to allow many modern materials to be examined. Because it provides dramatic sensitivity enhancement, solid-state Dynamic Nuclear Polarization (DNP) NMR is currently emerging as a powerful tool to study samples previously inaccessible to NMR.

We have recently shown how DNP could be used to selectively enhance the NMR signals from surfaces in a wide range of samples, including nanoporous and nanoparticulate materials (DNP SENS) (1). With the recent introduction of polarizing agents of high molecular weight like TEKPOL (2), enhancements greater than 100 are now routinely obtained at 9.4 T and 100 K for mesostructured materials. Such signal amplification factors enable multi-dimensional correlation experiments and thus offer the prospect of obtaining unprecedented quantitative structural information about species at the surface of these materials.

Here we will show that multi-nuclear correlation techniques can be applied to obtain measurements of ^{13}C-^{15}N and ^{29}Si-^{15}N distances in mesoporous silicas incorporating organic fragments as well as well-defined organometallic catalysts. These experiments lead to the determination of the three-dimensional structure of the surface species for both precursor and metal-organic catalyst.

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STRUCTURE OF DITHIOCARBAMATE COMPLEXES OF BISMUTH, YTTRIUM, AND LANTHANUM FROM X-RAY CRYSTALLOGRAPHY, SOLID-STATE NMR, AND DFT CALCULATIONS

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Dithiocarbamates ($R_2NCS_2^-$) are very versatile ligands that can coordinate to metals in several different ways and hence form complexes with most of the elements in the periodic table, including, lanthanides and actinides. Metal-dithiocarbamate complexes find wide-ranging applications in material chemistry, and have potential use as chemotherapeutic, pesticides, fungicides, and as additives to lubricating oils. Here we present structural investigations of dithiocarbamate complexes with bismuth, yttrium, and lanthanum of molecular formula $[Bi\{S_2CN(n\text{-}(C_4H_9)_2\}_3]$, $[Y\{S_2CN(C_2H_5)_2\}_3PHEN]$, and $[La\{S_2CN(C_2H_5)_2\}_3PHEN]$ (where PHEN=1,10-Phenanthroline). We report new single-crystal X-ray diffraction structures as well as high-resolution ^{13}C and ^{15}N solid-state cross polarization magic-angle-spinning (CP-MAS) NMR results of all three complexes. Our NMR results demonstrate a significant amount of structural disorder or polymorphism for bismuth di-n-butyldithiocarbamate complex.
The ^{13}C and ^{15}N CPMAS spectra of polycrystalline yttrium and lanthanum dithiocarbamate complexes show the presence of significant structural differences. In addition, diethyldithiocarbamato-phenanthroline complex of yttrium has a very similar structure type to

previously reported X-ray structure for [Nd{$S_2CN(C_2H_5)_2$}$_3$PHEN] whereas, the crystal structure of [La{$S_2CN(C_2H_5)_2$}$_3$PHEN] is considerably more complex. Finally, the experimental NMR results are complemented by chemical shifts obtained by DFT methods and suggested the spectral assignments. Overall, our work demonstrates how different experimental and theoretical methods can be combined, which can afford insights into the structure and bondings of metal complexes.

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P 172
CARBON DIOXIDE CAPTURE AND GEOSEQUESTRATION STUDIES VIA SOLID-STATE NMR

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We are studying in situ CO2 reactions for capturing it from flue gases, and for mineralizing it for geosequestration. Solid supported-amine adsorbents were reacted with CO2 and monitored with in situ static 13C NMR, to observe physisorption and ex situ 13C{1H} CPMAS NMR chemisorption reactions. CO2 can be sequestered through geologic mineralization reactions in which divalent cations (Fe2+, Mg2+, etc.) react with CO2 to form metal carbonates. Magnesium carbonate and other metastable magnesium carbonate minerals have been characterized via their distinct chemical shift anisotropy (CSA) lineshapes with static 13C NMR.

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AN OPEN ACCESS NMR DATABASE FOR ORGANIC NATURAL PRODUCTS "CH-NMR-NP"

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An NMR database for natural organic compounds "CH-NMR-NP" was opened as a freely accessible system from JEOL RESONANCE website. Total number of the compounds are about 30,500 compiled mainly from the published papers from 2000 to 2014. Items for a compound are Name, Molecular formula, Chemical structure with assigment, the Values of ^{13}C and ^{1}H shifts, ^{1}H-coupling information,Solvent, Shift reference and others.

CH-NMR-NP

^{1}H and ^{13}C NMR spectra are most important to determine a chemical structure of natural organic compounds, and newly found compounds in published papers are always accompanied by NMR data with their spectral assignments. To confirm chemical structure of compounds, NMR data are always essential. Then we started to compile an NMR database named "CH-NMR-NP" from research journals published in 2000. At that time, gradually two-dimensional NMR became popular and the reliability of the spectral assignments increased. The basic items for a compound in the DB are Name, Chemical structure with assignment, Molecular formula, Molecular weight, CAS registry number, Characteristics of the compound such as terpenoid, cyclic peptide, macrolide and so on. It is desirable to include the source from which the compound was extracted and a memo of the origin for the resources, such as sponge, whole plant, root, areal part and so on, based on the paper. For the data related to the NMR measurements, solvent, shift reference, ^{1}H frequency are included. To indicate the spectral assignment, numerical numbers are added in the chemical structure. Each carbon has a unique number.

The same number is given for chemically equivalent carbons. For each number, ^{13}C shift, carbon type (C, CH, CH_2 and CH_3), ^{1}H shift, ^{1}H-coupling information, assignment exchangeable, and a number of the equivalent carbons are given. From the spectral information, the spectral patterns are created in the Internet service.

Delta NMR software

A free NMR software "Delta" is well cooperative to "CH-NMR-NP". A comparison of a measured NMR spectrum with spectra included in the DB (similarity search) is easily accomplished on the "Delta". It is possible to export "CH-NMR-NP" data into the delta format. Alternatively, a peak list in a delta-format can be converted into the "CH-NMR-NP" format. The similarity search can be carried out in the both ways and successfully used for the determination of chemical structure.

URL for the access is:
http://www.j-resonance.com/en/nmrdb/

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STEREOCHEMICAL PURITY DETERMINATION BY NMR ANALYSIS OF BIOACTIVE PRECURSORS FROM ENZYMATIC KINETIC RESOLUTION

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The biological activity and the important role of enantiopure secondary alcohols containing heterocyclic ring systems as intermediates for the drug synthesis attracted many interests in the recent years. Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) have been proved suitable biocatalysts for the production of optically pure stereoisomers since these enzymes exhibit high stability and enantioselectivity in organic solvents. NMR analysis is a relevant method for the confirmation of enantiomeric purity of the reaction products.

In this work two compounds bearing furan [1,2-di(furan-2-yl)-2-hydroxyethan-1-one] or aromatic (1,5-Dihydroxy-1,2,3,4-tetrahydronaphthalene) rings and secondary hydroxyl groups were tested as substrates for the enantioselective kinetic resolution catalyzed by native and immobilized lipases. All the reactions were performed at 40˚C by using tetrahydrofuran as reaction media and vinyl acetate as acyl donor. The enantiomeric excesses of both substrate and product were determined by normal phase chromatography using the immobilized chiral stationary phase Chiralpak IC and IA. Among the tested substrates and lipases, the immobilized the sol-gel entrapped Burkholderia cepacia lipase by using a ternary mixture of silane precursors (PhTMOS : VTMOS : TMOS) for the matrix formation was the most efficient biocatalyst in term of stereoselectivity for the enantioseparation of 1,5-dihydro-1,2,3,4-tetrahydronaphthalene. The efficiency of the immobilized lipases was proved by the high values of substrate conversion, enantiomeric excess (ee) and enantiomeric ratio, after several reaction cycles.

The stereochemical purity of the reaction product was proved by using (S)–(+)-a-methoxy-a-trifluoromethylphenylacetyl chloride as derivative completed by the NMR analysis including: ^{1}H-RMN, ^{13}C-RMN. ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR (HSQC and HMBC).

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CLIP-ASAP-HSQC FOR FAST AND ACCURATE EXTRACTION OF ONE-BOND COUPLINGS FROM ISOTROPIC AND PARTIALLY ALIGNED MOLECULES

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We present the recently published CLIP-ASAP-HSQC[1] experiment that allows the detection of w_2-coupled 2D spectra in 25 seconds or less and from which $^1J_{CH}$-couplings can be determined with high precision. The experiment combines the previously published ASAP-HSQC[2] designed for fast acquisition with the commonly used CLIP-HSQC[3] which allows the measurement of couplings in the direct dimension without artefacts from incomplete coherence transfer. For best possible robustness of the pulse sequence, broadband excitation, inversion and refocusing pulses as for example the BEBOP[4], BURBOP[5] and BUBI[6] pulses are used, which are derived from Optimal Control Theory (OCT)**. The performance is demonstrated on three test samples including partially aligned molecules using chiral aligning media. Besides other structural NMR parameter the detection of RDCs from partially aligned samples is highly valuable for structure determination. Therefore speeding up the acquisition is desirable especially when samples undergo chemical reactions or dynamic processes are observed. Fastest 2D experiments can in principle be obtained in a single scan using gradient-encoding imaging-type schemes[7] - and the approach has already been applied to the measurement of RDCs[8] - but the method faces severe limits in terms of resolution, accessible bandwidth and sensitivity. In contrast spectra obtained from the CLIP-ASAP-HSQC can be recorded with no compromises. The extraction of couplings is

evaluated in comparison to the best available CLIP-HSQC sequence for the three test samples.

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LC-NMR ANALYSIS OF IMPURITIES IN A KEY STARTING MATERIAL OF ETODOLAC

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Tryptophols are derivatives of 2-(1H-indol-3-yl)ethanol, indole class bearing a C-3 hydroxyethyl side chain. 7-Ethyltryptophol, 2-(7-ethyl-1H-indol-3-yl)ethanol, is a key starting material in the synthesis of Etodolac, an important non-steroidal anti-inflammatory drug. The currently available synthetic procedures for 7-ethyltryptophol usually result with formation of a high-level of various impurities [1,2].

LC-NMR is a very powerful tool for obtaining detailed structural information of components in complex mixtures such as impurities and metabolites in pharmaceuticals, natural products and synthetic polymers [3,4]. In the present study, we developed LC-NMR methodology for separation and structural analysis of impurities in commercially available 7-ethyltryptophol. Several impurity peaks were detected in chromatograms and the structures of major impurities were elucidated by analyzing 1D and 2D LC-NMR spectra.

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NMR STUDIES OF DE NOVO-DESIGNED ANTIMICROBIAL PEPTIDES

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Antimicrobial peptides occur naturally in a wide range of organisms and are evolutionary conserved since a long period of time. They play an important role in the innate immunity of vertebrates to protect against pathogens like bacteria, virus and fungi. [1]

In the last years, a multitude of naturally occurring antimicrobial peptides has been discovered, they all have a more broadband or selective microbial growth inhibition in common, although their amino acid compositions and structures are highly dissimilar. Their properties, especially the selective inhibition, are of increasing interest due to the rising of pathogenic resistance to common antibiotics. [2]

A general mode of action for antimicrobial peptides has not yet been found, but one outstanding property is their amphipathic character, which enables the peptides to bind to microbial membranes, harming or destroying them. NMR is a highly appropriate tool to obtain a better understanding of their interactions, since rigid structures are not commonly found. [3]

We have recently started investigation of several de novo-designed antimicrobial peptides. Resonance assignments are obtained using tailored sets of 2D NMR experiments suitable for these unlabeled peptides of different size. Secondary structure propensities within the peptides could be derived from the NMR-parameters, using both algorithmic methods and a comparison to database structures. Insights into conformational selection and modified dynamics upon intermolecular interactions with proteins and peptides are thus obtained at atomic resolution. An important observation in this

context is that the conformation distribution can be significantly altered by common fluorescent tags.

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P 190
HOST-GUEST COMPLEXES OF PORPHYRINOGENS AND ORGANIC ACIDS

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Porphyrinogens are capable of forming complexes with organic acids. Some types of symmetrical (achiral) porphyrinogens can be used as a probe for NMR determination of enantiomeric excess of small chiral organic molecules. This method was introduced by Labuta et al. [1 – 3]. Particular organic chiral molecule (guest) and porphyrinogen (host) form host-guest complex while inducing nonequivalency of particular proton resonances in symmetrical host through chirality translation process. It causes splitting of NMR signals which is linearly dependent on enantiomeric excess of guest (regime of fast exchange of guests in the complex is required). Notably no diastereomeric species are formed in solution.

Here we present NMR and UV-vis measurements on both di-N- and tetra-N-benzylated oxoporphyrinogen. The phenomenon of chirality translation was confirmed. We also performed titration experiments as well as variable temperature measurements on some porphyrinogens derivatives (host) in the presence of camphorsulfonic acid or difluoroacetic acid (guests). It turns out that a few dynamic processes occur in the sample mixture. One of them is formation of host-guest complex and another is protonation of the porphyrinogen. Attachment of the proton at one of four porphyrinogen carbonyl sites causes rearrangement of its conjugated system with subsequent rotation of bulky side groups at the porphyrinogen periphery.

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THE SUMMIT MS/NMR METHOD FOR THE RAPID IDENTIFICATION OF NEW METABOLITES IN COMPLEX MIXTURES

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Many of the signals found in NMR and Mass Spectra (MS) of complex metabolite mixtures belong to molecules whose identification is notably hard. Traditionally, identification of unknown metabolites requires their isolation through time-consuming purification from complex mixtures by using separation techniques, such as chromatography, followed by extensive characterization by combining NMR, MS, X-ray, and other techniques. Here, we introduce an alternative metabolite identification strategy of complex mixtures by combining NMR with MS in a novel way. Since the method does not require purification, it is suitable for high-throughput identification of new molecules in crude metabolite extracts. We term this approach SUMMIT MS/NMR for "Structures of Unknown Metabolomic Mixture components by MS/NMR" [1].

The general workflow of SUMMIT MS/NMR is the following: for a sample of a complex metabolite mixture of unknown composition, the high-resolution mass spectrum is determined and accurate masses are converted to molecular formulas. For each molecular formula, all possible structures, which is the "structural manifold", are generated. The NMR spectrum (chemical shifts) of each structure is then predicted. Meanwhile, the experimental NMR spectrum is determined for the same mixture and deconvoluted into the NMR spectra of individual components. The NMR spectrum of each component is compared to the predicted NMR spectra of the structural manifold and the structures are rank-ordered according to their agreement to

identify those molecular structures that are most consistent with all available NMR and MS data.

The application of SUMMIT MS/NMR to an E. coli cell extract will be presented, which demonstrates the power of NMR chemical shift information as an effective filter to identify the correct structures among the large structural manifold belonging to MS-derived molecular formulas. SUMMIT MS/NMR neither requires purification nor the use of NMR and MS metabolite databases and it is applicable to identification of unknown metabolites in a wide range of complex metabolite mixtures ranging from biomedicine, biology, to food sciences.

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CYCLIC DIPEPTIDES – NMR, X-RAY AND THEORETICAL CALCULATIONS

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Cyclic dipeptides (CDP) belong to the important class of biologically active compounds, they can be used as simple heterocyclic scaffolds in combinatorial chemistry, model compounds in a crystal engineering and simple model compounds in quantum-chemical calculations. We have studied the structures of twenty-eight CDPs containing pipecolic acid or proline and their thia-analogs as one residue and phenylalanine or N-methyl phenylalanine as the second residue (both cis- and trans-CDPs) using NMR spectroscopy, X-ray structure analysis and theoretical quantum-chemical calculations. Different types of hydrogen-bonding patterns and conformations of central dioxopiperazine ring within a range of non-planar cis-amide bonds were found in crystal. Geometrically nonequivalent molecules, observed in the crystal unit cell of some CDPs, could be detected also in the solid-state ^{13}C NMR spectra. Crystal structures were compared with those calculated by DFT methods. Solution conformations of CDPs were derived from the experimental ^{1}H, ^{13}C and ^{15}N NMR data and theoretical DFT calculations of chemical shifts and coupling constants. Residual dipolar couplings (RDC), obtained from the NMR spectra of CDPs in water with disodium cromoglycate as an aligning medium at higher temperature (isotropic phase) and lower temperature (anisotropic phase), proved the power to check the correct geometry of CDPs in solution. Our attempts to find a correlation between non-planar peptide parameters (torsion angle w or pyramidicity at carbon or nitrogen atom of peptide bond) and chemical shifts of corresponding C=O carbon or amide nitrogen were

not satisfactory. CDPs containing thia-Pip or thia-Pro residue can be easily oxidized with meta-chloroperbenzoic acid to the epimeric sulfoxides. We have shown that configuration of sulfoxides can be determined by comparison of the experimental and calculated chemical shifts.

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H-1 NMR METABOLOMICS OF HALIOTIS DIVERSICOLOR RESPONSES TO ELEVATED TEMPERATURE AND HYPOXIA

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The small abalone Haliotis diversicolor is one of the most commercially important cultured abalones in southern coastal areas in China. However, the frequent occurrence of infectious diseases, especially during hot summer, is a major problem that has threatened the abalone aquaculture industry for a long time [1]. High temperature and hypoxia are two main causes of a large number of abalone deaths in summer, and they also induce various kinds of diseases frequently. For marine ecosystems, high temperature is the likely cause of some negative consequences, such as the blooming in microbial populations and the decreases in oxygen solubility, hence it is often accompanied with hypoxia [2]. In current study, ^{1}H NMR spectroscopy together with pattern recognition methods was used to investigate the response of small abalone to the exposure to elevated temperature and hypoxia. The abalone muscle was collected after 24 and 96 hours' exposure at elevated temperature (30 °C) and hypoxia (2 mg/L DO), then its extract was analyzed by NMR. The dominant metabolites in abalone muscle were found to comprise amino acids, organic osmolytes, neurotransmitter, and Krebs cycle intermediates. Following spectral preprocessing, orthogonal partial least-squares discriminant analysis (OPLS-DA) of the metabolite profiles was conducted. The analysis of the metabolome revealed significant differences between male and female organisms. Furthermore, males and females were shown to respond differently to environmental stress. In details, thermal and hypoxia stresses increased the levels of amino acids (including glycine, isoleucine, valine, leucine, tyrosine, phenylalanine, asparagine, tryptophan) and GABA, and decreased the level of ATP, homarine, succinate and trigonelline in male abalone muscle after 96 hours' exposure. In female abalone muscle, after 96 hours' exposure, taurine, isoleucine, leucine, phenylalanine and tryptophan were significantly elevated,

while asparagine, aspartate, acetone, glutamate, acetylcholine, GABA, homarine, and ATP were depleted. However, at 24 hours of thermal and hypoxia stresses the metabolic changes were insignificant. This study has important implication in understanding the strategy of metabolic phenotype modulation of abalone against environmental stresses.

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ULTRA-HIGH-RESOLVED PURE SHIFT NMR EXPERIMENTS FOR THE ANALYSIS OF COMPLEX MIXTURES OF COMPOUNDS WITH NEAR/IDENTICAL 1H AND 13C NMR SPECTRA

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NMR analysis is often limited by the lack of appropriate signal dispersion due to small chemical-shift differences (Dd) and the wide J_{HH} coupling patterns. In this work we present a useful experimental NMR strategy that greatly facilitates the analysis of highly congested spectral regions[1]. We will show here how a real sample consisting of a mixture of several unknown compounds with near-identical ^{1}H and ^{13}C NMR spectra can be distinguished and assigned using ultra-high-resolution NMR methods based on the combination of pure shift NMR[2] and spectral aliasing techniques.[3] We use a suite of modern pure shift 2D NMR methods based on the homonuclear decoupling band-selective (HOBS) technique[4](HOBS-HSQC[4a], HOBS-HSQC-TOCSY[1], and HOBS-HSQMBC[5] experiments) in order to obtain fully homodecoupled signals for a set of non-mutually J-coupled protons resonating in a selected region of the ^{1}H spectrum. Additionally, it is shown that using a reduced ^{13}C spectral width of a few ppm (spectral aliasing approach), optionally combined with nonuniform sampling (NUS), can produce ultra-high-resolved 2D HOBS spectra in conventional acquisition times.

The experimental results show that the full sensitivity and the excellent spectral resolution obtained from spectral-aliased 2D HOBS spectra makes it possible to enable the in situ distinction and assignment of similar organic compounds exhibiting near-identical ^{1}H and ^{13}C NMR

spectra into the same mixture. It is also shown that a complete set of extremely small Dd(^{1}H) and Dd(^{13}C) values, even below the natural line width (1 and 5 ppb, respectively), can be simultaneously determined and assigned. The proposed strategy could also be very useful in other applications, such as the analysis of crude reactions and detection of intermediates, reaction monitoring, or the analysis of complex mixtures.

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AN RDC BASED FORCE FIELD METHOD TO SOLVE THE CHIRAL CONFIGURATION OF COMPLEX NATURAL AND SYNTHETIC PRODUCTS

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Obtaining the total stereochemistry of complex natural products is very difficult by traditional NMR methods: chemical shifts, NOEs, and scalar (J) couplings. We used PMMA stretched gels[1] and PELG[2] liquid crystalline media to measure residual dipolar couplings (RDCs) of ecteinamycin, a novel polyether antibiotic with demonstrated potent activity against gram positive bacteria, including selectivity for Clostridium difficile (recently elevated to "Urgent" hazard level for infections by the CDC). Ecteinamycin was discovered by using LCMS-based metabolomics to investigate marine-invertebrate-associated bacteria.[3] This complex molecule couldn't be crystallized, and the configurations of its 14 chiral centers are largely unknown. Fitting the experimental RDCs (extracted from F1 ^{1}H-coupled BIRD HSQC with J-scaling and/or F2 ^{1}H-coupled CLIP-CLAP NMR experiments) to any of the 256 DFT models generated based on NOEs, J-couplings, and chemical shifts results in uniformly poor correlations with RMSDs of 10-13 Hz, whereas the experimental RDC precision is better than 0.5 Hz,

indicating that DFT models don't represent well the average solution conformations. We examine the capability of RDCs (together with standard NOE and J-coupling information) to provide the solution structures of complex synthetic and natural products with correct configurations of all their chiral centers using a modified XPLOR-NIH[4]protocol.

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P 208
NEW PYRROLO[1,2-a]QUINOXALIN-4-ONES

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Pyrrolo[1,2-a]quinoxaline skeleton is a constituent of several bioactive compounds that demonstrated anti-HIV and anticancer activities. These properties lead to a constant interest in developing more efficient ways for the synthesis of these heterocyclic systems.

We report here several new pyrrolo[1,2-a]quinoxaline derivatives obtained through a novel synthetic pathway. Thus, an one-pot three component reaction, starting from benzimidazoles unsubstituted at the five membered ring, alkyl bromoacetates and non-symmetrical electron-deficient alkynes in the molar ratio of 1:2:1, in 1,2-epoxybutane at reflux temperature, is leading to pyrrolo[1,2-a]quinoxalin-4-ones as solely reaction products.

The structures of newly synthesized pyrrolo[1,2-a]quinoxalin-4-ones were assigned by NMR spectroscopy. The ^{1}H, ^{13}C and ^{15}N NMR chemical shifts have been unambiguously assigned based on the following 2D NMR experiments: H,H-COSY, H,C-HSQC, H,C-HMBC, H,N-HMBC, H,H-NOESY. In the ^{1}H NMR spectra of pyrrolo[1,2-a]quinoxalines the protons from the phenyl ring and the annelated benzo ring are overlapping in the region of 7–8 ppm. Based on undecoupled H,C-HSQC spectra we assigned for the first time the individual aromatic signals, the multiplicity and the order of magnitude of the coupling constants for this class of compounds. The NMR spectra have been recorded on a Bruker Avance III 400 instrument operating at 400.1, 100.6 and 40.6 MHz for 1H, 13C, and 15N nuclei respectively. Samples were transferred in 5 mm Wilmad 507 NMR tubes and recorded with either a 5 mm multinuclear inverse detection z-gradient probe (1H spectra and all H,H/H,C/H,N 2D experiments) or with a 5 mm four

nuclei direct detection z-gradient probe for 13C spectra. Chemical shifts are reported in δ units (ppm) and were referenced to internal TMS for 1H nuclei, to the internal deuterated solvent for 13C nuclei (CDCl3 referenced at 77.0 ppm), and referenced to liquid ammonia (0.0 ppm) using nitromethane (380.2 ppm) as external standard for 15N nuclei.

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DECIPHERING MOLECULAR CHOREOGRAPHY OF TRANSITION METAL COMPLEXES IN SOLUTION: TOWARDS A BETTER UNDERSTANDING OF METALLO-ASSISTED CATALYSIS.

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The economic and ecologic context requires the development of new eco-compatible and cheaper chemical processes. Metallo-assisted catalysis appears to be a new domain in agreement with a lot of principles of a more and more virtuous chemistry [1].

In this field, the installation of a pendant methylamine arm at pyridine rings generates an ideal 1,2-N,N-bidentate ligand and confers to the so-called pyridylmethylamine (pma) scaffold interesting complexation properties. Such motif can be encountered in numerous bio-inspired metal-based coordination compounds. Recently, the combination of pma and palladium (II) has revealed especial value as catalytic systems in eco-friendly synthetic transformations [2].

Deciphering the complete molecular choreography of a pma-Benzylic (Bn)-Zinc (II) pre-catalyst involved in the Soai asymmetric reduction [3] is a challenge. Toward this aim, four stable conformations have been detected by EXSY experiments [4, 5] and exchange rates translated into free energy values. They are in agreement with the energetics of the molecular choreography described with DFT Intrinsic Reaction Coordinates data [6]. Moreover NOEs contacts deriving from the relaxation / exchange matrix have been extracted and allowed accessing structural constraints of all pma-Bn-Zn(II) conformers with a hydrated solvent.

Predicted chemical shifts and scalar couplings computed over each optimized solvent-dependent electronic structures related to stable conformers in solution were essential to disentangle the experimental NMR data. The latter were easily extracted from new fully resolved experiments able to generate sensitive and highly resolved signals [7] easily analyzable as the weighted sum of all stable conformers.

For the first time, the role of the amount of water on conformational equilibria of pma-Bn-Zn(II) families has been elucidated by the coupled NMR / DFT methodology [8] and could be used to orient the formation of products. New sensitive and highly resolved NMR techniques in combination with high level ab-initio calculations open the avenue for most complicated multi-catalytic systems involving several metallic entities looking forward the synthesis of one-pot valuable products of interest.

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APPLICATION OF HPLC-BPSU/NMR AND HPLC-SPE/NMR IN THE CHARACTERIZATION OF Euterpe Oleracea Mart. CONSTITUENTS.

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The isolation and structural elucidation of natural products continue to be challenging and a time-consuming task, since there is considerable effort to isolate each compound in a pure form, even the known ones. Furthermore, to obtain the required milligram quantities of all metabolites, even in the case of minor substances, large amounts of sometimes rare biological material and of expensive tools and supplies, like adsorbents and eluents, may be required. It is widely recognized that the LC-NMR coupled technology is an important tool in the natural products, pharmaceutical and organic synthesis research. This hyphenated technique has received considerable attention over the last three decades and has now been established as a powerful tool in analytical separations with a large variety of available operation modes, which include LC-BPSU-NMR and LC-SPE-NMR. Although the last one is the operation mode that is most applied to a very large variety of cases, it cannot be used in all situations. The flow system in LC-SPE-NMR is directed to the lines containing the cartridges for the compounds of interest according to the UV absorbance in the chromatogram. It is common to have samples with poor signal-to-noise ratio when working with this methodology since intensity of the UV absorption is not directly related to the compounds concentration and it can be very difficult to predict what the most efficient stationary phase for the retention of unknown compounds is. In the present work, a different approach to deal with the aforementioned situation was proposed by working with a combination of the LC-BPSU/NMR and LC-SPE/NMR systems in the analysis of Euterpe Oleracea Mart. fruit methanolic extract. The analyses were completely conducted in a HPLC (Agilent 1200 series) coupled to the BPSU (Bruker Peak Sampling 36/2) and the Prospekt 2 system with the use of a cryogenic NMR probehead (Bruker Avance III instrument, 14.1 Tesla/600 MHz, equipped with a TCI 5 mm triple

resonance ($^{1}H/^{13}C/^{15}N$) z-field gradient cryo-probe. After the optimization of the chromatographic parameters, the most intense peaks were first directed to the BPSU unit and analyzed by ^{1}H NMR spectroscopy. Based on the response concerning the compound class and the concentration of each compound in the extract, eight (8) fractions were selected to be absorbed by the Strong Hydrofobic (SH) resin while nine (9) fractions were directed to the General Phase polidivinylbenzen resin (GP). The signal-to-noise ratio was sufficient to the complete structural determination of these constituents and it shows that this methodology is a good alternative to the analysis of extracts containing a large variety of anthocyanins and flavonoids, which may not always have their UV absorption associated with their concentration.

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APPLICATION OF HPLC-SPE/NMR IN TIMBER WASTE AS A TOOL FOR THE RAPID CHARACTERIZATION OF ITS CHEMICAL PROFILE

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Some segments of the forest sector, government agencies and research groups are currently looking for ways to use timber waste. The Brazilian amazon rainforest is one of the regions that produce most of the tropical wood in the world and it moves about 5 billion BRL a year. The problem is that more than half of the timber becomes waste, which is burned or even discarded as trash. Some measures have been taken to minimize the disposal, such as the project developed at the Instituto Nacional de Pesquisas da Amazônia (INPA), in which young scientists use timber waste to make small objects, adding commercial value to this material. The production of small objects also generates residues, which have been analyzed through chemical studies of secondary metabolites. Therefore, the objective of this work was to use the HPLC-SPE/NMR hyphenated technique in the rapid identification and characterization of the largest possible number of chemical constituents of timber residues of Licaria airtu (Lauracea). The analyses were completely conducted in a HPLC (Agilent 1200 series) coupled to the Prospekt 2 unit (Automatic Cartridges Exchanger) with the use of a cryogenic NMR probehead (Bruker Avance III instrument, 14.1 Tesla/600 MHz, equipped with an automatic sample changer and a TCI 5 mm triple resonance ($^{1}H/^{13}C/^{15}N$) z-field gradient cryo-probe. After the optimization of chromatographic parameters, seventeen (17) UV absorption peaks in a chromatographic run were selected for the analysis. The compounds corresponding to these peaks were directed to SPE cartridges, stationary phase of polidivinylbenzen, during fifteen (15) consecutive runs. All the compounds had their chemical structures determined based on NMR and HRMS data. The high selectivity achieved in the chromatographic separation, together with an adequate SPE retention and the use of cryogenic probe in a

magnetic field of 14.1 Tesla, allowed for the obtaining of spectra with excellent quality and high sensitivity. According to literature review, seven (7) of the seventeen (17) identified compounds are unpublished. Among them, three (3) essential oils, one (1) sesquiterpene and thirteen (13) neolignans were identified. Since there are several reports in the literature describing the synthesis of neolignans due to their interesting pharmacological profile, this work shows that the timber waste of Licaria aritu could represent an important source of phytotherapeutic compounds, which have been taken for granted.

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CONFORMATIONAL ANALYSIS OF AN ANTIBIOTIC CYCLODEPSIPEPTIDE

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Griselimycin[1],[2] as well as Methylgriselimycin are cyclic depsipeptides which are composed of ten amino acids. The structural difference between these two peptides is the presence of L-trans-4-methylproline (Methylgriselimycin) instead of L-proline (Griselimycin) at position 8 in the amino acid sequence. This tiny variation involves some major anti-bacterial advantages over Griselimycin. To get a deeper insight in the structure-activity relationship we determined the conformations of Methylgriselimycin in $CDCl_3$ using nuclear Overhauser effect (NOE) distance measurements. Furthermore we prepared anisotropic NMR samples to get access to residual dipolar couplings (RDC) which contain in contrast to NOE data alone "global" structural information of the aligned molecule. The obtained RDC constraints are used in a subsequent refinement process of the previously NOE-derived structural ensemble.[3],[4],[5]

Primary structure of Methylgriselimycin:

Acetyl-O MeVal[1] Me_{4t}Pro[2] MeThr[3] Leu[4] Me_{4t}Pro[5] Leu[6] MeVal[7]

| |

-O Gly[10] Me-D-Leu[9] Me_{4t}Pro[8]-

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ULTRA HIGH-RESOLUTION HSQC: APPLICATION TO THE EFFICIENT AND ACCURATE MEASUREMENT OF HETERONUCLEAR COUPLING CONSTANTS.

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Digital resolution and signal resolution are two important concepts in multidimensional NMR spectroscopy. One of the more critical parameters defining the total acquisition time of a 2D NMR experiment is the number of variable t_1 evolution times required to achieve a satisfactory digital resolution in its indirect F1 dimension. In this study, the success in implementing spectral aliasing along the indirect F1 dimension of HSQC experiments[1-3] is demonstrated by the easy measurement of heteronuclear coupling constants from the indirect dimension of 2D HSQC spectra, without any significant increase of the experimental time.

It is also shown that the gains of introducing aliasing are further improved with the large signal resolution achieved by the collapse of the J(HH) multiplet structure by broadband ^{1}H homodecoupling in the F2 dimension.[4-5] The resulting 2D cross-peaks exhibit ultra simplified multiplet patterns from which the measurement of the active J values is determined in a straightforward manner. Experimental data will be provided for the simultaneous determination of the magnitude and the sign of J(CX) and J(HX) coupling constants (X = ^{19}F, ^{31}P or ^{2}H).[6]

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CHARACTERIZATION OF INTRAMOLECULAR HYDROGEN BOND OF β-DIKETONES BY NMR ISOTOPIC PERTURBATION METHODOLOGY

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β-Diketones are known to exist as two fast interchanging enolic tautomers (stabilized by intramolecular hydrogen bonding) through slow keto-enol tautomerism. The NMR spectra of the cis-enolic tautomer are the weighted average of the two forms. Therefore, both symmetric and non-symmetric cis-enolic forms give only one resonance for each type of nuclei, which makes it difficult to distinguish between these two forms.

To study the enol-enol tautomerism of the cis enol form of non-symmetric 1-phenyl-3-(3,5-dimethoxyphenyl)-propane-1,3-dione (model compound) and the ortho-dibromo derivative, the deuterium isotope effects on ^{1}H and ^{13}C chemical shifts of these compounds were measured. It is known that isotope effects on chemical shifts may occur in different ways: as more common the intrinsic effect or as perturbation of equilibrium.[1]

During deuteriation of the model compound, the methine proton and hydroxyl proton were exchanged to get monodeutero products. From the variable-temperature ^{1}H NMR spectra of the products we observed the coalescence temperature was20 °C. Additional information of the equilibrium was obtained from ^{13}C NMR spectra at low temperature. Eight signals in carbonyl carbon region were detected, which belonged to the enolic forms of two monodeutero products. All these NMR data of equilibrium deuterium isotope effects indicated that the intramolecular hydrogen bond was in the double-well potential type with one minimum of lower energy than the other.

The introduction of a steric group (bromine atom) at the ortho positions of the methoxy substituted phenyl ring changed the potential energy curve of the molecule, which was caused by the change of the electron density in the entire molecule. Contrary to the model compound, parent dibromo derivative that exists in only one localized cis-enolic tautomer exhibits intrinsic secondary deuterium isotope effect on ^{13}C chemical shifts.

This is a clear indication of two completely different types of intramolecular hydrogen bond potentials present in β-diketones.

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P 232
ECOMETABOLOMIC STUDY OF PLANT SHOOTS/ROOTS RESPONSES TO DROUGHT

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Ecometabolomics aims to analyse the metabolome of the plant, i.e. the total number of metabolites, and their shifts in response to environmental changes [1,2]. Ecometabolomics has recently been used to monitor the phenotypic changes of a particular genotype in response to the drivers of global change, particularly shifts in temperature [4,3,6]. When a stress treatment - or different stresses occurring simultaneously like in real field conditions - is applied to a plant, plant organs can respond differently. Shoots are autotrophic and roots heterotrophic organs of plants with different physiological functions. Roots and shoots can respond asymmetrically, as has been observed at the morphological level, e.g. shifts in the shoot/root biomass and growth-rate ratios occur when the availability of soil water changes [7].

In this work NMR- and LC-MS-based ecometabolomics [8] together with stoichiometric analysis were used to study and compare the metabolism of plant shoots and roots and their different responses to drought. The study was performed with two common grass species (Holcus lanatus L. and Alopecurus pratensis L.) ^{1}H NMR fingerprinting

of all 400 individuals and metabolic profiling – based on the assignment of peaks through 2D NMR spectroscopy – was carried out. Results pinpointed the differences between shoot and root metabolomes and stoichiometries. Principal component analyses (PCAs) of both metabolomic and stoichiometric data indicates the highest variability among species and seasons of shoot with respect to root metabolome. These results were confirmed by PERMANOVA analysis. In addition, the metabolic response of shoots to drought contrasts with that of roots; shoots decrease their growth metabolism (lower concentrations of sugars, amino acids, nucleosides, N, P, and K), while roots increase it in a mirrored response. Shoots are metabolically deactivated during drought to reduce the consumption of water and nutrients, whereas roots are metabolically activated to enhance the uptake of water and nutrients, buffering together the effects of drought, at least at the short term. The study shows the suitability of the combination of NMR- and LC-MS-based metabolomics.

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P 235

CROSS-LINKED POLY(ETHYLENE GLYCOL) DIACRYLATE – A UNIVERSAL ALIGNMENT MEDIUM FOR THE MEASUREMENT OF RESIDUAL DIPOLAR COUPLINGS

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The measurement of residual dipolar couplings (RDCs) requires an appropriate weak partial alignment in a so-called alignment medium. For small molecules two types of alignment media are commonly used to align solutes: liquid crystalline phases and stretched polymer gels. A major drawback of both media is the limited applicability to various solvents. However, previous work with cross-linked poly(ethylene glycol) gels (PEG) has shown that a large range of solvents can be covered with this polymer network[1]. Corresponding gels are formed from linear PEG either by irradiation with γ-rays and accelerated electrons, or by derivatization of their terminal hydroxyl groups followed by chemical cross-linking.

Here, we show that the bisacrylated derivative, poly(ethylene glycol) diacrylate (PEG-DA), can easily be cross-linked to yield homogeneous gels. These are equally applicable in a stretching[2] or compressing apparatus[3] or by direct swelling in NMR tubes[4] to provide uniaxially anisotropic phases. Due to their broad compatibility to solvents and their inherent homogeneity, PEG-DA gels allow extraction of RDCs with small line widths for almost any class of small organic molecules.

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P 238
RAPID IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES BY MULTINUCLEAR NMR SPECTROSCOPY

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New psychoactive substances (NPS), including cannabino-mimetics eg **1**, cathinones eg **2** and phenethylamines eg **3**, are currently exhibiting a surge in variety and popularity, in terms of production, availability and recreational use. This is in large part due to the ambiguities inherent in the existing legislation, which render the legality of such analogues uncertain, leaving the door open for unscrupulous importers to make these drugs available on the Australian market. There is an urgent need for rapid screening methods to be available to frontier bodies such as the Australian Customs and Border Protection Service and the Australian Federal Police for the classification and, ultimately, identification of these NPSs, knowledge which would undoubtedly be useful to Government agencies in other countries.

The NPS **1**, **2** or **3** may take the form powders of high purity, or incorporated in vegetative matter, or as paper tabs. Extraction and direct ^{1}H, ^{13}C and ^{19}F NMR analysis of the extract has proven to be most effective for determining the nature and full structural features of the actives. More recent work has demonstrated the practicality of indirectly-detected ^{15}N experiments, viz. ^{1}H-^{15}N HSQC and HMBC experiments, to resolve the nitrogen environments often present in these substances.

Overall, this approach serves as excellent indicator of the particular class of NPS to which a suspect compound belongs, and shows great promise as a rapid and effective first step in the identification of these quickly evolving compounds, without having to rely on the availability of analytical standards of the NPS.

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NEW INSIGHTS ON FAST-FIELD-CYCLING NMR ELECTROMAGNETS

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Field-cycling NMR instruments aimed for the fast switching of the magnetic field were mainly based on air-cored electromagnets (EM) [1-4]. The general performance of these instruments strongly depends on the EM design and the associated power management & control strategy. Typical technical requirements for this kind of EM are high field-to-power ratio (Fabry-factor), adequate electric performance for fast-switching of the field and NMR-compatible field homogeneity within the sample volume. At first glance, any improvement for EM technology should consider these requirements. However, reducing the thermo-mechanical stress of the magnet assembly, simplifications in the design and robustness are also considerable. We will describe the problem in general and show details on a recent prototype of an a-helical-cut notch–coil geometry with movable pieces having tunable homogeneity and uniformly distributed power dissipation. A more realistic model was used for the optimization process by considering a magnet geometry with broken azimuthal symmetry [5]. This work is the first attempt for an autoadaptive (electronically controlled) magnet system for fast-field-cycling NMR.

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ECCS FOR DOSY NMR AS VALUABLE TOOL IN UNDERSTANDING AGGREGATION OF GRIGNARD COMPOUNDS AND ALKALI CYCLOPENTADIENES

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Aggregate formation in solution determines the reactivity and reaction mechanism of many organometallic compounds (e.g. Grignard compounds or alkali cyclopentadienes).

Williard et al. introduced a method to predict molecular weights (MW) from diffusion coefficients via power law using Diffusion ordered spectroscopy (DOSY) which has been used frequently to correlate these weights to discrete aggregates.[1,2] In those studies at least three internal references per sample were needed. Recently our group has extended this ^{1}H-DOSY-NMR technique using external calibration curves (ECCs).[3] With this new method only one reference is needed to determine the MW of an unknown compound by fitting the recorded logarithmic diffusion coefficient to a prerecorded external calibration curve. These calibration curves have to be optimized for different solvents and solute geometries. By normalizing the diffusion coefficients of references and analytes they can be compared even when using different NMR devices or measuring at different temperatures. For toluene-d_8 and THF-d_8 calibration curves exist for compact spheres (cs), expanded discs (ed) and dissipated spheres and ellipsoids (dse).[3] We present a new calibration curve for chainlike structures to best describe alkyl Grignard compounds in THF-d_8.

We employed this method to determine the aggregation of the aforementioned Grignard compounds (Ethyl-MgCl, Butyl-MgCl, Hexyl-MgCl, Octyl-MgCl, Decyl-MgCl, Butyl-MgBr) and alkalimetallocenes (CpLi, CpNa, CpK, CpRb, CpCs) in THF-d_8. At room temperature all

alkyl Grignard compounds form monomeric species with two coordinating THF molecules ([RMgCl · 2THF]). Alkali cyclopentadienes (fitted to dse calibration curve) mostly form monomeric species in THF-d_8, except for CpCs, which turned out to be an aggregate with a MW of >1000 g/mol. The solvation number varied: CpLi/Rb are coordinated by two THF molecules whereas CpNa/K are solvated by three THF molecules. We are currently working on a calibration curve for DMSO-d_6 and investigating the interesting aggregate of CpCs forming in THF-d_8 more closely.

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A J-MULTIPLIED GSERF AND GETSERF EXPERIMENTS FOR MEASURING SMALL nJH-H AND nJF-H SCALAR COUPLING

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Fluorinated organic compounds have become increasingly important in the field of polymers, in pharmaceutical industry and for clinical applications [1]. To determine the structure of these compounds, triple resonance NMR experiments {1H, 13C, 19F} are highly important due to the favourable NMR properties of proton and fluorine nuclei and the relationship between the three-dimensional structure and homo- and hetero-nuclear scalar coupling [2,3]. As part of this work, in addition to the use of the most advanced technologies in 1D and 2D NMR for the measurement of homo- and hetero-nuclear scalar coupling [4-7], we proposed two new pulse sequences MJ-GSERF and MJ-GETSERF for measuring the weak homo- and hetero-nuclear coupling constants which has not been so far possible to measure. We used this series of experiments to determine the structure of two fluorinated compounds with rigid structures and we have shown that the range of experiments presented helps to simplify the interpretation of the most complex NMR spectra as well as open up alternative methods for determining all the homo- and hetero-nuclear couplings in small fluorinated molecules.

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P 253
EXPLORING THE LIMITS OF THE ENOE: PROBING THE WW-DOMAIN STRUCTURE

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Exact distance restraints (eNOEs) [1,2] are obtained by recording series of NOESY spectra at different mixing times and correcting the obtained NOE buildup rate for spin diffusion errors [3]. Using exact NOEs (eNOEs), a structure calculation protocol based on multi-state ensembles is reflective of the occupied conformational space [4-6] and reveals concerted conformational exchange on an atom per atom basis.

Here we present an analysis of the error of the extracted NOE buildup rate due to broken spectral symmetry if only one of the two symmetry-related cross peaks in a NOESY spectrum is available. This is in practice the case for a significant fraction of NOEs. The choice of a correct tolerance for errors is particularly important for multiple-state ensemble calculation, since otherwise incorrect spatial sampling may result. Our analysis yields a similar error tolerance when normalizing the cross peak intensities to the intensities of the destination spin rather than the origin spin of magnetization. This finding allows for an extended set of unidirectional eNOEs, which offers the prospect of eNOE analysis of more challenging systems.

WW domain folds into one of the smallest β-sheet structures known. The domain unfolds and refolds reversibly both thermally and chemically [7] with very fast folding kinetics in the two-state limit. With the developments in eNOE techniques described above, we have an enlarged set of tools available to dissect the dynamics and long-range correlations of the WW domain structure at 5C. In the process we analyze the conformational network in the calculated multi-state ensembles with tools of the trade such as cross-correlated relaxation rates, multiple simultaneous super-positions with statistical analysis

calculation of backbone correlation (Theseus [8]) and principal component analysis (PCA).

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THE INSTRUMENT SET FOR GENERATING FAST ADIABATIC PASSAGE

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The design and construction of a high-performance, low-cost, and easy to assemble adiabatic extension set for homebuilt and commercial spectrometers is described. Described apparatus set was designed for the fast adiabatic passage generation and is based on direct digital synthesizer DDS. This solution gives the generator high signal to noise ratio and phase stability even during frequency change, which is only possible in expansive commercial high-end hardware. Critical synchronization and timing issues are considered and solutions are discussed. Different experimental conditions and techniques for the measurements are briefly discussed. The proposed system is very flexible and might be used for the measurement of low-frequency nuclear magnetic resonance [1].

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P 259
TOWARD EFFICIENT SPIN NOISE DETECTED 2D NMR

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Recent NMR methods, which exploit transverse nuclear spin noise [1], have been improved for efficient detection and processing. Noise detected NMR can be superior over classical thermal polarization based detection for minute sample amounts (≤ 10^8 spins) [2]. This corresponds to the nano-liter nano-molar regime. With current commercial hardware, experiments with such low spin numbers are not possible. Hence large spin numbers are used (0.6M ^{13}C-labelled glucose) to develop noise detection based experiments, which bear the potential of enhanced sensitivity, once applicable to low spin numbers. We recently achieved a first proof of principle for transverse spin noise detected 2D NMR spectroscopy [1] by implementing a strategy based on the principle of CONQUEST [3] introduced in the context of MRFM. [4]This was a major breakthrough as this experiment is ca. 10^{16} times less sensitive compared to conventional pulse NMR. In spin noise detected spectroscopy, instead of detecting longitudinal magnetization fluctuations, which is normally practiced in MRFM, rf-detection of transverse spin noise was employed. We have now improved the acquisition and data processing scheme to accelerate the development of new techniques. The pulse sequence is derived from the constant time-HMQC experiment without any ^{1}H pulses. Two^{1}H-noise direct-detection periods with ^{13}C decoupling bracket the mixing and evolution periods containing^{13}C pulses. The noise blocks acquired during the acquisition periods are cross-correlated to extract the coherent response. In the new implementation we make use of the T1-independence of spin noise and repeat the experiment head-to-tail without recovery delays and can use a noise block twice as a reference and as a detection block. After summing up all the correlations, a single noise block is generated for each t_1-value. The

double usage of the noise blocks increases S/N theoretically by a factor √2. In addition, the fast repetitions highly increase the time-efficiency. Based on this progress and new processing software, development of quadrature detection and phase cycling in spin noise detected NMR has started. The same basic principles may be employed for spin noise detected multi-dimensional spectra as well as pulse-free Fourier MRI [5].

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ADVANTAGES OF THE ROTATED AND MODULATED MAGNETIC FIELD GRADIENT IN 2D SPATIAL AND SPECTRAL-SPATIAL EPR IMAGING

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Methods for fast 2D spectral and spectral-spatial electron paramagnetic resonance imaging (EPRI) are presented. The aim of the measurements was to reduce acquisition time of the sinogram by combining rapid scan of Zeeman magnetic field using high frequency sinusoidal modulation with simultaneously applied magnetic field gradient, which orientation or amplitude are changed at low frequency. The correctness of the method is confirmed by studies carried out on a phantom consisting of two LiPc samples. The images from the acquired data are reconstructed using iterative algorithms. The proposed methods allows to reduce the image acquisition time up to 10 ms for 2D spatial [1], and 200ms for 2D spectral-spatial EPRI [2]. The linewidths obtained from 2D spectra-spatial imaging and phantom shapes obtained from 2D spatial imaging were in excellent agreement with the values expected for the phantoms used.

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DYNAMIC NUS TO MONITOR MAGNETIZATION TRANSFER

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Time-resolved non-uniform sampling (TS-NUS) has been previously shown as a method to monitor chemical reactions on the fly.[1,2,3] The idea behind (TS-NUS) is to apply Compressed Sensing to overlapping subsets of the NUS dataset collected using dynamically changing sample. In the resulting set of 2D spectra, time acts as the third pseudo-dimension.This enables one to monitor the changes occurring in a sample with time. In this project we have further extended the idea by implementing it in a NOESY pulse sequence to monitor the change of magnetization transfer with varying mixing time. Mixing time in the pulse sequence is incremented linearly and in parallel to the random sampling of the FID in indirect t1 dimension. Subsets of these NUS measurements are formed and several spectra corresponding to these subsets are reconstructed using CS. This stack of spectra enables one to plot the NOE build-up curve in a continuous manner and effectively calculate the interproton distances. The advantage of the method is the reduced time required to acquire TS-NUS experiment in comparison to several short conventional NOESY experiments. Application of TS-NUS in a spectrum with high dynamic range of intensities required special optimizations of the sampling schedule and a pulse sequence in order to minimize t1-noise. Both method and its optimizations are discussed on the example of the rigid organic molecule strychnine. The resulting interproton distance values were found to be remarkably close to literature [4].

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P 268
PULSE SEQUENCE TOOLS FOR BRUKER NMR SPECTROMETERS

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We present an original approach for the setup and sharing of complex NMR experiments on Bruker spectrometers. Each experiment consists in a python setup script and a Bruker pulse sequence program. In particular, shaped pulse parameters are computed on the fly within the pulse sequence from user defined input values for the desired excitation band. The different experiments are accessible from the TopSpin menu bar via a series of "IBS templates" buttons. Clicking a particular button executes the corresponding Python script and sets up the experimental parameters of the chosen experiment.

This approach makes it possible for a non hard-core NMR spectroscopist to run complex NMR experiments at optimal performance without particular knowledge about the experimental details. In addition, the library, consisting in Python scripts, pulse sequence programs, and pulse shapes can be centralized on a single NMR machine or an external computer disk that is accessible to all NMR spectrometers, ensuring easy maintenance and further development. As a consequence, these tools can be easily exchanged between different spectrometers in the same lab, as well as with other NMR laboratories around the world. Our tools are fully compatible with the Bruker sequence library using prosol relation files and parameters.

Currently, the IBS library currently contains more than 40 NMR experiments for protein applications, including the whole series of SOFAST, BEST-HSQC, BEST-TROSY and HADAMAC experiments developed at IBS over the last decade. The pulse sequences have also been optimized for application at high magnetic field spectrometers using broadband RF pulses on the 15N channel. Signal improvements of up to 50% are observed for certain correlation peaks at 950 MHz.

Moreover the IBS experiment library contains many useful additional tools for advanced data processing, setup of composite decoupling, … . It also includes a Python script allowing the user to create and add its own favorite experiments to the library by creating the corresponding Python setup scripts.

The complete library has been developed and tested on Bruker Advance III HD spectrometers running TopSpin version 3.2. The IBS library is freely available for academic users from the IBS web page (http://www.ibs.fr/science-213/scientific-output/software/pulse-sequence-tools/)

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AN OPTIMISED DOUBLE STRIPLINE TRANSMISSION LINE DETECTOR COMPATIBLE WITH MICROFLUIDIC LAB-ON-A-CHIP DEVICES

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In order to enable NMR observation of biological processes on microfluidic lab-on-a-chip devices, a parallel-plate transmission line detector that accommodates microfluidic devices as sample holders has been developed. A method of simulation has been developed to optimise the design of this detector using finite element method simulations in combination with bench top experiments to maximise sensitivity.

Stripline detector geometries have been shown to provide good rf-homogeneity, sensitivity and spectral resolution.[1] In addition to this a design using two parallel stripline conductors has been used for Magnetic Resonance Imaging.[2] This design is similar but a microfluidic chip, which can be easily removed and replaced, separates the two plates. The conductors are connected so the current passing through them is antiparallel. The constriction in the centre of the transmission line creates a region of high current density, concentrating the magnetic flux density and focussing the sensitivity on the sample area. The detector is attached vertically to the probe, parallel with the B_0 magnetic field, minimising susceptibility broadening effects. It creates a homogeneous B_1 field in the plane of the chip, perpendicular to the static magnetic field.

The transmission line probe was fabricated from copper-laminated polyimide sheet material with a copper thickness of 18 µm, glued to a glass support. Microfluidic chips are manufactured by removing a channel from 0.2 mm thick PMMA with a laser cutter and sandwiching between two 0.5 mm PMMA panels using adhesive tape.

Initial experiments were conducted using a 20 x 1 mm constriction, giving a sample volume of 4 µL. This design yielded a sensitivity of 10.6 nmol $s^{1/2}$ enabling metabolite concentrations of 0.1 mM from MCF-7 cells seeded in the chips to be observed in a 20 minute experiment time. Due to the non-destructive nature of NMR these cells could be studied over 24 hours in hypoxic conditions with the changes in metabolite concentration monitored over this time.

Finite element method simulations were used to simulate the resistive and dielectric losses in order to quantify the sensitivity of the detector. Current is restricted to the surface of the detector as an approximation of the skin depth. Solutions to the electronic wave equation are used to assess the electromagnetic properties of the detector including the eigenfrequency, Q factor and magnetic field distribution. The geometry of the detector was then optimised, enabling smaller concentrations of metabolites to be observed. The simulations are validated by comparing resonance frequencies and Q factors of resonators of varying geometry with experimental values.

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P 274
PSYCHE NMR: PURE SHIFT YIELDED BY CHIRP EXCITATION

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Resolution and sensitivity are longstanding challenges in NMR spectroscopy. In the case of high-resolution ^{1}H NMR spectroscopy, a ubiquitous tool for chemical structural elucidation, a major limitation is the presence of homonuclear scalar couplings between protons, which split signals into multiplets. Because of the narrow range of proton chemical shifts, multiplet overlap is very common and can severely complicate the analysis and assignment of spectra. In the last decade, "pure shift" NMR, in which the multiplet structure of signals due to homonuclear coupling is collapsed to singlets, greatly improving resolution, has become a practical tool. Such experiments generally use a pulse sequence element that selectively refocuses the effect of evolution under homonuclear couplings.

Very recently, a new pure shift pulse sequence element, Pure Shift Yielded by Chirp Excitation (PSYCHE), has been introduced which relies on statistical separation of spin populations by the use of low flip angle (β) swept-frequency chirp pulses. PSYCHE offers a significant improvement in performance over existing methods, in terms both of sensitivity and of tolerance of strong coupling, as demonstrated for 1D ^{1}H NMR [1] and 2D ^{1}H-^{1}H TOCSY [2] experiments. The theory, practical aspects, and some recent developments of the PSYCHE method will be discussed, showing its potential to find wide application in many areas of NMR including small molecules, metabolomics and biomolecular applications.

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P 277
MAGNETIC RESONANCE PORE IMAGING AS A TOOL IN POROUS MEDIA RESEARCH

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Imaging of the micro-structure of porous media such as biological tissue or porous solids is of high interest in health science and technology, engineering and material science. Various Nuclear Magnetic Resonance (NMR) techniques are applied to study porous media on different length scales. Among them diffusive-diffraction Pulsed Gradient Spin Echo (PGSE) NMR [1] is able to resolve features of the pore space on the micrometer scale. However, this technique only measures the modulus square of the structure factor (a shortcoming it shares with other widely used diffraction methods such as x-ray), which leaves the exact pore structure obscured due to the lack of phase information.

Magnetic Resonance Pore Imaging (MRPI) on the other hand is a recent technique based on diffusive-diffractive PGSE NMR [2] which allows one to preserve the phase information and therefore to acquire images of the average pore shape in a given sample [3]. We will discuss selected details on the experimental design, challenges and requirements of MRPI including its calibration procedures. We will report that the method is indeed able to acquire the diffractive signal including its phase which allows the direct image reconstruction of the pore space, averaged over all pores. We furthermore demonstrate that this approach may combine the advantages of MRI, namely its robust and straightforward image reconstruction via Fourier Transformation with the much improved spatial resolution of Pulsed Gradient Spin Echo (PGSE) NMR.

The method was successfully applied to various samples such as cylindrical and triangular micro capillaries. Images of these closed pore systems have been obtained even in the presence of NMR relaxation effects at the pore walls [4]. We therefore show that MRPI is applicable to porous samples without a priori knowledge about their pore shape and symmetry. Furthermore, we introduce "MRPI mapping" which combines MRPI with conventional magnetic resonance imaging (MRI). This enables one to resolve microscopic pore sizes and shapes spatially, thus expanding the application of MRPI to samples with heterogeneous distributions of pores.

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P 280
IMPROVED ELECTROPHORETIC NMR – A SYSTEMATIC STUDY COMPARING IONIC TRANSFERENCE NUMBERS IN IONIC LIQUIDS

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In the last years the characterization of Ionic Liquids (IL) has become very important for potential application in energy storage devices [1]. Therefore, diffusion, conductivity and viscosity are often measured to investigate the intermolecular interaction inside of IL. Nevertheless, these methods can not identify the conductivity contribution of a specific ion species. To overcome this lack of information, the electrophoretic mobility μ has to be known. With this information, the amount of current which is carried by one ion species can be determined and transference numbers can be calculated. Electrophoretic NMR (eNMR) is one of the few methods to measure the electrophoretic mobility μ of non-metal ions. During a diffusion experiment (Pulsed Field Gradient (PFG)-NMR)) an electric voltage is applied and the electrophoretic mobility of one ion species can be determined by observing one specific nucleus (e.g. ^{1}H, ^{7}Li, ^{19}F) of the ion [2]. The investigation of IL is difficult due to convection caused by resistive heating occurring in the sample [3]. However, so far for two IL mobilities in the range of 10^{-9} m^2/Vs could be determined [4].

Here we employed a self-built eNMR electrode configuration suitable for highly conductive samples (10^{-3} S/cm) for 5 mm NMR-tubes and a pulse generator (max. voltage 1kV at 50 mA). With our eNMR setup we show a systematic mobility study of seven different IL in the range down to 10^{-10} m^2/Vs for the first time. The electrophoretic mobilities depend strongly on the cation and anion structure and increase for the cation type with shorter alkyl chainlength and increasing delocalization of the positive charge, which weakens the ion-ion interaction in the IL. The transference numbers of cation and anion in each of the IL indicates that each ion carries at least one-third of the current. The comparison of the electrophoretic mobilities with self-diffusion coefficients provides insights in the intermolecular dynamics of the IL.

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AN ARRAY OF ACTIVE TX/RX 19F NMR FIELD PROBES FOR GRADIENT TRAJECTORY MAPPING

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Increased static field strengths of MR scanners in combination with advanced pulse sequences such as EPI or spiral MRI enable imaging with increased resolution in shorter imaging times. However, those advancements impose more stringent requirements on the gradient hardware which can currently not be met by existing instrumentation thus leading to image artifacts. To mitigate this problem, we present an active NMR sensor array which can be used for a real-time monitoring of the gradient trajectories during MR imaging experiments in order to perform a post-correction of the distorted image data. By using active sensor nodes, we eliminate the need for any high-frequency cable connection to the sensor nodes and due to the autonomous TX/RX operation the NMR sample excitation is independent of the scanner's RF pulse. This both allows for an arbitrary pulse sequence selection and avoids blocking any channels of the MR scanner.

Each miniaturized sensor node consists of a probe head with a hexafluorobenzene sample within a solenoid TX/RX coil, which is in turn encapsulated in an ellipsoidal susceptibility-matched epoxy casing. The tuned probe heads are directly connected to the transceiver electronics which are integrated into each sensor node. These electronics comprise a frequency synthesizer, which generates the local oscillator (LO) signals for the downconversion of the detected signals and the RF excitation pulse, a low-noise amplifier and a quadrature demodulator in the RX path and a power amplifier in the TX path. Due to the local generation of the excitation pulse and the downconversion of the NMR signal, only low frequency signals have to be transmitted to and from the sensor nodes, greatly reducing

interferences between the trajectory mapping and the imaging experiment. The sensor nodes are connected via shielded twisted-pair cables to a signal-conditioning box which interfaces to a commercial ADC (NI PXIe-6368) with 2 MS/s and 16 bit per channel.

In the current implementation of the sensor array, the transceiver electronics are realized as PCB-prototypes using commercially available IC components. These sensor nodes cover a frequency range of 175-660 MHz corresponding to field strengths of 4.4-16.4 T for ^{19}F samples and have a noise figure of 2.7 dB for quadrature detection. They have been successfully characterized in a 9.4 T human scanner and an 11.7 T small-animal scanner where they displayed a frequency resolution better than 4 ppb. Furthermore, in these experiments, the sensors proved to be robust against gradient switching and recovered from the scanner RF pulse within a few microseconds. An array of four sensors has been successfully employed for the mapping of first-order gradient trajectories and is capable of measuring the unwrapped phase evolution for more than 100 ms with a single excitation. The number of sensor nodes can easily be scaled up to allow for a correction of higher-order spherical harmonics.

Currently, we are testing an ASIC version of the transceiver electronics which has a size of only 1 mm^2, reduces the power consumption by two orders of magnitude and increases the frequency range to 30-550 MHz.

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PARAMAGNETIC SHIMMING FOR WIDE-RANGE VARIABLE-FIELD NMR

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High-resolution NMR and MRI experiments require a strong, stable, and homogeneous magnetic field over the sample volume. For typical superconducting magnets used widely nowadays, field homogeneity is not satisfactory, so that shimming is essential for high-resolution measurements. Usually, shimming is performed in a fixed magnetic field. However, operation of the magnet over a wide range of magnetic fields is also of interest in NMR spectroscopy. In principle, the existing shimming schemes, electric (active) shimming and ferromagnetic (passive) shimming, would work by changing the shim currents or configuration of the shim pieces every time the magnetic field is altered. However, this would be quite formidable and not practical. What is required is a new shimming strategy that is convenient and works over a wide range of magnetic fields. In this work, we present one such. Our approach is categorized into passive shimming, in the sense that no electric current is applied. Instead of the ferromagnetic steel pieces, we employ pieces of paramagnetic material. Here, we exploit one important property of the paramagnetic material; the magnetization and thereby the produced magnetic field are proportional to the main-field strength. It follows that, once field correction by paramagnetic shimming has been done for one given field strength, the identical shim configuration should also work at any other field strengths. In this work, we demonstrated field correction using pieces of Dy_2O_3 in a cryogen-free superconducting magnet, examining NMR spectra measured in different magnetic fields.

First, we obtained the field distribution in our magnet by monitoring the peak positions of ^1H NMR spectra measured using a microcoil attached to an xyz stage. Then, we calculated the shim configuration that compensates the field inhomogeneity, fabricated paramagnetic pellets, and placed them accordingly. Paramagnetic shimming was

demonstrated in ^{7}Li, ^{87}Rb, and ^{45}Sc NMR of a liquid solution sample in magnetic fields of 3.4, 4.0, and 5.4 T at a fixed carrier frequency of 56.0 MHz. The resonance lines of all spectra showed significant narrowing by the improved field homogeneity with an identical configuration of the paramagnetic shim pieces.

Paramagnetic shimming presented in this work opens the possibility of high-resolution variable-field NMR experiments.

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COMPARISON OF PELDOR AND RIDME FOR Fe(III)-NITROXIDE DISTANCE MEASUREMENTS

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Distance measurements by pulsed electron paramagnetic resonance (EPR) techniques are widely used to gain information about the structure and dynamics of proteins and oligonucleotides [1]. These measurements are usually carried out between two spin centers, which can either be an intrinsic paramagnetic center of a biomolecule or an artificially attached spin label. One of the intrinsic spin centers present in many proteins is the Fe^{3+} ion. Therefore, it is of interest to establish a procedure of distance measurements between the Fe^{3+} ion and a spin label. Here, we present such a procedure for the Fe^{3+}-nitroxide distance measurements in spin-labelled cytochrome P450cam. Two EPR methods, relaxation-induced dipolar modulation enhancement (RIDME [2]) and double electron-electron resonance (DEER [3]), are applied and compared to each other. A special emphasis is made on the optimization of the five-pulse RIDME experiment [4]. The parameters of the pulse sequence, such as the lengths and frequency of microwave pulses and the inter-pulse intervals, are varied in order to achieve a better RIDME signal. Several methods of suppression of ESEEM in the RIDME signal were tested and compared to each other. The effect of the protein buffer on the RIDME signal was also investigated.

Our experiments revealed several advantages of RIDME over PELDOR, when applied to the Fe^{3+}-nitroxide spin couple. The major advantage stems from the fact that the RIDME experiment allows avoiding orientation selectivity as compared to the PELDOR experiment. This makes the extraction of the distance distribution from the RIDME data more easy and reliable. Another benefit concerns the signal-to-noise ratio, which was found to be considerably higher for RIDME.

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TUNEABLE COMPLEX FORMATION IN A BIOMIMETIC MODEL SYSTEM – A PROOF-OF-PRINCIPLE STUDY FOR ASSESSING DIMERISATION BY PULSE EPR

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Pulsed electron-electron double resonance (PELDOR, also called double electron-electron resonance or DEER) is a spectroscopic technique for the reliable determination of inter-spin distances in the nanometre range (typically, between 2 and 8 nm).[1,2] In recent years, this method has become increasingly applied as complementary technique in structural biology,[3] specifically in combination with site-directed spin-labelling in proteins.[4] Many proteins form dimers or even higher oligomers in their biologically active state. However, the application of PELDOR for a quantitative assessment of the dimer- or multimerisation process has so far been limited,[5] presumably due to the lack of benchmarking studies on appropriate model systems.[6]

Here, we demonstrate a biomimetic small-molecule model system for tuneable, templated dimerisation.[7] The model system is based on spin-labelled terpyridine (tpyNO) ligands and Zn(II) metal ions as the template for complex formation. Thus, the bis-complex $Zn(tpyNO)_2$ resembles the dimeric state, while the mono-complex and the tpyNO alone resemble the monomeric state in our dimerisation model. A nanomole-scale titration series was performed for assessing dimerisation degree and cooperativity by EPR, whereby the amount of ligand was kept constant and the metal/ligand ratio was varied from 0.0 to 1.0. PELDOR data were processed using DeerAnalysis2013.[8] The modulation depth gradually increased with the addition of Zn(II) until a metal/ligand ratio of 0.5, at which point each metal ion is present in the bis-complex, while further addition of Zn(II) led to a successive decrease in modulation depth. Quantitative

assessment of the observed changes suggested a non-cooperative binding of the Zn(II)-ligand complex.

The potential application of the quantitative assessment of the PELDOR modulation depth for dimer or multimer formation will be addressed with respect to two proteins currently under investigation, the bacterial surface protein M3, and the archaeal single-stranded DNA binding protein (SSB). We suggest that further studies on homologous synthetic model systems will ultimately improve our understanding of the PELDOR characteristics of dimer-forming proteins.

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PULSED EPR DIPOLAR SPECTROSCOPY ON A TRITYL BIRADICAL

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Pulsed EPR dipolar spectroscopy[1-4] is a valuable technique for the precise determination of distances between paramagnetic centres in the range of 1.5-8 nm.[5] Determination of such distances elucidates the conformational dynamics and folding of biomolecules. Nitroxide spin species attached via site-specific spin labelling[6,7] are mostly used for this kind of measurements. Recently, another type of organic radicals, triarylmethyl[8] or shortly called trityl, has been used as alternative spin marker for dipolar spectroscopy.[9-12] These paramagnetic centres exhibit longer electron relaxation times and reveal higher stability toward redox processes under in-vivo conditions compared to nitroxides. Despite the advantages of trityls, only a few examples for their applications on biological systems[9,10] and fundamental studies[11,12] with dipolar spectroscopy have been presented so far.

In this work we studied a trityl model compound by dipolar spectroscopy at Q - (33.8 GHz) and G - (180 GHz) band frequencies. The trityl EPR spectrum obtained at Q-band frequencies revealed a narrow spectral width of about 30 MHz. Hence, the usage of single-frequency dipolar spectroscopy techniques is beneficial. SIFTER[2] and DQC[3] experiments were carried out and the performance of these experiments was compared with each other. The distances extracted from the dipolar evolution functions using Tikhonov regularization[13] are in agreement with literature.[11,12] The trityl EPR spectrum obtained at G-band frequencies revealed the axial symmetric anisotropy of the g-tensor and a spectral width of about 160 MHz. This allowed the performance of PELDOR experiments with high sensitivity. The time

traces obtained at different pump-probe positions across the EPR spectrum exhibited orientation selection. By using a fit algorithm,[14] additionally to the distance, information about the flexibility of the molecule was extracted.

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PARAMAGNETIC METALLOPROTEINS WITH MAS OVER 100 KHZ: NMR FINALLY GETS ONTO THE METAL CENTRE

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The presence of metals containing unpaired electrons causes severe difficulties in solid-state NMR: signals with large shifts and shift anisotropies are spread over a huge spectral window, possess extremely short relaxation times, and are broadened by inhomogeneous susceptibility effects.

In this work, we show how the application of a set of NMR experiments, recently developed for the study of complex paramagnetic inorganic battery materials, can be adapted to the solid-state NMR analysis of paramagnetic metalloproteins, and can be used to increase the information obtainable from these systems.

These experiments combine ultra-fast (>60 kHz) magic-angle spinning frequencies and short high-powered adiabatic pulses (SHAPs), and are applied to triply-^{2}H,^{13}C,^{15}N-labelled, fully back-exchanged microcrystalline metalloenzyme superoxide dismutase (SOD). SOD has two high-affinity binding sites for metal cations, and the metallation state can be experimentally controlled to obtain samples with the desired paramagnetic effects with minimal structural perturbation. The most common physiological form contains Cu^{2+} and Zn^{2+} at the active site: however, the presence of the slowly-relaxing Cu^{2+} ion causes severe line broadening, hindering the detection of signals surrounding the active site. Here we show that by replacing Zn^{2+} with fast-relaxing Co^{2+} - thereby increasing the relaxation rate of the Cu^{2+} ion - and by spinning the sample at rates of up to 111 kHz, the line broadening is dramatically reduced, allowing the detection of ^{15}N ^{13}C and ^{1}H signals from residues directly coordinating the metal centres.

Under these experimental conditions, the coherence lifetimes of these signals are long enough to allow the acquisition of well resolved multidimensional spectra, dramatically improving the quality of information obtainable from these systems, and opening up a new avenue for the study of structure and reactivity of metal centres in complex insoluble systems.

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TOWARDS 'TRUE' PULSE EPR DISTANCE DISTRIBUTIONS IN MULTIPLY SPIN-LABELLED SYSTEMS

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Distance measurements by pulsed EPR spectroscopy are an emerging complementary tool for structural biology [1]. However, in systems bearing more than two paramagnetic centres the analysis is complicated by the simultaneous excitation of several coupled spins [2]. This situation becomes increasingly significant for increasing numbers of spins per molecule or aggregate and for large excitation bandwidths [3,4]. It has been shown that for up to four spins or in case of reduced excitation bandwidth these effects can be diminished during post-processing [3].

We have been striving for a methodology to allow extraction of all distances from symmetric homo-oligomers up to heptamers and octamers [5]. Numeric simulations predict significant improvements of distance distributions by reduction of pump excitation bandwidth and sparse labelling as well as post-processing [3]. In this contribution we will evaluate these approaches on synthetic models [6] as well as on the heptameric mechanosensitive channel of small conductance (MscS) of E.coli [5a].

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THE EFFECT OF THE VARIOUS GD3+ EPR TRANSITIONS ON GD3+- GD3+ DEER DISTANCE MEASUREMENTS OBSERVED USING A DUAL MODE CAVITY

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Since their introduction in 2007, Gd^{3+} tags have been proven to be a good alternative of the nitroxide spin labels for DEER distance measurements of biomolecules in frozen solutions at high microwave (MW) frequencies such as W-band (95 GHz) and Q-Band (35 GHz) [1]. Gd^{3+} tags allow shorter pulses, more rapid signal averaging and has the advantage of high sensitivity and no orientation selection. However, as has been recently shown, that under typical Gd-DEER conditions in which the pump pulse is positioned at the peak of the Gd^{3+} spectrum covering the central -1/2 → 1/2 transition and the detection pulse is positioned 100 MHz away such that the main contribution to the signal is from the -3/2 → -1/2 transition, the Pake pattern obtained from the Fourier transform of the DEER trace is distorted for short distances (2-3 nm) [2, 3]. Simulations have shown that the distortions are a result of the breakdown of the weak coupling approximation assumed in the standard analysis of DEER spectra [3]. Moreover, it predicted that by reducing the contribution of the -3/2 → -1/2 transition to the observed echo the distortions are reduced as well. In this work we demonstrate this experimentally. A dual mode cavity based on the design of Tkach et al [4] was fabricated to fit the configuration of our home made W-band spectrometer. A series of DEER measurements were performed on a bis-Gd-DOTA model with a Gd-Gd distance of 2.3nm, with the detection frequency ranging from 100 MHz up to 1.09 GHz away from the central transition. This increase is associated with increasing the contribution of the -5/2 → -3/2 and -7/2 → -5/2 transitions to the signal at the expense of the -3/2 → -1/2 transition. The results show a recovery of non-distorted Pake pattern for an increased shift of the detection frequency, confirming the origin of the distortions.

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EFFECT OF THE YB/MN RATIO ON THE ESR SPECTRUM OF YBMNO3

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Polycrystalline samples of ytterbium manganites $YbMnO_3$ were synthesized according to two different ceramic technologies ($YbMnO_3$-I and $YbMnO_3$-II). These technologies differ in the sintering time and annealing temperature. The X-ray analysis of the synthesized manganites (I and II) showed that both samples belong to the space group $P6_3cm$ and they are in single-phase state. The analysis of the XRD peak intensities demonstrated only the slight deviation in oxygen content and Yb/Mn ration between two samples. Electron spin resonance (ESR) was much more sensitive to such at first glance small differences in the crystal structure.

ESR measurements were carried out in the temperature range of 100 - 320K at the frequency of 9.48GHz. The ESR spectrum of ytterbium manganite $YbMnO_3$ (I and II) consists of one broad exchange-narrowed resonance line in all temperature range for both samples.

At the same time the fitting of the ESR spectrum of YbMnO3-I gives the g-factor above 2.1, which is unusual for Mn^{3+} ions, and 1.99 for $YbMnO_3$-II. The ESR linewidth is about 800 Oe in both cases, that is 2.3

times less that in $La_{0.95}Sr_{0.05}MnO_3$ or in $GdMnO_3$, where the linewidth of the ESR line at room temperature is several thousand oersted, and the effective g-factor is less than 2 [1, 2]. Moreover, in W-band (94 GHz) the ESR spectrum of $YbMnO_3$-I splits in two lines, when the ESR spectrum of $YbMnO_3$-II still consists of one exchange-narrowed resonance line. The possible reasons of the phenomenon are under discussion.

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ROOM TEMPERATURE PELDOR MEASUREMENTS WITH RIGID NITROXIDE SPIN LABELS ON DUPLEX-DNA

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Although pulsed EPR techniques, such as Pulsed Electron Electron Double Resonance (PELDOR or DEER) provide highly accurate distance information in the nanometer range and enable measurements of conformal changes even of flexible biomolecule regions, these experiments are commonly carried out in frozen solutions at ~50 K. Pushing this to a physiological temperature implies reducing the rotational correlation time of the spins by decreasing the local and global motion. This has been demonstrated by using trityl-labels with intrinsic slower relaxations rates attached to a protein.[1] Immobilization of trityl-labeled DNA, bound to a surface, allowed measurements of distances up to 4.6 nm.[2] More recently spirocyclohexyl-nitroxides have been utilized to achieve distance informations by pulsed EPR at 295 K in a dry glassy trehalose matrix.[3]

Here we present the first PELDOR data of nitroxides attached to DNA at room temperature (RT), using rigid spin labels that have slow relaxation rates and distinct distance distribution.[4] A number of rigid spin labled duplex DNAs, differing in their label position, have been synthesized and immobilized using a rigid matrix. This indeed resulted in a extended coherence time of the nitroxide spin labels, allowing distance measurements at RT. When the spin-labeled duplex DNA was adsorbed to a surface through electrostatic interactions, we were able to measure distances by PELDOR even in liquid solutions. Thus offering a direct access to the time scale of conformer dynamics.

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ON THE POTENTIALITY OF FIELD-CYCLING NMR RELAXOMETRY FOR THE CHARACTERIZATION OF MICROSCOPIC AND MESOSCOPIC PROPERTIES IN BIOMEMBRANES

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Lipid dynamics in biomembranes are known to influence the viscoelastic and hydrodynamic properties. The microscopic and mesoscopic fluctuations occur over a broad time scale, covering almost 20 decades from fast molecular internal processes to slow trans-bilayer flipflop process. While many experimental techniques have been used for the characterization of membrane dynamics (including NMR spectroscopy), most of these are only capable of monitoring a single aspect of the dynamics and/or a very limited time scale. On the contrary, fast-field-cycling (FFC) NMR relaxometry allow scanning the molecular dynamics in a broad time-scale, although the obtained information is strongly model dependent. FFC relaxometry studies in 1,2-dimyristoyl-sn-glycero-3-posphocholine (DMPC) and 1,2-dioleoyl-sn-glycero-3-posphocholine (DOPC) liposomal samples revealed the high potentiality for the technique when a consistent model can be derived [1,2]. The success of the approach strongly suggests its general applicability for the study of lipid dynamics in more complex membrane compositions. As a first case of this sort, the effect of cholesterol on the local ordering and dynamics of the lipid molecules were evaluated. The results are found to be most consistent with the partitioning of the lipids into affected and unaffected populations, allowing to speculate about the quantity of lipid molecules that are ordered by the cholesterols, depending on relative concentrations [3]. This result is the first evidence providing experimental backup to the findings of many computational molecular dynamics studies. In this work we will outline the general

problem and show how the technique can be used to determine the elastic properties of the biomembrane using a simplified model, and measurements in a restricted frequency range. We will also advance new results in a different liposomal formulation based on DMPC and sodium deoxycholate.

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NMR RELAXATION OF POLAR AND NONPOLAR MOLECULES PARTIALLY SATURATING A HARDENED CEMENT PASTE

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The cement paste is a complex porous structure which represents the "glue" for the materials mixed to form concrete and mortar samples. It is produced by the hydration of cement grains in a complex and irreversible chemical reaction with water which is influenced by a variety of factors such as: temperature, water-to-cement ratio, the presence of additives, the phase composition of the clinker, the type and the particle packing, the specific surface of the mixture, the compatibility between superplasticizers, etc. [1]. Low field nuclear magnetic resonance (NMR) techniques have been extensively used for the investigation of different water reservoirs in cement paste both during the hydration process and under hardened conditions [2-5]. Note however that, most NMR studies on liquids confined inside cement samples refer to saturated conditions and to water molecules. Consequently, the information extracted about the pore surface effect on molecular dynamics is incomplete. In the present work we have extended these studies to partially saturated cement paste containing polar (water, ethanol) and nonpolar (cyclohexane) molecules inside the porous structure. To reveal the effect of magnetic impurity content on NMR relaxation measurements, two types of cements were considered for the sample preparation: white cement and gray cement. The samples were prepared at room temperature with a water-to-cement ratio of 0.5 and allowed to hydrate for one year in the same conditions. Transverse relaxation measurements were performed using the classical Carr–Purcell–Meiboom–Gill (CPMG) radiofrequency pulse sequence [2, 3] versus the filling factor for all the samples and filling fluids. The longitudinal relaxation time measurements as a function of frequency were performed using the Fast Field Cycling (FFC) technique [4]. The results have indicated a completely different relaxation behavior of polar molecules as compared with the nonpolar ones both as a function of filling factor and frequency.

Moreover, information about the transverse correlation time and the surface diffusion coefficient of confined molecules was extracted revealing a strong dependence of the polarity. The use of cyclohexane and ethanol as test molecules appears to better reveal all classes of pores in cement paste as compared with water molecules [5].

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P 319
QUANTUM COHERENCE IN POTENTIAL MOLECULAR QUBITS

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The development of a working quantum computer would enable a new era of computing concerning speed and security issues. Quantum computers differ from classical ones in the smallest information unit: a quantum bit (qubit) cannot only be in the states |0> and |1> like a classical bit, but also in an arbitrary superposition of those two.

The quest for qubits can be tackled by rational design of molecular compounds. An electron spin embedded in a molecular shell possesses crucial advantages over other systems, e. g. because the surrounding of the quantum mechanical entity can be chemically modified at will to tune the physical properties. Electron Spin Qubits can be investigated with EPR and the critical parameter for quantum computing is a phase memory time (decoherence time) which is at least 10000 times longer than the time required for an individual quantum operation.

Systematical investigations on decoherence in transition metal complexes are sparse, but crucial for understanding the accounting processes. We aim to contribute to this knowledge in order to enable a rational design of molecular qubits. This presentation will unravel the

influences of molecular structure, sample matrix as well as experimental conditions on electron spin relaxation.

Here we show our results on quantum coherence in potential molecular nanomagnets, especially **1CuD**, $(d_{20}\text{-}PPh_4)_2[Cu(mnt)_2]$. The compound was studied in detail by the means of EPR spectroscopy at various temperatures and frequencies. As previously published[1], the compound **1CuD** exhibits extraordinarily slow electron spin dynamics, which was detected in the temperature range 7-300 K. At low temperatures the decoherence time is 3400 times longer than the duration of a single manipulation, which is much higher than previously reported values for this kind of systems.

The frequency dependence of spin-spin-, spin-lattice-relaxation and influence of (super)hyperfine coupling on electron spin dynamics was studied in detail for **1CuD**. Furthermore we were able to see electron spin echo envelope modulation (ESEEM) due to $^{2}D/^{14}N$ nuclei at X/S-band frequencies.

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DYNAMICS OF HLA-B27 MOLECULES ARE HIGHLY DEPENDENT ON SUBTYPE AND PEPTIDE LIGAND

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INTRODUCTION. For some immunoproteins coded by the highly polymorphic Major Histocompatibility Complex (MHC, HLA in humans), a single-residue exchange can dramatically increase the risk of contracting the autoinflammatory disease Ankylosing Spondylitis (AS). AS patients typically experience painful inflammation of joints and spine. Although the association of AS with HLA-B27 is known for more than 40 years, its causes remain unexplained.

HLA class I complexes are 45 kDa heterotrimers consisting of a heavy chain, the non-covalently bound beta-2 microglobulin and a peptide which is bound by residues of a binding groove formed by the alpha1/2-superdomain of the heavy chain. Peptide and heavy chain interact with T cell receptors on effector cells.

Using NMR spectroscopy, we explore dynamic differences between the AS-associated HLA-B*27:05 and the non-associated HLA-B*27:09 subtypes, which differ in an Asp116His exchange at the bottom of the peptide binding groove. We examined the two self-derived peptides TIS (RRLPIFSRL) and pVIPR (RRKWRRWHL) in complex with each HLA subtype. Our analysis is based on protein backbone amide data, collected at two fields, at physiological buffer conditions and at 310K. For the first time, we are able to pin down dynamic differences to specific protein regions.

RESULTS & CONCLUSIONS. HLA-B27 subtypes provide another example that small changes in protein sequence can have large effects on protein dynamics.

Large areas of the helical portions of the alpha1/2-superdomain exhibit a great degree of mobility on an intermediate-to-slow timescale, which is reflected by diminishing signal intensities and the occurrence of multiple conformational states. The dynamic properties are subtype-specific, with HLA-B*27:05/TIS being the most plastic and HLA-B*27:09/pVIPR the least mobile protein complex. In some complexes (HLA-B*27:05/TIS/pVIPR, HLA-B*27:09/TIS) we observe signals vanish or split in the middle of the beta-sheet. This could be an indication for a destabilization of the bottom of the binding-groove in a subtype- and ligand-dependent manner.

Furthermore, our relaxation measurements show that the alpha-3-domain of the heavy chain is less rigidly attached to the rest of the complex than the crystal structures suggest. However, the degree of inter-domain mobility seems to be highly subtype- and peptide-specific, while the internal dynamics of the alpha-3 domain are very similar in all examined cases. Intriguingly, the NH signals of heavy chain residues 223-226 are not visible. These are located in a loop providing part of the binding sites for both the CD8 coreceptor on effector cells as well as the chaperone tapasin which is participating in peptide loading and complex assembly within the endoplasmatic reticulum.

Our study is a first step to examine the consequences of the HLA-B*27:05/09 polymorphism on protein dynamics at atomic-level detail. Understanding the differences in dynamics between the two subtypes will help explaining how only one of two almost identical proteins becomes a risk factor for a disease.

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POPULATION OF LONG-LIVED STATES AND LONG-LIVED COHERENCES THROUGH INCOHERENT CROSS RELAXATION

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In a pair of coupled nuclei, long-lived states (LLS) represent a population imbalance between singlet and central triplet states. In the same way, long-lived coherences (LLC's) are defined as coherences between the singlet state and the central triplet state. LLS and LLC's are immune to inhomogeneous broadening and exhibit longer relaxation time constants compared to T_1 and T_2, respectively. Provided two J-coupled nuclei do not sense strong magnetic interactions with outside spins, the LLC relaxation time, T_{LLC}, can reach nine times T_2 while the LLS relaxation time, T_{LLS}, can reach tens of minutes. [1] LLC's and LLS are insensitive to inhomogeneities in the magnetic field, B_0, [2,3] and their favourable relaxation properties afford sharper resonances in the indirect dimension of multi-dimensional NMR experiments and extend the time scale of transverse magnetization trajectories in NMR sequences.

The excitation of LLC's and LLS requires frequency-dependent methods relying on their chemical shift evolution in case their frequencies are resolved [4,5]. For quasi-equivalent magnetic nuclei, complex pulse schemes involve repeated spin echoes [6] or a spin-lock period [7]. Nevertheless, if B_0 inhomogeneities are non negligible and the chemical shift difference is low, these methods may fail to produce LLS or excite LLC's.

We investigate LLS and LLC's excitation in presence of an asymmetric incoherent interaction with a third nucleus using incoherent NOE-like effects. The magnetization transfer relies on the asymmetry in the interaction between the remote nucleus and the J-coupled nuclear pair. LLS population obtained in this manner can be transferred to either LLC's or single-quantum coherences for detection. Applications of LLC's and LLS to DNP and hyperpolarized NMR experiments will also be discussed. [8,9]

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USING NMR SPECTROSCOPY TO PROBE CONFORMATIONAL DYNAMICS OF PROTEIN DRUG TARGETS AND LIQUID-LIKE STATES OF PROTEINS

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Two separate applications of NMR-based studies of protein dynamics will be presented: 1) concerning the ligand: complexes of oncogenic MdmX and 2) the motional properties of Nucleophosmin (NPM1) in a liquid-like state. MdmX (also known as Mdm4) is a negative regulator of p53 whose overexpression has been linked to the tumorigenesis of retinoblastomas and other human cancers. The N-terminal domain of MdmX binds with nanomolar affinity to the first transactivation domain of p53 (p53-TAD1) and promotes p53 ubiquitination and degradation. Therefore, the inhibition of this interaction by small molecules could stabilize p53 and induce either cell cycle arrest or cell death. New compounds developed at St. Jude (SJ) bind MdmX at least 6 fold more weakly than p53-TAD1 even though the solution structures of MdmX in the different complexes are nearly identical. Given the structural similarities, we hypothesized that the differences in affinities arise from differences in the conformational dynamics of the various MdmX:ligand complexes. To test this hypothesis, we constructed a reference energy landscape for the MdmX:p53-TAD1 complex using NMR relaxation dispersion spectroscopy and compared this with that of several MdmX:SJ compound complexes. We observed a slow motional event for the N- and C-terminal tails of MdmX that is distal from the p53-TAD1 binding pocket and when manipulated perturbs

the affinity of MdmX for p53-TAD1. This motion was altered in the MdmX:SJ complexes, suggesting that the ligands induce differences to MdmX associated conformational dynamics and that these differences can cause the low affinity of the SJ compounds. We seek to understand the mechanisms through which p53-TAD1 and the SJ compounds modulate MdmX dynamics and to apply this knowledge to optimize MdmX inhibitors in the future. Nucleophosmin (NPM) is a major constituent of the liquid-like granular component of the nucleolus, a membrane-less organelle. Acidic tracts within the oligomerization domain (74 kDa) and disordered domain of NPM bind to arginine rich motifs within disordered regions of ribosomal proteins, sequestering them within the nucleolus. Interestingly, however, these interactions are associated with phase separation into liquid-like droplets. We used NMR experiments that probe fast timescale motion (nanosecond range) to understand the dynamic features of NPM in solution, prior to phase separation and within liquid-like droplets in order to elucidate the mechanism of phase separation. These studies illustrate the abilities of solution NMR to monitor the backbone dynamics of high molecular weight proteins both in solution as well as within a meso-scale, fluid structure. Analysis of data from NMR, as well as from single molecule fluorescence and small angle neutron scattering, has provided detailed insights into the structural organization of NPM-containing liquid-like droplets.

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CONFORMATIONAL DYNAMICS OF DOTA-LIKE Eu(III) COMPLEX

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Complex compounds based on polyaminocarboxylates with f-element central ions are widely used as contrast agents in MR imaging, shift agents for protein studies and agents for relaxation enhancement. Major attention is focused on compounds based on a DOTA-like ligand scaffold.[1]

These complexes exist in two diastereomeric pairs of enantiomers differing in twisting angle of the coordinated pendant arms and in distortion of ethylene parts of the aza-cycle. Each of diastereomers has different MRI-relevant properties and therefore, an exchange between them significantly affects effectiveness of the contrast agent.[2]

Despite the fact that these processes were thoroughly studied by quantum-chemistry calculations [3], experimental data for comparison are rare in literature. Moreover in the case of EuDOTA complex, remarkable inaccuracies were found in published experimental data.[4]

In the presented study, these dynamic processes were studied using selective 1D-EXSY based technique which was optimized for usage in systems with fast relaxation induced by presence of the paramagnetic central ion. Complete Solomon equation-based model was used for exchange and T_1 relaxation rates determination. Main advantage of this method is its universal applicability even in systems with low symmetry (C_1), where various-temperature line-shape analysis cannot be used due to signal overlaps.

Using this technique, Eu(III) complexes with DOTA and DO3AP (one carboxylate replaced by phosphonate) were studied. In the case of DO3AP complex, ^{31}P-NMR lineshape analysis was used for determination of exchange activation parameters as well. Data from both method are in a good agreement.

Significant increase of pendant arm movement rate was determined for the DO3AP complex compared to the DOTA complex which can be associated with higher fluxionality of phosphonate pendant arm. It is possible that this mobility of pendant arms implicates faster coordinated water exchange of the DO3AP complexes compared to the DOTA ones.[5]

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SPIN RELAXATION STUDY OF 7LI DYNAMICS IN POLYMER GEL ELECTROLYTES

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In order to analyse the mobility of the ions in electrolyte systems, the local ion dynamics can be studied by using spin-lattice NMR relaxation measurements (T_1) and by describing the temperature and frequency dependent data by different models [1]. Ternary polymer gel electrolytes consisting of an ionic liquid, a polymer and a lithium salt are promising materials for a compromise between sufficient conductivity and mechanical stability in lithium ion conducting battery electrolytes. In particular, poly(ionic liquids) such as for example poly(diallyldimethylammonium) bis(trifluoromethanesulfonyl) imide (PDADMATFSI) were recently introduced as the polymeric component [2,3,4]. Here, we compare two ternary polymer electrolyte systems, containing either PDADMATFSI or polyethylene oxide (PEO), which are based on the ionic liquid 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide (P_{14}TFSI) and LiTFSI. We study the influence of the polymers on the local lithium ion dynamic at a constant lithium ion concentration using T_1 relaxation measurements which were performed at different frequencies and at different temperatures. From the relaxation experiments we can conclude that the local lithium mobility and the local lithium environment is influenced by the type of polymer and by the polymer concentration. It is possible to describe the data by a motional model of the Li dynamics: We fit the lithium data assuming the Cole-Davidson model with Arrhenius behavior and a temperature dependent prefactor. The local activation energy is slightly increasing for higher amount of PEO and slightly decreasing for higher amount of PDADMATFSI. The results show that PDADMATFSI has a more beneficial effect on the local Li

dynamics in comparison to PEO, as it hinders Li motion to a lesser extent.

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THE N-TERMINAL DOMAIN OF POLYPYRIMIDINE-TRACT BINDING PROTEIN: A DYNAMIC FOLDING PLATFORM FOR ADAPTIVE RNA RECOGNITION

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Polypyrimidine Tract Binding (PTB) protein[1] belongs to a class of RNA binding proteins involved in a myriad of essential processes in RNA biology. They are faced with the conundrum of how to recognize a given RNA target in the appropriate context, for example the same RNA sequence can appear in different secondary or tertiary structure. and perhaps remodel it to perform essential tasks such as enhancing or repressing splicing, or IRES mediated translation activation. Defining the changes in dynamics of both RNA and protein binding partners is likely to play an important role in understanding this process.

Structural studies have demonstrated that the RNA recognition motif domains (RRMs) of PTB interacts with RNA through the β-sheet surface and the loops connecting the β-strands.[2] Interestingly the N-terminal RRM of PTB forms an additional a-helix α3 with its C-terminal extension that docks to the β2-strand upon binding to a stemloop RNA containing a UCUUU pentaloop conserved in picornavirus type II Internal Ribosomal Entry Sites (IRES). This is not the case when RRM1 binds ssRNA.[3] We therefore performed ^{15}N and ^{13}C NMR relaxation studies to characterize the dynamics changes of RRM1 and the SL-RNA in this system.

We found that the free protein is highly dynamic exhibiting motions in the C-terminal half of the domain which are characteristic of partly folded proteins Millisecond timescale motions detected in α1, β2 and α3 suggest the C-terminal helix transiently folds and docks to α1 and β2 both of which contact α3 in the SL-RRM complex. Dynamics measurements performed with a site-directed mutant L151G,

designed to abolish the a3-RRM domain contact eliminate millisecond timescale motions in the affected residues supporting this interpretation. On-resonance and off-resonance relaxation dispersion measurements performed with the PTB RRM1-stemloop RNA complex revealed µsec dynamics at the binding interface of the protein and RNA apical loop that could be fitted to a global exchange model. On the other hand, much of the msec exchange observed in the free protein outside of the core binding interface were quenched upon RNA binding. In conclusion our study suggests that PTB RRM1 is quite labile in the free state exploring a range of partially unfolded conformation and that upon interaction with a structured RNA target some of this motional freedom is lost during a cooperative folding process allowing it to adapt to a wide range of structured RNA targets by acting as a dynamic folding platform.

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THE DYNAMICS OF TETHERED POLYISOPRENE CHAINS IN ASYMMETRIC POLY(STYRENE-B-ISOPRENE) DIBLOCK COPOLYMER: THE CASE OF PI BLOCKS CONFINED IN SPHERICAL DOMAINS

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Poly(styrene-b-isoprene) diblock copolymers (SI) are formed by linking two chemically different and mutually immiscible homopolymers (poly-isoprene PI and polystyrene - PS) via covalent bond. SI diblock copolymers are a classical example of polymeric self-organizing systems revealing the existence of unique microdomain structures (e.g. spheres, cylinders, bicontinous double-diamonds, and lamellae). The type of morphology is determined by a minimum value of the free energy of the system reached under given conditions and reflects the balance of various thermodynamic requirements (the elastic, interfacial and osmotic requirements). The formation of regular structures on the nanometer scale makes diblock copolymers extremely valuable for novel applications in the material science, nanotechnology, microelectronics and lithography [1,2].

The aim of the study was to evaluate the effect of spherical morphology of PI domains on the dynamics of the PI blocks (M_w=10000) embedded in a matrix constructed by the PS blocks (M_w=40000). The nuclear magnetic resonance dispersion (NMRD) and broadband dielectric spectroscopy (BDS) methods were used to investigate the segmental and global dynamics of the PI block in the SI diblock copolymer in a wide range of temperatures and frequencies. Results were compared with those of obtained for the bulk PI homopolymer having a molecular weight equal to the weight of the copolymer PI block (M_w=10000). An application of the frequency-temperature superposition to NMRD and BDS susceptibility

data allowed one to obtain spectra in an extended frequency range [3,4].

The results have shown that the spherical shape of PI domains embedded in a stiff PS block matrix significantly affects molecular mobility of the polyisoprene block. In comparison to the glass transition temperature (T_g) of bulk PI the increase in the T_g value of PI blocks was observed. It is a consequence of presence of constrains caused by the stiff polystyrene matrix being in direct contact with mobile PI blocks in SI copolymer. Additionally, the considerable suppression of global dynamics and broadening in the distribution of correlation times connected with the PI normal mode process in copolymer was revealed.

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NMR RELAXATION STUDIES OF RECEIVER DOMAIN OF WILD-TYPE CYTOKININ RECEPTOR CKI1RD AND ITS MUTANTS FROM ARABIDOPSIS THALIANA

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In our project, we studied the signaling pathway known as the multistep phosphorelay signaling system in the plant Arabidopsis thaliana. The multistep phosphorelay signaling system serves as a mechanism allowing organisms to sense and respond to changes in many environmental conditions. This signaling system is based on phosphate transfer in a cascade of proteins localized in membrane, cytosole, and nucleus. In the plant Arabidopsis thaliana, histidine kinase is phosphorylated upon signal recognition, and forwards the phosphate group through histidine phosphotransfer proteins to a response regulator protein located in nucleus, where the response takes place.

Aspartic acid 1050 present in the active centre of the sensor histidine kinase CKI1RD plays an important role in the signaling pathway. This residue binds phosphate and transfers it to the Arabidopsis histidine phosphotransfer proteins (AHPs). Wild-type protein CKI1RD and two CKI1RD proteins with mutations in their active sites (CKI1RD - D1050A and CKI1RD - D1050E) were expressed in E.coli and labelled by stable isotope 15N and studied by NMR relaxation experiments.

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DIFFUSION OF CO2 IN DMOF-1, AN ANISOTROPIC MICROPOROUS METAL-ORGANIC FRAMEWORK

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DMOF-1 is a microporous metal-organic framework (MOF) composed of (Zn04)2 secondary building units and two types of organic linkers – 1,4-benzene dicarboxylate in two crystallographic directions and 1,4-diazabicyclo[2.2.2]octane in the third one. The crystal structure thus contains 1-dimensional channels of roughly nanometer size interconnected by smaller windows. The compound displays a favorable adsorption capacity for carbon dioxide as well as a good selectivity for CO2 with respect to methane.

We studied diffusion of CO2 in DMOF-1 by means of 13C NMR spectroscopy, diffusometry and relaxometry complemented by simulations of molecular dynamics. In this way, it was possible to localize the binding sites for CO2 and to characterize the self-diffusion CO2 inside of the MOF [1, 2]. Furthermore, we have analyzed the contributions of different mechanisms leading to relaxation 13C spins of the adsorbed CO2.

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NMR DYNAMICS OF THE INFLUENZA A VIRUS RNA

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Retinoic acid-inducible gene I (RIG-I) recognizes pathogen-associated molecular patterns (PAMPs) such as viral RNAs. The C-terminal domain (CTD) of RIG-I binds to double-stranded RNA (dsRNA) with the 5'-triphosphate (5'-PPP), which induces a conformational change in RIG-I to an active form for the interferon synthesis. It has been suggested that RIG-I detects infection of influenza A virus by recognizing the 5'-triphosphorylated panhandle structure of the viral RNA genome. Influenza panhandle RNA has a unique structure with an internal loop and sharp helical bending. In spite of extensive studies of how viral RNAs activate RIG-I, whether the structural elements of the influenza panhandle RNA confer the ability to activate RIG-I signaling has not been explored. Here, we investigated the dynamics of the influenza panhandle RNA in complex with RIG-I CTD using NMR spectroscopy. In the detection of the hydrogen exchange rate, it showed the significant differences between influenza RNAs with and without 5'-triphosphate moiety.

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PRELIMINARY STUDIES OF THE ENZYMATIC PENTOSE PHOSPHATE PATHWAY USING DISSOLUTION DYNAMIC NUCLEAR POLARIZATION

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NMR spectroscopy of low abundant substrates have benefitted from the recent introduction of hyperpolarized techniques, which aim at enhancing the nuclear spin polarization well beyond the Boltzmann equilibrium, and lead to considerably enhanced sensitivity. Dissolution Dynamic Nuclear Polarization (D-DNP) has proven a very efficient hyperpolarization technique, providing dramatic, up to four or five orders of magnitude, enhancement. It has been applied to a very broad variety of molecules and nuclear spins. As a result, many applications have emerged, among which the study of fast time-varying processes is of particular interest.

Several groups have recently demonstrated the possibility to probe metabolic processes both in solution ("in vitro") and in cell suspensions or living tissues. To date, only a limited number of in vitro D-DNP NMR studies aiming at the investigation of protein-substrate interactions or enzyme kinetics have been performed. These very promising results open new avenues for the investigation of fast kinetic processes, where short experimental times prevent product re-association or enzyme inhibition by reaction products that may skew quantitative measurements.

Such pathways as glycolysis or the pentose phosphate pathway (PPP), represent crucial metabolic pathways. Therefore kinetic studies of the individual enzymes contributing to the metabolism of glucose are of crucial interest for the detailed investigation of these metabolic

chains. We have recently undertaken the kinetic study of Glucose phosphorylation by Hexokinase, the first metabolic step, common to glycolysis and the PPP, using D-DNP experiments. Glucose decay and Glucose-6-Phosphate (G6P) buildup curves were observed. Experiments were also performed in the presence of G6P, the reaction product, known to be an inhibitor of the enzyme. We used a simple kinetic model to describe the reaction, taking into account the inhibiting action of G6P, through which the relevant kinetic parameters could be extracted.[1]

More recently, we have pursued our investigations of the PPP and focused on the next stage of the pathway, the dehydrogenation of the G6P. The preliminary G6P dehydrogenation results will be presented, as well as several technical and methodological issues raised by these experiments.

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HETEROGENEOUS PARAHYDROGEN-INDUCED POLARIZATION OF PROPANE GAS FOR MRI APPLICATIONS

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Heterogeneous parahydrogen-induced polarization (HET-PHIP) was employed to prepare propane gas with non-equilibrium population of spin energy levels. The theoretical approach was developed in order to examine factors lowering the maximum attainable propane ^{1}H hyperpolarization yield and the percentage of pairwise hydrogen addition. It was found that the maximum attainable polarization is ~7% for propane produced by heterogeneous hydrogenation of propylene over Rh/TiO_2 [1]. Improvement of catalytic system (e. g. the use of single-atom catalysts) may help to further overcome this number [2].

Finding the optimum conditions for production of highly polarized propane gas promotes its biomedical use. Propane is a non-toxic gas, and HET-PHIP allows to produce gas which is free from the catalyst contamination. The Rh/TiO_2 catalyst used here can be recycled for hundreds of times, and it is stable over the years. Therefore, the results presented here enable low-cost high-resolution and high-speed MRI of gases for functional imaging of lungs and other applications [3]. The only drawback is the short propane relaxation time (T_1~0.6 s). Deuteration of molecular precursor (i. e. the use of propene-d_6) does not significantly improve T_1 in high magnetic field. Nevertheless, it was shown that in the low magnetic field (0.0475 T) parahydrogen partially preserve its long-lived nuclear spin order even after addition to propene, and hyperpolarized state has much longer relaxation time (4.7±0.5 s for propane and 6.0±0.3 s for propane-d_6) [4]. While the direct detection of hyperpolarized propane "pseudo singlet state" at

low-magnetic field (0.0475 T) is challenging [5], Spin-Lock Induced Crossing (SLIC) was used to transform this state into observable nuclear magnetization [6]. The signal obtained was suitable for MRI with sub-millimeter and sub-second spatial and temporal resolution respectively for propane at 0.0475 T (with signal enhancement > 5,000 times).

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P 358
HYPERPOLARIZED EQUIVALENT LONG-LIVED STATES (HELLS)

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Dissolution dynamic nuclear polarization (D-DNP) [1] allows one to enhance NMR signals by up to five orders of magnitude. Unfortunately, the lifetimes of hyperpolarized spin states are usually limited by T_1 relaxation times. The lifetimes can be extended by storing the hyperpolarization in the form of long-lived states (LLS) [2]. Levitt and co-workers have shown how long-lived states can be populated by D-DNP in a pair of inequivalent spins [3].

We have shown recently [4] that low-temperature DNP allows one to create a triplet-singlet imbalance (TSI) in molecules such as $CD_3C\mathbf{H}_2OD$ that contain two magnetically equivalent protons, as highlighted by bold letters. An imbalance between the population of the anti-symmetric singlet state and the average population of the three symmetric triplet states has a lifetime T_{TSI} that can be much longer than the longitudinal relaxation time T_1. We refer to this type of triplet-singlet imbalance as a "Hyperpolarized Equivalent Long-Lived State" (HELLS). Like in para-hydrogen, a HELLS cannot be observed directly but can be "revealed" by a chemical reaction that breaks the symmetry. For example, the enzyme fumarase catalyzes the addition of D_2O onto the double bond of fumarate (HOOCC**H**=C**H**COOH) to yield malate (HOOCC**H**DC**H**OHCOOH). This addition reaction breaks the magnetic equivalence of the two protons highlighted by bold letters, so that the invisible HELLS of fumarate is converted into a hyperpolarized NMR signal of malate. Since fumarate plays a crucial role in the Krebs cycle, it may be of interest for studies of cellular necrosis [5].

HELLS may find applications in other areas of magnetic resonance such as metabolic imaging where hyperpolarization by D-DNP has become a technique of choice, and where the short lifetimes of

hyperpolarization are still a major limitation. We are currently trying to widen the scope of our HELLS approach to more challenging molecules containing magnetically equivalent pairs of spins, such as CH_2RR', CH_2Cl_2, and ultimately H_2O.

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INSIGHT INTO THE SUPRAMOLECULAR ARCHITECTURE OF DIATOM BIOSILICA FROM DYNAMIC NUCLEAR POLARIZATION (DNP)-SUPPORTED SOLID-STATE NMR SPECTROSCOPY

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Biomineralization is the formation of inorganic materials by biological processes. Examples are calcium phosphates like bones and calcium carbonates such as nacre or coccoliths. The most beautiful and abundant biosilica formation process occurs in diatoms, organisms of global ecological importance. They are responsible for about 20 % of the global primary production and major players in the earth's silicon cycle. Furthermore, diatom biosilica is an interesting inorganic/ organic hybrid with promising materials properties. Diatom cell walls exhibit a species-specific micro- and nano-patterning and contain a certain amount of tightly bound organic molecules, typically 1 – 15 wt.-% [1].

Here, we demonstrate the usefulness of DNP solid-state NMR to investigate biosilica. The gain in signal intensity allows the detection and characterization of biosilica-associated long-chain polyamines (LCPAs), polysaccharides, and peptides. In situ insight into the secondary structure elements of tightly biosilica-associated native peptides is given for the first time. Selective signal enhancements allow establishing a model for the location and supramolecular arrangement of these biomolecules. The following conclusion can be drawn:

(i) The peptides/proteins and polysaccharides are located on the biosilica surface thus "shielding" polyamines and silica. This is in line with ^{1}H-^{13}C-^{29}Si triple resonance studies indicating the intimate contact between polyamines and silica [2].

(ii) Using the enhanced sensitivity provided by AMUPol, we could even apply 2D techniques such as ^{13}C-^{15}N and ^{15}N-edited ^{13}C-^{13}C correlation spectroscopy. For the first time, we could obtain direct insight into the backbone fold of native peptides associated with diatom biosilica. The solid-state NMR analysis in combination with MD simulations suggests a mixture of random coil and β-strand conformations that may help to establish compactness as well as intermolecular network.

(iii) Clearly resolved signals due to the N-methyl-propyleneimine repetitive unit in the LCPAs could be detected. The polyamine structure determined previously for LCPAs isolated from diatom cell walls could thus be confirmed on intact biosilica.

(iv) 2D DNP solid-state NMR showed the presence of an oxygen-linked glucuronic acid as a fraction of silica-associated polysaccharide material. N-linked saccharides as well as N-acetylglucosamine can be excluded.

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P 364
FIELD-CYCLING PHOTO-CIDNP MAS NMR. DESIGN OF A SHUTTLE SYSTEM

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The solid-state photo-chemically induced dynamic nuclear polarization (photo-CIDNP) effect has been shown to occur in the photosynthetic reaction centers (RCs) studied so far [1,2]. In addition, it has been detected in a blue-light photoreceptor demonstrated that the effect is not a peculiarity of photosynthetic systems [3]. All these results led to the idea that the occurrence of high-field solid-state photo-CIDNP is correlated to efficient electron transfer in photosynthetic RCs [4]. Nevertheless, all experiments done to date have been performed in a high-field regime, which is not associated with biologically relevant spin dynamics.

Theoretical studies have shown that the solid-state photo-CIDNP effect may be common in biological systems at low magnetic fields (e.g. Earth's magnetic field) [5]. Those low- and medium-field regimes can be obtained with the use of field-cycling techniques, moving the sample mechanically between positions of different magnetic flux densities (i.e. sample shuttle technique).

In order to develop the solid-state photo-CIDNP effect into a more generally applicable method for signal enhancement in solid-state NMR, a shuttle system has been proposed. The shuttling device combines the advantages of low fields (high nuclear spin polarization) and high fields (high chemical shift dispersion). With this shuttle system we expect to have more signal enhancement, measure other nuclei and to do experiments with a larger number of accessible systems.

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P 367

PROPOSALS FOR CHIRALLY INDUCED SYMMETRY BREAKING IN LONG-LIVED STATE NMR

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Nuclear magnetic resonance (NMR) experiments are limited by relaxation dynamics. Observing non-equilibrium magnetization is restricted to timescales governed by the longitudinal relaxation time T_1, which limits potential applications such as hyperpolarization or transport phenomena. Long-lived states (LLS) have relaxation times much longer than T_1 [1,2], providing a possible approach to overcome relaxation constraints. Often the duration of information capture is extended by an order of magnitude over T_1 [3]. LLS commonly exist in symmetry-constrained homonuclear pairs termed singlet states, with some multi-spin variants established. Molecular systems exhibiting LLS include; parahydrogen [4], parawater [5], gamma-picoline [6], peptides [7], fumarates [8] and naphthalenes [9].

However, few of these molecules have significant biological application. The ability to selectively embed LLS into biologically relevant substances could be advantageous. A ubiquitous molecular motif, such as a methyl group, could be target for LLS implantation. Lactate is a small, simple biologically interesting molecule with a methyl group and thus provides a strong initial model. Deuteration at a single position in the methyl group forges the required spin-pair. For convenience, Lactic Acid esters were synthesised. The end groups have negligible experimental effect. In general, the proximal asymmetric centre breaks coherent Hamiltonian symmetry allowing singlet state access. However, the effect may be small in the case of a CH_2D group. We describe our experimental attempts to demonstrate this effect.

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APPLICATION OF HETERONUCLEAR CROSS-RELAXATION EFFECTS FOR THE IN VITRO CHARACTERIZATION OF ENZYMATIC REACTIONS BY HYPERPOLARIZED 13C→1H NMR

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Dissolution dynamic nuclear polarization (dDNP) has been used in the recent years to enhance the sensitivity of magnetic resonance (MR) methods enabling the monitoring of in-vivo metabolism, proteins folding or chemical reactions. dDNP involves the hyperpolarization of nuclear spins in a molecule put jointly with a polarizing agent at cryogenic temperature and high magnetic field. The MR experiment performed in liquid state then requires the rapid sample dissolution for subsequent signal detection. So far, most of the studies have focused on hyperpolarization of long-lived nuclear spin species such as ^{13}C, in small molecules. While advantages could also arise from hyperpolarized ^{1}H, the lag time between sample dissolution and liquid-phase spectral acquisition limits the utility of hyperpolarizing fast relaxing nuclei. However a recent study has reported enhancement in ^{1}H NMR peaks in solution phase of ^{1}H covalently bound to ^{13}C nuclei following ^{13}C DNP (1). Heteronuclear cross-relaxation enabling a spontaneous $^{13}C \rightarrow ^{1}H$ polarization transfer is the main contributor for this observation.

In this work, the potential of using spontaneous $^{13}C \rightarrow ^{1}H$ polarization transfer to obtain insight on an enzymatic process that could only be resolved by hyperpolarized ^{1}H MR, was demonstrated at an in-vitro level. The pyruvate decarboxylase (PDC) enzymatic reaction catalyzing the decarboxylation of pyruvate into acetaldehyde, a key step in the alcoholic fermentation pathway of yeast and other microorganisms, was monitored and characterized by ^{1}H NMR at 500

MHz. Sodium [U-$^{2}H_3$,2-^{13}C] pyruvate was hyperpolarized and injected into suspensions of PDC holoenzyme following which arrays of ^{1}H NMR spectra were recorded. In situ production of acetaldehyde was observed for more than a minute while acetaldehyde from thermal pyruvate was not detected in this time scale, attesting for cross-relaxation process within the product of the enzymatic reaction. In addition the dynamic feature of the data enabled to obtain the apparent rate constant k_{cat} of PDC. Acetaldehyde signal intensities over time were fitted to Bloch-McConnell differential equation modeling both the build up and the decay of the ^{1}H NMR signal to extract k_{cat}, while the constant of polarization transfer and the T_{1s}were measured independently and included in the equation. The reaction was monitored at high initial pyruvate concentration (50 mM) to ensure full saturation of the enzyme as well as at low substrate concentration (6 mM) and the respective apparent k_{cat} were obtained. In addition to the enhancement of acetaldehyde's ^{1}H NMR signal from cross-relaxation effects, single-shot experiments induced by $^{13}C \rightarrow ^{1}H$ polarisation transfer via INEPT (insensitive nuclei enhanced by polarization transfer) sequence, were also carried out. These resulted in a ten times higher signal enhancement –yet were not amenable to multi-shot kinetic studies. Complementary potential in-vivo applications of both approaches are thus evidenced.

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HETEROGENEOUS PARA-HYDROGEN INDUCED POLARIZATION IN WATER UTILIZING LIGAND-CAPPED NANOPARTICLES

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Para-hydrogen induced polarization (PHIP) is a technique that utilizes the singlet state of para-hydrogen to generate nuclear spin polarization at magnitudes far greater than state-of-the-art superconducting magnets.[1,2] An emerging application of PHIP is to generate molecular imaging agents for biomedical applications. Contrast agents that are clinically viable and effective require high levels of polarization with minimal toxicity. So far, high levels of polarization in water were only achieved utilizing homogeneous catalysts with potential toxic effects and the challenge of removing a substrate of interest from the catalyst.[3] It is therefore desirable to design a heterogeneous catalyst that can easily be removed before a substrate of interest is injected into an organism. A recent study showed first evidence that the pair-wise addition of para-hydrogen to dissolved substrates is feasible in water utilizing nanoparticles.[4] We demonstrate the first heterogeneous catalyst based on ligand-capped nanoparticles that can be used to generate significant levels of polarization.[5] It consists of platinum particles with an average size of 2.0 nm and is capped with glutathione. Hydroxyethyl propionate, a contrast agent for magnetic resonance angiography, was hyperpolarized and a ^{1}H polarization of P = 0.25% was achieved. In subsequent steps the ligands covering the nanoparticle were

optimized resulting in an increase in ^{1}H polarization up to P=0.65%. We will furthermore present a mechanistic investigation of PHIP on ligand-capped nanoparticle surfaces in water and demonstrate that the particles can be recycled up to five times without loss of polarization. We expect that heterogeneous catalysts that can be removed and recycled from potential contrast agents will play an important role in the expansion of PHIP for biomedical molecular imaging applications.

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SIGNAL ENHANCEMENT BY MULTIPLE-CONTACT CROSS-POLARIZATION UNDER MAGIC-ANGLE SPINNING

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Cross-polarization (CP) of rare spins with a low gyromagnetic ratio such as ^{13}C and ^{15}N from abundant spins with a higher gyromagnetic ratio (e.g., ^{1}H) is a popular way of obtaining high sensitivity NMR spectra of solids. We have previously shown that multiple equilibrations-re-equilibrations with the proton spin bath improves the polarization transfer efficiency at short contact times and provides higher signal enhancements than state-of-the-art techniques such as adiabatic passage through the Hartmann-Hahn condition CP (APHH-CP) [1] when slow magic-angle spinning (MAS) is applied [2]. Indeed, the chemical shift powder spectra resulting from the so-called multiple-contact isotropic CP (MCI-CP) experiment [2] have been found to be identical to the ones obtained by using APHH-ROtor-Directed Exchange of Orientations CP (APHH-RODEO-CP) [3] with intensity gains of a factor 1.1-1.3. In this work, we show that the MCI-CP technique requiring no pulse-shape optimization also yields higher polarizations than optimized APHH-CP when the MAS frequency is comparable to the heteronuclear dipolar coupling, i.e., when APHH-CP through a single sideband matching condition is impossible or difficult to perform [4].

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Poster Session 2

P 002
MECHANISM OF KINETIC REGULATION BY THE 2'-DEOXYGUANOSINE SENSING RIBOSWITCH

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The 2'-deoxyguanosine sensing (2'dG) riboswitch regulates the expression of ribonucleotide reductase subunit a on the level of transcription.[1–3] In the presence of 2'dG, the expression platform folds into a terminator stem and transcription is aborted. In the absence of 2'dG, the formation of an antitermator allows the transcription to proceed. We have found that, in the thermodynamic equilibrium of the full-length mRNA, the 2'dG riboswitch adopts the terminator conformation regardless of whether the ligand is present. Therefore, the 2'dG riboswitch is controlled kinetically and on-state folding must occur co-transcriptionally. To further investigate this mechanism of kinetic regulation, we developed a method for simultaneous screening of RNA secondary structure of a multitude of transcriptional intermediates at single nucleotide resolution by NMR, which can be performed within two days only. The approach combines PCR using 2'-methoxy modified reverse primers[4] to generate various DNA templates from a single DNA plasmid with the use of DMSO as a cosolvent during transcription to obtain highly homogeneous RNA transcripts. The high purity of these transcripts facilitates direct buffer exchange of the transcription mixture, while time-consuming purification steps typically required for NMR studies can be avoided. We applied this approach to monitor the transcriptional progress by screening the secondary structure of 30 different transcriptional intermediates and by characterizing their ligand binding properties. Ligand binding by transcriptional intermediates turned out to be highly dependent on the Mg^{2+} concentration. While all intermediates with a folded aptamer domain bind 2'dG at low Mg^{2+} concentrations,

intermediates adopting the on-state can bind 2'dG and switch to the off-state but require increasing Mg^{2+} concentrations with increasing stability of the antiterminator. We further determined segments on the riboswitch, in which a particular conformation is adopted in both absence and presence of ligand. In particular, at a Mg^{2+} concentration of 3 mM, ligand binding can occur over a stretch of 41 nt, while the kinetically stabilized on-state is stable for a stretch of 22 nt in the absence of ligand and for a stretch of 15 nt in the presence of ligand. We intend to combine this knowledge with pause site analysis and on-state folding kinetics in order to understand how the speed of transcription couples with folding kinetics to fine-tune gene regulation for kinetically controlled riboswitches.

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P 005
INFLUENCE OF GLYCANS ON DRUG BINDING STUDIED IN AGP1

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Serum proteins play a central role in ligand transport and the response against pathogens. The heavily glycosylated alpha-1-acid glycoprotein (AGP1) represents one of the two major serum proteins in mammals. It mainly targets basic ligands but has some affinity for neutral and acidic small molecules. Binding to a wide range of targets from endo- and exogenous origin, the interaction of AGP1 with drugs are crucial for determining the correct doses of pharmaceuticals that are to be administered. AGP1 consists of 183 amino acids and five glycan chains; the glycans contribute almost half to the total molecular weight. Based on an X-ray structure of the unglycosylated AGP1 (1) the binding site is assumed to be a broad groove, however, specific binding residues are not known. Further, previous studies suggested an influence of the glycans on the structure of the protein (2) as well as on the binding to some drugs (3). Using NMR spectroscopy we want to further analyse the binding site of AGP1 and the role of the attached glycans in drug binding. The glycosylation pattern of AGP1 changes, depending on physiological and pathological conditions. Thus, the sugar moieties may have an important influence on the pharmacokinetics of the drugs.

For the study of the interaction of glycans with the protein and their role in ligand binding, we expressed recombinant wild type AGP1, AGP1-wt, and a mutant, AGP1-NQ, in Pichia Pastoris. In AGP1-NQ the five Asn residues involved in N-linked glycosylation were mutated to Gln. Further, we purchased AGP1 extracted from human blood, hAGP1. For a first characterization of the 3 variants [^{13}C,^{1}H] and [^{15}N,^{1}H]-HSQC spectra were measured; the latter only for AGP1-wt and AGP1-NQ. Chemical shift differences between the glycosylated and the non-glycosylated forms clearly indicate interactions between the glycans and the polypeptide chain, and thus, possibly conformational differences between AGP1 in solution and in the crystal. To investigate the influence of these changes on drug binding

we performed NMR titration experiments with the three AGP1 variants and monitored the chemical shift changes of the protein and the carbohydrates. All our results confirm the influence of glycans in drug binding to AGP1 and clearly demonstrate, that the glycopattern of the protein needs to be considered when measuring pharmacokinetic data on drugs of interest.

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P 008

AN EQUILIBRIUM-BASED MODEL FOR -1 PROGRAMMED RIBOSOME FRAME-SHIFT STIMULATOR

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The functional determinant of RNA pseudoknot for -1 programmed ribosome frame-shift (PRF) stimulation has been inconclusive. Recently, NMR analysis of the MLV readthrough pseudoknot revealed a pH-dependent conformation change that has been linked to readthrough efficiency. It was proposed that pH-dependent base-triple formation facilitate S1-L2 interaction crucial for read-through competence. This model was further proposed to extend to the pseudoknot stimulator of -1 PRF. We use the human telomerase pseudoknot (hTPK) DU177 as a model system to examine the pH-dependent -1 PRF efficiency. The UAU triples of DU177 can be replaced by isomorphic CGC triples while retaining the -1 PRF stimulation activity. The CGC triple can harbor an extra protonation-mediated tertiary hydrogen-bond to form a C+GC triple. The -1 PRF efficiencies of the CGC variants exhibit strong pH-dependency that correlates very well with their thermal stability. Two distinct thermal transitions were observed for the CGC variants with the first one being more pH-sensitive. Such a systems may serve as a platform for examining the role of S1-L2 interaction (involving conserved AACAA in L2 in several viral -1 PRF pseudoknot stimulators) in -1 PRF stimulation and its coupling to the base-triple formation. Particularly, it will be very informative to analyze the S1-L2 configurations in different pH values that can tune the -1 PRF efficiency to different levels to link a specific pseudoknot configuration to -1 PRF stimulation activity.

P 011

THE DYNAMICS OF THE G PROTEIN-COUPLED NEUROPEPTIDE Y2 RECEPTOR IN PHOSPHOLIPID MEMBRANES INVESTIGATED BY SOLID-STATE NMR SPECTROSCOPY

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In contrast to the static snapshots provided by protein crystallography, G protein-coupled receptors constitute a group of membrane proteins with highly dynamic properties, which are required in the receptors' function as signaling molecule. Here, the human neuropeptide Y2 receptor was reconstituted into a model membrane composed of saturated or monounsaturated phospholipids and solid-state NMR was used to characterize its dynamics. Static ^{15}N CP NMR spectra showed the superposition of an axially symmetric powder pattern and very narrow signals indicative of receptor sites that undergo large amplitude motions. ^{15}N CP NMR spectra acquired as a function of the CP contact time revealed that about 40% of the backbone amides of the receptor undergo large amplitude motions. This was confirmed by directly excited static ^{15}N NMR spectra. ^{13}C MAS NMR experiments showed relatively narrow lines and enabled also detection of NMR signals of highly mobile groups using ^{13}C INEPT NMR experiments under MAS. Quantitative determination of ^{1}H-^{13}C order parameters through measurement of the ^{1}H-^{13}C dipolar couplings of the CH, CH_2 and CH_3 groups in separated local field experiments (DipShift) revealed axially symmetric motions of the whole molecule in the membrane and molecular fluctuations of varying amplitude from all molecular segments. Wirth increasing CP contact times lower order parameters were detected as also the more mobile receptor segments were sufficiently cross polarized. The molecular order parameters ($S_{backbone}$ = 0.59-0.67, S_{CH2} = 0.41-0.51 and S_{CH3} = 0.22) obtained in directly polarized ^{13}C NMR experiments demonstrate that the Y2 receptor is highly mobile in the native-like membrane. Interestingly, according to these results the receptor was found to be slightly more rigid in the membranes formed by the monounsaturated phospholipids (POPC) than by saturated phospholipids (DMPC). This

could be caused by an increased chain length of the monounsaturated lipids, which may result in a higher helical content of the receptor. Furthermore, the incorporation of cholesterol, phosphatidylethanolamine, or negatively charged phosphatidylserine into the membrane did not have a significant influence on the molecular mobility of the Y2 receptor.

P 014

THE SOLUTION STATE NMR INSIGHTS IN THE CYTOSOLIC TAIL OF THE TUMOR MARKER PROTEIN-TROP2

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The tumor-associated calcium signal transducer 2 (Trop2) is a transmembrane type-1 glycoprotein expressed in multistratified epithelia and several stem/progenitor and carcinoma cells. Trop2 transmits extracellular signals via its extracellular and transmembrane (Trop2TM) domains to a short (26 aa) cytosolic tail (Trop2IC). Proliferation-enhancing signaling is triggered by intramembrane proteolytic release of a short cytoplasmic fragment. This part is later engaged in a cytosolic signaling complex. Our research interest was focused on phosphorylation of a specific serine residue within the cytosolic region (Ser303). Our experimental results suggest that function of peptide is triggered by serine phosphorylation and by proximity effects exerted on the cytosolic tail by Trop2 dimerization. Structural characterizations of both the transmembrane (Trop2TM) and cytosolic regions (Trop2IC) support this hypothesis, and presents that the central region of Trop2IC forms an short a –helical structure. Comparison of NMR structures of both structures suggest that phosphorylation of Trop2IC triggers salt bridge reshuffling, resulting in significant conformational changes including ordering of the C-terminal tail. In addition, we demonstrate that the cytosolic regions of two Trop2 subunits can be brought into close proximity via transmembrane part dimerization. Finally, we show that Ser303-phosphorylation significantly affects the structure and accessibility of functionally important regions at the cytosolic tail. These observed structural features of Trop2 at the membrane-cytosol interface could be important for regulation of Trop2 signaling activity.

P 017

AN NMR APPROACH TO PROBE THE ROLE OF FAST PROTEIN MOTIONS IN PETNR-CATALYSED HYDRIDE TRANSFER

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Pentaerythritol tetranitrate reductase (PETNR) is a monomeric 40 kDa flavoenzyme that uses NADPH/NADH to reduce a wide variety of α,β-unsaturated compounds. The hydride transfer from NAD(P)H coenzyme to the flavin cofactor in PETNR has been extensively explored by using enzyme variants, substrates and by studying pressure effects and temperature dependencies of kinetic isotope effects. From these studies, an important role for fast protein motions in PETNR-catalysed hydride transfer has been inferred. These motions might have a significant contribution to H-tunnelling in the enzymatic reaction cycle [1]. Thus, PETNR has arisen as an excellent model system for studying H-transfer reactions.

To further test the hypothesis of coupled intrinsic protein motions to the reaction coordinate, the binding effect of $NADPH_4$ and $NADH_4$ (ground state mimics) and progesterone (inhibitor for the oxidative reaction) on the structure of PETNR was observed [2]. Upon addition of progesterone, $NADPH_4$ or $NADH_4$ to the holoenzyme, residues located within 4 Å from FMN and substrate present a significant chemical shift change. Moreover, the frequencies and amplitudes of dynamical modes of groups in specific residues that contribute to cofactor and coenzyme/substrate barrier compression were observed by recording ^{1}H-^{15}N TROSY resonances as a function of pressure, from 1 to 1500 bar. The comparison of pressure responses between different PETNR complexes indicated some key residues with different degree of compressibility.

The results provide a platform for the future determination of a more complete dynamic profile of the enzyme. Compressive modes can act to transiently compress the activation energy barrier and enable quantum mechanical tunnelling between the flavin and the coenzyme. The fast exchange motions which can intrinsically facilitate the tunnelling of the hydride group will be atomistically characterized by undertaking a selective study of side chain groups as a function of pressure. In tandem, backbone amide dynamics will be studied by using ^{15}N relaxation experiments in conjunction with pressure, which will enable a more detailed study of fast (ps-ns timescale) fluctuations.

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P 020
WATER - PROTEIN INTERACTION : ENGRAILED HOMEODOMAIN MUTANT K52E

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The homeodomain fold is a 60-amino-acids helix-turn-helix structural domain that binds DNA or RNA. This fold is commonly found in transcription factors important during embryonic development, regulating cell fate and development plan of the body. The drosophila engrailed transcription factor controls the cell segregation during embryonic development. It is known that Homeodomains use a specific lone-pair-π interaction between water molecule and some amino acid sidechain (i.e Trp, Arg) to recognize their cognate DNA. A specific interaction of W48 and the sugar-phosphate backbone of the DNA the intermediated by two water molecules has been observed in crystal. This interaction exists also in the Engrailed protein without DNA. An engrailed mutant where lysine has been replaced by glutamate (EnHD-K52E) has shown an additional stabilization of the water molecule implicated in the lone-pair-π interaction with W48, due to hydrogen bonding to the carboxylate of glutamate 52.

In order to study water – protein interaction we prepared a doubly labelled sample of mutant protein EnHD-K52E. The protein backbone assignment has been obtained using the standard approach utilizing 3D HNCACB and CBCACONH spectra. For detailed analysis of water-protein interactions we measured homonuclear 2D NOESY and TOCSY spectra with high resolution in indirect dimension on unlabeled protein sample. For water molecules that are significantly slowed down via tight interaction with the protein we typically observe a NOESY cross peak, therefore detailed analysis of 2D NOESY spectrum enables identification of such protein sites [1]. However, if the water molecule is more labile, the NOESY cross peak will not be observable and more

elaborated experiment, CLEANEX [2] is a current method of choice for detection of such contacts.

NMR experimental data reporting about water-protein interaction will be presented along with a hydration analysis obtained from molecular dynamics simulation of the protein in explicit solvent.

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P 023
STRUCTURAL STUDY OF ETR1 USING PROTEIN TRANS-SPLICING

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Ethylene was the first gaseous biological signaling molecule discovered. As a plant growth regulator factor, it is involved in several developmental processes like seed germination, seedling growth, leaf, root, stem and flower development, fruit ripening, senescence, and responses to variety of stresses. Perception of ethylene and the signal transduction into the cell is achieved by membrane receptors, to which ethylene receptor 1 (ETR1) belongs. ETR1 is a multi-domain protein which contains a hydrophobic N-terminal trans-domain that encompasses the ethylene-binding site, followed by a large cytosolic domain consisting of GAF domain, transmitter histidine kinase and response regulator (or signal receiver). We successfully expressed the receiver domain of ETR1, which monomeric form resembles the bacterial receiver domain. Although the crystal structure of $ETR1_{RD}$ has been already determined, its intermolecular interactions and the role of divalent ions are experimentally not well defined. For this purpose, we designed an $ETR1_{RD}$construct with an intein. The intein sequence has been added to the N-terminus of the receiver domain and expressed in ER2566 E. coli host strain. By using protein trans-splicing as a post-translational modification, we ligated two flanking N- and C-terminal segments (N-extein and C- extein) by a peptide bond and concomitantly cut the sequence of interest out from the precursor protein and achieved to obtain a ligated soluble protein. The results showed that the protein trans-splicing was successful and can be used for the upcoming segmental isotopic labeling of our multi-domain protein.

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P 026

ENSEMBLE MODELS OF DISORDERED PROTEIN DOMAINS BASED ON LONG-RANGE DISTANCE DISTRIBUTION CONSTRAINTS FROM EPR MEASUREMENTS

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In many cases regulation of biological processes and the interaction of proteins with other biomacromolecules depend on conformational flexibility of certain protein domains. Such domains are often, but not always, located at a terminus of the peptide chain. They are often substantially disordered in at least one of the functional states and thus invisible by structure determination methods that depend on well-defined electron density or nuclear density. Depending on the extent of disorder and the size of the protein or complex, NMR techniques may or may not be able to yield information on conformation distribution. Distance distribution constraints in the 1.5-8 nm range, as they are available from double electron electron resonance (DEER) measurements, provide such information for any width of the conformation ensemble and for a broad range of protein environments. Further restraints can be obtained from water and oxygen accessibility and mobility measurements by continuous-wave EPR spectroscopy. Local secondary structure can be detected from periodic changes in accessibility and mobility parameters.

This contribution discusses the computation of ensemble models for such domains by a Monte-Carlo approach based EPR-based constraints and restraints as well as residue-specific Ramachandran plots. It will be discussed how true distribution of conformations can be distinguished from uncertainty of conformation caused by a lack of constraints. The approach is implemented in the open-source program Multi-scale Modelling of Macromolecules (MMM) available at www.epr.ethz.ch/software/index. It is demonstrated for an underconstrained ordered linker in a soluble protein,for an at least partially ordered domain in a secondary transporter, and for the disordered N-terminal domain of major plant light harvesting complex LHCII.

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P 029

A NOVEL APPROACH TO PROTEIN ASSIGNMENT IN SOLID STATE NUCLEAR MAGNETIC RESONANCE

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Here we describe a 3D-NCOCA that enables the sequential assignment of the entire nitrogen backbone of a 56-residue protein GB3 in the solid-state in an single experiment. The method exploits the Mixed Rotational and Rotary Resonance condition[1] (MIRROR) to drive polarisation transfer between the $^{13}CO/^{13}C_\alpha$ under moderately high spinning speeds (35 kHz). Due to the band selective nature of this transfer, polarisation is transferred not only to the $^{13}C_\alpha^{i-1}$ but also the $^{13}C_\alpha^{i}$ despite the stronger coupling to the $^{13}C_\alpha$adjacent to the CO. The geometry of this $C_\alpha CONC_\alpha$ results in couplings between the C_α^{i-1}/CO and CO/C_α^{i} that remain invariant irrespective of the proteins structure. This results in characteristic patterns in the 2D-NcoCA correlation spectra, with two resonances arising from each nitrogen site, the stronger representing the C_α of the preceding amino acid, the weaker that of the current amino acid. In the context of a 3D-NCOCA experiment, this permits the assignment of the protein backbone by tracing the connectivities between $^{13}C_\alpha^{i-1}$ and $^{13}C_\alpha^{i}$ in a manner analogous to that employed in the analysis of HNCA experiments in the liquid-state.

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P 032

RECOMBINANT EXPRESSION, REFOLDING AND INITIAL NMR STUDIES OF THE EXTRINSIC PHOTOCHEMICAL PROTEIN PSBO

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PsbO (33 kDa), an extrinsic protein of photosystem II (PS II), is present in all oxygenic organisms. Together with other extrinsic proteins it protects and stabilizes part of PS II called oxygen-evolving complex, where water is split by light driven oxidation into protons, electrons, and molecular oxygen. In the absence of PsbO, the oxygen evolution capability of PS II is reduced significantly. PsbO exhibits a high contents of β-sheet secondary structure. Neither crystal nor solution structures of PsbO from higher plants are currently available. Thus the current structural model including the association of the protein within PS II is mainly based on the crystal structure of cyanobacterial PS II.[1]

Preparation of recombinant PsbO for NMR measurements and initial NMR characterization has been achieved. Both single (^{15}N) and double labeled (^{15}N, ^{13}C) recombinant PsbO from Spinacia oleracea were expressed in Escherichia coli and the preparation was optimized to obtain a well folded non-aggregating protein. Similarity between native and recombinant PsbO was tested by fluorescence spectroscopy and secondary structure information was confirmed by NMR and FT-IR. Both the native and the recombinant protein contain a disulfide bond which is vital for the PsbO structure stability.[2] The formation of the native disulfide bond within the recombinant protein

sample used for NMR was verified by Raman spectroscopy. Initial sets of 2D and 3D NMR spectra were recorded in order to assess the existence of a stable fold allowing to start the resonance assignment.

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P 035

STUDY OF CISPLATIN DERIVATIVE BY NUCLEAR MAGNETIC RESONANCE (NMR), RAMAN SPECTROSCOPY, SMALL ANGLE X-RAY SCATTERING (SAXS) AND THEORETICAL APPROACHES

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Since its discovery some 40 years ago, cisplatin has evolved for its efficacy in one of the most used drugs in treatment of various cancer types. Huge effort was invested in understanding the action of cisplatin and development of more potent platinum, osmium, and ruthenium based drugs. These potential drugs target mainly neighboring purine bases of nuclear DNA forming covalent intra- or inter-strand cross-links that affect inhibition of replication and transcription, cell cycle arrest, and attempted repair of the damaged nucleotides. If such damage cannot be removed the cell dies[1]. Several structures determined by NMR or X-ray crystallography are now available in the PDB database containing 1,2d(GpG) cisplatin or oxaliplatin (e.g. 1AIO, 3LPV, 1A84). Common structural features of all these structures are: a significant roll (25-60°) of the guanine bases involved in the cross-link, bending and unwinding of the double helix at the site of cross-link and orientation towards the major groove. Also, the platinum-guanine plane angle varies between 19-54°. Although the experimental structures were often used as the starting models for molecular dynamics (MD) simulations[2,3], results of these MD still leave

many questions unresolved. In our contribution, we will present an NMR study of a DNA cross-linked with a new highly potent cisplatin-based derivative. Details of the binding site have been cross-examined using complementary solution techniques used in modern structural biology, including Raman spectroscopy with DFT calculations aided interpretation of the obtained vibrational spectra. Moreover, the calculated structure of the DNA duplex was verified using SAXS (Small Angle X-ray Scattering) curve that has been measured on an in-house bioSAXS.

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P 038

MATRIX PROTEIN OF MASON-PFIZER MONKEY VIRUS AND ITS MUTANTS INTERACTIONS WITH MEMBRANES

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Mason-Pfizer Monkey Virus (M-PMV) is a simple retrovirus which belongs to the genus of Betaretroviruses. In a retroviral life-cycle, matrix proteins play a key role particularly in the transport of viral proteins inside the infected cells and for their interaction with cellular membranes. The majority of retroviral matrix proteins are N-terminally myristoylated and this modification serves as a membrane targeting signal and as an anchor for the membrane interaction.

In this work, we focused on the wild-type matrix protein of M-PMV and its two budding deficient double mutants, i.e. T41I/T78I and Y28F/Y67F. The structures of the mutants were determined using solution NMR and structural changes in respect to the wild-type were compared. Further, the interactions of the proteins with water-soluble dioctanoyl phospholipids were studied. Dioctanoyl phospholipids are widely used as a model for the study of membrane interactions; however, this approach might lead to artificial results due to the hydrophobic interactions caused by non-membrane-like form of the phospholipids. Consequently, a new approach for the observation of the interactions was used. We measured the loss of the signal intensity in the ^{1}H NMR spectra of the protein after addition of the liposomes which consisted of phospholipids with naturally long fatty acid residues. The interactions of Human Immunodeficiency Virus (HIV-1) matrix protein and Mouse Mammary Tumor Virus (MMTV) matrix protein with liposomes were also measured for comparison. We found that neither the M-PMV matrix proteins nor the MMTV matrix protein interacted with the liposomes in the same manner as the HIV-1 matrix protein.

Therefore, we conclude that Betaretroviruses might adopt different membrane binding mechanism than HIV-1.

P 041
SPECIFIC ISOTOPE LABELING OF THE NEUROPEPTIDE Y RECEPTOR TYPE 2 VIA CELL FREE EXPRESSION

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The neuropeptide Y receptor type 2 (Y2R) belongs to the family of rhodopsin-like G protein-coupled receptors. Its cellular responses are mediated by tyrosine rich peptides and influence the inhibition of neurotransmitter release, the regulation of memory retention, circadian rhythm and angiogenesis. Furthermore, investigation of Y2R signaling has high relevance in treatment of obesity, since activation of Y2R induces satiety while other receptors of this family show opposite effects. NMR is a powerful tool to provide information about the function of Y2R in terms of the molecular structure and its intramolecular dynamics. However, preparation of functional receptor in sufficient amounts and NMR signal assignment are still challenging tasks. Here, we present a cell free expression system for the production of Y2R. Besides an accelerated production, easier purification of the expressed protein and no toxicity concerns, this system offers the great potential of selective isotope labelling without being hampered by the metabolism of a living host cell environment. The resulting NMR spectra show a reduced spectral complexity and allow the investigation of specific amino acid sites, e.g. with respect to receptor activation.

P 044

31P CODEX NMR WITH POWDER-AVERAGE MODELLING FOR MEASURING LATERAL DIFFUSION IN LIPID BILAYERS

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Lateral diffusion of phospholipids is a process essential to membrane function, and its accurate determination can provide insights into kinetics of membrane-associated biochemical reactions. Here we describe the application of CODEX (Centerband-Only Detection of Exchange)[1] to measure lateral diffusion of phospholipids in lipid bilayers assembled into large unilamellar vesicles (LUV)[2]. CODEX is an ideal experiment for these systems because ^{31}P NMR can be measured in natural abundance, eliminating the need for synthetic labels.

The ^{31}P CODEX spectrum for LUV composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) consists of a narrow resonance in both the liquid crystalline and gel phases. With increasing mixing times, the resonance exhibits a monoexponential decay from which the correlation time for lateral diffusion can be extracted, provided the LUV size and size-distribution has been established, for example using dynamic light scattering. We also employ a simulation model based on the powder-average distribution of lipids on a sphere to obtain the lateral diffusion coefficient from experimental data. Lateral diffusion coefficients determined in this fashion agree with established literature values. Another advantage of ^{31}P CODEX is the ability to multiplex; since different phospholipid headgroups appear as separate resonances in an NMR spectrum, their individual decays can be monitored. Lateral diffusion coefficients have been measured in the gel phase, which would prove useful in studying membrane heterogeneities, such as those induced thermotropically, by various membrane-associating proteins or in lipid rafts.

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P 047
HYDRATION PROPERTIES OF CARRAGEENANS ANALYZED BY MULTI-NUCLEAR MIXED-PHASE MAS NMR

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Simultaneous observation of multiple phases (e.g. liquid, gel and solid) is one of the unique qualities of NMR spectroscopy. This asset is highly relevant for detailed structural analysis of polysaccharides/hydrocolloids such as their structural modifications due to interactions with water. Mixtures of water and polysaccharides often form suspensions and are examples of real and complex multiphase systems of relevance for both food and biofuel production. In this context the food hydrocolloids comprise eg. carrageenans, starch, pectin and dietary fibers, whereas cellulose and plant stems are relevant in relation to bioethanol.

Carrageenans is a versatile group of polysaccharides extracted from red seaweed (Rhodophyta) and they are used as texturizing ingredients in the food industry (turnover of 527 million US $ in 2009 [1]). Carrageenans are composed of sulphated galactans consisting of repeating units of disaccharides of 3-linked β-D-galactopyranose (G-units) and 4-linked α-D-galactapyranose (D-units) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA) units. A number of different carrageenans with different patterns of sulphate substituents exist and in the present study the hydration properties of three of the most used carrageenans: λ-, κ- and ι-carrageenan are explored. As κ-carrageenans can be partly methoxylated on C6 in the G-units, two κ-carrageenans with different degree of methoxylation were included in the study. All samples were ion-exchanged such that the counter ion was Na^+ in all samples.

Samples were analysed as dry powers as well as suspensions/gels at different degrees of hydration using D_2O as the source of hydration. Subsequently, ^{13}C CP/MAS and SP/MAS NMR spectra were recorded to assess the hydration impact on the immobile as well as the

immobile and mobile parts of the sample, respectively. In addition ^{2}H SP/MAS NMR spectra were used to assess the interactions/gelling on the water environment, whereas the hydration effects on the counterion were evaluated by ^{23}Na SP/MAS NMR spectra.

As also observed previously for pectin, modified celluloses and starches [2-5], hydration induces narrower resonances and reduces the amount of immobile regions in the carrageenans but the hydration process differs according to the specific type of carrageenan due to their different sulphate substitution pattern.

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P 050

NMR STUDIES ON HUMAN MELANOCORTIN-4 RECEPTOR FOR THE FUNCTIONAL IMPLICATION OF THE DISEASE CAUSING MUTANT

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The melanocortin receptors (MCRs) are members of the G protein-coupled receptor (GPCR) 1 superfamily with seven transmembrane (TM). Melanocortin-4 receptor (MC4R) has been highlighted recently by genetic studies in obese humans. Previous studies have shown that extracellular (ecto) domain is related to obesity disease. Extracellular domain of human MC4R (hMC4R) has critical region for interacting with hMC4R ligand. We observed specific interactions between hMC4R ecto-domain and SHU9119, which is well known potent antagonist of hMC4R. Antagonist, SHU9119 binds to Val2, Arg7, Trp16, Asn17, Leu23 and Lys33 residues of hMC4R ecto-domain in 200mM DPC micelle. In addition, mutations in the MC4R gene are the most frequent monogenic causes of severe obesity and are described as heterozygous with loss of function. We performed NMR studies on TM2 domain of MC4R and Asp90 mutant in a micelle environment. Data shows that TM2 of MC4R forms a long a-helix with a kink at Gly98. Interestingly, the disease-related mutant also has an a-helical conformation with a kink; however, the thermal stability and homogeneity of MC4R mutant are dramatically different from those of wild-type. The structure from molecular modeling and NMR suggests that Asp90 plays a key role in allosteric sodium ion binding. Our data concludes that the dynamic nature of MC4R and the sodium ion interaction in the allosteric pocket of receptor molecule together with antagonist binding are essential to its function, explaining the loss of function of the MC4R mutant.

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P 053

THE MOLECULAR ARCHITECTURE OF Aβ PROTOFIBRILS INVESTIGATED BY SOLUTION AND SOLID-STATE NMR

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The aggregation and fibril formation of the amyloid-β peptide (Aβ) are central in the pathology of Alzheimer's disease and accumulating evidence suggest that the neurodegenerative process is triggered by prefibrillar aggregates, so-called oligomers and protofibrils. Molecular characterization of these species has been impeded by difficulties to isolate pure and stable material. We have recently engineered a variant of Aβ, Aβcc, that allows for preparation of large amounts of protofibrils with properties that are indistinguishable from those of the wild type peptide.[1,2] This allows us to investigate the structural and biochemical properties of Aβ protofibrils.

We have used solid-state NMR spectroscopy to investigate the structure of Aβcc(1-42) protofibrils.[3] Our results show that the peptide adapts a β-hairpin conformation with high β-strand content in the C-terminal segment. We also detect a significant number of intermolecular contacts involving the C-terminus of the peptide. The data is consistent with packing of the hyrdophobic C-terminal part of Aβ into hexamer barrels that constitute the building blocks of protofibrils. The model provides molecular explanations for several biochemical observations of Aβ aggregation, including the differences in aggregation pathways observed for Aβ(1-42) and Aβ(1-40), resepectively.

Furthermore, we found that the N-terminal part of the peptide is still flexible in the protofibrils and resonances from this segment can be observed in solution NMR experiments. This gives us the possibility to combine solution- and solid-state methods to obtain a more complete picture of the structure and dynamics of Aβ protofibrils.

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P 056
PROTEIN ASSIGNMENT USING PARAMAGNETIC EFFECTS WITH PARASSIGN

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The first step of protein NMR study is the assignment of the NMR spectrum. Nevertheless, NMR spectra assignment remains challenging, especially for big proteins. The large number of peaks and faster relaxation caused by dipolar interactions give crowded spectra. The methyl groups are attractive probes from a spectroscopic point of view to improve NMR spectrum quality. A specific 13C labeling on the methyl groups of selected residues (Valine, Isoleucine and Leucine) enables to work on a more sparse and resolved spectra (1) and ensures a nice overview of the protein thanks to a good dispersion of these latter residues all over the protein. However NMR assignment remains challenging and time-consuming. Besides it requires high protein concentration to counteract the use of multidimensional NMR experiments that induces more important relaxation. We aim here to introduce a new assignment strategy combing methyl group labelling and paramagnetic effects. Paramagnetic effects and precisely pseudo-contact shifts (PCS), which is a perturbation of the chemical shift under the effect of an unpaired electron, carry interesting distance information. Indeed a PCS depends only on the distance between the observed nucleus and the paramagnetic centre. PARAssign (2) software uses PCS from several paramagnetic centres positions and the protein crystal structure to assign protein NMR spectrum. PCS datasets are obtained from simple 2D-HSQC NMR experiments by tagging the protein with a paramagnetic probe on the protein surface. PARAssign has already been successfully used to obtain amide assignment for a small 14 kDa protein and on synthetic data for a 47 kDa protein on the methyl groups. We present here the assignment of the methyl groups on the Leucine, Valine and Isoleucine of a bigger protein of 25 kDa (the N terminal domain of Hsp90) using PCS experimental data. We show that with only PCS extracted from 2D-HSQC spectra, this new

PARAssign strategy enables to assign up to 85% of the observed PCS of Leucine, Isoleucine and Valine methyl groups. Therefore PARAssign strategy is a promising method to be applied on big proteins that couldn't be assigned with traditional NMR methods assignment.

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P 059

COMBINING SPECIFIC-METHYL LABELING, 3D & 4D NUS, SAXS AND ADVANCED COMPUTATION TO UNDERSTAND ARFS AND ARFGAPS IN THE RAS SUPERFAMILY

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ADP-ribosylation-factors (Arfs) are small guanine nucleotide binding proteins that belong to the Ras superfamily of GTP-binding proteins. ASAP1 is a member of the Arf-specific GTPase-activating proteins (ArfGAPs), which can regulate Arfs by stimulating the hydrolysis of GTP bound to Arf. ASAP proteins are involved in cell migration and invasion. The overall goal of our research is to examine the structural biology of Arf1, ASAP1, the complex of Arf1:ASAP1, and membrane surface activation via interaction with phosphoinositide lipids (PIP2). The enzymatically active fragment of ASAP1, which we call PZA, is comprised of three domains, the PH domain (P), and the Arf GAP domain and Ankyrin repeat domain (together referred to as ZA). The crystal structure of related family member ASAP3:Arf6 have been determined; however, the functionally essential PH domain was excluded in the structure. We utilize a multi-disciplinary approach combining NMR, small-angle X-ray scattering (SAXS), and X-ray crystallography to examine the domains and interactions. The 32 kDa dual-domain ZA construct was optimized for stability and solution behavior. Relaxation properties and SAXS data suggested an unusual, highly anisotropic shape of the molecule, thus indicating the necessity of full deuteration. Complex isotopic labeling schemes were employed, consisting of uniform ^{15}N and ^{13}C labeling, combined with selectively protonated $^{13}CH_3$ methyl groups of I, L, and V residues (including stereospecific labeling of L and V). Non-uniform sampling (NUS) methods combined with our efficient reconstruction software (NESTA-NMR, JBioNMR-online 2015) were used to obtain ultra high-resolution triple-resonance, 4D methyl-methyl-NOESY, and 4D N/N

NOESY NMR spectra. These data provided nearly complete resonance assignments and sparse structural restraints. These sparse restraints were combined with NH residual dipolar coupling (RDC) data and used in a novel structure calculation protocol designed to improve structure calculation for medium-to-large proteins from sparse data sets, Pomona/CS-Rosetta-CM (Shen and Bax, Nat. Methods, in press). This approach yielded a high-resolution (rmsd = 1.5 Å) structure of ASAP1-ZA, which was significantly improved compared to Xplor-NIH calculations based on the same restraint and chemical shift data. The ZA structure is highly asymmetric and correlates well with the known inactive structures. The PH domain structure has been determined by both solution NMR (also using the CS-Rosetta-CM protocols) and X-ray crystallography. Interactions between PH and ZA, as well as between PH and PIP2, combined with SAXS studies are addressing the solution structure of the complete three-domain PZA construct. These methods demonstrate that high-quality structures of multi-domain proteins (>30-40 kDa) and complexes are accessible via solution NMR and SAXS technologies.

P 065

H/D EXCHANGE OF A 15N LABELED TAU FRAGMENT AS MEASURED BY A SIMPLE RELAX-EXSY EXPERIMENT

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We present an equilibrium H/D exchange experiment to measure the exchange rates of ^{15}N labile amide protons in intrinsically unfolded proteins. By measuring the contribution of the H/D exchange to the apparent T1 relaxation rates in solvents of different D_2O content, we can easily derive the rates of exchange for rapidly exchanging amide protons. The method does not require double isotope labelling, is sensitive, and requires limited fitting of the data. We demonstrate it on a functional fragment of Tau, and provide evidence for the hydrogen bond formation of the phosphate moiety of Ser214 with its own amide proton in the same fragment phosphorylated by the PKA kinase.

P 068

DETERMINATION OF THE KINETICS OF PHOSPHORYLATION OF TYROSINE HYDROXYLASE AND ITS INTERACTION WITH 14-3-3 ZETA ISOFORM ELUCIDATED BY NMR

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Human tyrosine hydroxylase 1 (hTH1) activity is regulated by phosphorylation of its N-terminus and by an interaction with modulatory 14-3-3 proteins. In order to monitor structural changes within the regulatory domain of hTH1 (RD-hTH1, region of first 169 residues) caused by phosphorylation of S19 and S40 we have assigned NMR spectra by two different approaches. The non-uniform sampling approach based on sparse multidimensional Fourier transform allowed efficient acquisition of high dimensional NMR spectra. Increased dimensionality (5D) provided significant speed up of backbone and side-chain assignment of the unstructured RD-hTH1 region (~70 residues). The rest (structured parts) of RD-hTH1 was assigned by conventional set of 3D NMR experiments.

The quantification of the binding mechanism between the doubly phosphorylated hTH1 and 14-3-3zeta was achieved by applying of the phosphorous NMR. The proof of principle of our approach was shown for the interactions between 14-3-3zeta and minimalistic system comprising first 50 residues of hTH1 (hTH1_50), containing both phosphorylation sites of our interest (S19, S40). Analysis of the NMR titration data revealed that a 14-3-3zeta dimer and the S19_S40-doubly phosphorylated hTH1_50 interact in multiple ways, with three major complexes formed: (1) a single peptide bound to a 14-3-3zeta dimer via the S19 phosphate with the S40 phosphate occupying the other binding site; (2) a single peptide bound to a 14-3-3zeta dimer via the S19 phosphorous with the S40 free in solution; or (3) a 14-3-3zeta dimer with two peptides bound via the S19 phosphorous to each binding site.

As the principle of this methodology was successfully proved for hTH1_50 we will apply it for revealing the binding scenario between 14-3-3zeta and phosphorylated RD-hTH1 protein.

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P 071

"FILTERING" INTERMOLECULAR NOES IN A 50 KDA RNA-PROTEIN COMPLEX BY COMBINING PROTEIN PERDEUTERATION WITH SELECTIVE, AMINO ACID-TYPE AND RNA LABELING

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Protein interactions with mRNA regulate gene expression post-transcriptionally from mRNA splicing to mRNA transport and translation. TDP-43 (TAR DNA binding protein - 43 kDa) binds in two copies to a UG-repeat sequence upstream of exon 9 of CFTR (cystic fibrosis transmembrane conductance regulator) and this leads to skipping of the exon associated with severe forms of cystic fibrosis. In principle, NMR spectroscopy is a powerful tool to determine the structure of such regulatory RNA-protein interactions, but structure determination of larger RNA-protein assemblies still remains challenging. Especially the detection and assignment of intermolecular contacts is difficult in larger RNA-protein complexes (50kDa) since 2D and 3D filtered/edited NOESY experiment suffer from short transverse relaxation times.

We therefore eliminate the need for lengthy filtering and editing elements by using selective amino acid-type labeling (both ^{13}C,^{15}N-labeled and unlabeled) in a fully deuterated protein background. Simple 2D NOESY spectra recorded on fully deuterated TDP-43 with selected protonated amino acids in complex with unlabeled RNA yield intermolecular NOEs between amino acid sidechains and RNA ribose and base protons with high sensitivity. Similarly, 3D ^{13}C-edited HMQC NOESY spectra acquired on fully deuterated TDP-43 with selected ^{13}C,^{15}N-labeled amino acids or ^{13}C ILV-labeling in complex with unlabeled RNA allows unambiguous NOE assignment of amino acid sidechains to RNA protons. To confirm the assignments of RNA ribose and base proton NOEs to the protein sidechains, we record 3D ^{13}C-edited NOESY HSQCs using fully or segmentally ^{13}C-labeled RNA in complex with fully deuterated TDP-43 with selected, protonated amino acids. Using this array of labeling schemes, we could recover over 600 intermolecular NOEs between the two TDP-43 copies and the UG-rich RNA. Our approach should also enable

structure determination of other large RNA-protein complexes. The preliminary structure will be presented and the implications for splicing regulation will be discussed.

P 074

ATOMIC - RESOLUTION CHARACTERIZATION OF PHOSPHORYLATED MICROTUBULE ASSOCIATED PROTEIN 2C (MAP2C) AND ITS EFFECT ON INTERACTION WITH 14-3-3

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Microtubules are flexible polymers constituting the eukaryotic cytoskeleton and taking part in fundamental cellular processes such as morphogenesis, cytokinesis and mitosis. Microtubule-associated proteins (MAPs) regulate the microtubule assembly and disassembly, the process termed dynamic instability, in a phosphorylation dependent manner. The microtubule dynamics in neuronal cells is mainly modulated by two members of the structural MAPs, MAP2 and tau, which play fundamental roles in the development of dendrites and axons. MAP2 bind and stabilize microtubules, but phosphorylation by protein kinase A induces dissociation of MAP2 from microtubules, which leads to microtubule destabilization. The MAP2 proteins are predominantly present in the dendrites of neuronal cells. All MAP2 proteins are transcribed from a single gene through alternative RNA splicing. The individual MAP2 proteins are distinguished according to their size and developmentally regulated expression levels. The high-molecular weight MAP2a and MAP2b contain approximately 1830 amino acids, and low molecular weight MAP2c and MAP2d consist of 467 and 498 amino acids, respectively, in rats. The smallest isoform, 49 kDa MAP2c, is predominantly expressed perinatally, during the time of main dendritic outgrowth and synaptogenesis. Postnatally, only regions exhibiting postnatal plasticity, such as the olfactory bulb, continue to express MAP2c at a high level, suggesting that MAP2c plays an important role in the synaptogenesis and dendritic outgrowth. Interestingly, independent studies have shown high expression of the regulatory 14-3-3 protein in the same regions, and speculations about its importance in the synaptogenesis appeared in the literature.

The MAP2c is a 49 kDa intrinsically disordered proteins (IDPs). Intrinsically disordered proteins (IDPs) are macromolecules interesting both from biophysical and physiological point of view, but difficult to

study by the current biophysical methods. Nuclear magnetic resonance (NMR) is a key technique for atomic-resolution studies of IDPs, but its applicability is limited by a spectral overlap in case of long or highly repetitive amino-acid sequences. Our group recently developed high-resolution NMR methodology that overcomes this limitation and makes studies of large IDPs possible [1].

In this study, we investigated the effects of phosphorylation by protein kinase A on MAP2c, with a combination of mass-spectrometry and NMR. We determined the kinetics of phosphorylation, and identified the phosphorylated residues. We also studied the influence of phosphorylation on the affinity of MAP2c for the regulatory protein 14-3-3, and determined the binding sites of 14-3-3 on MAPc.

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P 077

NMR INVESTIGATION OF TRANSMEMBRANE AND JUXTAMEMBRANE DOMAINS OF HER2 RECEPTOR KINASE IN MONOMERIC AND DIMERIC STATES.

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Receptor tyrosine kinases of the human epidermal growth factor receptor (HER or ErbB) family transduce biochemical signals across plasma membrane, playing a significant role in vital cellular processes and in various cancers. Inactive HER/ErbB receptors exist in equilibrium between the monomeric and unspecified, so-called, pre-dimeric states. After ligand binding, the receptors are involved in a strong lateral dimerization with proper assembly of their extracellular ligand-binding, single-span transmembrane and cytoplasmic kinase domains, and it was shown that the transmembrane domain plays an important role in that processes. The dimeric conformation of the HER2 transmembrane domain that is believed to support the active orientation of cytoplasmic kinase domain configuration corresponding to the receptor active state was previously described in lipid bicelles. In addition antiparallel dimerization of short juxtamembrane cytoplasmic helical domains was also shown to correspond to the active state of the EGFR receptor. Here, using another membrane-mimicking micellar environment we identify by high-resolution NMR spectroscopy an alternative HER2 transmembrane domain dimerization mode, coupled with the self-association of membrane embedded cytoplasmic juxtamembrane region in a fashion, possibly allowing the effective inhibition of the receptor kinase activity. In other words, the dimeric structure of HER2 transmembrane and juxtamembrane domain is reported, which corresponds to the receptor inactive state. We show that such inactive state is characterized by the helix-helix-interaction with small (~20 deg.) angle between the helical aces and extended contact

interface, and differs substantially from the interaction mode, observed previously in lipid bicelles.

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P 080

STRUCTURAL CHARACTERIZATION AND INTERACTION STUDIES OF PEPTIDES REPRODUCING THE ODIN-SAM1 BINDING REGION FOR EPHA2-SAM

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Odin is a cytosolic adapter protein which belongs to the ANKS (Ankyrin repeat and Sterile alpha motif domain protein) family (1) and includes in its primary sequence two Sam (Sterile alpha motif) domains in tandem. Sam domains are small helical protein binding modules which can be considered highly versatile as concerning their binding preferences (2). It has previously been reported that Odin exerts a regulatory role towards EphA2 receptor endocytosis and consequent degradation and likely influences receptor pro-oncogenic functions (3). Odin is recruited at the receptor site by means of a heterotypic Sam-Sam interaction. In detail, the first Sam domain of Odin (Odin-Sam1) binds the Sam domain of EphA2 receptor (EphA2-Sam) by forming a heterodimer with a dissociation constant in the low micromolar range (4). The two domains interact via the "Mid-Loop/End-Helix" topology of binding, in which the central regions of Odin-Sam1 constitutes the Mid-Loop interface whereas the C-terminal portion of EphA2-Sam provides the End-Helix surface.(4)

Herein, we report on peptide fragments -of different lengths- encompassing the Odin-Sam1 interacting portion for EphA2-Sam. Peptide structural properties have been analyzed by means of circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies in different solvent systems. These studies indicate that peptides lack of an ordered secondary structure in water, but in mixtures of water/trifluoroethanol they assume helical conformations.

Moreover, surface plasmon resonance (SPR) studies reveal that peptides interact weakly with EphA2-Sam (dissociation constant in the high micromolar range). Taken together, our results represent the starting point for a future design of molecules with enhanced binding affinity for EphA2-Sam and with possible therapeutic applications.

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P 083
STRUCTURAL INSIGHTS INTO EGCG-INDUCED AMYLOID-B OLIGOMERS

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Protein misfolding and aggregate formation are linked to a variety of so-far incurable human diseases, such as Alzheimer's disease. Fibrils formed by the β-amyloid (Aβ) peptide are the major component of plaques found in human brains affected by Alzheimer's disease. In addition to the fibrillar forms of Aβ, there are a multitude of smaller species, including spherical oligomers that may be formed transiently during the aggregation process. These oligomers are now thought to play a key role in the toxicity mechanism of the disease. In recent years, several classes of small molecules that can interfere with Aβ aggregation have been identified. The polyphenol compound epigallocatechin gallate (EGCG), which is found in green tea, prevents fibril formation by Aβ, and redirects it to form oligomeric particles that are non-toxic. We are studying the nature of the interaction of EGCG with Aβ, in order to understand how it can alter Aβ assembly and mitigate cell toxicity. We are employing both solution and solid-state NMR spectroscopy, in concert with other biophysical techniques such as light scattering, small-angle X-ray scattering and atomic force microscopy, to study the interaction at multiple scales. The two most common forms of Aβ – Aβ40 and the more toxic Aβ42 – are being studied concurrently and compared. This integrated approach will shed light on small molecule:amyloid interactions, as well as on structural pathways of amyloid aggregation.

P 086

ISOLATED VOLTAGE-SENSING DOMAIN OF HUMAN NAV1.4 CHANNEL: TOPOLOGY IN MEMBRANE MIMICKING ENVIRONMENT AND INTERACTION WITH SPIDER TOXIN HM-3

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Voltage-gated Na+ channels (Nav) are essential for signal transduction in nervous, cardiac, and muscle systems of multicellular organisms. The variable functional regions of Nav are prime targets of natural toxins from venom of scorpions, spiders, snakes and cone snails. These toxins serve as useful molecular tools to study channels structure and for design of novel biomedical compounds for treatment of a number of neurological diseases. Unfortunately, the structure of Nav channels and mode of its interactions with polypeptide toxins presently are poorly studied. Polypeptide chain of eukaryotic Nav (a-subunit) encloses 24 transmembrane (TM) helices, which form five quasi-independent domains: four voltage-sensing and one pore domains. This makes expression, purification and structural studies of full-sized Nav extremely difficult.

Here we present the NMR structural study of second voltage-sensing domain (VSD, 153 a.a.) from human Nav1.4 channel and its interaction with the inhibitory toxin Hm-3 from the crab spider (Heriaeus melloteei). ^{15}N- and ^{13}C,^{15}N-labeled VSD samples were produced using cell-free expression. The micelles of different detergents, lipid-detergent bicelles, and lipid-protein nanodiscs were assayed to find optimal membrane mimicking environment for the

NMR study. The membrane mimetics containing lipid bilayer (bicelles, nanodiscs) did not provide enough stability to the VSD and induce fast protein aggregation. Signals in NMR spectra of VSD in zwitterionic micelles (e.g. DPC) were broadened, probably due to extensive μs-ms time-scale motions, and some of the backbone VSD resonances were unobservable. High quality NMR spectra were obtained in the partially cationic LDAO micelles, although the sample stability was insufficient for structural studies. In addition ^{15}N-relaxation data revealed significant ps-ns time-scale mobility of VSD backbone in this environment. Finally, the VSD secondary structure and backbone dynamics were determined in the micelles of anionic lyso-lipid LPPG. The obtained data were in general agreement with the expected VSD topology. The domain involves four conservative TM helical regions (S1-S4) and additional short helical element S23 (Tyr84-Gln88) located between S2 and S3 helices. Moreover, in the micellar environment S4 helical structure is disrupted around one of the conservative Arg residues (R121). Short S45 helix is supposed to be a part of the linker to the pore domain.

^{15}N-labeled Hm-3 toxin (35 a.a.) inhibiting voltage-gated activation of Nav1.4 channel was produced by bacterial expression. Spatial structure of Hm-3 was determined by NMR. Hm-3 was found to adopt "inhibitor cystine knot" fold stabilized by three disulfide bonds. Its molecule is amphiphilic with a hydrophobic ridge on the surface enriched in aromatic residues and surrounded by positive charges. The ability of Hm-3 to interact with zwitterionic and anionic lipid vesicles was demonstrated, and the binding sites to the LDAO and DPC micelles on the surface of Hm-3 were determined. Surprisingly, the specific binding of Hm-3 to VSD in the LPPG environment was not observed. Nevertheless, the specific interaction of Hm-3 with VSD was observed in DPC micelles. The VSD binding site on the Hm-3 surface is located just above the site involved in the interaction with micelles.

P 089

APPLICATION OF MICELLES AND NANODISCS FOR STRUCTURAL STUDIES OF P75 NEUROTROPHIN RECEPTOR - MULTIDOMAIN INTEGRAL MEMBRANE PROTEIN

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P75NTR is a type I integral membrane protein, which plays a key role in neurotrophin signaling. The structural data, describing the receptor in various functional states is sparse and controversial. The transmembrane domain (TM) domain of p75NTR plays a key role in receptor function. This domain stabilizes receptor dimers through a disulfide bond essential for p75NTR. Using NMR spectroscopy we solved the three-dimensional structure of p75-TM in lipid micelles. We describe two different dimer conformations: the wild-type forms a covalent dimer stabilized by a disulfide bond, while the C257A mutant forms a non-covalent dimer via an interface on the opposite side of the a-helix. These p75 dimers differ in terms of crossing angle and γ-secretase cleavage.

We additionally investigated the connection between the states of TMD and intracellular domain (ICD) of p75NTR, in order to get an insight into the details of the molecular activation mechanism of the receptor. For that purpose, we used two constructs on the basis of the p75NTR: (1) construct, containing TMD and juxtamembrane "chopper" domain, and (2) construct, containing TMD and full-size ICD of the receptor. These constructs were incorporated into various membrane mimetics, including lipid/protein nanodiscs of different size and composition. We managed to obtain NMR spectra of constructs with folded "Death Domain" (DD), assign chemical shifts and study the intramolecular mobility of p75NTR ICD or "chopper" domain in lipid/protein nanodiscs and in almost physiological conditions. Our

data reveal that the whole ICD is extremely mobile and partially disordered, which results in a loose linkage between the motions of the ICD and motions of the TMD helix. Moreover, our experiments with lipid/protein nanodiscs, containing several copies of constructs on the basis of p75NTR, demonstrate that DDs of the receptor have no propensity to interact tightly with each other or to bind to the membrane. As a result, previously suggested activation mechanism of p75NTR is brought into question with our observations. With this respect, we discuss our data in the context of the receptor activation and suggest two possible mechanisms that contradict neither the reported NMR data, nor the previously published results of functional assays and other in vivo studies.

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P 092
IMPORTANCE OF THE SPECIFICITY OF TOLAIIIV.CHOLERAE / PIII-N1CTXΦ COMPLEX DURING BACTERIAL PHAGE INFECTION

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Vibrio cholerae is responsible for cholera, a widespread disease in countries without safe drinking water. Vibrio cholerae colonizes the small intestine using a type IV pilus called the toxin co-regulated pilus (TCP). Virulent V. cholerae secrete cholera toxin (CT). The genes of the toxin are acquired via horizontal gene transfer during Vibrio cholerae phage infection by CTX^{Φ}. Phage infection is a two-step process involving the minor coat protein pIII located at one end of the phage and the TolAIII protein of the gram negative bacteria. First the N2 domain of $pIII^{CTX}$ binds to the pilus, then the N1 domain of $pIII^{CTX}$ interacts with the TolAIII domain of V. Cholerae Tol-Pal system. The second step was demonstrated to be the limiting step of the infection [1] and our aim is to characterize the structural parameters involved in pIII-N1/TolAIII recognition to understand the phage / bacterium specificity necessary for phage infection.

Using NMR and two-hybrid experiments, we have first established that bacterial TolAIII proteins are specific to phagic pIII-N1 domains. $TolAIII^{E.coli}$ cannot interact with $pIII\text{-}N1^{CTX}$ and $TolAIII^{V.cholerae}$ does not interact with $pIII\text{-}N1^{Fd}$. The X-ray structure of two TolAIII / pIII-N1 complexes have been solved [2] [3] and revealed that the interacting sites are not conserved in the two complexes. The E. coli complex involves the beta 3 strand and the V. cholerae complex the beta 2 strand. Moreover the two complexes contain intermolecular salt bridges formed by unconserved residues. Mutations performed on these residues demonstrate that salt bridges are essential for the complex formation and probably the driven force for the complex formation.

During complex formation a conformational change of $TolAIII^{V.cholerae}$ is observed by NMR. A NMR structural study of the free TolAIII protein is performed to observe the conformational changes

between the free and bound structures. We will analyze these structural changes in terms of flexibility of TolAIII proteins.

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P 095

THE NEDD4-1 WW3* DOMAIN RECOGNIZES THE ALPHA-ENAC PY MOTIF PEPTIDE VIA A COUPLED FOLDING-BINDING EQUILIBRIUM

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The four WW domains of human Nedd4-1 (Neuronal precursor cell expressed developmentally down-regulated gene 4-1) interact with the poly-proline (PY) motifs of the epithelial Na+ channel (ENaC) subunits, with the third WW domain (WW3*) showing the highest affinity. We have previously shown that the alpha-ENaC PY motif binding interface of WW3* undergoes conformational exchange on the millisecond time-scale, indicating that conformational sampling plays a role in peptide recognition [1]. To further understand this role, we have investigated the structure and dynamics of hNedd4-1 WW3*. The NOE-derived structure of the apo-WW3* domain is very similar to the domain in complex with the alpha-ENaC peptide, although analysis of 3JNbeta and 3Jalphabeta couplings reveals side-chain chi1 rotameric averaging for several residues in the apo-WW3* domain, which was further investigated by molecular dynamics simulations of the apo- and alpha-ENaC peptide-bound WW3* domain. Modelfree analysis of the 15N spin relaxation reveals that the apo- and alpha-ENaC peptide-bound states of the WW3* domain also have very similar backbone ps-ns time-scale dynamics. However, the apo-WW3* domain exhibits pronounced chemical exchange on the millisecond time-scale and this chemical exchange is quenched upon peptide binding, indicating that conformational selection is responsible for peptide interaction. 1H and 15N CPMG relaxation

dispersion experiments at five different temperatures reveal that the apo-state of the WW3* domain exists in an equilibrium between the natively folded peptide-binding competent state and a random coil-like denatured state. The thermodynamics of the folding equilibrium was determined by fitting a thermal denaturation profile monitored by CD spectroscopy in combination with the CPMG data, concluding that the unfolded state is populated to about 20% at 37°C. The results show that the binding of hNedd4-1 WW3* domain to the alpha-ENaC peptide follows a coupled folding-binding equilibrium.

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P 098

STRUCTURAL CHARACTERISATION OF COMPLEX BETWEEN PROTEIN-TYROSINE PHOSPHATASE A (MptpA) AND PROTEIN-TYROSINE KINASE A (PtkA) FROM M. TUBERCULOSIS BY NMR SPECTROSCOPY

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Protein-tyrosine phosphatases (PTPs) and protein-tyrosine kinases co-regulate cellular processes. In pathogenic bacteria, they are frequently exploited to act as key virulence factors for human diseases. Mycobacterium tuberculosis, the causative organism of tuberculosis, secretes a low molecular weight PTP (LMW-PTP), MptpA, which is required for its survival upon infection of host macrophages. PtkA, the kinase complementary to MptpA, belongs to the haloacid dehalogenase (HAD) superfamily [1] and phosphorylates two key tyrosine residues in MptpA, thereby co-regulates cellular function [2, 3]. Previously, we have reported the structure of MptpA and also characterized the interaction interface of the MptpA-PtkA by NMR spectroscopy [4]. Here, we aim to determine the three-dimensional structure of PtkA and the MptpA-PtkA binary complex.

NMR backbone assignment of the 216 amino acid protein D_{1-75}PtkA is essentially complete to the extent of 86% for the non-proline residues. Secondary chemical shifts and NMR-based relaxation studies revealed that the protein PtkA possess an N-terminally located intrinsically unstructured region (IUR, M1-G80). The biological function of this disordered tail is unknown. Using the paramagnetic spin labelling studies, we present current insights gained into the dynamics of this IUR and its interaction with the structured core domain of the PtkA.

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P 101
NMR STUDIES ON INTRINSICALLY DISORDERED PROTEINS

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An efficient approach to NMR assignments in intrinsically disordered proteins is presented making use of the good dispersion of cross peaks observed in [^{15}N,^{13}C']- and [^{13}C',^{1}H^{N}]-correlation spectra. The method involves the simultaneous collection of {3D (H)NCO(CAN)H and 3D (HACA)CON(CA)HA} spectra for backbone assignments via sequential H^{N} and H^{alpha} correlations and {3D (H)NCO(CACS)HS and 3D (HS)CS(CA)CO(N)H} spectra for side-chain ^{1}H and ^{13}C assignments, employing sequential ^{1}H data acquisitions with direct detection of both the amide and aliphatic protons. The efficacy of the approach for obtaining resonance assignments with complete backbone and side-chain chemical shifts is demonstrated experimentally for the 61 residue [^{13}C,^{15}N]-labelled peptide of a voltage-gated potassium channel protein of the Kv1.4 channel subunit. The alpha subunit of the voltage-gated K^{+} channel gives rise to inactivating A-type K^{+} currents. The process of channel inactivation is mediated by its N-terminal protein structure ("ball-and-chain" domain), which occludes the intracellular entry of the channel pore to terminate K^{+} conductance and has potentially to be intrinsically disordered to reach its receptor site in the internal vestibule of the channel pores.

We further present results of the structure determination of RecQL4 which belongs to the family of RecQ helicases, a class of proteins initially identified in E. coli and involved in several aspects of genome

maintenance. This molecule consists of structured domains and intrinsically disordered protein regions. Most RecQ helicases carry a conserved carboxyterminal domain (RQC) involved in dsDNA interaction and scaffolding. RecQL4 plays a role in the initiation of DNA replication and mutations in RecQL4 are linked to the Rothmund-Thomson, the RAPADILINO and the Baller-Gerold syndromes characterised by e.g. skeletal abnormalities or pre-disposition to cancer (osteosarcoma). The N-terminal part of the human RecQL4 protein adopts a homeodomain fold, binds DNA in a non-sequence specific manner and interacts with TopBP1. The N–terminal region of RecQL4 bears a further potential zinc-binding motif (ZBM). Here we report the structure of this second motif from the mouse RecQL4 sequence where the Asn of the human CxxN motif is replaced by the more canonical CxxC motif. The additional Cys is highly conserved in most other species but replaced by Asn in primates.

P 104
BEHAVIOR OF NATIVE AND PHOSPHORYLATED MYOSIN II COILED-COIL FRAGMENTS

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Non-muscle myosin IIA heavy chain (NMIIA) is a 1960 residues long protein which has mainly coiled-coil structure. NMIIA coiled-coils form filaments in the cellular cytoplasma and are responsible for locating cells. Phosphorylation by CK2 kinase at Ser1943 probably alters the filament formation according to the current literature, which can be an important step in developing tumor metastasis in tumor cells. Our studies are focused on molecular basis characterization of coiled-coil fragments of myosin IIA to investigate the effect of phosphorylation on the myosin filaments, and comparing with its isoform, non-muscle myosin IIB.

We investigate the aggregation tendency of the fragments as a function of temperature, pH and ionic strength using a combined approach of MS, CD spectroscopy, diffusion NMR and multinuclear NMR spectroscopy. Six protein fragments and their phosphorylated forms were characterized: a disordered 67 residues long fragment (M67), a 111 residues long fragment containing both coiled-coil and disordered regions (M111), and a M111Leu mutant with three alanine to leucine mutations. CD temperature-dependent melting curves showed no significant difference between the native and phosphorylated species, however, M111Leu coiled-coil has significantly increased stability compared to the wild type protein. Temperature dependent translational diffusion measurements are in agreement with these findings, moreover there are differences in the aggregation tendencies: M111Leu is more prone to filament formation than the wild type M111. The M67 behaved as a disordered fragment in the whole temperature range, as expected. The unfolding of M111 also can be monitored from the diffusion measurements.

Residue specific characterization of the above mentioned fragments allow calculation of secondary chemical shift (SCS) values. Thus, it is possible to detect the existence of disordered regions and the nascent helical tendencies. On the other hand the predicted coiled coil sequence is undetectable in the spectra, regardless of the chosen experimental conditions. We found no significant difference between the relaxation properties of the native and phosporylated fragments. Our investigation disproves the previously suspected effect of phosphorylation on NMIIA filament assembly.

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P 107
MOLECULAR-MECHANICAL LINK IN A SHEAR-INDUCED SELF-ASSEMBLY OF A FUNCTIONALISED BIOPOLYMERIC FLUID

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Kappa-carrageenan is a linear sulphated anionic polysaccharide extracted from edible red seaweeds. It is widely used in food sciences, pharmaceutical industry, biotechnology, tissue engineering, medical applications and is also used in some unconventional areas like marbling [1]. Aqueous kappa-carrageenan solutions demonstrate shear-thinning properties at lower polymer concentration in a solution and form transparent gels at higher polymer concentration.

Macromolecules of kappa-carrageenan solutions also undergo conformational changes with temperature. The molecules are in random coil conformation at 313K and adapt isolated rod conformation upon cooling to room temperature. Further cooling leads to intermolecular synergy between rods resulting in gelation of a 0.5% kappa-carrageenan solution at 283K.

Mechanical behavior of the 0.5% carrageenan fluid changes from Newtonian (313K) to shear-thinning (283K). This mechanics is presumably correlated with conformational changes experienced by these biomacromolecules upon cooling. Therefore novel ^{23}Na MQF rheo-NMR [2] methods were applied to establish this molecular-mechanical link.

The fluid was sheared in 1 mm gap at different flow rates in a Couette cell mounted inside 9.4Tmagnet. In addition, temperature in the Couette cell was varied from 285K to 313K with a range of flow rates sampled at each temperature. Na-23 nucleus frequency was used for the NMR signal detection in all experiments. We were able to demonstrate that Na-23 DQF MA and TQF signals were observed only in the presence of shear, however at 313K Na-23 MQF signals were absent with and without shear field. We have also shown a significant change in sodium dynamics under shear with the increase of flow rate

and temperature. We also performed bulk rheological measurements of this fluid for all temperatures used in this study. We were able to correlate the detection of Na-23 MQF signals with the presence of flow-induced molecular alignment occurring only with macromolecules present in rod conformation.

This result is significant as to the best of our knowledge the correlation between shear-thinning, conformation of molecules in a fluid and mechanically induced behavior has never been shown experimentally. The work has been done without adding extra sodium and using cations that naturally compensate negatively charged sites of this anionic polysaccharide. Sodium is a very important nucleus as it is naturally present in many biological systems including human bodies. The elucidation of these phenomena with sodium may result into shifting these studies towards in vivo to better our understanding about the mechanics of body fluids and their molecular behavior under shear. This will have huge impact on anti-cancer drug delivery and could improve our management of cancer and other life-threatening diseases.

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P 110
ANALYTICAL DESCRIPTIONS OF CROSS-POLARIZATION DYNAMICS: RELAXING THE SECULAR APPROXIMATIONS [1]

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In this work, analytical expressions of the cross-polarization (CP) dynamics under both static and magic-angle spinning (MAS) conditions are obtained by solving the generalized Liouville-von Neumann quantum mechanical equation beyond the standard approximations, i.e., reintroducing neglected non-secular terms in the system superoperator. Although the simple model of a two-spin system interacting with a spin-bath gives a rather crude description of CP dynamics it accounts well for the orientation dependence of CP in a static sample of ferrocene powder and permits to detect slight departures from the Hartmann-Hahn matching condition. This approach also has the advantage of yielding manageable analytical expressions that can be used even by less inclined or experienced workers to obtain results that are good enough in an operational sense. Moreover, the resulting spin diffusion rate constants containing the different sources of anisotropy of the system-environment interaction as well as their dependence on the MAS frequency are related semi-quantitatively to the local network of dipolar interactions. Finally, it is shown that non-secular solutions improve significantly the analysis of CPMAS-based separated-local-field spectroscopy experimental data in the absence of homonuclear decoupling.

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P 113
IMPROVING STRUCTURE QUALITY FROM SPARSE NMR DATA SETS USING A FRAGMENT-BASED APPROACH IN CYANA

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NMR protein structure determination is mainly driven by restraints from NOE measurements. Different factors like increasing molecular weight or embedding in membranes lead to line broadening and signal intensity decrease. Methods to overcome line broadening like deuteration come at the price of eliminating numerous observable NOEs. These and other effects lead to sparse datasets, which are responsible for a considerable decrease in the structure quality. Thus, there has been notable interest in determining global folds based on a minimal number of NOEs. At a local structure level, similarities of the protein backbone occur that can be found in fragments of other proteins. Thus, it is possible to assemble the local protein backbone fold of a given protein using suitable fragments selected from a pool of various available proteins. Since chemical shifts are highly sensitive to their local environment, methods like TALOS+ are able to predict protein backbone torsion angles by selecting fragments based on chemical shifts.

In this study we implemented a new CYANA potential that is able to select suitable fragments from a predefined fragment ensemble in combination with a sparse NOE dataset to improve the overall structure quality. Here, the predefined fragment ensemble is automatically assembled based only on chemical shift and sequence information available. The approach was tested on a dataset of NOE signals of eight different proteins where the effects of both NOE sparseness and chemical shift sparseness were analyzed. A second dataset of 41 proteins, for which NMR data and a crystal structureare available, was obtainedfrom the Northeast Structural Genomics consortium (NESG). Here the distance restraints available were

progressively thinned out from using all to none of the distance restraints.

Results from structure calculations with and without the usage of fragments where compared. We evaluated the formation of the native fold in terms of rmsd, hydrogen bond network, and secondary structure conservation compared to a reference structure. Overall we observe a general improvement of the structure quality in terms of rmsd using the fragment potential. Calculations employing the fragment potential in many cases lead to reasonably good predictions of the native fold where the conventional structure calculation based only on sparse data lead to misfolded or even completely unfolded structures. Furthermore, we observe strong improvements in the prediction of the native hydrogen bond network and a concomitant improvement of the secondary structure prediction for a majority of the datasets with a high degree of sparseness. These results show that our fragment potential can improve the structure quality especially in cases of sparse datasets. The fragment potential can be employed during a structure calculation and can therefore be used directly in combination with all NMR restraints available to drive the calculation towards more native structures.

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ONE-DIMENSIONAL MODELS IN ANALYSIS OF MULTIPLE-PULSE EXPERIMENTS AND SOLID STATE NMR ABSORPTION LINES

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Modern applications of nuclear magnetic resonance and electron spin resonance, and especially quantum computing problems call for more effective formalisms to describe relaxation and evolution of various orders of coherence in the presence of various control effects.

Our work is devoted to the possibilities of one-dimensional models in the analysis of the NMR absorption line shape.

It is motivated by the fact that in isolated quantum system in d =1 spatial dimension, breakdown of equilibration is captured by the phenomenon known as many-body localization (MBL). Recent experimental advances make it possible to produce isolated, strongly interacting ensembles of disordered particles. In the MBL case, injections of energy propagate at most a finite distance even after a long time.This length is about 1-2 lattice parameters for the limiting case. It allows to solve the problem by considering a group of spins in the open translational-invariant lattice.

On this basis, one type of quantum control experiments has been established: multiple pulse experiments which were formalized by A.A. Nevzorov and J. H. Freed within the method of direct-product formalism [i]. As an extension of the above approach we begin with the solid-echo experiment in terms of the ensemble-averaged isochromats. If one were off-resonance, an additional factor of cos(wt) appears in equation, representing oscillations between the first-order coherence, and the zeroth- and second-order coherences. The method is easy to extend for describing the behavior of the system in a multiple-pulse sequence WHH-4.

In the high-temperature approximation, as a testing the detailed comparison of the known theoretical and experimental data on the shape of the absorption lines for the equidistant linear chain of spins has been carried. The region of existence of so-called cross-singular dips [ii] in the absorption spectra of polycrystalline two-spin systems is found unexpectedly extensive in one-dimensional case. In particular, a qualitative explanation is achieved to some of the experimental data on ^{13}C NMR in a completely substituted diamond [iii]. Here we suggest a formulation for the description of many-body dynamics based on structures that take into account the permutation symmetries and quantum coherences of a multispin system.

The line shape expression in the thermodynamic limit is obtained. Computational experiment have been used too with a direct diagonalization of the spin Hamiltonian along with theoretical calculations.

In general, we demonstrate wider applicability of one-dimensional models than they are considered presently.

Finally the quantitative analysis of the dipole-dipole MNR experimental spectra with cross-singular dips has been made for the particular compound: the polycrystalline trichloroacetic acid.

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SYSTEM-LEVEL SIMULATION OF MAGNETIC RESONANCE IMAGING MICRO SENSOR

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Miniaturized Faraday-induction based magnetic resonance sensors are radio-frequency micro-devices that operate within strong magnetic fields. In magnetic resonance imaging, such devices can be used for study of biological samples in a non-destructive manner [1]. To extract the information from the detected signal, the sensor is connected to a signal processing circuit. The development of these device-circuit systems often requires elaborate simulation methods for design, optimization and control. The full-scale finite element (FE) models cannot be directly used for co-simulation with the circuitry, due to high computational cost. Therefore, compact sensor models with high accuracy and low dimension are sought-after. Lumped element models are commonly used for this purpose, however, this approach often requires an experienced designer and may not be accurate enough. An alternative approach is to use mathematical model order reduction (MOR), which is highly accurate and can be automated [2]. In this work we use parametric model order reduction (pMOR) to reduce the size of the FE model and thereby lower the computational cost of a transient simulation. Furthermore, the pMOR allows to vary certain material parameters (e.g. the electrical conductivity of the coil's wire) at the level of the compact model, i.e., without the need to repeat the model reduction process for different parameter values. Simulations show an excellent match between the full-scale model and the parametrically reduced model.

Realistic signal processing circuits to extract information from detected signal are generally complex. As an example from [3], the signal induced in the MRI coil is first boosted using a low noise amplifier (LNA). The use of pMOR allows the transient co-simulation of the LNA

circuit with the compact model of the receiver coil. The results of the device-circuit co-simulation shows an excellent match between the pMOR model and conventional lumped element models (both estimated from the same FE model), but the pMOR model is valid for each applied frequency, whereas the lumped-element model does not take frequency changes into consideration.

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INTERACTION OFMETAL COMPLEXES CONJUGATED TO PITTSBURG COMPOUND B WITH THE MOMOMERIC AND AGGREGATED ABETA1-40 PEPTIDE IN SOLUTION BY NMR

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In vivo visualization of the amyloid plaques is a critical issue for early diagnosis of Alzheimer's disease (AD), and also important for monitoring new therapies. The most significant progress has been made in nuclear imaging using Positron Emission Tomography (PET) using the ^{11}C-labeled benzothiazol Pittsburgh compound B (PiB). Metal-complexes are increasingly explored as imaging probes in amyloid peptide related pathologies. In an effort towards the visualization of b-amyloid plaques by T_1-weighted MR imaging for detection of AD, we report the first detailed study on the mechanism of interaction between a metal-complex and both the monomer and the aggregated form of the Ab_{1-40} peptide in aqueous solution using a variety of NMR techniques.

We first characterized in vitro the stable neutral Gd^{3+} complexes of three different DO3A-monoamide derivatives (L1, L2 and L3) and of a negatively charged complex of a DOTA derivative (L4), conjugated to PiB, a well-established marker of Ab amyloid plaques. [1, 2, 3] The ligands differ in the nature and size of the spacer linking the macrocyclic chelator and the PiB targeting moiety. Their lipophilicity varies according to the nature of the spacer and the overall charge of the complexes (log $P_{Oct/H2O}$, -0.15 to +0.32). They form micelles in aqueous solution with cmc values of 0.50-1.49 mM, as determined by water proton NMR relaxometry. The parameters determining their r_1 relaxivity, including the water exchange rate and rotational correlation times, were assessed for the monomeric and micellar forms

of the complexes by combined ^{17}O NMR and ^{1}H NMRD studies. Upon binding to human serum albumin (HSA) or to the aggregated $A\beta_{1-40}$ amyloid peptide in solution, their relaxivity (40 MHz) increases 2-4 fold. These complexes bind to HSA with K_A values of 250 - 910 M^{-1}, as obtained by PRE techniques. The interaction of the corresponding La^{3+} analogue complexes with Ab_{1-40} in the aggregated form were studied by STD NMR (K_D ~ 67-190 mM), and the affinity constants obtained were compared with Surface Plasmon Resonance (SPR) results. The group epitope mapping (GEM) for the La^{3+} complexes, obtained by STD NMR, shows that the interaction occurs primarily via the benzothiazole unit.

The interaction of the La^{3+} and Gd^{3+} Li (i =1-4) complexes with ^{15}N-labeled Ab_{1-40} in the monomeric state was studied by ^{1}H-^{15}N HSQC NMR, evidencing a relatively specific weak interaction of Ab_{1-40} with LnL1 involving mainly the hydrophilic R5-L17 peptide region with a high concentration of charged residues, which includes the unstructured R5-Y10 region, the PII-helix prone E11-Q15 region, as well as K16 and L17 at the beginning of the first b-strand prone region. The interaction of monomeric Ab_{1-40} with LnL2 is scarcely detectable by NMR while the interaction with with LnL4 is the strongest of all the probes studied. These data were complemented by CD, DLS and TEM.

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P 131

CHARACTERIZATION OF SILICA-BASED MATERIALS BY HYPERPOLARIZED 129XE-NMR

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Hyperpolarized-^{129}Xe-NMR (HP-^{129}Xe-NMR) has developed into a powerful tool to characterize porous materials and surfaces. The chemical shift of Xe is very sensitive towards the environment and the measured chemical shift values in general reflect the interaction between the gas and the sample material exclusively.[1] Compared to other surface characterization techniques, such as N_2-adsorption and Hg-intrusion, it both provides information about the pore structure and geometry and can be used to probe the interconnectivity of the voids. Furthermore, one can detect defects of the pore architecture.[1,2]

Silica-based materials such as CPG (Controlled Porous Glasses) and MCM (Mobil Composition of Matter) materials can be synthesized with various textural properties, whereby geometry and pore structure can be modelled well-defined. This makes them to attractive construction materials in biotechnology, micro-reaction engineering and heterogeneous catalysis.[3]

Here we present the use HP-^{129}Xe-NMR to study pore properties of CPG samples and the transformation of those glasses into MCM materials.

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THE CURING STATE ANALYSIS OF THE ACRYLIC POLYMER FOCUSED ON THE EXTRACTS SEPARATED WITH THE SEMIAUTOMATIC COLLECTION DEVICE

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The UV curing of the polymer solution is generally used in various fields such as the ink, the varnish and the coating solution. As far as we know, it is difficult to evaluate the UV curing process. Thus, it is very important to build the effective method to trace the UV curing process. Fourier transform infrared (FT-IR) spectroscopy is well known as one of the most famous method to evaluate the UV curing process of the thin film substrate. However, the FT-IR spectroscopy cannot trace the whole UV curing process because the information obtained from the FT-IR spectroscopy is limited to the specific functional group. To understand the UV curing process in detail, the other approach was needed.In this study, we report on the UV curing resin consist of the acrylic polymer. For tracing the UV curing process, we focused on the extracts collected from the thin film on substrates. To estimate the degree of the UV curing process and the curing state, we measured the extracts by the solution NMR. To evaluate the extracts quantitatively, semiautomatic collection device for the thin film on substrates was used. We will discuss the UV curing process and curing state by comparing the extracts with the solid state after curing.

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MAGNETIC PROPERTY AND X-RAY CRYSTALLOGRAPHY OF bis[(μ2-CHLORO)CHLORO(1,10-PHENANTHROLINE)COPPER(II)] COMPLEX

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The dinuclear complex bis[(μ2-chloro)chloro(1,10-phenanthroline)copper(II)] (A) was synthesized, and characterized by X-ray and magnetic susceptibility measurements. The solid-state structure of complex (A) consists of centrosymmetric [CuCl(phen)μ-Cl]2dimers bridged by chloride anion. The 1,10-phenanthroline ligand, terminal Cl, and bridging Cl are coplanar and the square pyramidal Cu atom is displaced slightly (0.023 Å) out of this basal plane, towards the axial bridging chloride. The studies of the magnetic susceptibility and magnetization measurements of (A) reveal a weak antiferromagnetic interaction between copper(II) ions with an exchange coupling J = -0.65 ± 0.02 cm^{-1}. The electronic structure has been also determined by density functional theory (DFT) method.

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XENON SHIELDING TENSOR IN A NEMATIC LIQUID CRYSTAL CONFINED TO CYLINDRICAL CAVITIES

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NMR of dissolved atoms and molecules has successfully been used to obtain information about the properties of variety of materials, including liquid crystals. NMR spectra of solutes in liquid crystals can reveal information on, for example, the phase transitions and the orientational order as well as the structures of the liquid crystalline phases. The large and extremely polarizable electron cloud of xenon makes it an especially sensitive probe for the materials research. In a recently published theoretical study the orientational order of liquid crystals confined into cylindrical cavities of different sizes was studied with molecular dynamics simulations [1] and the results were later combined with electronic structure calculations on the nuclear shielding of xenon gas dissolved into the same systems [2]. In addition to the temperature dependence of the xenon nuclear shielding parameters, the theoretical works predicted cavity size dependent paranematic behaviour in the average ordering related to the interplay of the wall-induced orientational order and the self-organisation of the liquid crystal.

In this study we have carried out experiments on the nuclear shielding parameters of xenon dissolved in liquid crystal Phase 4 confined to cylindrical cavities of two different sizes. As model systems of cylindrical cavities we used aluminium oxide membrane (Anopore™) with cavity diameter of 20 nm as well as mesoporous silica particles (SBA-15) with cavity diameter of 7 nm. The isotropy and the anisotropy of the nuclear shielding tensor of xenon was studied as a function of temperature. The experimental results are compared to the results obtained with the calculations [1,2].

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P 143
NMR STUDY OF THERMORESPONSIVE BLOCK COPOLYMERS IN AQUEOUS SOLUTIONS AND SUSPENSIONS

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Stimuli-responsive polymers are class of materials that respond to small changes in chemical or physical condition. Along with the changes of conditions at the macromolecular level dramatic phase or property changes of polymer chains occur. These are changes in conformation, solubility, hydrophilic-to-hydrophobic balance, bond cleavage and degradation. In last decades various temperature responsive polymers and their derivatives, which exhibit a temperature-induced phase transition and form globules upon heating of their aqueous solutions, has attracted considerable attention[1].

We used NMR spectroscopy to study two types of amphiphilic block copolymers containing thermoresponsive component (poly(N-isopropylacrylamide) (PNIPAm) or poly(2-ethyl-2-oxazoline) (PEtOx). First type were the AB and AB_2 block copolymers poly(ethylene glycol)(PEG)-b-PNIPAm and PEG-b-$PNIPAm_2$, with constant length of PEG block (114 monomer units) and different length of PNIPAM block (47-249 units). Second type were ABC_2 block copolymers PEG-b-PEtOx-b-(poly(caprolactone)(PCL))$_2$ which were synthesized with constant length of PEG blocks (44 monomer units) and PEtOx blocks (252 monomer units), and various length of PCL blocks (87-131 monomer units). Lengths of blocks in both cases were determined by ^{1}H NMR, and both systems were investigated in D_2O solutions. Additionally, micellar particles were prepared in advance by nanoprecipitation method[2] in systems containing PEtOx.

Increase in temperature results in investigated systems in formation of micellar nanoparticles; this can substantially affect mobility of some blocks. Measurements of temperature dependences of integrated

intensities in high-resolution ^{1}H NMR spectra enabled us to determine the fraction p of polymer units with significantly reduced mobility.[3] By series of ^{1}H NMR measurements we obtained temperature intervals of the phase transition for all studied samples. For systems of the first type we found that phase-transition temperatures depend on the length of PNIPAm block and polymer concentration of the solution. For systems of the second type we found that temperature dependence of the p-fraction for PCL blocks shows a minimum around 320 K. At the same time, at highest temperatures the values of the p-fraction for PCL blocks are larger than those for PEtOx blocks. To obtain information about behavior of hydrophilic blocks and water molecules during the phase transition we used for both types of investigated systems spin-spin relaxation time T_2 measurements. Additionally, ^{1}H-^{1}H two-dimensional NOESY spectra were also recorded in some cases.

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P 149
FUNCTIONAL GROUP ANALYSIS OF TECHNICAL LIGNINS BY 31P NMR

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Depletion of fossil raw materials, concerns about man-made climate change have led to an increased interest in the utilization of lignin as a possible feedstock for value added products and chemicals. As it is a by-product in Pulp and Paper industry, lignin is produced in vast amounts. Its variety of chemical properties is based on a large density of functional groups und its highly cross linked aromatic backbone. Additionally to its naturally highly complex composition, lignins are further chemically altered by the pulping processes. Among others, like organic sulfur groups in kraft lignins and carboxyl groups, the majority of functionalities are hydroxyl groups. Despite the interesting chemical features lignin has not yet lived up to its full potential as a resource and is mostly used for important, but rather low value applications. Hence, the major part of the lignin is burnt in the mills for recovery of the pulping chemicals and energy production. Furthermore, lignosulfonates originating from sulphite pulping are used as dispersants and emulsifiers, due to their amphiphilic properties. A limiting factor for application is the challenge to adequately characterize and ensure a stable quality for lignin as a raw material.

Among other techniques NMR spectroscopy has proven to be a valuable tool to gain deeper insight into lignin structure and functional group composition. In particular phosphorous NMR has been used widely in lignin analytics (Argyropoulos et al. 1993). Phosphorous is therefore introduced by phosphitylation into the lignin, providing information about different types of hydroxyl functionalities as they appear in lignin and its technical counterparts. By adding suitable internal standards the hydroxyl moieties are also quantified. However, lately concern has been raised about the lab to lab reproducibility of the method (Balakshin and Capanema 2015).

This work revisits the main parameters of this method, such as the relaxation times and long term stability of most common the internal standards. In the case of technical lignins an application aims at a higher sample throughput experimental time, while still providing reliable and reproducible results. Also the applicability for technical lignin, in terms of interferences unique to this sample type, will be discussed. Therefore a comparison with quantitative data of total aromatic and aliphatic hydroxyl groups, determined by ^{1}H NMR after acetylation (Zakis 1994) will be presented, as well as a comparison of in-lab measurements to external data. Finally, the suitability of the method for various technical lignins and the influence of sample preparation on the analysis of lignosulfonates will be addressed and summarized.

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P 152
57Fe NMR IN MAGHEMITE

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Maghemite (γ-Fe_2O_3) is iron oxide, which is used in magnetic resonance imaging (MRI) as contrast agents for diagnostic purposes, location, tracking of labelled cell or drug delivery studies and for information storage in magnetic discs and tapes. It has a spinel structure and is closely related to magnetite Fe_3O_4. Maghemite can be considered as an Fe(II)-deficient magnetite with vacancies located in octahedral positions.

Two sample series of maghemite (γ-Fe_2O_3) fine particles were synthesized by the thermal decomposition of either iron(II) oxalate dihydrate or iron(II) acetate dihydrate. The ^{57}Fe NMR spectra were measured in the temperature range of 4.2-355 K in zero magnetic field and at the temperature of 4.2 K also in external magnetic fields of 0-2 T. The spectra consisted of partly overlapped signals of iron nuclei in both tetrahedral and octahedral sites. The obtained spectral shapes depended on the preparation protocol including the temperature of decomposition.

The temperature dependences of magnetization of octahedral and tetrahedral sublattice were determined from temperature dependences of NMR spectra. Analysis of the dependence on external magnetic field enabled separation of subspectra corresponding to tetrahedral and octahedral sublattices. Resulting spectral shapes of the sublattices were interpreted in terms of vacancy distribution in the vicinity of resonating nuclei.

P 158
EPR STUDY OF SPIN FLUCTUATIONS AND SPIN ORDERING IN CHEMICALLY DISORDERED FE-BASEDDOUBLE PEROVSKITE MULTIFERROICS

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Multiferroics are materials having two or more order parameters (for instance, magnetic, electric or elastic) coexisting in the same phase. Among them, magnetoelectric materials that exhibit coupling of electric polarization and magnetization are very promising for spintronic and magnetic random access memory applications.

In the present study we report a detailed study of magnetic resonance in new magnetoelectric multiferroic $PbFe_{1/2}Sb_{1/2}O_3$ (PFS) as well as in $PbFe_{1/2}Nb_{1/2}O_3$ (PFN) and $PbFe_{2/3}W_{1/3}O_3$ (PFW) doped with Ba and Ti. All these materials belong to the rich family of Fe-based double perovskites $A(FeMe)O_3$ with nonmagnetic ions A=Pb,Ca,Sr,Ba, and Me=Nb,Ta,Sb. In these compositions, Fe^{3+} and $Me^{5+/6+}$ cation positions may be ordered or disordered within simple cubic B-sublattice of the perovskite structure ABO_3. They exhibit interesting multiferroic properties [1] which can be tuned by means of doping [2] and changing of the chemical order. However, the microscopic origin of this doping or chemical order influence is still unclear.

The magnetic resonance measurements were performed at 9.4 and 34 GHz microwave frequencies at temperatures from 3.5 to 300 K on ceramic and single crystals samples. In the paramagnetic phase, all studied samples show the same absorption line attributed to normal electron paramagnetic resonance (EPR) of Fe^{3+} ions. This EPR line is exchange narrowed that allowed us to estimate spin-spin exchange constants as a function of composition, chemical order and doping.

They are in the range 50-75 K and 2-12 K for the nearest and next-to-nearest neighbor exchange interactions, respectively. When temperature approaches the Neel temperature, on cooling, the linewidth critically broadens and the EPR line becomes invisible indicating the freezing of the spin fluctuations at EPR time scale. In the solid solutions with Ba and Ti ions, the Fe^{3+} EPR spectrum only partly decreases at the Neel temperature. This fact indicates that the long-range antiferromagnetic (AFM) phase is created only in a part of sample's volume, so the rest of the volume remaining in the paramagnetic and superparamagnetic phases. The superparamagnetic (superspin) phase is seen well in PFS, where it is characterized by strong magnetic relaxation and separate magnetic resonance line. By combining the magnetic resonance data with data of magnetic measurements (magnetic susceptibility, magnetization) we have built phase diagrams describing the magnetic properties of the studied systems.

The results obtained clearly show that both A- and B-site dilutions of $Pb(Fe_{1/2}Nb_{1/2})O_3$ result in the breaking of the infinite magnetic percolation cluster responsible for the long-range AFM order. Under this doping, the long-range AFM state disappears and the super AFM or spin cluster glass state becomes dominant. The role of chemical order on magnetic and polar ordering is discussed as well.

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P 161
PHOTOCATALYTIC AND PARAMAGNETIC PROPERTIES OF PURE AND DOPED NANOCRYSTALLINE TITANIA

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In recent years the use of semiconductor nanomaterials in air and water purification systems is under a great interest. Titanium dioxide (TiO_2) is one of such promising materials. But some difficulties take place in wide usage of this material. TiO_2 is a semiconductor with wide band gap and UV light is required for the activation of it's photocatalytic properties.

In this work the nature of paramagnetic centers was identified and photocatalytic properties were investigated in pure and doped TiO_2 (N-TiO_2 and C-TiO_2) obtained by sol-gel method and supercritical flow reactor method. We have used the EPR technique for our investigations because it is sensitive to the detection of radicals and reactions between them.

The EPR spectra were detected by a standard CW Bruker EPR spectrometer ELEXSYS-500 (X-band, sensitivity around 10^{10} spin/G). Photocatalytic properties were observed using the method of IR spectroscopy, by recording the change in intensity of the characteristic absorption bands of formaldehyde during period of time. For this purpose, a standard FT IR - RX I spectrometer Perkin Elmer (spectral range 400 – 6000 cm^{-1}, with a resolution of 2 cm^{-1}) was used. A surface area values were determined by low-temperature nitrogen adsorption (BET Bruner-Emmett-Teller) method. A single-point mode on the Chemisorb 2750 device (Micromeritics) was used.

The measurements of EPR spectra were performed at two temperatures: 295°K and 77°K. The samples were illuminated in situ with high pressure tungsten lamp in the spectral range between 400 and 1000 nm.

All samples under investigation showed similar forms of signals with different intensities. The parameters of the signal, calculated during a

computer simulation, were the same as in the literature, so it was defined that the signal is due to $NO^{\bullet}$ radicals in N-TiO_2samples, and CO_2^- and $OH^{\bullet}$ radicals in C-TiO_2 samples. We can also say, that the intensity of a signal of pure samples increased at low temperature. All samples give different signal, which depends on the method of preparation.

To clarify the roles of the different paramagnetic centers in the photocatalytic decomposition of organic compounds the example of formaldehyde was used. The EPR signal was sharply reduced under illumination. The effect of exposure was fully reversible. The sharp decrease in the intensity of the EPR signal in the process of photocatalysis with doped TiO_2 is correlated with a decrease in the amount of toxic contaminants in the air. Such correlation naturally is attributed to the degradation of organic substances on the surface of titanium dioxide and proceeds via a radical mechanism. Photocatalytic data was prepared by recording the change in intensity of the characteristic absorption bands of formaldehyde during period of time using an IR spectrometer.

Also several cheap methods of sample's deposition on the different kinds of surfaces were observed. The obtained results can be useful for photocatalytic applications. The experiments were performed using the facilities of the Collective Usage Center at Moscow State University.

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COHERENT CONTROL OF SINGLE SPINS IN SILICON CARBIDE AT ROOM TEMPERATURE

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Spins in solids can serve as a two-level quantum system, which is useful for quantum information processing (QIP) [1], and various quantum applications like quantum metrology [2]. This is possible thanks to their large spin signal, the tunable quantum properties by external magnetic and electric field, and the easiness in the fabrication of their host material. One of the well-known examples is the nitrogen-vacancy (NV) center in diamond. Since the success of the first single spin detection of the NV center in diamond by optically detected magnetic resonance (ODMR) about two decades ago, researchers have proven that these centers can be used as a quantum bit for QIP [3] and single spin sensors operating at ambient conditions with the outstanding sensitivity approaching single proton sensitivity [2]. One of the similar quantum systems, silicon vacancies (V_{Si}) in silicon carbide (SiC) also recently has attracted a great amount of attention especially because of the electrical properties of the host material which stands out the diamond. This advantage, together with the mature fabrication from the modern silicon technology may allow scalable spintronic devices, resembling modern electronics devices,

in contrast to the diamond-based quantum devices which mostly rely on optical-circuits.

In order to test whether the creation of isolated defects in SiC is possible, we created isolated V_{Si} defects in a pure SiC single crystal sample by 2 MeV electron irradiation. We found the diluted defect concentration by detecting the fluorescence emission of individual defects. As a next step to test whether single spin detection at room temperature is possible, we detected the ODMR spectra of single V_{Si} defects whose ensemble spectra were reported earlier. We confirmed that the optical spin polarization can also be realized in a single V_{Si} which allows a strong spin signal at room temperature. In addition we found a long longitudinal spin relaxation time (T_1~500 microseconds) and a slow spin pair flip-flop rate. The latter originates from the diluted electron paramagnetic impurity concentration and the suppression of the hetereonuclear spin flip-flop process by applying a small external magnetic field. In the end, we found these enables long single spin coherence times (T_2>160 microseconds) at room temperature.

Our results show that SiC can host single-point defects with long spin coherence times, making it promising for long-lived quantum bits and quantum metrology [4]. This, combined with mature fabrication methods, also strongly suggests that SiC is a promising platform for integrating spintronics and electronics in a single quantum system.

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ELECTRON PARAMAGNETIC RESONANCE AS A TOOL TO STUDY THE SIZE DEPENDENCE OF MAGNETIC PHASE TRANSITION IN Ni0.2Zn0.8Fe2O4 NANOPARTICLES

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Spinel ferrite nanoparticles (NPs) have been the focus of an increasing amount of research work because of their unusual magnetic behavior and applicability in various technological fields, such as magnetic recording media, microwave devices, ferrofluids and so on. Among the spinel ferrites, nickel-zinc ferrite (Ni, Zn)Fe_2O_4 is widely studied due to its high resistivity, low eddy current losses and excellent soft magnetic properties for applications in electronic devices. From a fundamental perspective the study of magnetic nanoparticles shed light on how bulk properties transform to atomic as size decreases. When the dimension of particles is reduced to nanoscale, the fraction of surface materials becomes dominant; hence bulk properties may either give way to surface properties and /or be significantly perturbed by the surface. Furthermore, the large surface fraction opens the opportunity for manipulation of properties via interfacial interactions. In this paper, $Ni_{0.2}Zn_{0.8}Fe_2O_4$ NPs with different mean crystallite sizes (d) ranging from 15 to 50 nm were synthesized by the combustion method. Magnetic measurements showed that the magnetic ordering temperatures T_C and coercivity H_C increase significantly when the crystallite size decreases. However, the saturation magnetization M_S displays non-regular change with the crystallite size. Temperature dependences of the electron

paramagnetic resonance (EPR) spectra were recorded between 120 K and 460 K. The changes in the peak-to-peak linewidth ΔH_{PP}, resonance field H_r and intensity as functions of temperature were studied to understand the nature of spin configurations in the system of $Ni_{0.2}Zn_{0.8}Fe_2O_4$ NPs. The EPR spectrum at 460 K shows a relatively narrow sharp line, but as the temperature decreases the linewidth ΔH_{PP} considerably increases and their apparent resonance field H_r shifts towards lower magnetic fields. The broadening and shift of spectra to lower magnetic field with the decrease of temperature is typical of superparamagnetic behavior of NPs. Moreover, $Ni_{0.2}Zn_{0.8}Fe_2O_4$ NPs with crystallite size $d \leqq 40$ nm, demonstrate a resonance anomaly near 140-150 K that could indicate the presence of a magnetic phase. The anomalous variation of saturation magnetization and resonance spectra are explained by the redistribution of the cations on the tetrahedral and octahedral sites.

P 170
INNOVATIVE ECO-COMPATIBLE MgO-BASED CEMENTS: A SOLID-STATE NMR AND RELAXOMETRY STUDY

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The development of eco-compatible cements represents a relevant issue in the field of building material research. To this aim, cement based on reactive periclase (MgO), which in the presence of a silica source forms a binder phase, M-S-H (Magnesium Silicate Hydrate), is one of the most promising emerging technologies, allowing to greatly reduce CO_2 emissions involved in the production of the traditional CaO-based cements. Although the increasing research interest in MgO-based cements, a thorough investigation of their properties, such as hydration kinetics, the nature of the hydrated products, the structural and morphological properties of the obtained phases, is still lacking. Nevertheless, this understanding is fundamental to achieve the industrial breakout of these systems, whose production is today limited to pilot plants.

In this work, a detailed and systematic study of MgO-based cement formulations was carried out by means of multinuclear solid-state NMR (SSNMR) and relaxometry. Both these techniques already proved to be very powerful for the investigation of the structural and morphological properties and hydration kinetics of the traditional CaO-based cements.[1-3] In this study, ^{29}Si and ^{1}H one- and two-dimensional experiments were performed on pastes freeze-dried after up to 28 days curing, in order to characterize the structural properties of the M-S-H binder phase also in dependence on the hydration time. In particular, ^{29}Si MAS spectra allowed to identify and quantify the different silicon sites in M-S-H, characterized by different connectivity

to -OSi, -OH and -OMg groups. Moreover, in order to shed light on the hydration process and the morphological evolution of these systems, the status of water in pastes at different hydration times was investigated by analyzing ^1H T_1 relaxation times measured by FFC NMR relaxometry and ^1H T_2 relaxation times determined by low resolution experiments at 20 MHz. The obtained results were combined with complementary data obtained by DSC, TGA, XRD and SEM measurements.

This work was financially supported by MIUR (FIR2013 Project RBFR132WSM).

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P 173
CHENOMX BASED NMR METHOD FOR ORGANIC AEROSOL ANALYSIS

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A novel NMR approach to the analysis of organic aerosol composition was tested. The method is focused on the water soluble organic compounds (WSOC) analysis, which is the least examined group of organic aerosols. So far, the use of NMR technique was limited to so called Functional Group Analysis, where measured ^{1}H NMR spectra of the WSOC are divided into parts and the entire area is integrated without or with very little identification of individual compounds [1]. Recently, the employment of 2D NMR techniques (COSY, HSQC) was published, however the analysis is rather challenging [2].

The proposed NMR technique exploits the NMR metabolomic approach, in which the ^{1}H NMR spectra of individual compounds are fitted into the complex ^{1}H NMR spectra. The assignment is based on precise chemical shift of dominant signals of given compound. The library spectrum is subsequently subtracted from the aerosol sample spectrum. For this purpose software ChenomX 8.0 is employed. The key to the detail analysis lies in an extensive database. ChenomX database is primarily intended for metabolite analysis, however ca. 60 compounds can be found also in WSOC fraction. Additionally, the software allows database extension and new compounds can be added.

The suitability of ChenomX was performed on both real atmospheric aerosols and model samples of coal combustion and up to 30 compounds were found in analyzed samples. Most abundant substances were mono- and dicarboxylic acids (formic, acetic, succinic acid) and their derivatives (lactic acid), followed by carbohydrates, anhydro saccharides and sugar alcohols (levoglucosan, fructose, D-threitol) and amines (methylamine, dimethylamine). The real aerosol sample was analyzed also on four different NMR spectrometers (500, 600, 700, 800 MHz) in to order to

enhance the resolution and find less abundant substances. The frequencies of the spectrometers were chosen according to the ChenomX database. In the 800 MHz spectrum ca. 50 compounds were identified.

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A METABOLOMIC APPROACH TO ANALYSIS OF HEN EGGS ACCORDING TO DIFFERENT TYPE OF HEN FARMING.

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In Poland hen eggs are divided into four groups, which are related to four kind of hen farming: caged (3PL), bedding (2PL), free run (1PL) and ecological farming (0PL). Due to different origin and supposedly the content, eggs have different price depending on the assigned number. In principle the healthiest eggs should come from ecological farming, because hen eat organic feed and can move freely.

The aim of this study is to create metabolic profile of hen eggs in order to evaluate changes in their chemical composition depending on the type of hen farming.

In above described research we used metabolomics approach, which plays an important role in systems biology. It enables exploration and analysis of small molecular weight compounds called metabolites (MW<1500 Da) such as: amino acids, organic acids, carbohydrates, alcohols or nucleosides. Therefore it is a great tool for qualitative and quantitative food analysis. Moreover, metabolic profiling allows to observe changes in the samples in real time as the response to external conditions. In the literature so far there are many examples of metabolomics platform utilization in evaluation of the quality, authenticity and food analysis.

In our study we applied NMR spectroscopy combined with chemometrics methods (Principal Component Analysis – PCA, Partial Least Squares Discriminant Analysis - PLS-DA and Orthogonal Partial Least Squares Discriminant Analysis OPLS-DA) and statistical analysis. In this study the eggs were investigated with separation into yolk and white fraction, where yolk turned out to be "more informative". Performed statistical analysis provide detailed information on metabolites, which bring differentiation between all groups of eggs.

The used methodology allows as to visualize the similarities and differences in molecular composition of all type of eggs and enable to compare the impact of environmental factors on their composition.

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RECONSTRUCTION OF TOP-RESOLUTION SPECTRA USING SPECTRAL ALIASING. APPLICATION TO 2D HSQC AND 1D HOMODECOUPLED PROTON SPECTRA WITH SCALAR COUPLING INFORMATION

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Improving resolution of 2D spectra by one to two orders of magnitudes using spectral aliasing is straightforward. But the analysis of the resulting spectra is not always trivial because the aliasing order, the information lost because of the violation of the Nyquist condition, needs to be recovered in one way or another. We present two cases of reconstructions of high-resolution spectra solving this problem. In the first application, normal full-width 2D HSQC spectra with a top resolution were obtained from a single aliased spectra recorded using modified HSQC sequences encoding the aliasing order. In the second applications, the reconstructed spectra are high-resolution homodecoupled 1D proton spectra with the lists of the first-order scalar coupling constants. They are obtained from a special homonuclear 2D spectrum where only the diagonal is recorded using either the PSYCHE or the nemo-ZS homodecoupling schemes to obtain singlets along the indirect dimension.

P 182
COCON - NEW ASPECTS ON THE STRUCTURE ELUCIDATION OF SMALL MOLECULES

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The structure elucidation of natural products is particularly challenging because of the novel or non-standard structural features they often contain. Usually, if a crystal structure is not accessible, the molecular constitution of a new natural product is elucidated by application of different spectroscopic (IR, NMR, and UV) and spectrometric (MS) methods. In course of the analysis, NMR spectroscopy is of special importance, as it is the only technique which delivers structural information on constitution, configuration, and conformation.

The 2D NMR-guided computer program COCON is extremely valuable for the constitutional analysis of unknown compounds. In particular, structural proposals made on the basis of the molecular formula and of 2D NMR experiments can be analyzed for the existence of alternative constitutions being in agreement with the same data set. Already back in the 1990s, John Faulkner stated: "Rather than defining a structure that can be shown to fit the data, it is best to examine many possible structures (we would sayevery) and to treat each proposed structure as a hypothesis that cannot be proved but can only be disproved by incompatible data." [1]. Proton-poor compounds are particularly challenging for an NMR-based structure elucidation. The constitutional analysis of natural products by NMR spectroscopy is usually based on 2D NMR experiments like COSY, HSQC, and HMBC. This connectivity information is used as input for the NMR-based structure generator COCON which both improves and accelerates the process of constitutional assignment.

This contribution will focus on the following topics: 1) consideration of 4J and 5J HMBC correlations within the structure elucidation process of COCON, 2) incorporation of 1,1- and 1,n-ADEQUATE correlations in

the automated structure elucidation of small organic molecules, 3) evaluation of the COCON output by C-13 chemical shift calculation, 4) ranking of COCON structural proposals by energy (smi23d), 5) statistical filtering of the COCON structural proposals (smi23d), and 6) consideration of symmetry. Furthermore, COCON was used to find structural alternatives for about 450 natural products published in Journal of Natural Products. It turns out that for about 25% of the published molecules at least one alternative constitution can be proposed.

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P 185
A CLEAN IN-PHASE COSY-EXPERIMENT

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The COSY-experiment is one of the oldest 2D NMR experiments; yet, it is still invaluable for the assignment of signals in ^{1}H spin system as it only shows coherences between directly coupled spins, providing cross peaks of high explanatory power.

We present a novel experiment to overcome its major drawback: the anti-phase line shape. The clean in-phase COSY (CLIP-COSY) yields purely absorptive cross peaks for the same coherences. It allows short acquisition times in both dimensions and is less prone to mismatched processing parameters while providing full resolution of phase-sensitive spectroscopy.

The line shape is strongly dependent on the properties of a z-filter element applied. To obtain insight into the limits of such elements, we utilized the GRAPE algorithm, an implementation of optimal control theory on quantum systems.

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EVALUATION OF REPORTED 13C NMR DATA AND CHEMICAL STRUCTURES BY USING CAST/CNMR SHIFT PREDICTOR AND STRUCTURE ELUCIDATOR

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The CAST/CNMR system is composed of two functions: one is CAST/CNMR Shift Predictor[1] for the prediction of accurate ^{13}C NMR chemical shift values for a query chemical structure by using a database (DB) containing reported/observed ^{13}C NMR chemical shift data and three-dimensional structure information including relative stereochemistry and information of ring systems, described by the CAST (CAnonical-representation of STereochemistry) coding method. The other is a new function for chemical structure elucidation, called CAST/CNMR Structure Elucidator.[2] The same DB is shared in this component to obtain fragment structures by mapping query chemical shift values to the chemical shifts in DB, which are associated with partial structures. The collected fragment structures are assembled into larger structures, which are merged at common parts to give final candidate structures. This protocol is implemented by using several graph algorithms, such as convex bipartite matching (CBM) and weighted bipartite matching (WBM). If all fragments that are needed to elucidate a full structure are contained in the DB, the Structure Elucidator can suggest a correct structure for the query of ^{13}C NMR chemical shift values.

Those two functions are complementary to each other in the use of structure elucidation. In the present study, we would like to show some of the results from the evaluation of reported ^{13}C NMR data and chemical structures by using the CAST/CNMR system. Most of the DB contents are selected from published peer-reviewed journals. However, mistaken assignments or incorrect structures are contained

even in such authorized journals. A wrong structure is often reported as a new compound. The CAST/CNMR Shift Predictor can be used to locate the mistaken assignments of ^{13}C signals by comparing the predicted values with the reported data. The CAST/CNMR Structure Elucidator can be used to revise the incorrect structures by looking into another possibility suggested by the elucidator. We have been continuing the evaluation for the improvement of the DB by removing or correcting the error data. For example, we revised the structure of aldingenin C, which was originally proposed as a brominated sesquiterpenoid, to a known caespitol. In this case, from the reported ^{13}C NMR data for aldingenin C, the elucidator suggested the structure of caespitol, which is very different in molecular formula and structure from those of aldingenin C. We will demonstrate also some other results obtained for several natural products.

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P 191
THERMORESPONSIVE BEHAVIOR OF PORPHYRINS AND THEIR SUPRAMOLECULAR COMPLEXES

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Porphyrins (derivatives of porphin) have attracted a great deal of attention due to their many interesting properties. They can complex with metal cations and molecules capable of hydrogen bond formation, detect chirality [1] (upon complexation the chirality is transferred from the chiral molecule (guest) to the achiral porphyrin (host)) which makes them sensors of enantiomeric purity. The macrocyle is aromatic and upon complexation, it changes its shape from planar to the saddle-like, which is manifested by a significant change of colour. Thus they can be used in colorimetric visualization of acid-base equilibria or naked-eye discriminator of methanol from ethanol.

We study a new group of porphyrins that, in addition to these properties, exhibits property similar to phase separation in temperature-responsive polymer solutions - at room temperature these porphyrins are dissolved, above certain temperature (lower critical solution temperature (LCST)) phase separation occurs. Stimuli-responsive materials have found numerous applications in biomedicine and nanotechnology due to their ability to undergo large property changes triggered by a relatively small change in various external stimuli. Polymers represent majority of known substances that show the LCST phenomenon by undergoing a coil-globule transition [2]. Although, in principle, nonpolymeric assemblies could be developed to show this property, there are still only a few examples of small molecular systems with LCST [3, 4]. Design of novel temperature-sensitive porphyrins could combine their attractive features with properties and applications complementary to those of

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P 194

NMR EXPERIMENTS WITH PARALLEL 1H AND 19F DETECTION FOR SCREENING AND MOLECULAR STRUCTURE IN DRUG DISCOVERY

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From NMR point of view fluorine-19 is similar to protons in terms of abundance and sensitivity. In addition it is highly sensitive to the smallest changes in its surroundings. The NMR chemical shift of the ^{19}F-nuclei strongly depends on its molecular environment and the NMR signals of fluorine show pronounced line broadening upon weakest binding interaction with bio-macromolecules, such as proteins. This allows high dynamic range in ligand based screening where potential binders to target protein are searched among many low molecular weight compounds in so called fragment based screening. Today, a number of ^{1}H-detected NMR experiments are used routinely, whereas ^{19}F-detection appears as a promising expansion of tools available to NMR users. Here we propose a set of parallel experiments for ligand screening and small molecular structure elucidation that use simultaneous ^{1}H and ^{19}F-detection by using two receivers. This allows the experiment time to be reduced by half as compared to the corresponding conventional experiments. The most common two-dimensional experiments that are routinely used in small molecule research have been adapted for use in systems equipped with two receivers. In addition to previously published H,F-X HSQC and H,F-X HMBC experiments (X = ^{13}C or ^{15}N [1]) we also show the utility of two versions of the PANSY COSY experiment [2,3] - H,F-H COSY and F,H-F COSY. We also show that such spectra with ^{19}F chemical shifts spred over 40 kHz wide spectral bandwidth in F1, can be recorded in as little as 14 seconds using Hadamard encoding technique [4].

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P 197

HCNMBC: A METHOD FOR MEASUREMENTS OF 13C-15N COUPLING CONSTANTS AT NATURAL ISOTOPIC ABUNDANCE

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Recently, we have introduced a new experiment, HCNMBC[1], which permits ^{1}H-^{15}N correlation via the natural abundance ^{13}C-^{15}N coupling. The experiment produces data which is highly complementary to direct ^{1}H-^{15}N HMBC type correlations, if available and can provide ^{15}N chemical shift data for nitrogens that may not show up in the HMBC. Examination of a variety of azole systems reveals consistent and diagnostic patterns of correlations among these heterocycles.[2] We have further examined this result to understand the dependence of cross peak intensity on the setting of the J_{CN} delay by direct measurement of the ^{13}C-^{15}N coupling constant at natural isotopic abundance. There are two major ways of determining the ^{13}C-^{15}N coupling – (1) direct measurement in F1, for instance by using the J-doubling technique and (2) analyzing signal intensities as a function of the J_{CN} delay. The first technique relies on direct measurement of ^{13}C-^{15}N splitting in F1 by omitting the 180˚ carbon-13 pulse in the middle of the t_1-evolution period of the HCNMBC.[1] The results of direct measurement performed on a sample of 1-methyl imidazole are shown.

In addition it is also possible to monitor the intensity of the signal as a function of the J_{CN} delay. The advantage here is that several measurements are carried out and therefore the chances of observing the signal in at least some of them are greater than with the 'classical' approach. Furthermore, the zero crossing is sharp increasing the accuracy of the coupling measurement .The most accurate estimation of the coupling constant in this case can be determined by fitting the modulation and we are currently exploring this technique. Results from the examination of a series of azoles and the applicability of ^{13}C-^{15}N coupling constants to structure elucidation will be presented.

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P 200
1H NMR PROFILING OF GEOREFERENCED OLIVE OILS.

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1H NMR profiling is nowadays a consolidated technique for the identification of geographical origin of food samples. The common approach consists in correlating NMR spectra of food samples to their territorial origin by multivariate classification statistical algorithms.

In the present work we propose an alternative perspective based on the creation of GIS (Geographic Information System) maps according to the information contained in the NMR spectra. This approach permits to identify territorial regions having strong similarities in the chemical content of the produced olive oil.

To set up the method we have analyzed more than 190 georeferenced olive oil samples produced in the Abruzzo Region in Italy.Suitable features are created from the NMR spectra which are then traduced in GIS maps.

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ANISOTROPIC NAD 2D-NMR OF BIOMARKERS: FROM THE SITE-SPECIFIC ISOTOPE FRACTIONATION (D/H) TO THE UNDERSTANDING OF BIOSYNTHETIC PATHWAY

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The proton-decoupled ^{2}H 1D/2D NMR spectroscopy in chiral liquid crystals (oriented solvents) using helical polymers (polypeptides) is a powerful analytical approach to study isotopically-enriched chiral and prochiral solutes [**1**]. The mechanisms of molecular orientation and enantiodiscrimination lead to orient differently enantiomers or enantiotopic directions on the average. It then becomes possible to separate their NMR signals on the basis of a difference of residual quadrupolar coupling (RQC) observed for nuclei with spin I > I/2 [**1**,**2**].

Many applications based on this concept but detecting deuterium at natural abundance level (NAD = 0.0155%) have been proposed, particularly in the field of the analysis of molecular stereochemistry [**1**] but also for the study of site-specific, natural isotope fractionation $(D/H)_i$ of biomolecules such as unsaturated and saturated fatty acids [**3**].

In the framework of the potential paleoclimate or paleoenvironmental studies, we apply this tool to analyze the isotopic distribution $(D/H)_i$ of biomarkers like Miliacin, a pentacyclic triterpene [**4**]. Indeed, preserved in sedimentary archives, these specific compounds (via their intramolecular isotopic composition) can provide clues to interpret the evolution of past climate conditions.

The analysis of the distribution of deuterium in methyl groups of Miliacin (from the millet oil) provides key information for a better

understanding / interpretation of biosynthetic process leading to its formation [**5**].

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DEGRADATION OF BISPHENOL A: SPECTROSCOPIC STUDY AND QUANTUM CHEMISTRY CALCULATIONS

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Bisphenol A (BPA) is widely used to harden plastic containers. Yet, due to its xenoestrogenic properties BPA can act as inappropriate estrogen and may disrupt normal estrogenic signalling even at a very low concentration (μg·L^{-1} and ng·L^{-1} range). Thus, the method of efficient removal of BPA from the environment is needed. Various advanced oxidation processes have been reported to be effective for the degradation of xenoestrogens. However, in these studies only removal efficiency is reported, whereas all possible metabolites are not identified, and the esterogenicity of these identified has not been studied. This information is vital as the degradation of BPA can lead to the formation of substances with even higher estrogenic activity.

In this work the reaction path of BPA degradation by Fenton reaction has been studied in detail. Radicals formed as a result of degradation of BPA by Fenton reaction, by Fenton reaction in the presence of DMSO and in the presence of ethanol were detected with ESR spin trapping utilizing 4-hydroxy-5,5-dimethyl-2-trifluoromethylpyrroline-1-oxide (FDMPO), (a -4-Pyridyl-1-oxide)-N-tert-butylnitrone (POBN) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) spin traps. Simulation-based fitting of ESR spectra was performed utilizing Easyspin toolbox in Matlab. It revealed new FDMPO radicals adducts with a_N=14.1G and a_F=3.7G in Fenton in the presence of BPA. We have attempted to identify these new radical adducts with DFT calculations. Total energy analysis of the reaction pathway was performed by DFT calculations at different levels and basis sets (B3LYP/6-31G(d,p), MO6X/6-3111+G(d,p)) in gas phase and in water as a solvent using the PCM model. It appears that 4-hydroxyphenyl and 2-(4-hydroxyphenyl)-propan-2-yl radicals are preferably formed during the degradation leading to the formation of the phenol, p-hydroxyphenol, 4-(1-hydroxy-1-methylethyl)phenol and 4-(1-methylethyl)phenol. The xenoestrogenity of these BPA metabolites has been evaluated based

on their binding to the estrogen receptor as studied with molecular docking method. The calculations revealed that all 4 studied metabolites have lower binding affinity strength than BPA.

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APPLICATION OF "AQARI: ACCURATE QUANTITATIVE NMR WITH INTERNAL REFERENCE SUBSTANCE" TO THE JAPANESE PHARMACOPEIA

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QNMR using the internal reference substance with traceability to the International System of Units (SI), named as AQARI (Accurate QuAntitative NMR with Internal reference substance), recently attracts attention as one of absolute purity determination methods, because of its efficiency and reliability.

Since 2008 we have conducted research on AQARI to determine the absolute purity of analytical marker compounds used for standardization of natural medicines. As the results, we found that AQARI was able to determine the purity of the natural compounds with the accuracy of approximately 2 significant digits and established the supply system of magnol, paeonol, geniposide, magnoflorine iodide rosmarinic acid, (E)-cinnamic acid, rhein and saikosaponin b_2. The former four compounds have been used for standardization of natural medicines in JP16 from March 2014. The latter four will be used in JP17 from March 2016.

P 212
CAN NMR METHOD BE USEFUL IN THE METABOLOMICS STUDIES OF FILAMENTOUS FUNGI?

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Serious infections caused by yeast and filamentous fungi become a major global healthcare problem in the 21st century. Pathogenic fungi of the genus Candida, Asperigillus, Fusarium and Geotrichum represent systematic human pathogens. These infections are commonly associated with immune dysfunction and increasing incidence of drug resistance phenomenon. From this point of view it is necessary to know the molecular grounds of fungi drug resistance processes, possibilities of their diagnostics and elaborating mechanisms for new potential antifungal drugs.

In our studies we used 1H NMR-based metabolomics approach to study of drug susceptible and drug resistance strains of Candida albicans and Candida glabrata exploring the mechanisms responsible for drug resistance of both species. Moreover we used also the 1H NMR for differentiation of three various species Aspergillus fumigatus, Fusarium oxysporum and Geotrichum candidum belonged to three various classes of filamentous fungi. Finally we demonstrated antifungal properties of grapefruit essential oil (GEO) extracted from Citrus paradisi against Aspergillus fumigatus with characterization of perturbed biochemical pathways during time of GEO action.

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QUANTITATIVE STRUCTURAL CONSTRAINTS FOR ORGANIC POWDERS AT NATURAL ISOTOPIC ABUNDANCE VIA DYNAMIC NUCLEAR POLARIZATION

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Diffraction from single crystals has revolutionized our knowledge of crystalline matter by providing atomic-scale images of countless samples and leading to landmark achievements in science. However, when crystals of sufficient dimensions cannot be grown, structure can hardly be retrieved using currently available methodologies. This hampers our understanding of the physico-chemical behavior of numerous samples occurring as powders, such as pharmaceutical products, precluding the design of new materials with tailored properties and leading to possibly harmful consequences. Solid-state NMR has the potential to be the key to access the crystal structure of powders. However, the inherently low sensitivity of NMR constitutes the main barrier to retrieve valuable constraints such as interatomic distances from spin-spin couplings involving rare nuclei on organic samples at natural isotopic abundance.

We present a straightforward methodology to quantitatively relate structural constraints based on ^{13}C-^{13}C double quantum dipolar build-up curves obtained by Dynamic Nuclear Polarization (DNP) solid-state NMR to the crystal structure of organic powders at natural isotopic abundance [1]. This methodology relies on the tremendous sensitivity enhancement provided by DNP (about 50 here, reducing the experimental times from a few years to a few days) and is sensitive to both the molecular conformation and the crystal packing of the studied powder sample. The method is demonstrated on the challenging sample of theophylline [2]. We show here how the proposed methodology allowed the correct crystal structure of

anhydrous theophylline to be rapidly and effectively identified among a set of both existing and virtual theophylline polymorphs.

These encouraging results suggest that the this methodology could pave the way to three-dimensional structural elucidation of powders via the combination with powder X-ray diffraction, crystal prediction [3] and density functional theory computation of chemical shifts [4].

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STABILISATION OF METASTABLE POLYMORPHS AS CONFINED NANOCRYSTALS. NMR INSIGHT INTO STRUCTURE, DYNAMICS AND SELF-ASSEMBLY MECHANISM OF CONFINED CRYSTALS

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Crystallisation and molecular self-assembly processes are not fully understood important phenomena in chemistry and biology. Control over molecular self-assembly in discovering materials with new and improved properties is an important and vital problem in many areas of material science. From the perspective of pharmaceutical industry control over crystallisation processes within complex drug delivery systems is of paramount importance as molecular alignment within a crystal (i.e. polymorphism) determines resulting physical properties of solids including dissolution, fusion temperature and physicochemical stability.

Recently, mesoporous (2-50 nm) silicas have attracted growing attention in pharmaceutical drug delivery due to their synthetically tailored pore diameter, large pore volume and surface area. Possible functionalisation of internal surfaces of the silica host may lead to the synthesis of a broad spectrum of smart drug delivery systems. [1] Due to the narrow distribution of the tailored pores, mesoporous silicas are exciting as nano-size crystallisation chambers for studies of molecular aggregation and drug polymorphism. Determination and understanding of structure and dynamics of nano-size confined solids in complex materials is a significant analytical challenge due to lack of long range order (broadening of PXRD peaks), changes of the melting points and other properties. In this work we present how solid state NMR can gain molecular insight into the structures and transitions which are not accessible using another techniques due to its sensitivity to the local environment of atoms. Using NMR as a probe for local mobility we demonstrate differences in dynamics of confined pre-

nucleating species as compared to nano-crystals or molecular liquids embedded in uniform composites in two dynamically different regimes.

Three poorly soluble drugs indomethacin, tolbutamide and flufenamic acid were chosen as model systems and loaded into porous solids. All compounds differ significantly in structural flexibility which leads to a large number of polymorphs and difficulties in controlling phase transitions. Firstly crystallisation process from amorphous state into the confined solvate and then into the stable form V of indomethacin were monitored inside the pores of ca. 30 nm. For the first time, through ^{13}C and ^{1}H solid-state NMR we monitored the formation and stabilisation of ultra-metastable tolbutamide form V inside the host with pores sizes as small as 3 nm, followed by its phase transition into the most stable form I^{H}. Furthermore, applying ^{19}F NMR and ^{19}F T_1 relaxation measurements we were able to gain a molecular level insight into the crystallisation mechanism of confined crystals as we showed the formation of "molecular liquid-like" layer on the silica surface prior to the build-up of confined crystal of flufenamic acid. All NMR findings were corroborated with PXRD, DSC and N_2 adsorption proving that all confined crystalline forms show properties of nano-size solids.

Such combined application of nano-size crystallisation methodology and solid-state NMR spectroscopy is essential in directing molecular aggregation and answering fundamental questions on self-assembly of crystalline solids.

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APPLICATION OF CP/MAS NMR AND ISOTPE LABELING TO RECOGNIZING DIFFUSION SPECIES IN SOLID-STATE REACTION OF QUINHYDRONE

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In this work, we apply solid-state high-resolution NMR to examine solid-state reaction of benzoquinone and hydroquinone to form quinhydrone. It has been suggested by Rastogi[1] and Kuroda[2] groups that a molecular crystal forming mechanisms predominantly involves surface diffusion, assisted to varying degrees by diffusion through gas phase, when one or both reactants exhibit significant vapor pressures in the solid state. So far, quinhydrone formation without grinding was reported by Patil et al., who noted hydrogen transfer.[3]

Here, we show that one can recognize atomic and molecular diffusion in the solid-state reaction of quinhydrone by combining isotope labeling and cross-polarization (CP). Firstly we confirmed the solid-state reaction of quinhydrone without grinding by monitoring the heating-time dependence of the reactants and the product. Signals from reactants decreased with time and finally disappeared completely with concomitant growth of hydroquinone signals. This result showed totally formation of quinhydrone without grinding. There are two possible diffusion processes for crystal formation of quinhydrone. One is the atomic diffusion of hydrogen and the other is molecular diffusion of benzoquinone and/or hydroquinone.

We show that atomic and molecular diffusion can be recognized on the basis of CP measurements of quinhydrone prepared from normal benzoquinone (BQ) and totally deuterated hydroquinone (HQ-d_6) as follows. If hydrogen atoms transfer from hydroquinone to benzoquinone crystalline, the resultant quinhydrone consists of BQ-d_4-HQ-d_6 and BQ-HQ-d_2. If benzoquinone molecules migrate into hydroquinone crystalline, the resultant hydroquinone is BQ-HQ-d_6. The ^{1}H-^{13}C CP/MAS NMR signals from the HQ-d_6 moiety in BQ-HQ-d_6 can not be observed if the contact time is short. Thus, atomic and

molecular diffusion in quinhydrone formation can be distinguished by combining deuterated benzoquinone and CP.

A ^{1}H-^{13}C CP/MAS NMR spectrum of quinhydrone made of BQ and HQ-d_6 at the contact time of 0.1 ms showed a quite weak signal at the peak position of the HQ moiety in quinhydrone and strong peaks at the peak positions corresponding to BQ moiety in quinhydrone. This result shows that either hydrogen atoms or benzoquinone molecules diffuse during reaction. By analyzing the CP signal intensities, we concluded that benzoquinone molecular diffusion in solid state reaction of quinhydrone is concluded.

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NMR MOLECULAR REPLACEMENT, NMR2

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X-ray crystallography molecular replacement (MR) is a highly versatile tool for the detailed characterization of lead compounds and binding modes in the pharmaceutical industry. The two major limitations of its application to drug research are (i) the availability of a similar protein structure, which, in the area of structure-based drug design, is most often a complex of the protein with a lead compound, and (ii) obtaining well-diffracting crystals of the ligand-protein complexes of interest. While nowadays the first point is often not a limitation anymore, obtaining well-diffracting crystals might be difficult. In such situations structure determination of protein-ligand complexes by liquid-state NMR is a good option. Unfortunately, the established standard structure determination protocol is in general time-consuming, and a shortcut using available structural data as in the case of MR in X-ray crystallography is not available.

Here, we present NMR^2, a MR-like approach in NMR to determine the structures of the binding pockets of ligands at atomic resolution. The calculation of structures of protein-ligand complexes relies on the collection of unassigned semi-quantitative inter-molecular NOE distance restraints and on previously solved structures. The NMR^2 method uses a high throughput structure calculation protocol, rather than a docking-scoring simulation. It is fast since it requires only a few days of measuring time and bypasses the time-consuming sequential assignment steps for the protein. When applied to the cancer-relevant HDMX protein, the NMR^2 method yielded the structure of a ligand protein complex with an accuracy below 1 Ångstrom for the binding pocket irrespective of the starting protein

structure templates used. We will present multiple NMR^2 applications covering a peptidomimetic inhibitor and small molecules that bind strongly or weakly to protein receptors. Our findings demonstrate that NMR^2 may open an avenue for the fast and robust determination of the binding pocket structure of ligand-protein complexes at atomic resolution without the need of diffracting crystals and high affinity ligands.

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REDOR NMR ANALYSIS OF A MICROTUBULE-BOUND EPOTHILONE B DERIVATIVE

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Epothilones are promising microtubule-targeting agents for antitumor drugs to overcome multidrug resistance and limited supply of taxane derivatives [1]. However, the binding conformations of most of their prominent members are yet unknown [2]. Herein, a deuterium and fluorine labeled analog of epothilone B was designed and synthesized in order to investigate its microtubule bound-conformation employing solid-state rotational-echo double resonance (REDOR) NMR techniques [3]. Our $^{2}H\{^{19}F\}$ REDOR results indicated that the macrolide conformation of epothilone B was different from the one previously reported for tubulin-bound epothilone A in zinc-stabilized 2D sheets but, instead, be similar to the conformation found in the crystal structure of epothilone B-bound P450epoK complex. AutoDock calculations of epothilone B with tubulin dimer was also carried out, which suggested that epothilone A and B share in common the hydrogen bond interactions between the hydrophilic side of the ligands and the Thr274 residue of b-tubulin, but their binding modes, especially the conformations of macrolides, are different from each other.

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A SERUM NUCLEAR MAGNETIC RESONANCE-BASED METABOLOMIC SIGNATURE OF ANTIPHOSPHOLIPID SYNDROME

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Autoimmune diseases are among the unsolved, chronic, widespread diseases affecting the quality and the duration of the life, with important consequences concerning the economic sustainability of the sanitary system.

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by venous and arterial thrombotic events, due to the production of autoantibodies directed against the blood vessel layers (1). The laboratory abnormalities of APS include the presence of anticardiolipin antibodies, lupus anticoagulant, anti-phospholipid β_2-glicoproteina I. However, in view of the heterogeneity of autoimmuno diseases, more sensitive biomarkers for early detection and molecular targets for better treating of APS are urgently needed. Metabolomics, diagnosis based on 'metabolic fingerprinting' may provide clinically useful biomarkers applied toward identifying metabolic alterations and introducing new insights for prevention and therapeutic application.

We report here a ^{1}H NMR-based metabolic phenotyping study of APS patient sera aimed at identifying coordinated metabolic serum changes associated with APS syndrome in comparison to healthy blood donors. A model discriminating APS patients vs healthy blood donors is obtained, and validated with an independent cohort; several metabolites distinguishing healthy blood donors from APS patient sera are identified.

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P 233
IN VITRO AND IN VIVO METABOLOMIC STUDIES FOR THE EVALUATION OF BREAST CANCER NANOMEDICINES

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Nanomedicine is a rapidly growing field that is significantly contributing to anticancer treatment strategies by enabling site-specific release of chemotherapeutic agents based on their physicochemical characteristics and biological attributes [1]. Polymer therapeutics are considered the first generation of polymeric nanomedicines [2] and can significantly improve anticancer therapies mainly due to their ability to prolong the drug circulation time in blood, and promote passive drug accumulation in the tumour via the enhanced permeability and retention (EPR) effect.

Metabolomics is a systems biology approach concerned with the high-throughput identification, characterization and quantification of small molecule metabolites [3]. Although scarcely explored in the nanomedicine field, Nuclear Magnetic Resonance (NMR)-based metabolomics can potentially contribute to the global evaluation and understanding of the biological effects of new drug delivery systems [4]. Moreover, given its applicability to biological systems of different complexity (cell cultures, animal models, humans), metabolomic profiling is suitable for making direct connections between in vitro and in vivo data, thus facilitating further progress in translational research.

In this study, a HPMA conjugate of doxorubicin for the treatment of breast cancer [5] has been chosen as a model system to evaluate the potential of metabolomics for characterizing the effect of controlled drug delivery. To this end, the metabolic fingerprint of samples from cell cultures and animal models after drug-treatment was determined by NMR. We observed that characteristic metabolic profiles could be

associated with the treatment of the conjugate and the free drug in in vivo and in vitro preclinical models. In addition, metabolite levels could be related to key parameters such as efficacy, toxicity and mode of action, and associated with the different forms of drug release.

In summary, our study shows that NMR-based metabolomics is a powerful tool for studying controlled drug delivery. Together with gene expression data and proteomic analyses, it could be implemented on a regular basis for the evaluation of drug therapies, and especially for the development of new drug delivery systems at the preclinical stage.

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P 236
APPLICATION OF NMR SPECTROSCOPY TO MARINE NATURAL PRODUCTS

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In the interest of identifying natural substances from marine invertebrates collected off the waters of Taiwan, we studied the gorgonian coral Echinomuricea sp. for its organic extract showed interesting chemical constituents by NMR data analysis. In this presentation, including three new compounds, echinoclerodane A (**1**), echinohalimane A (**2**), and echinolabdane A (**3**), were isolated from gorgonian coral Echinomuricea sp. The structures of these compounds and their derivatives were established primarily on the basis of nmr spectral analysis and chemical derivatization. The application of NMR techniques included ^{1}H NMR, ^{13}C NMR, DEPT,COSY, HMQC, HMBC, NOESY and so on.

Keywords: NMR, Echinomuricea sp., echinoclerodane, echinohalimane, echinolabdane

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IMPROVING SIGNAL SEPARATION FOR OLIGOMERIC STRUCTURES IN PURE SHIFT HSQC SPECTRA

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Repetitive motives in oligomers intrinsically carry a high risk of signal overlap in NMR spectra when signals are clustering in specific spectral regions due to structural similarity. Such a situation was identified throughout the structural analysis of a novel oligourea, built out of one monomer type. Oligoureas belong to a peptidomimetic class of helical foldamers[1a] and have potential in design of self-assembled structures[1b].

The high potential of broadband homodecoupling methods to reduce signal overlap in HSQC spectra in such cases has recently been presented[2], though techniques achieving decoupling of diastereotopic protons in methylene groups remain scarce[3]. Robust approaches providing clean signals even in the case of strongly coupled signals are required, to avoid missing out signals of such protons that may have very weak intensities in homodecoupled spectra.

To improve signal separation for the hexamer oligourea, in particular in the backbone region, we combined the perfectBIRD HSQC experiment[3b] with the RESET processing approach[2a]. The homodecoupling strategy chosen allows the collection of broadband homodecoupled spectra of high quality over the full ^{1}H and ^{13}C offset range, even for diastereotopic methylene groups. While homodecoupling artifacts cannot be avoided by this method, the RESET processing approach of the data can provide a strong reduction of the artifacts associated with strongly coupled signals. High quality correlation maps can therefore be obtained that provide good signal separation even for the pivotal backbone signals.

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MR-COMPATIBLE MINI-INCUBATOR FOR IN VITRO STUDIES OF EPILEPTOGENESIS IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

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Epilepsy is one of the most prevalent neurological disorders, which is accompanied by recurring seizures. Epileptogenesis are the morphological, molecular and electrophysiological changes of the brain associated with Epilepsy. This process of Epileptogenesis is not yet fully understood. An established in vitro model to examine Epileptogenesis are **organotypic hippocampal slice cultures (OHSC)** [1,2]. OHSC contain the physiological composition of all cell types found in the hippocampus, retaining the cytoarchitecture.

Using **magnetic resonance microscopy (MRM)** with a resolution under 10 µm3 [3,4], for examining artificially induced Epileptogenesis in OHSC, has the potential to identify early biomarkers of Epileptogenesis. To achieve a high resolution in MRM, measurement times in the range of a couple hours within an MR scanner are necessary. To resolve the temporal development of Epileptogenesis, which can last from days to weeks, it is useful to measure the same OHSC repeatedly within one study. Therefore, for an in vitro measurement, the **OSHC have to be viable during the whole MR scan period.** We have developed an MR-compatible mini-incubator to allow for these measurements.

We introduce an incubator that enables the use of **common protocols** to cultivate OHSC for up to 6 months [5]. The MR-compatible incubation chamber enables to control the **gas atmosphere** (here: 95%/5% O2/CO2). An external humidifier for the gas minimises the drying effect to avoid demoisturing of the OHSC. The incubator is **temperature stabilised** by connecting it to a temperature bath. The basis of the incubator is made from a 3D-printed ABS-holder, which is dimensioned for use in a Bruker CryoProbe. Nurturing medium is filled into an exchangeable custom-made PMMA container, into which a **commercial 12 mm Millicell-HA cell culture plate insert** can be mounted. This Millicell insert is compatible with commercial cell culture plates, which enables the cultivation of the OHSC between MRM measurements in standard lab equipment. The first MRM experiments with fixed OHSC in the incubator in a**7 Tesla Bruker BioSpec 70/20 small animal scanner with a two-element Bruker CryoProbe demonstrated the MR compatibility and principle functionality of the system,** and measurements on acute and cultivated OHSC are on the way.

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COMPARISON OF THE 3 MM AND THE 5 MM 800 MHZ CRYOPROBE IN LIFE SCIENCE APPLICATIONS

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For many years, the 5 mm cryoprobe has been the golden standard in high-fleld NMR. The 5 mm probe geometry represents a good balance between sample size and sensitivity. However, with increasing magnet field strength and with increasing ionic strength, the S/N in cryoprobes is adversely affected by the E-field. To overcome this, optimal buffer components and composition for biological macromolecules were suggested [1] but this approach cannot be used in e.g. metabolomics applications involving biofluids such as urine or serum. The use of shaped sample tubes has been described [2] and is frequently applied. A simpler solution involves the use of 3 mm sample tubes in the 5 mm probe geometry.

We here present results from the first 3 mm 800 MHz Bruker TCI cryoprobe. The 3 mm probe geometry requires smaller sample volumes, offers shorter pulses at the same power levels and allows better water handling compared to the 5 mm probe geometry. In one-pulse experiments on 2mM sucrose, the S/N of the 3/3 (3mm probe / 3 mm sample) is slightly lower than for the 5/5 case. In "real" multi-pulse experiments (e.g. 2D ^{15}N HSQC, 2D ^{13}C HSQC, 3D HNCA, 3D HNCO) on "real" protein solutions, we obtain similar or better S/N for the 3/3 compared to the 5/5. Similarly, in metabolomics applications on biofluids such as urine or serum, the performance of the 3/3 is as good or better than the 5/5.

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P 248
MEASURING MOLECULAR TRANSLATIONAL DIFFUSION COEFFICIENTS BY PFG-NMR USING BAND-SELECTIVE RF PULSES

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Molecular translational self-diffusion, a measure of diffusive motion, provides information on the effective molecular hydrodynamic radius as well as information on the properties of media or solution through which the molecule diffuses. Pulsed-Field Gradient NMR (PFG-NMR) based methods have seen increased application in following the changes of protein/peptide effective hydrodynamic radius due to self-association and aggregation, folding and unfolding processes, ligand binding and protein-protein interactions, and structural characterization of drug metabolites in mixtures.

Here we describe translational diffusion coefficients measured by PFG-NMR using a modified STimulated Echo (STE) sequence where band-selective pulses are employed for all three ^{1}H RF pulses. Firstly, translational diffusion measurements using BEST-STE are reported and the results compared with values obtained from the conventional non-selective Bipolar Pulse Pair Longitudinal Eddy-current Delay (BPP-LED) sequence[1]. The BEST-STE sequence was subsequently used to measure translational diffusion coefficients of Ab42 in the presence of significant molar excess of phospholipid (non-deuterated) in micelles. The dynamic range problems caused by intense resonances arising from the micelles, as experienced in conventional BPP-LED sequence, are avoided and the translational diffusion coefficients of Ab42 in the presence of phospholipid (molar ratio 1:200) across the temperature range of 278 – 313 K are reported[2].

Compared with conventional non-selective sequence, e.g. the BPP-LED sequence, the advantage of this modified Band-selective Excitation Short Transient (BEST) version of STE (BEST-STE) sequence is

multi-fold, namely: (i) potential sensitivity gain as in generalized BEST-based sequences, (ii) water suppression is no longer required as the magnetization of solvent water is not perturbed during the measurement, and (iii) dynamic range problems due to the presence of intense resonances from molecules other than the protein or peptide of interest, such as non-deuterated detergent micelles, are avoided.

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P 251
CHARACTERIZATION OF PARTIALLY DISORDERED DELTA SUBUNIT OF RNA POLYMERASE FROM B. SUBTILIS

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RNA polymerases of gram-positive bacteria of Bacillus subtilis differ from well studies RNA polymerases of gram-negative bacteria in the presence of two additional subunits interacting with the core enzyme, delta and omega1. Their role in the transcription machinery is not well understood. The well-structured N-terminal domain of delta (N-delta) is proposed to bind to the RNAP core and probably orients the unstructured and highly negatively charged C-terminal domain (C-delta) on the surface of RNAP. That might mimic nucleic acidsand compete with DNA/RNA for binding on RNAP. The 3D structures of the ordered N-terminal domain as well as the full length molecule has been determined previously by our group [1, 2].

NMR spectroscopy provides a possibility to obtain several experimental parameters, which are sensitive to different aspects of the structural and dynamical behavior of the disordered state. In our case, the experimental data comprise chemical shifts (CS's),residual dipolar couplings (RDC's), and paramagnetic relaxation enhancements (PRE's) obtained previously in our laboratory on the full length molecule of delta subunit.

Our studied system delta subunit of RNAP has unstructured (C-delta) and structured part (N-delta). We handled with structured part as rigidbody while we used recently developed protocols for flexible-meccano and ASTEROIDS [3] on unstructured part (C-delta). Representative sub-ensemble based on experimental data from a large generated ensemble of structures generated by flexible-meccano can be selected by ASTEROIDS. Experimental data of CS, RDC, and PRE were used together for selection of sub-ensemble,

which was further analyzed. RDC's data was separated to provide information about local structural component fitted together with CS's and about global orientation component calculated from PRE. The final result showed propensities to adopt different structural motives for the unstructured C-terminal domain (C-delta).

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P 254
HADAMARD NMR WITH MULTIPLE RECEIVERS

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Use of cryoprobes and multiple receivers can significantly alleviate the sensitivity issues that are usually associated with the so called direct detection experiments. In addition to parallel acquisition, further time savings are feasible due to significantly smaller F1 spectral windows as compared to the indirect detection experiments. In cases where experiments are sampling limited rather than sensitivity limited even greater reduction in experiment time is achieved by using Hadamard encoding [1].

We have adapted two PANSY (Parallel NMR SpectroscopY) experiments – PANSY COSY and PANSY-TOCSY for Hadamard encoding [2, 3]. The PANSY-TOCSY-Q experiment has been modified to allow for simultaneous acquisition of three different types of NMR spectra - 1D C-13 of non-protonated carbon sites, 2D TOCSY and multiplicity edited 2D HETCOR. In addition the J-filtered 2D PANSY-gCOSY experiment records a 2D HH gCOSY spectrum in parallel with a ^{1}J-filtered HC long-range HETCOR spectrum as well as offers a simplified data processing. The spectra of pamoic acid recorded using the Hadamard encoded PANSY COSY pulse sequence are shown. In favourable cases the total recording time for the two PANSY experiments can be reduced to just 40 seconds. The proposed Hadamard encoded PANSY experiments provide sufficient information to allow the CMC software package (Bruker) to solve structure of small organic molecules.

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P 257
EXPERIMENTAL REALIZATION OF A QUANTUM SUPPORT VECTOR MACHINE

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The fundamental principle of artificial intelligence is the ability of machines to learn from previous experience and do future work accordingly. In the age of big data, classical learning machines often require huge computational resources in many practical cases. Quantum machine learning algorithms, on the other hand, could be exponentially faster than their classical counterparts by utilizing quantum parallelism. Here, we demonstrate a quantum machine learning algorithm to implement handwriting recognition on a four-qubit nuclear magnetic resonance test bench. The quantum machine learns standard character fonts and then recognizes handwritten characters from a set with two candidates. Because of the wide spread importance of artificial intelligence and its tremendous consumption of computational resources, quantum speedup would be extremely attractive against the challenges of big data.

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ESTABLISHING TWO-DIMENSIONAL HETERONUCLEAR NMR CORRELATIONS BY OFFSET-SENSITIVE RECOUPLING

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Two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy is a powerful tool for the elucidation of molecular structure and dynamics. 2D NMR correlations typically require a full sampling of two time-domains according to Nyquist criteria.[1] As one of these domains involves a t_1 delay within a pulse sequence, this necessitates multiple repetitions and extended experimental timescales. This abstract presents an approach for enabling the fast – in principle single-scan– acquisition of 2D heteronuclear single quantum coherence (HSQC) spectral information. In the simplest 13C-1H version of this new sequence,

1H: (90°) ---------- (180°) ---------- acquiring

13C: (adiabatic π pulse) (adiabatic π pulse)

the traditional t_1 evolution is replaced by a pair of linearly frequency swept adiabatic pulses [2], possessing identical durations T and spectral sweep-widths $\Delta\omega$. The aim of this pair, together with a proton 180° pulse in their middle, is to introduce an offset-dependent recoupling of the ^{13}Cs and the ^{1}Hs. The accumulated phase of a proton attached to a ^{13}C with frequency offset ω_C and a heteronuclear coupling constant J_{CH} among these spins, will thus be at the end of these adiabatic pulses

$$\theta = 4\pi J_{CH} T \omega_C / \Delta\omega \quad (1)$$

The ^{1}H magnetization can thus be described as a mixture of in- (IP) and anti-phase (AP) components modulated by and respectively:

$$H_y \cos\theta - 2H_x C_z \sin\theta \quad (2)$$

From Eqs. (1) and (2), the relation between the ^{13}C frequency offset ω_c and the intensities of ^{1}H J-coupled multiplets can thus be obtained as

$$\omega_c = (\Delta\omega/4\pi J_{CH}T)\arctan(S_{AP}/S_{IP}) \quad (3)$$

where and are the intensities of the IP and AP components respectively, and is the equilibrium magnetization of ^{1}H spin. Experimental results demonstrate the usefulness of this approach in different scenarios. Standard deviations of the errors between the calculated ^{13}C frequency offsets and real frequency offsets dependent on the spectral bandwidth being addressed (standard deviations being ca. 3 and 30 Hz when spectral widths are 2 kHz and 20 kHz respectively).

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P 263
HETERONUCLEAR SPIN DECOUPLING IN SOLID-STATE NMR: FEATURES OF rCW SCHEME

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Heteronuclear spin decoupling is essential in most of the solid-state NMR experiments for resolution and sensitivity of rare spins dipolar coupled to abundant spins. We have witnessed a variety of methods to achieve decoupling. Most of them involve modulations in phase and frequency. Here, we will present advances made in one of the recently introduced method, the refocused continuous-wave (rCW) scheme. We will focus on the improvement in efficiency, robustness to various experimental parameters, and the ease in experimental implementation of this scheme.

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NOVEL 3D HETERONUCLEAR EXPERIMENTS TO RESOLVE OVERLAPPED RESONANCES IN 1H/1H DQ/SQ CORRELATION SPECTRA UNDER ULTRAFAST MAS

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$^{1}H/^{1}H$ DQ/SQ (Double Quantum/Single Quantum) MAS (Magic Angle Spinning) chemical shift correlation experiment is one of the most popular and most important homonuclear correlation experiments in contemporary solid-state NMR spectroscopy, especially under fast and ultrafast MAS rates [1]. This experiment probes spatial proximity of proton nuclei which is important to get insights into intra/intermolecular interactions and crystal structure of various chemical compounds and materials. Although, resolution of DQ/SQ spectra can be greatly enhanced by the ultrafast MAS rates up to 120 kHz [2] and ultrahigh magnetic fields up to 24 T (1.02 GHz) [3], overlapped signals still remain in 2D correlation spectra of complex chemical compounds and materials. Low intensity intra/intermolecular cross peaks of interest may be still masked by other, less informative cross peaks of high intensity even at the fastest MAS and highest magnetic field currently available. Therefore, there is a need to develop more advanced techniques for spectral analysis of overlapped regions.

To simplify complex $^{1}H/^{1}H$ DQ/SQ spectra we introduce a ^{13}C (^{15}N, ^{31}P, ^{29}Si, etc.) dimension and optimize $^{1}H/^{13}C$ transfers to select directly bound $^{1}H/^{13}C$ pairs. This approach has a twofold effect: firstly, protons that are not attached to carbons, such as NH, OH and SH protons, are suppressed; secondly, eventual overlaps of CH, CH_2 and CH_3 protons are resolved by ^{13}C chemical shifts. To accomplish this, we have combined $^{1}H/^{1}H$ DQ/SQ and $^{1}H/^{13}C$ double-CP (Cross Polarization), also known as CP-based HSQC (Heteronuclear Single Quantum Coherence) [4,5], steps. In this regard, we have designed $^{13}C/^{1}H/^{1}H$ SQ/DQ/SQ and $^{1}H/^{13}C/^{1}H$ DQ/SQ/SQ pulse

sequences. As these pulse sequences differ by the position of ^{13}C-filter, results obtained by these two experiments are not completely identical and both contain unique spectral features. For example, the ^{1}H/^{13}C/^{1}H DQ/SQ/SQ pulse sequence eliminates NH, OH and SH signals more efficiently, while the ^{13}C/^{1}H/^{1}H SQ/DQ/SQ experiment also detects two-bond ^{1}H/(^{1}H)/^{13}C interactions.

In this contribution, we will demonstrate the 3D experiments described above utilizing BaBa-xy16 recoupling [6] and ^{1}H-^{13}C double-CP pulse sequence on uniformly ^{13}C-labelled organic molecules at ultrafast MAS rate of 70 kHz.

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P 269
INEXPENSIVE SITE SPECIFIC 13C LABELING OF AROMATIC SIDE CHAINS

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Solution studies of large macromolecular systems and membrane proteins have benefited from selective isotope labeling. In particular, site specific isotopic labeling of methyl groups has helped overcome the traditional size barrier. However useful, the number of reporter sites can be limiting leaving need for additional probes. Aromatic amino acids, often found at important interaction interfaces and playing significant structural roles, have many of the same advantages as methyl containing residues. Aromatic side chains have distinct ^{13}C chemical shifts, multiple magnetically equivalent ^{1}H positions, and in most cases ring flipping results in reduced effective correlation times. This allows for observation in large, slow tumbling systems. Herein we report inexpensive bacterial production of phenylalanine and tyrosine with ^{13}C incorporation at the Ca, Cg and Ce positions. We also present methodology to maximize incorporation of aromatic amino acids into recombinantly overexpressed proteins in bacteria with greater than 95% efficiency. Site specifically labeled phenylalanine and tyrosine provide new tools for NMR studies, opening new avenues for the study of large macromolecular systems and membrane proteins. Studies using these new tools are underway for the novel antibiotic target peptidyl-tRNA hydrolase in complex with peptidyl-tRNA, the antimicrobial lectin Scytovirin bound to high mannose moieties, and the transmembrane domain of notch signaling receptors.

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QUICK AND EASY NMR TITRATION USING SLICE-SELECTIVE EXPERIMENTS TO STUDY CONCENTRATION GRADIENTS IN AGAROSE GELS

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NMR titration is a long recognised method for determination of equilibrium constants or thermodynamic parameters of reactions, but suffers from the drawback of being time consuming. Recently, spatial-selective NMR spectroscopy for reaction monitoring was proposed[1]. The method relies on slow diffusion of one of the reaction components into a polystyrene gel, containing the other, thus resulting in a spatially dependent sample composition along the NMR tube. Following a similar approach we present the use of agarose gels as the medium for single-experiment NMR titrations in water. It was used to study the inclusion of paracetamol in cyclodextrine macrocycle as a model reaction. The agarose gels benefits from a very simple and reliable sample preparation. Moreover, we observed no interaction between the matrix and the compounds under study and obtained high-resolution spectra, identical to those obtained from solution samples.

One of the main advantages of the proposed method is its speed achieved by performing the slice selective experiments in an interleaved manner, thus affording the acquisition of quantitative spectra in 1-10 minutes. In addition to the room temperatures measurements, the variable temperature NMR titration has been also investigated, as the agarose gels possess the attractive property to lower the freezing point of water making it possible to study water solutions up to -8 C.

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CRYOCOIL MAS-NMR PROBE TO ENHANCE THE SENSITIVITY ON LOW-GAMMA NUCLEI OF INORGANIC SOLIDS

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A cryocoil MAS-NMR probe which we have developed is compatibly realized both a sample spinning system around room-temperature and a high-Q detection coil with its temperature of < 20 K by cryogenic refrigerator, which results in higher S/N gain for high-resolution solid-state NMR. We have demonstrated to apply the single-tuned cryocoil MAS-NMR probe for observing some low-sensitive nuclei such as ^{6}Li, ^{29}Si, ^{43}Ca, and so on included in inorganic solids under 14 Tesla. The typical sensitivity enhancement factor compared with the sensitivity in a conventional probe has been attained at 4.4 times.

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NMR CORRELATION SPECTROSCOPY BETWEEN TWO CONFORMERS OF PEPTIDE BY PHOTO-ISOMERIZING AN AZOBENZENE CROSS-LINKER ON THE SUBSECOND TIMESCALE

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Property changes of protein solution by chemical and physical jump are able to modulate structure and function of a protein. In area of NMR methods, stopped flow, pressure jump (P-jump), and temperature jump (T-jump) are known. A typical method of stopped flow acquires a milliseconds-order time resolution. However, the jump is progressed in one direction and is irreversible. On the other hand, P-jump and T-jump are reversibly controlled and the time resolution of both methods is suitable to NMR acquisition. In this presentation, we utilized an azobenzene derivative[1] as a molecular device to control a peptide structure. Photo-irradiation isomerizes azobenzene between trans and cis reversibly, followed by a structure change of the cross-linked peptide. We used the structural conversion of azobenzene to acquire of correlation spectrum between two conformers of azobenzene cross-linked peptide. In order to detect correlation signals, the photo-conversion has to be much faster than nuclear spin relaxation. In our case, the structural conversion is induced during a mixing time in NOESY pulse sequence; therefore, the conversion time is made shorter than T_1 relaxation time. This method is originally reported as SCOTCH (spin coherence transfer in chemical reactions)[2]. Here, we show a novel photo-irradiation device to promote rapid structural conversion and photo-induced correlation spectroscopy of an azobenzene cross-linked peptide. The photo-irradiation device with a high power laser diode achieved 90% structure conversion from cis to trans of an azobenzene derivative within 80 ms. Thereby an azobenzene cross-linked peptide in 20% trifluoroethanol was converted from coil to helix according to an azobenzene structure change from cis to trans within a mixing time of NOESY. Consequently, correlation signals between two conformers appeared on NOESY spectrum. These results are shown that this method allows

incorporating a protein structure manipulation into the NMR pulse sequence.

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P 281
ACCURATE MOLECULAR WEIGHT DETERMINATION OF SMALL MOLECULES VIA A NEW DOSY-NMR-METHOD

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Particularly in s-block chemistry the aggregation /deaggregation of the starting materials plays the leading role on the reactivity and hence on the product distribution. The solid state structure and theoretical calculations from the gas phase both are of limited use to predict the complex constitution in solution, because solvation effects are either not present or difficult to mimic.[1] The method of choice is NMR spectroscopy from solution. Here the formula weight analysis using DOSY NMR spectroscopy, pioneered by Paul Williard at Brown, determines the molecular weight of a small molecule with an error bare of ±30%.[2] Many internal standards, which might interfere with the reactive alkaline metal complexes are required. Recently we developed a new method for accurate MW-determinations that relies on external calibration curves (ECC) with normalized diffusion coefficients. The addition of multiple internal references is not required anymore. One internal reference (that also can be the solvent) is sufficient. If the solvent signal is not accessible, 16 other internal standards (aliphatics and aromatics) are available to avoid signal overlapping problems and provide flexible choice of analytes. This method is independent of NMR-device properties and diversities in temperature or viscosity and offers an easy and robust method to determine accurate MWs in solution with a maximum error of < ±9%.[3] With this publication we contribute a mayor improvement to the method that will further push the limits to structural insights on solution structures. In that context we analyzed the aggregation of lithium diisopropyl amide (LDA) in toluene. LDA is a very prominent reagent that plays a key role in organic synthesis, serving as a base par excellence for a broad range of deprotonation reactions. However, the state of aggregation in solution in the absence of donor bases was unclear. With the new ECC-MW-determination method we were able to solve this problem: At room temperature, toluene solvated LDA adopts trimeric and tetrameric aggregation in a 2:1

ratio. This equilibrium ranges from trimers and tetramers through pentamers to higher oligomers as the temperature decreases. The lower the temperature, the closer the solution structure approaches the polymeric solid-state structure.[4]

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SLICE-SELECTIVE NMR SPECTROSCOPY – SURPRISINGLY VERSATILE

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Slice-selective NMR spectroscopy is a straightforward and easy-to-use technique: A magnetic field gradient along the axis of the NMR tube and shaped radiofrequency pulses with variable frequency offsets allow the excitation of discrete horizontal sample slices. These experiments can be performed on standard solution NMR equipment: A whole scan through the active spectrometer volume (~2 cm height for a 5 mm standard probe) in steps of 1 mm thick slices is executed in less than 2 min (for abundant nuclei like ^{1}H and ^{7}Li). We found that the spatial information acquired by slice-selective NMR spectroscopy can be exploited in a number of surprisingly variable methods:

Initially, we applied this technique to observe the diffusion of solvents and solutes into polymers.[1]

These experiments prepared the ground for the application of slice-selective NMR spectroscopy to reaction monitoring:[2] The organic base PMDTA was added on top of crosslinked polystyrene swollen with nBuLi/toluene-d_8 inside the NMR tube. As PMDTA diffuses into the inert polymer it gets lithiated in an exothermic reaction. The polymer matrix prevents convection and largely immobilizes nBuLi. Consequentially, a clear reaction front can be observed by slice-selective ^{1}H and ^{7}Li NMR spectra. Within these spectra distinct additional signals appeared that can be attributed to the x-ray structurally characterized intermediate $[(nBuLi)_2PMDTA]_2$.[3]

In a titration-type application of slice-selective NMR spectroscopy[2] we froze 12-crown-4 at the bottom of the NMR-tube and layered a solution of the $LiClO_4$ in acetonitriled_3 on top of it. The solid ether melts and builds up a smooth concentration gradient as it diffuses into the metal-solution. Slice-selective ^{7}Li and ^{1}H scans provide specific ether/lithium ratios for each slice to which ^{7}Li chemical shifts

can be assigned. The resulting titration curve fully agreed with previous reports.[4]

Currently we are extending the versatility of slice-selective NMR spectroscopy to the concept of NMR-chromatography. This analytical method is usually conducted as DOSY type experiments.[5] We are hoping to take advantage of slice-selective measurements as a robust and rapid technique which additionally offers powerful combination with classical 2D experiments yet to exploit.

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INVESTIGATION OF PRE OF HIGH SPIN IRON-LANTHANIDE COORDINATION CLUSTERS UP TO 1.4 GHZ

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Paramagnetic relaxation enhancement (PRE) in nuclear magnetic resonance (NMR) is an important subject for magnetic resonance imaging (MRI). The dispersion of longitudinal and transverse paramagnetic relaxivity up to highest magnetic fields is of interest in application as well as in basic research.

In this contribution PRE of a series of new ultrahigh-spin magnetic cluster in water is examined up to highest magnetic fields (32.9 T) and frequencies (1.4 GHz): The 3d/4f cyclic clusters $[Fe^{III}_{10}Ln^{III}_{10}(Me\text{-}tea)_{10}(Me\text{-}teaH)_{10}(NO_3)_{10}]$, abbreviated as $Fe_{10}Ln_{10}$, in which Ln stands for Gd, Dy, Tb, Er or Tm, and Me-tea is the triethanolamine ligand which is singly substituted with CH_3-group

The characterization of ^1H PRE induced relaxivity dispersion is especially interesting, showing a pronounced increase with magnetic field [1,2]. The experimental results comprise data from time-domain NMR instruments using permanent magnets, NMR spectrometers equipped with super-conducting magnets, and, for the data above 850 MHz up to 1.4 GHz, an ultra high-field NMR equipment using a 24 MW resistive magnet.

The longitudinal (r_1) and transverse (r_2) relaxivities and their dispersion depend on the nature of the Ln^{III}. Except for $Fe_{10}Gd_{10}$, where r_1 is almost field independent, r_1 and r_2, monotonically increase for $Fe_{10}Ln_{10}$ up to the highest fields. The results are qualitatively discussed in the context of PRE theory.

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EPR AND NMR CHARACTERIZATION OF TI HYDRIDES

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Early transition metal (Ti) hydrides stabilized on the surface of silica attract the attention of researchers thanks to their unique ability to catalyze not only the polymerization of olefins but also the splitting of linear and branched alkanes and polyolefins in the presence of hydrogen under mild conditions. Catalysts are usually prepared by the deposition of an organometallic compound: Ti and neopentyl on the surface of partially dehydroxylated silica followed by heating in the atmosphere of hydrogen. Under these conditions, a mixture of surface hydrides of the Ti metal is formed. According to the electron paramagnetic resonance (EPR) data, trivalent metal compounds are present. Their content is up to 30% for titanium, also these compounds were expected to include hydrides, although no unequivocal proof for a Ti(III)-H bond had been reported. Likewise, direct bonds between Ti(III) and carbon, as they are postulated in the mechanism of Ziegler-Natta polymerization catalysis, had not yet been proved to exist. In this study, these problems were addressed by synthesis of and pulsed EPR measurements on well-defined surface species.

Pulsed EPR measurements, in particular with the two-dimensional hyperfine sublevel correlation (HYSCORE) experiment, are useful not only for quantifying the distribution of the unpaired electron in the complex, but also for guiding synthesis efforts. In the example of surface-bound Ti(III) species that are related to Ziegler-Natta polymerization catalysis, local elemental analysis by HYSCORE revealed a high reactivity of certain surface species towards nitrogen, whereas reaction with trace amounts of oxygen was already revealed by continuous-wave (CW) EPR.

$TiNp_4$ was synthesized by reacting $Ti(OEt)_4$ with four equivalents of NpLi, followed by sublimation. The silica-supported titanium hydrides were prepared by grafting $TiNp_4$ on $SiO_{2-(700)}$ followed by a treatment under H_2 and first characterized by classical techniques such as Infrared (IR) and Nuclear magnetic resonance (NMR) spectroscopies. The formation of titanium hydride is confirmed by the appearance of bands which correspond to Ti-H along with bands associated with the concomitant formation of Si-H and SiH_2. An additional band is attributed to (N-H) and consistent with the formation of NH_x species via activation of traces of N_2 in H_2.

HYSCORE experiments at the X-band frequency revealed the presence of Li in the starting material. To prevent this contamination, at least two additional recrystallizations from pentane were necessary to afford a Li free material. After reacting the surface species with ^{13}C isotope labeled ethylene, the coordinated nascent polymer chain could be detected via large ^{13}C hyperfine couplings. Successful synthesis of Ti(III) hydride surface species could be proved by preparing the sample with deuterated hydrogen and observing in the HYSCORE spectra deuterium hyperfine couplings that were consistent with the expected Ti-H (or Ti-D) bond distance of about 1.7 Å. These couplings and the corresponding proton hyperfine couplings in a sample prepared with natural isotope abundance hydrogen were not resolved in the CW EPR spectra, which are broadened by substantial g strain for such surface species.

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CHARACTERIZATION OF METAL CHELATE COMPLEXES FOR ELECTRON-ELECTRON DIPOLAR SPECTROSCOPY

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Recently a lot of interest emerged with respect to the use of metal centres as spin labels for long-range site-to-site distance measurements in biomacromolecules and biomolecular complexes. Herein we report on the characterization of a series of metal complexes with the focus on their spectroscopic properties that are important for electron-electron dipolar spectroscopy. To concentrate on the intrinsic properties of the metal centres, the work was conducted in a deuterated matrix.

The ligands studied are TAHA, PyMTA, PCTA-12, NO3Py and PyDTTA. Among the tested metal ions, iron (Fe(III)) and cobalt (Co(II)) exhibit rather short transverse relaxation times, thus giving a very low signal-to-noise ratio, so that their complexes might be inefficient as spin labels. Manganese (Mn(II)), gadolinium (Gd(III)) and cupper (Cu(II)) exhibit relatively slow transverse relaxation thus being, in principle, suitable for double electron-electron resonance (DEER) or relaxation induced dipolar modulation enhancement (RIDME) experiments. The zero-field splitting of the different Gd(III)-complexes covers a range of 360-2080 MHz. Furthermore, a rather strong dependence of the transverse relaxation time on the type of ligand is observed. With Gd(III) complexes, potentially, dipolar evolution time traces up to 30 to 40 µs can be measured. For the Mn(II) complexes the transverse relaxation is in about the same range as for the Gd(III) complexes, however the signal-to-noise ratio is reduced as the Mn(II) hyperfine coupling results in a splitting of the central electron spin transition into six lines and the longer longitudinal relaxation time of Mn(II) requires a longer repetition time of the experiment. For the same ligand, the transverse relaxation of corresponding Cu(II)-complexes is slower than for Gd(III)-complexes, which may allow for longer dipolar evolution

traces and, thus, longer detectable distances. However, the broader width of the Cu(II) spectra and relatively long longitudinal relaxation reduces the signal-to-noise ratio. Furthermore, the broad anisotropic spectrum of Cu(II) might lead to orientation selection artefacts in measurements of the dipole-dipole coupling. However, in combination with nitroxide or trityl, all these problems might be alleviated by observing RIDME at about 50 K at the organic radical.

In summary, out of the tested metal ion/ligand combinations the complexes of Gd(III), Mn(II) and Cu(II) are most suitable for the pulse dipolar measurements. The Gd(III)-based spin labels seem to have clear spectroscopic advantages over Mn(II). The Cu(II)-based spin labels might be interesting as a low spin (S=½) system, with spectroscopic properties sufficiently different to the most common nitroxide-based as well as Gd(III)-based spin labels.

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NITRIC OXIDE ADSORPTION IN AMINO-MODIFIED Cu3(btc)2 -TYPE MOFS STUDIED BY SOLID-STATE NMR

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Adsorption and delivery technologies of the biological signaling molecule nitric oxide (NO) using porous solid storage materials are at the forefront of research [1]. NO delivery from a storage material is attractive for many in vitro and in vivo antibacterial, antithrombotic, and wound healing applications [2]. Robust NO drug delivery methods are needed, one of the most promising candidates is to use porous materials, specifically MOFs (Metal-Organic Frameworks). We studied NO adsorption on $Cu_3(btc)_2$ and its amino derivative, $Cu_3(btcNH_2)_2$, and their interaction with NO by solid-state NMR. The presence of unsaturated metal sites in both MOFs has a pronounced effect on NO adsorption and $Cu_3(btcNH_2)_2$ has additionally the potential to store NO covalently, forming N-diazeniumdiolates (NONOates) [3-4]. Solid-state NMR is a powerful technique to probe disordered gas loaded systems, providing information on the interaction of guest molecules with the host material, adsorption sites as well as the dynamics of NO within the pore. Samples with different amounts of adsorbed NO were investigated by ^{1}H MAS NMR. Changes to the electronic properties of the antiferromagnetically coupled copper ions in the MOFs by adding the paramagnetic molecule NO is evident in ^{1}H NMR shift, hyperfine coupling, and spin-lattice relaxation times (T_1). NO interaction with $Cu_3(btc)_2$ follows a linear trend for ^{1}H shift, hyperfine coupling, and spin-lattice relaxation time that can be explained by a decrease of unpaired electron density by interaction of the unpaired electron at the copper sites with nitric oxide. For $Cu_3(btcNH_2)_2$, clear evidence is found that NONOate formation occurs, as the ^{1}H shift, the hyperfine coupling, and the ^{1}H T_1 remain fairly constant with NO loading.

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PARAMAGNETISM IN GRAPHENES: WHAT CAN EPR AND PARAMAGNETIC NMR PARAMETERS TELL?

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Chemical modifications by adatoms and other kinds of point defects in graphene alter the electronic band structure by creating local magnetic moments into the nonmagnetic, pristine graphene. An adatom on graphene surface may carry a magnetic moment causing spin-half paramagnetism. This theoretically predicted phenomenon has recently also been experimentally verified [1].

The present periodic density-functional theory (DFT) study [2] demonstrates that electron paramagnetic resonance (EPR) g-tensor and the hyperfine coupling tensors, as well as the paramagnetic nuclear magnetic resonance (pNMR) shielding tensor can be used to identify the paramagnetic centers in graphenes. These directly measurable parameters give insight to the electronic and atomic structures of these defects. The obtained results should encourage pioneering experimental verification. Such experiments would contribute in a hitherto unexplored way to understanding carbon-based magnetism and characterization of the defect centers in graphenic materials.

We show that missing hydrogen and fluorine atoms in the functionalized graphane and fluorographene, respectively, constitute very local defect centers, in which the magnetic resonance parameters are greatly enhanced. Instead, slowly decaying adatom-induced magnetic resonance parameters with the distance from the defect, are found in pure graphene.

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SINGLE-MOLECULE MAGNET BEHAVIOR FROM PARAMAGNETIC NMR: A TRIGONAL PRISMATIC COBALT(II) COMPLEX WITH LARGE MAGNETIC ANISOTROPY

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Single-molecule magnets (SMMs), the term referring to chemical compounds exhibiting slow magnetic relaxation and magnetic hysteresis of purely molecular origin, have been discovered in the early 1990. Since then, they emerged as perspective components for information storage, quantum computing, spintronics, and magnetic refrigeration. A necessary condition for a compound to be an SMM is a large axial magnetic anisotropy D that splits the energy levels of a metal ion under zero magnetic field and gives rise to an energy barrier between the states with opposite directions of the magnetic moment $U = |D|S^2$ (or, for non-integer S, $U = |D|(S^2 - 1/4)$).

The method of choice for detecting a large magnetic anisotropy is magnetometry, which often allows determining both the D value and the effective barrier height U. This technique, however, is quite demanding and requires accumulation of large array of data at different values of magnetic field at very low temperatures. If the purity of the compound is not ideal, which is often the case for complexes with metal atoms in unusual oxidation states, the obtained results should also be interpreted with extreme care.

For the same purpose, we suggest to use NMR spectroscopy coupled with DFT calculations for evaluating the magnetic anisotropy of transition metal complexes in solutions based on a linear dependence of pseudocontact paramagnetic shifts on the anisotropy of the magnetic susceptibility. Paramagnetic shifts extracted from experimental NMR spectra have two components: the dipolar component, which depends on polar coordinates of the

corresponding nuclei in the coordinate frame of the unpaired electron and the value of the magnetic anisotropy, and the contact Fermi component, which is governed by the distribution of the density of the unpaired electron. As modern DFT calculations allow obtaining a reliable picture of spin density distribution, they have been used to estimate Fermi shifts with the remaining part of the paramagnetic shifts considered dipolar in origin and thus to extract the value of magnetic anisotropy at different temperatures. The temperature dependence of the magnetic anisotropy was fitted using van Vleck formula to get the D value.

The measurements only require a high resolution NMR spectrometer and a reasonable number of ^{1}H NMR spectra to be collected at different temperatures. The proposed approach is also immune to the presence of any impurities, as long as they allow detecting the signals of the compound under study.

In particular, this method allowed us to analyze a large library of cobalt(II) cage complexes to identify those with the largest magnetic anisotropy (25.1×10^{-32} m^3 at room temperature), which corresponds to a very large negative D value of −90 cm^{-1}. The magnetochemical measurements confirmed the large magnetic anisotropy and a resulting SMM behavior (with an effective barrier U = 156 cm^{-1}) of a chosen cobalt(II) compound. Therefore, this NMR-based approach paves the way towards fast and inexpensive prescreening of possible SMM candidates.

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STRUCTURAL STUDY OF PARAMAGNETIC LITHIUM MANGANESE TITANATE BATTERY MATERIALS FROM COMBINED BROADBAND SOLID-STATE NMR SPECTOSCOPY AND DFT CALCULATIONS

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The lithium manganese titanate spinels $LiMn_{2-x}Ti_xO_4$ are promising cathode materials exhibiting fast Li^+ transport, high theoretical capacity, and low toxicity[1-3].

The local structure of these materials is complex, due to the solid solution of the paramagnetic $Mn^{2+}/Mn^{3+}/Mn^{4+}$ ions and diamagnetic Ti^{4+} ions. We studied the local lithium structure using ^7Li MAS NMR. The isotropic shifts and shift anisotropies of these materials are dominated by the Fermi-contact and spin-dipolar hyperfine interactions of the nuclei with the unpaired electrons of the Mn ions. The acquisition of the resulting broad spectra required the combined use of fast sample rotation, and state-of-the-art adiabatic pulse sequences to achieve broadband excitation[4]. Multiple lithium sites were identified due to the distribution of the Li^+ ions amongst the tetrahedral and octahedral sites in the structure. The individual isotropic shifts were measured using the recently-proposed adiabatic magic-angle turning (aMAT) experiment[5].

In order to determine the variation with transition-metal composition of the distribution of the Li^+ ions amongst the different sitesstructural Mn^{2+}/Mn^{3+} cation ordering within the $LiMn_{2-x}Ti_xO_4$ structure DFT calculations were performed. A comparison of the different cation arrangements based on thermodynamics and electrostatics was performed, and the Fermi-contact shifts and individual contributions of each Mn—O—Li delocalization pathways were calculated and compared to the NMR data in order to identify the local environments and the structure[6,7].

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CHEMICAL SHIFT AND SHIFT ANISOTROPY IN PERIODIC PARAMAGNETIC SYSTEMS: A COMBINED SOLID-STATE DFT AND NMR STUDY.

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Many electrode materials contain paramagnetic transition metal (TM) ions, the redox reactions of which govern the electrochemistry of the battery. Solid-state Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for analyzing the related changes in the local structure, and quantum mechanical studies provide a detailed theoretical description of the process. However, the unpaired electrons of the metal ions induce large paramagnetic shifts and shift anisotropies in the NMR spectrum which, whilst potentially providing an invaluable source of information on the local chemical environment of the observed centres (OCs), can be extremely difficult to interpret.

The presented study is based on the formalism for the paramagnetic shift of molecular systems in the presence of spin-orbit coupling derived by Pennanen and Vaara [1, 2], combined with the theoretical approach described by Grey and co-workers [3, 4] to express the paramagnetic Fermi Contact (FC) shift in terms of the bulk magnetic properties. However, for solid systems of arbitrary electronic spin state the effect of spin-orbit coupling needs to be included so to reproduce the paramagnetic shift correctly. One effect is the anisotropic contribution to the g-tensor which, when coupled to the dipolar component of the hyperfine tensor, gives rise to the Pseudo Contact (PC) contribution to the paramagnetic shift.

This work aims to extend the calculation of the paramagnetic shift to solids of arbitrary spin state. An analysis of spin-orbit coupling and its effect on the g-tensor in periodic systems treated via linear response [5] is presented, based on symmetry and group theory. Also, together

with the FC term [3], the evaluation of the PC shift is obtained by calculating the contributions due to each neighbouring TM site separately, and by summing the results so to obtain the total PC contribution to the paramagnetic shift. The method is applied to interpret the ^{7}Li and ^{31}P NMR shifts of $LiFePO_4$ and the computational results are compared with the experimental shifts obtained for $LiFePO_4$ via solid-state Magic Angle Spinning NMR [6].

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SIMULATION OF 2D EDNMR SPECTRA EVALUATING THE HYPERFINE AND QUADRUPOLE PARAMETERS OF THE 33S LIGAND IN THE BLUE COPPER AZURIN

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ELDOR (electron-electron double resonance) detected NMR (EDNMR) is an electron double resonance technique used to determine hyperfine and quadrupole interactions by measuring frequencies of nuclear spins coupled to electron spins. In this experiment a high turning angle (HTA) pulse excite forbidden transitions of mixed electron-nuclear states while simultaneously observing the change of the echo intensity generated by a detection sequence in resonance with an allowed EPR transition. To achieve better resolution and obtain correlation between signals that assist their assignment this experiment has been extended into a two dimensional experiment called 2D EDNMR. The resulting spectrum is a 2D pattern showing correlations between nuclear frequencies belonging to different electron spin manifolds. In 2014 Kaminker et al. [1] introduced the 2D EDNMR experiment and demonstrated its utility by applying it to nitroxide spin labels observing correlations between ^{14}N nuclear frequencies. This new sequence was used to determine the ^{33}S hyperfine coupling in the Cu(II) site of Azurin in order to resolve the overlapping ^{33}S signals with those of the ^{14}N from the histidine ligands in the standard EDNMR experiment. The 2D EDNMR spectra of the Cu(II) site of the ^{33}S enriched protein Azurin was already reported but not analyzed [1]. To account for the observed spectra

we present spectral simulations of 2D EDNMR recorded at two magnetic field positions and determined the ^{33}S hyperfine and quadrupole interactions using starting spin Hamiltonian values obtain from DFT calculations [2] The simulations are based on the Liouville equation that gives the evolution in time of the populations of the various energy levels during the 2D EDNMR experiment.

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EFFECT OF MOLECULAR ARCHITECTURE ON STRUCTURE AND MOLECULAR DYNAMICS IN POLYSTYRENE-B-POLYETHYLENE OXIDE COPOLYMER SYSTEMS

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Block copolymers (BC) have attracted much attention during the last years as a hybrid materials obtained by connecting together polymers with different properties [1]. Linear block copolymer molecules comprise two or more polymer chains attached at their ends. The incompatibility of BC blocks causes the formation of ordered structures at the nanometer scale below the so-called order-disorder temperature. By changing the volume fraction of one component in an diblock copolymer one may obtain various structures. Symmetric diblock copolymers exhibit a lamellar morphology consisting of alternating layers of the components, while an increase of one component leads to formation of a cylindrical morphology (hexagonally-packed cylinders of minor component in the matrix of major component). In turn the most asymmetric systems have spherical morphology (body centred cubic spheres of the minor component embeded in the matrix of the major component). The phase separation in semicrystalline block copolymers is more complicated due to the occurrence of a crystallization process, resulting in both morphology richness and dynamics complexity.

Star polymers consist of several arms which are attached by one end to a central core [2]. The arms number (f) is called functionality of the star. A linear polymer may be regarded as a star polymer with the value of f equal to 2. Star-shape polymers have a lower viscosity in comparison with their linear analogues as a consequence of their compact structure and globular shape. Homo-arm star molecules

contain polymer chains of identical chemical composition and a similar molecular weight. The unique architecture of star polymers facilitates ordering phenomena. The block copolymers are known as self-assembled systems. It is interesting to investigate how the introduction of block copolymers into the star molecules influences the self-organisation ability of the homo-arm star system.

The goal of the study is to determine the effect of the molecular architecture of block copolymers on their phase structure and molecular dynamics. The materials under investigations have been the polystyrene-b-polyethylene oxide (PS-b–PEO) block copolymers of linear and star architectures.The phase structure of the studied systems has been revealed using differential scanning calorimetry (DSC) and wide angle x-ray scattering (WAXS) methods. The nuclear magnetic resonance (NMR) and broadband dielectric spectroscopy (BDS) methods have been used to study the influence of the architecture and chain length on the relaxation processes of soft PEO blocks in linear and star-type PS-b-PEO copolymers.

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CONFORMATIONAL DYNAMICS OF THE AUTOPHAGY-RELATED PROTEIN GABARAP ON MULTIPLE TIME-SCALES

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Understanding the function of a protein usually requires knowledge of its tertiary structure and conformational dynamics. NMR spectroscopy is a powerful tool for studying structure and dynamics on virtually all time-scales at atomic resolution. In particular, sub-nanosecond dynamics determine spin relaxation rates, whereas the biochemically often more relevant dynamics on the micro- to millisecond time-scale causes line broadening, which can be quantified by Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion (RD) experiments [1]. As a complementary technique the fluorescence spectroscopic toolkit provides insight into long-range dynamics. Thus, minor conformational states and local flexibility of proteins on the microsecond to sub-millisecond time-scale are accessible by single-molecule multiparameter fluorescence detection (MFD) [2], correlation techniques (filtered FCS) [3], and ensemble lifetime-resolved fluorescence (eTCSPC).

The 117-residue $GABA_A$ receptor-associated protein (GABARAP) is implicated in vesicle transport and fusion events in autophagy [4]. To this end, GABARAP is enzymatically lipid-conjugated to allow membrane anchoring, which has been reported to facilitate hemifusion of membranes upon oligomerization. Structure determination of GABARAP by NMR [5] and X-ray crystallography [6] suggested significant conformational heterogeneity. Intriguingly, crystallization under high salt conditions resulted in an alternate conformation in which the N-terminal region is associated with the hydrophobic binding pockets of a neighboring molecule [6]. Unfortunately, it remains unclear whether this alternate conformation indeed facilitates oligomerization during membrane fusion and/or

tubulin polymerization or is merely a crystallization artifact. The structural details, kinetics and thermodynamics as well as the functional relevance of the conformational heterogeneity are still poorly understood.

To gain insight, we have measured ^{15}N relaxation rates, $\{^{1}H\}^{15}N$ heteronuclear NOEs, and ^{15}N RD profiles, which reveal conformational dynamics in various regions of the tertiary structure. Residues in the termini and loop regions are highly mobile on the nanosecond time-scale as indicated by low order parameters and lifetime-resolved fluorescence anisotropy. CPMG RD experiments reveal two distinct conformational exchange processes on the millisecond time-scale. Specifically, resonances in the N-terminal helical subdomain exhibit separate resonances as a result of exchange on a time-scale of several milliseconds between two conformations with similar equilibrium populations. By contrast, residues lining the hydrophobic binding pockets reveal a slightly faster exchange process with an excited state population of about 1-2%. Investigation of the structural details of these conformational exchange processes is tackled by CPMG RD experiments on a variety of different nuclei. In addition, filtered FCS and eTCSPC suggest large-amplitude structural rearrangements of the N-terminal part of GABARAP occurring on the microsecond time-scale. The fact that dynamics are prominent over virtually all time-scales emphasize the importance of characterizing conformational changes for achieving a complete understanding of GABARAP.

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MONITORING AND QUANTIFYING DEFECT FORMATION OF A NON-IONIC SURFACTANT VIA DIFFUSION NMR

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Nuclear Magnetic Resonance (NMR) is well established as method for investigating diffusion. Applications are ubiquitous in porous media research and clinical studies. However, the majority of these studies still relies on the simple Stejskal-Tanner diffusion pulse sequence[1], which loses its unambiguity if the probed systems become more complex. For microscopically anisotropic systems a distribution of diffusion coefficients is observed, which depends on the orientation of the individual domains with respect to the diffusion encoding direction. Subsequently the introduction of double pulsed diffusion pulse sequences[2] allowed the detection of microscopic anisotropy. Despite unambiguous results for anisotropic systems, NMR-diffusometry was applied to characterize defects in non-ionic surfactants which exhibit multiple different (anisotropic) phases depending on the temperature and surfactant concentration.[3] While the dependence of the ^2H line splitting of ^2H_2O on the phase of the these systems has long been established, the influence of defects on the line splitting could only be presumed.[4] Attempts have been made to measure the temperature dependence of these defects for manually parallel aligned domain directors, but it is unlikely that perfect alignment and equilibrium conditions were achieved.[5] In 2013 the q-MAS pulse sequence (magic angle spinning of the q-Vector – in analogy to the solid state magic angle spinning) enabled isotropic weighting of the diffusion tensor and the distribution of diffusion coefficients for randomly aligned domain directors was established.[6] A further development of q-MAS is the in 2015 introduced TriPGSTE- (Triple Pulsed Gradient Stimulated Echo) pulse sequence that grants the quantification of the anisotropy parameter of the diffusion tensor, isotropic weighting, and enables the studies of systems with lower T_2^{eff}.[7-8] The TriPGSTE pulse

sequence is set up as a pseudo-4D experiment: While the first and second dimension are well known as the spectroscopic dimension and the magnitude of the diffusion encoding, the type of diffusion encoding is varied in the third dimension and the direction of the diffusion encoding is varied in the fourth dimension.

In this study the temperature dependent defect behaviour of the lamellar phase of the non-ionic surfactant $C_{14}E_5$ (Tetradecylpentaglycol) in $H_2O/^2H_2O$ was monitored and quantified. The presented measurements show a decrease of the diffusion coefficient orthogonal to the 2D lamellae with increasing temperature and concentration. This corresponds to a reduction of mesoscopic holes in the lamellae with increasing temperature/ surfactant concentration. With the TriPGSTE pulse sequence it is now possible to determine the diffusion tensor for a random orientation distribution of microscopically anisotropic domains. Therefore no manual reorientation of domains is needed anymore.

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SURFACE DIFFUSION OF IMIDAZOLE CATIONS AT SOLID/LIQUID INTERFACE IN GEL POLYMER ELECTROLYTE

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The object of the present study is a gel polymer electrolyte (GPE) based on ethoxylated bisphenol A dimethacrylate and 1-butyl-3-methylimidazolium tetrafluoroborate ionic liquid (IL), [BMIm][BF_4], prepared by in situ photopolymerization in a polymerization-induced phase separation process. The obtained material shows an exceptional behaviour, i.e., its ionic conductivity at room temperature (6.5 mS/cm) is almost twice higher than that of pure IL (3.76 mS/cm). To explain this effect the role of phase separation on the ionic diffusion at the polymer/IL interface forming on the border of the solid polymer phase and separated ionic liquid phase was examined. The self-diffusion coefficients of IL cations were determined by means of fast field-cycling (FFC) proton (^{1}H) nuclear magnetic resonance (NMR) relaxometry. The spin-lattice relaxation time of proton in cations were measured for neat IL and the IL involved in the polymer matrix in the temperature range from 248 to 343 K. The relaxation data obtained for bulk IL were analysed based on the force-free-hard-sphere (FFHS) diffusion model, which allows to determine the self-diffusion constants. A very important result was that IL confined to polymer matrix revealed a low-frequency dispersion (not observed for bulk liquid) which is the fingerprint of the ionic liquid cations/polymer matrix interactions. Therefore, the NMR relaxation data in GPE were analysed assuming the reorientation mediated by translational displacements (RMTD) mechanism. This dynamic process allows to explain a very long correlation time of the order of 10^{-5} s calculated for the cations at the polymer/IL interface and determine their diffusion coefficient. Finally, applying the NMR relaxometry method and proper theoretical models for the relaxation data (i.e., the FFHS diffusion model and RMTD mechanism) one can distinguish the translational dynamics of the IL

cations within the separated IL phase of GPE and of the cations interacting with the polymer matrix at the polymer/IL interface.

The obtained result is very interesting and important: in the phase separated GPE material the diffusion of the IL cations along the polymer/IL interface is characterized by significantly higher diffusion constants (by more than two orders of magnitude) than inside the bulk of the separated IL phase. In this context, we discussed the possibility of existence of an interphase, formed between the polymer phase and the IL separated phase, within which some new ion conducting pathways may be created in the nanometric scale enhancing the ionic conductivity effect in the GPE.

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HYDROGELS OF POLY(N-ISOPROPYLACRYLAMIDE)-POLYACRYLAMIDE THERMORESPONSIVE INTERPENETRATING NETWORKS

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Phase transition in hydrogels of interpenetrating polymer networks (IPNs) of thermoresponsive poly(N-isopropylacrylamide) (PNIPAm) and hydrophilic polyacrylamide (PAAm) was studied by a combination of NMR spectroscopy and differential scanning calorimetry (DSC). With increasing content of PAAm component in IPNs the fraction p_{max} of polymer units with significantly reduced mobility detected by NMR as well as the enthalpy change DH in DSC measurements are reduced and the transition is shifted towards higher temperatures. No phase transition was detected for IPN hydrogels containing at least 40 mol% of PAAm units. Reversed order of adding components during IPNs preparation also significantly affects parameters of the phase transition and collapsed structures. From ^{1}H NMR spectra it follows that in PNIPAm/PAAm hydrogels all groups of PNIPAm units participate in collapse with the same extent. On the other hand, for PAAm/PNIPAm hydrogel fraction of polymer units with reduced mobility as determined for main chain PNIPAm protons is smaller in comparison with isopropyl protons.

For all PNIPAm/PAAm and neat PNIPAm collapsed hydrogels certain amount of bound water remains in the globular structures above the phase transition. In most cases a slow exchange regime between bound and free water was revealed and for the residence time t of bound HDO it holds t >> 70 msec. Spin-spin relaxation times T_2 for bound HDO are up to one order of magnitude smaller in comparison with “free” HDO. The bound HDO appears already at temperatures when polymer segments just begin to form collapsed structures but on the hour scale the bound water slowly releases.

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EVIDENCE FOR DYNAMIC CROSS-LINKING IN SELF-HEALING MATERIALS BY MULTINUCLEAR PFG NMR SPECTROSCOPY

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Hybrid organic-inorganic elastomers have been elaborated from a common organic monomer, butyl acrylate, that was co-polymerized, under classical thermally initiated free radical polymerization, with a difunctional organotin oxo-cluster, $\{(BuSn)_{12}O_{14}(OH)_{16}\}(AMPS)_2$, used as cross-linker. The so-obtained elastomers exhibit remarkable self-healing properties: when cut, they can be mended by simply pressing the two pieces together. Raising the temperature accelerate the mending process.

The well-defined nano-size cross-linker was selected because of the electrostatic interactions that the macrocation $\{(BuSn)_{12}O_{14}(OH)_{16}\}^{2+}$ exchanges with its two polymerizable counter anions (AMPS : Acrylamido-2-methyl-1-propanesulfonate). It provides a cross-linking strong enough to yield rubber-like elasticity behaviour at RT but labile enough to enable bonds recombination after severe mechanical damages.

The preservation of the organotin oxo-clusters in the final material was first confirmed by ^{119}Sn NMR. Then the dynamic character of the cross-linking was studied by PFG NMR spectroscopy. The elastomer was first swollen with a solution of chloroform containing a small amount of free organotin macrocation associated with non-functional anions, p-toluenesulfonates (pTs). The diffusion coefficient of the organotin oxo-clusters, dispersed inside the swollen material, was then measured by ^{119}Sn PFG NMR. Similarly, the diffusion coefficient of pTs anions was determined by ^{1}H PFG NMR. The observed difference between these two diffusion coefficients was explained by an exchange process, which involves the cross-linking nodes and is at the origin of the self-healing properties.

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VELOCITY, POROSITY AND TRANSPORT OF NANOPARTICLE MEASUREMENTS IN ROCKS USING MRI

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Nanoparticles have a wide range of applications such as cosmetics, textiles, paints and drug delivery. Once nanoparticles are released in to ground waters they might have toxic effects. To protect groundwater from this new threat we must be able to predict nanoparticle movement within the aquifer. Critically, however, we are at present unable to do this due to significant limitations in current experimental datasets which lack spatial resolution. To overcome this fundamental knowledge gap, we have used MRI to collect spatially resolved data on NP concentration [1] and three dimensional (3D) velocity in a sandstone core [2]. From such data we will seek to develop more robust NP transport models.

An epoxy encapsulated Bentheimer sandstone sample was used to make all measurements. The porosity was determined from the weight of the column before and after saturation of water and used to calibrate MRI porosity maps . The MR imaging experiments were carried out on a 7T Bruker Avance BioSpec system. The rf volume resonator used for all experiments has a inner diameter 72 mm. A series of T_2-weighted images were recorded for the transport of Carboxyl NP in sandstone with a time interval of 8 min. The pulse sequence used in this work was a combination of an Alternating-Pulsed-Gradient Stimulated-Echo (APGSTE) sequence with a RARE imaging module. The 3D velocity images at two different flow rate 1 and 2 ml/min were acquired with a repetition time T_R of 5000 ms and measuring time of 16 hours and 32 s. The resulting images have a matrix of 60 × 45 × 45, a field of view (FoV) of 60 × 45 × 45 mm^3, giving a pixel size of 1 mm^3.

Our results shows that MRI is able to image the transport of paramagnetically tagged NPs, inside the Bentheimer sandstone by

using T_2- weighted images. In addition the APGSTE-RARE pulse sequence proved to be a powerful tool with which to investigate 3D velocity map in rock core.

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INTERACTIONS BETWEEN INCLUSIONS MEDIATED BY LIPID MEMBRANES

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We aimed to probe the interaction induced by lipid membranes between included molecules (and other nano-objects). This study presents both a fundamental interest, from the point of view of soft matter physics, and a relevance to understanding the activity of important biological molecules, such as native membrane proteins and antimicrobial peptides. In particular, the cytotoxic activity of the latter is directly related to the composition of the membrane (charge, thickness, presence of cholesterol) rather than to specific chemical recognition. It has long been posited that highly simplified descriptions (e.g. in terms of 'hydrophobic matching') capture the essence of the process, but the extensive body of theoretical and numerical work cannot be validated and refined due to the lack of experimental results. We intend to observe and propose a model for interactions between different matrices and oxo-cluster inclusions.

Ideally we aim to study interactions between highly aligned lipid multilayers but some other matrices such as liquid crystal are also considered. One of the main limitations encountered in such systems is the oxo-clusters is present only in very small concentrations with respect to the matrix they are coated in making there mobility behavior (relaxation, diffusion) challenging. The tin and gold nanoparticles will be used as 'membrane probes' within the bilayer, in order to extend the investigation to a wider range of parameters (especially to lower concentrations of inclusions). From the results of this investigation combining e.g ^{1}H, ^{119}Sn, ^{13}C PFG liquid and solid-state NMR will emerge a clearer picture of the lipid membrane as a complex two-dimensional fluid (diffusion) and of the way it influences the interaction between included objects, in particular membrane proteins.

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NMR INVESTIGATION OF THE WATER TRANSPORT THROUGH THE CELL MEMBRANE IN SELECTED SPECIES OF YEASTS

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Exchange rates of the water transport through the plasma membrane was investigated for following species of yeasts: Sacharomyces cerevisiae 288c, Sacharomyces pombe, Candida albicans, and Zygosacharomyces rouxii. The temperature dependence of the exchange rates was investigated for Sacharomyces cerevisiae 288c between 5 and 35 °C. Filter-exchange PGSE NMR sequence [1] was used to determine the effective exchange rates, apparent diffusion coefficients of extra- and intracellular water, extra- and intracellular water fractional population at equilibrium in the yeast suspension, intracellular lifetime and membrane permeability of the yeast. The exchange rates are found species-specific, fastest for Candida albicans (11.8 s^{-1}), slowest for Sacharomyces cerevisiae 288c (3.9 s^{-1}).

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NMR DIFFUSION AND RELAXATION STUDIES OF WATER AND METHANOL IN MATERIALS RELEVANT FOR ADSORPTIVE HEAT TRANSFORMATION

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For adsorptive heat transformation applications the heat of adsorption of a fluid in a nanoporous host (adsorbent) is utilised for heating, cooling or energy storage[1]. In these applications the transport rate of the molecules is one of the critical parameters for the efficiency of the respective process. Furthermore, a high heat of adsorption and, therefore, a strong host-guest interaction is favourable in order to achieve high energy densities. Here we present the results of pulsed field gradient (PFG) nuclear magnetic resonance (NMR) studies on the dynamics of water and methanol in materials which are promising candidates for adsorptive heat transformations, namely SAPO-34, aluminium fumarate and MIL-100(Al). This work is done within the frame of a collaborative research project, where we aim towards the exploration of the fundamental molecular transport mechanisms and a comparison of these materials[3].

The measurements were performed with home-build FEGRIS NMR spectrometers[4,5] operating at ^{1}H resonance frequencies of 125 MHz and 400 MHz. ^{1}H NMR relaxation studies yield short relaxation times, indicating strong interactions between the guest molecules and the respective host framework. The longitudinal relaxation times of the guest molecules are in a range from 20 ms to 100 ms, while the transverse relaxation times do not exceed 1 ms. Therefore, the diffusion measurements were performed with ultra-high intensity pulsed magnetic field gradients and the stimulated echo sequence.

The diffusion coefficient of water in the silicon aluminophosphate SAPO-34 is around 4E-11 m^2s^{-1} at room temperature and increases to 9E-11 m^2s^{-1} at 85°C. From the diffusion data the activation energy of the transport process of (11 ± 2) kJ mol^{-1} was calculated. Methanol diffuses slower (D=1E-12 m^2s^{-1} at room temperature) and has a higher activation energy of (29 ± 4) kJ mol^{-1}.

In the microporous metal-organic framework MIL-100(Al) water diffuses much faster. Its diffusion coefficient is about 1E-9 m^2s^{-1} at room temperature and decreases slightly with decreasing loading.

The metal-organic framework aluminium fumarate was investigated as a bed of small crystals and as a dense coating as used in adsorption heat exchangers. In both cases the self-diffusion coefficient is in the order of 4E-10 m^2s^{-1}, which means that the morphology of the material is not the main limiting factor of the water transport. However, we assume that the value of 4E-10 m^2s^{-1} represents the long-range self-diffusion coefficient and not the intracrystalline.

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TRIPLE RESONANCE NMR RELAXATION EXPERIMENTS FOR STUDIES OF INTRINSICALLY DISORDERED PROTEINS

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Intrinsically disordered proteins (IDP's) do form a significant portion of proteome and the crucial need for understanding their biological functions based on detailed description of various physico-chemical properties is widely recognized. The traditional paradigm of direct interconnection between protein structure and it's function has to be revisited for these systems. It has been clearly demonstrated by a number of experimental techniques, that IDP's under native condition do not have a unique, well defined conformation, which could be reliably represented in terms of single (average) 3D structure. Instead, we face a highly heterogeneous system existing in a multitude of conformations which are interconverting on a broad range of timescales forming a huge spatiotemporal conformational space. Development of NMR methods for spectral assignment and structural characterization of IDP's has been rapidly progressing during past decade,however the physical background of their high conformational heterogeneity lies in local flexibility. The residue--specific information about dynamics is a key in understanding the fundamentals of IDP's design. A direct characteristics of local motions of protein backbone on ps-ns timescale is obtained from 15N relaxation rates. Relaxation studies of structured proteins are nowadays well established part of the NMR spectroscopy of ordered proteins.

Here we present a suite of triple resonance HNCO-type NMR experiments for measurements of five relaxation rates (R1, R2, NOE, transversal and longitudinal cross-correlated relaxation rates) in doubly 13C/15N labelled IDP's. These experiments provide a solution to the aforementioned complications arising in NMR dynamics studies

of IDP's. The first step, which makes use of favorable relaxation properties of IDP's is utilization of higher dimensionality (3D) experiments, however traditional sampling methodology would lead to impractically long experiments.

The second step is then utilization of non-uniform sampling (NUS), where we benefit from two aspects: i. we can use long evolution periods in order to maintain or even significantly increase spectral resolution, ii. we keep the experimental time in reasonable range (2-3 times longer) as only a fraction of hypercomplex points needs to be acquired.

The application of these experiments is demonstrated using partially disordered RNAP delta-subunit, a very challenging protein from NMR spectroscopic point of view. The C--terminal 91aa long domain is highly acidic and repetitive -- in addition to 25 aspartic and 23 glutamic acid residues it contains a positive stretch of 8 lysines.

A comparison with 15N relaxation data obtained previously by standard methods will be provided.

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TOWARDS ENDOR-DNP: W BAND ENDOR STUDIES OF N@C60

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For physical reasons the transfer of the orders of magnitude higher electron spin polarization by ordinary DNP work best at low magnetic fields which is a substantial drawback of this approach. It is known theoretically that electron nuclear double resonance (ENDOR) DNP should be able to overcome this disadvantage [1]. ENDOR-DNP enhanced EPR spectra of $^{15}N@C_{60}$ have already been generated showing that the nuclear spin polarisation can be increased by a factor of ×1100 [2].

We aim to transfer the ENDOR-DNP approach to liquid-state NMR which would be an enormous breakthrough in DNP research as it will further boost DNP signal enhancement at high magnetic fields.

An interesting system to study the potential of ENDOR-DNP is $N@C_{60}$ as it provides advantageous electron spin properties (sharp and resolved EPR lines, high symmetry, large electron spin $S = {}^3/_2$, long T_{1e}). We report pulsed ENDOR measurements in a 94 GHz EPR spectrometer at a range of temperatures. The materials we investigate are unenriched as well as ^{15}N and ^{13}C enriched $N@C_{60}$ as a solid and in solution.

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INVESTIGATION OF 0Q-TO-MQ EXCITATION IN SINGLE CRYSTALS LED TO NEW SFAM SCHEME PROVIDING EFFICIENT MQ EXCITATION IN MQMAS EXPERIMENTS

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The main idea behind MQMAS is to obtain indirectly detected spectra from multiple quantum evolution, since these undetectable symmetric transitions contain signals less broadened from anisotropic second order quadrupolar coupling than the 1Q Central Transition. The basic MQMAS sequence consists of two hard pulses, one excites the equilibrium population to multiple quantum coherence, and the other converts back to detectable coherence after some evolution time t1 [1].

Unfortunately the MQ excitation and conversion processes are very inefficient due to the nonlinear nature of these MQ processes. MQ conversion efficiency can significantly be enhanced with DFS (Double Frequency Sweep) or FAM (Fast Amplitude Modulation) type pulses instead of rectangular pulse irradiation [2]. Although several efforts have been taken to improve MQ excitation, still no generally applicable scheme exists.

First, the effect of RF irradiation on single crystals experiencing a quadrupolar coupling was investigated. Invoking density matrix formalism and numerical simulations it was shown how MQ probability densities can be created in single crystals from equilibrium state involving technically available amplitude modulated pulses. This resulted in a pulse scheme that can perform a 3Q filtered coherence pathway with triple the efficiency compared to 1D CT spectra of spin-3/2 nuclei. The excitation/conversion scheme was tested experimentally on a Bruker AVANCE III. 500 MHz spectrometer, and the results agreed with the expectations.

Combining this excitation scheme with the FAM technique yielded to a method that can be used to enhance the MQ excitation of powders

as well. This method involves a cosine type amplitude modulation of the RF pulse shifted with a constant factor, hence the name SFAM (Shifted FAM). Numerical simulations were performed with SIMPSON to compare whole MQMAS experiments, including the SFAM enhanced scheme. Finally SFAM was tested experimentally on an $^{87}RbNO_3$ powder sample, under MAS conditions. A sensitivity enhancement of 1.6 was observed compared to the rectangular pulse excitation at the maximal available RF power (300 W).

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RATIONAL DESIGN OF NITROXIDE BI-RADICALS FOR EFFICIENT CROSS-EFFECT DYNAMIC NUCLEAR POLARIZATION

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Dynamic nuclear polarization (DNP)[1-3] currently attracts considerable attention as one of the most efficient methods to increase the sensitivity of NMR experiments. In particular, it can boost the sensitivity of magic-angle spinning (MAS) solid-state NMR experiments at low temperatures by several orders of magnitude.[4] Typically, the samples are dissolved or impregnated with a solution of stable bi-radicals, acting as polarizing agents capable of transferring the electron hyperpolarization through the cross-effect. In this study, a series of 37 bi-nitroxide radicals has been prepared and their performance studied as polarizing agents in cross effect DNP NMR experiments at 9.4 T and 100 K. We observe that in this regime the DNP performance is strongly correlated with the electron and nuclear spin relaxation times, with longer relaxation times leading to better enhancements. We also observe that deuteration of the radicals generally leads to better DNP performance. One of the new radicals introduced here provides the best performance obtained so far under these conditions. In similar conditions we show that over a two-fold improvement in DNP enhancements can achieved be simply incorporating solid particles into the sample. Enhancements up to ε_H = 515 are obtained in this way, corresponding to 78% of the theoretical maximum.[5]

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P 365
DEVELOPMENT OF IMMOBILIZED SABRE CATALYSTS AND THE HYPERPOLARIZATION LOSS CAUSED BY DIFFERENT SUPPORT MATERIALS

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The SABRE (signal amplification by reversible exchange) approach is an efficient hyperpolarization method enhancing NMR signals by repeatedly transferring polarization from para-hydrogen to target molecules using homogeneous Iridium complexes.[1]Unfortunately, these complexes are expensive, usually inseparable from the reaction mixture for reuse and potentially disadvantageous for biological applications. Therefore immobilizing these well-known homogeneous catalysts seems to be a straight forward approach for new catalytic systems.[2,3]

The aim of this project is to obtain a defined heterogeneous catalyst, which is still able to continuously transfer polarization from para-hydrogen molecules to a target substrate without alteration of its chemical structure. Investigating the hyperpolarization loss caused by different supporting materials, these were added stepwise to the known homogeneous system. Furthermore immobilized Iridium catalysts are synthesized by adding Iridium precursors such as [Ir(COD)(IMes)]Cl under hydrogen pressure to a polymer-bound phosphine.[4]

These catalysts and their homogeneous analogues are compared regarding the efficiency of their polarization transfer to pyridine in a low-field NMR spectrometer (42.5 MHz SpinSolve by Magritek Ltd.) in high pressure NMR-tubes at 6 bar. The promising results shown in this study enable the combination of an immobilized SABRE catalyst with a microfluidic system. Current research focuses on the design of a continuous flow hyperpolarization set-up with separated chambers for the polarization transfer and the acquisition.

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P 368
PULSED DYNAMIC NUCLEAR POLARIZATION WITH TRITYL IN GLYCEROL/WATER FROZEN SOLUTION AT 0.34 T

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Most dynamic nuclear polarization (DNP) mechanisms thatinvolve continuous microwave irradiation become considerably less efficient athigh magnetic fields. Certain forms of pulsed DNP, however, do not have aninherent drop in efficiency and could potentially provide high enhancements athigh fields.

A promising form of pulsed DNP is Nuclear Spin Orientationvia Electron Spin Locking (NOVEL). [1,2] In NOVEL, polarization is transferred fromelectrons to nuclei coherently via an alternative Hartmann-Hahn matchingcondition: $\gamma_e B_1 = \gamma_n B_0$,where $\gamma_e B_1$is the electron nutation frequency and $\gamma_n B_0$is the nuclear Larmor frequency.

Due to the high microwave power required forthe NOVEL matching condition, application of NOVEL for sensitivity enhancement in magic-angle spinning (MAS) NMR at high fields is technically challenging. In addition, up to now NOVEL has only been demonstrated in specializedsingle crystal systems. [1-4] A first step in the direction of generalapplicability in MAS NMR would therefore be to show polarization transfer byNOVEL using a readily available and stable polarizing agent in a generalsolvent matrix.

We report NOVEL DNP with the narrow-line radical trityl inwater/glycerol at 80 K at 0.34 T (15 MHz or 9.5 GHz). Polarization transfer wasfound to be highly efficient with an enhancement as high as ~330 and a build-uptime of only 2 s at a trityl concentration of 10 mM.

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P 371

A BENZYL ALCOHOL DERIVATIVE OF BDPA RADICAL FOR FAST DISSOLUTION DYNAMIC NUCLEAR POLARIZATION NMR SPECTROSCOPY

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The synthesis, structural characterization and the successful application of a carbon centered radical derived from 1,3-bisdiphenylene-2-phenylallyl **[1]** (BDPA), its benzyl alcohol derivative (BA-BDPA), as a polarizing agent for fast dissolution Dynamic Nuclear Polarization **[2]** (DNP) are described. The reported BA-BDPA **[3]** radical meets all the requirements to become a promising candidate for its use in in-vivo DNP-NMR experiments: it is soluble into neat [1-^{13}C]pyruvic acid, insoluble in the dissolution transfer solvent and effective as a polarizing agent in fast dissolution DNP-NMR applications, without the need of using glassing agents. Moreover, it enables a simple but effective in-line radical filtration to obtain hyperpolarized solutions of [1-^{13}C]pyruvic acid free of radicals, that offer a much better polarization performance.

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P 374
OPTIMISING SABRE FOR MRI APPLICATIONS

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The use of Signal Amplification By Reversible Exchange (SABRE) [1] as a method for increasing the sensitivity and speed of NMR and MRI experiments has rapidly gained momentum in the past few years, due to the technique's versatility, applicability to a wide range of biocompatible molecules and low cost. These advantages, together with the fact that the method does not require any chemical modification of the substrate, can make SABRE hyperpolarised molecules highly efficient contrast agents for in vivo MRI investigations.

However, the use of hyperpolarised substrates for in vivo studies faces many challenges, from both the clinical and the technical points of view. The substrate needs to be biocompatible, soluble in a non-toxic solvent and efficient in the limited amounts approved for i.v. administration. ^{1}H MRI experiments have the additional issue of low contrast due to the strong background and relatively short lifetime of the hyperpolarised signal.

In this work we present the optimisation of the complex formed with [IrCl(COD)(IMes)] (IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene) [2] in the presence of para-H_2 and 5-methylpyrimidine (a well-known building block of several nucleobases and nucleosides) in terms of the hyperpolarised signal's amplitude and lifetime. ^{1}H, ^{13}C and ^{15}N NMR data acquired on the activated complex dissolved in methanol, ethanol and water/ethanol mixtures in various conditions are reported. The results show that by varying the concentration of the complex in solution, the substrate to catalyst ratio and by changing the physical conditions in which the polarisation transfer takes place (such as temperature, pressure and magnetic field) significant improvements in signal enhancement, resolution and

contrast can be obtained using relatively small amounts of catalyst and substrate.

Furthermore, we show that, by using the optimised complex and conditions, we can obtain ^{1}H MRI images of phantoms with a resolution of the order of tens of microns, as well as images of biological media (exemplified by a series of lung images obtained ex vivo).

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P 377
THE BROCODE OF NMR: BROADBAND COOPERATIVE DECOUPLING

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In NMR spectroscopy, heteronuclear decoupling sequences are a key element in every correlation experiment where resolution or spectral dispersion and sensitivity are of higher importance than the information provided by resonance lines that are split due to heteronuclear couplings between spins. Pulse sequences are needed which provide high signal intensity paired with low artifact levels for a wide range of resonance offsets. Up to recently it was best-practice to pursue these goals in three steps:

1) Find a robust inversion pulse
2) Expand this inversion pulse by phase cycling
3) Dynamically alter the mode of execution (e.g. the timing) of the sequence in order to achieve cancellation of artifacts.

Maybe the best standard implementation addressing artifacts originating from the three-step approach is adiabatic bilevel decoupling [1]. It relies on adiabatic frequency sweeps as inversion elements and a temporal variation at the beginning of the sequence for each of the successive scans of an NMR experiment that ultimately cancels the most spurious artifacts that are introduced by the repetitive sweeping scheme.

Recently, methods based on Optimal Control Theory [2] have been introduced that tackle all of the three above-mentioned tasks simultaneously. By combining the Optimal Tracking algorithm [3] with multi-scan cooperativity [4], it is possible to derive a complete set of decoupling sequences that compensate each other's imperfections

de novo. In this work, we compare the results of the Optimal Control approach with the adiabatic bilevel technique in cases where the radio frequency power levels are high enough as well as too low to fulfill the adiabatic condition.

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Poster Session 3

P 003
STRUCTURAL STUDIES ON SCRAPIE SEEDED OVINE PRION PROTEIN AMYLOIDS BY HIGH RESOLUTION SOLID-STATE NMR

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Neurodegenerative diseases such as transmissible spongiform encephalopathies (TSEs) are not always due to inheritance, but can also be caused by proteinaceous infectious particles (prions). The aggregation of the prion protein (PrP) leads to accumulation of β-sheet-rich amyloid fibrils resulting in fatal neurodegenerative diseases like Creutzfeldt-Jakob disease (CJD) in human, scrapie in sheep and goats, or bovine spongiform encephalopathy (BSE) in cattle. A detailed structure of prions is yet unknown. Due to the fact that PrP fibrils are insoluble and non-crystallizable, they are not amenable to X-ray crystallography and liquid-state NMR. Based on low resolution techniques, however, different structural models have been proposed[1,2]. Furthermore, a first high resolution solid-state NMR study could already show the location and arrangement of the β-sheet core[3]. However, even solid-state NMR spectroscopy is limited by sample preparation due to the polymorphic character of de novo generated amyloids. Recently, we could show that seeding with brain derived prion amyloids may improve the homogeneity, and thus the resolution in solid-state NMR spectroscopy, of in vitro generated prion amyloids[4]. Also, previous solid-state NMR studies for β-amyloid fibrils showed, that with a repeated seeding protocol it is possible to selectively amplify single structures from an initial polymorphic state[5].

In our contribution, we will report on our experimental progress in characterization of seeded amyloids of full-length ovine recombinant

(ovrec) PrP by high resolution solid-state NMR. At first, we will show our new results investigating the structure of a first seeded amyloid prion generation, which will include the identification of secondary structure elements in the amyloid core region based on partial preliminary sequential assignments. Additionally, we will present our results on a following generation derived from the former parental generation, which also includes the identification of secondary structure elements by partial preliminary sequential assignments. These findings are supported by results obtained for amyloids prepared with different isotopic labeling schemes. Subsequently, we will state on the comparison of these two amyloid generations based on the high resolution results of the secondary structure elements.

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P 006
SPIN LABEL CONFORMATIONS ON A TRANSMEMBRANE PEPTIDE STUDIED BY PULSE EPR AND MOLECULAR MODELLING

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Spin labels are used very extensively in studying structure and function of various kinds of biomacromolecules ranging from small proteins or peptides in solution to large proteins in bilayers or protein complexes. Introduction of spin labels at desired positions on a protein allows to specifically address particular protein regions or to follow its functional rearrangements. Such studies nowadays are often facilitated by methods of pulse dipolar spectroscopy.

Accounting for spin label flexibility during such studies is important because spin label size is not insignificantly small comapred to experimentally measured distances. The rotamer library approach proved to be a simple and effective method to model conformational freedom of spin labels with satisfactory precision. Here we show, how by using a large experimental data set collected on water -soluble protein, properties of various rotamer libraries as well as the parameters of the effective potential function governing spin label /protein interaction can be optimized. Until now, no distinction within the rotamer library approach has been made between soluble and membrane proteins. In order to be able to address the latter more specifically, we attempted to create a corresponding rotamer library using very extensive MD simulations and molecular modelling. The spin labelled hydrophobic peptide WALP23 was used as a model system in order to collect experimental distance information necessary for calibration of the rotamer library. Experimental data were collected with the DEER experiment at Q-band frequencies. The new MD-based library is compared to libraries based on simpler approaches and

advanatges as well as limitations of the new approach are pointed out.

P 009

DETERMINATION OF THREE-DIMENSIONAL STRUCTURE OF M-PMV CAPSID PROTEIN C-TERMINAL DOMAIN

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Mason-Pfizer Monkey virus (M-PMV) is a betaretrovirus and one of the important model organisms for the study of retroviral life-cycle. The assembly of immature virus particles is driven by multimerization of the main structural polyprotein Gag. Gag is composed of several domains that are cleaved to single proteins during virus maturation. From these proteins only matrix protein, capsid protein (CA) and nucleocapsid proteins are conserved among all retroviruses. CA is the largest one and after the maturation of the virus it forms the viral core. It is consists of two structurally independent domains: N-terminal domain (NTD) and C-terminal domain (CTD). NTD has an arrow shaped structure, it is essential for the formation of immature and mature particles and its structure has already been determined[1]. CTD is stabilizing the hexamers in the immature and mature particles, but its structure has not yet been determined (only homology-based models are available). There has been an attempt to determine the structure using X-ray crystallography resulting in the structure of a domain-swapped dimer which turned out to be an artificial result. Therefore, to confirm or rule out whether this structure exists also in solution, we decided to determine its structure by NMR spectroscopy.

We prepared uniformly ^{13}C- and ^{15}N-labeled protein. Chemical shifts of protein atoms were assigned using the standard set of three-dimensional NMR experiments. For comparison we also tried to use APSY (automatic projection spectroscopy) for the assignment of backbone atoms together with the automatic assignment implemented in UNIO 10, which finally yielded over 70 % correctly assigned backbone resonances. The structure was then calculated using Xplor-NIH software. The structure has proven that in solution the protein exists exclusively in the monomeric state without any signs of

domain-swapped dimer. The solved structure is highly similar with structures of other retroviral CA-CTDs and can be successfully fitted into electron density maps of immature virus particles obtained by cryo EM[2].. These maps show that some CA molecules are in close proximity of viral RNA, therefore we wanted to test whether this is caused by RNA – CA interaction, or these molecules are close to each other just due to the conformation of RNA. The interaction was observed by monitoring of backbone NH groups chemical shift changes and we found out, that CTD does not interact with RNA.

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P 012
THE FOCAL ADHESION ADAPTOR PROTEIN PAXILLIN – NMR-SPECTROSCOPIC INVESTIGATION OF 3D-STRUCTURE, INTERACTION AND REGULATION

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Paxillin is an essential multi-domain adaptor protein present in early focal adhesions where it plays a key role in initiating and regulating processes during cell migration. This fundamental function includes the recruitment of various regulatory and structural proteins as well as integrin-mediated signaling. Therefore, paxillin is implicated in embryonic development but also in cancer metastasis and tissue remodeling. To date numerous binding partners of paxillin have been described, however it still remains elusive how this multifaceted protein itself is recruited to focal adhesions.

We focused our NMR studies on the LIM2/3 tandem domains since it was shown that the LIM3 domain is critical and LIM2 domain supporting for focal adhesion targeting[1].

We solved the three-dimensional structure of the LIM2/3 construct by multidimensional NMR spectroscopy which is, to our knowledge, the first example of a structure of a free LIM tandem domain.

Using chemical shift perturbation mapping we identified and characterized the interaction with the cytoplasmic domain of β integrins providing a possible explanation of focal adhesion targeting of paxillin.

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P 015
STRUCTURAL CHARACTERIZATION OF THE rS1-PROTEIN AND ITS mRNA COMPLEXES

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In gram-negative organisms the rS1-protein plays a key role in the formation of the translation initiation complex [1]. As part of the translational machinery it recognizes mRNAs even with weak Shine-Dalgarno sequences and hence facilitates their interaction with the 30S subunit of the ribosome. The rS1-protein is a modularly organized protein composed of six imperfect OB-fold repeats, with varying sequence homology [2]. They provide the rS1-protein with two functionally specialized regions, enabling interactions with proteins and RNAs. Previous studies revealed that the RNA-binding region is located within the four C-terminal domains, whereas the two N-terminal domains are involved in ribosome binding [3]. Recently solution NMR structures of individual domains were solved [4], [5]. However, to date, there is no structure available regarding the RNA-binding region.

Our research is focussed on the RNA binding region of the rS1-protein from the organsim Vibrio vulnificus. We aim to structurally characterize the rS1 RNA binding region and to investigate its interaction with the translation initiation region (TIR) of mRNAs using solution NMR. Here, we use the 5´-UTR and TIR of an mRNA from the same organism, comprising an adenine dependent riboswitch (ASW) as substrate [6].

The rS1-protein alone has a molecular weight of 61 kDa, rendering the structural analysis of the protein and its mRNA complexes challenging by NMR-spectroscopy. Therefore, in accordance to Bisaglia et al. [7] we designed five multidomain constructs, by stepwise truncating the RNA binding region from the N- and C-terminal ends of the protein. Our electrophoretic mobility shift assays indicate that constructs containing at least the third and fourth domains are capable of RNA

binding. On the basis of triple resonance experiments we were able to assign 60% of the backbone resonances of the smallest RNA binding construct, rS134 composed of the residues V180 to Q361. In addition, ^{1}H, ^{15}N-BEST-TROSY based titration experiments revealed the formation of a rS134-ASW complex. The timescale for this interaction appears to be within the intermediate to fast exchange time regime. This finding indicates dynamic interaction between protein and RNA, characterized by relative high k_{off}-values and does not contradict previous studies [3]. As a mediator of translation initiation, the rS1-protein has to bind and prepare the mRNA for the interaction with the ribosome. Subsequently it has to dissociate from the mRNA, to enable the formation of the translation initiation complex.

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P 018

TOWARD A SOLUTION STRUCTURE OF PSBP, AN EXTRINSIC PROTEIN OF HIGHER PLANT PHOTOSYSTEM II

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PsbP (23 kDa) is an extrinsic protein from Photosystem II (PS II), which has been found to be indispensable for maintaining stable oxygen production during photosynthesis. PsbP is located on the outer part of the thylakoid membrane together with other extrinsic proteins PsbO, PsbQ, and PsbR as outer part of the oxygen evolving center. Cooperation between PsbP and other accessory proteins ensures stable Ca^{2+} and Cl^- concentrations, cofactors of the water splitting reaction. Upon binding PsbP causes structural changes in the thylakoid membrane, which are essential for the proper function of PS II. [1] The exact nature of interplay between the extrinsic proteins has yet to be elucidated. The recently resolved crystallographic structure of PsbP has brought valuable structural information, but the functionally most important parts – the N-terminus and inner dynamic loops – are not resolved. [2] We are using solution NMR to pin down those regions and to determine the exact loci of interactions between the extrinsic PS II proteins from higher plants.

PsbP from Spinacia oleracea was prepared as a His_6-tagged protein from E. coli with uniform ^{13}C and ^{15}N labeling and subjected to a standard set of 2D and 3D NMR experiments in order to obtain resonance assignment. In total 87% of backbone and side chain

resonances have been assigned and an initial 3D solution structure has been derived. [3] To address the most important question of protein-protein interactions titration experiments including PsbP and PsbO as well as PsbQ have been carried out. Chemical shift and relaxation time changes reveal the interaction sites.

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P 021

SOLUTION NMR REVEALS A SHORT HELIX WITHIN THE DISORDERED N-TERMINAL REGION OF PSBQ FROM HIGHER PLANT PHOTOSYSTEM II

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The extrinsic proteins PsbO, PsbP, PsbQ and PsbR are essential in order to sustain oxygen production in the oxygen evolving center of photosystem II in green algae and higher plants. [1] In the most up-to-date X-ray crystallographic structure of higher plant PsbQ residues S14-Y33 are missing. [2] Based on the backbone NMR assignment of PsbQ, which includes this "missing link" [3], we report extended resonance assignment including side chain atoms. Building on 3D nuclear Overhauser effect spectra a high resolution structure of PsbQ with a backbone RMSD of 0.81 Å was derived using torsion angle dynamics CYANA. [4] While the four-helix bundle core found in the crystal structure is present in near identical conformation in the solution structure, the flexible N-terminal tail shows significant differences. A short α-helix occurs at the location where one strand of a short two-stranded β-strand had been proposed in the crystallographic study. R1, R2 and NOE data as well as unrestrained MD confirm that this single turn α-helix is a persistent secondary structural motif embedded between two intrinsically disordered regions.

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P 024

STRUCTURAL AND DYNAMIC STUDY OF THE RESPONSE REGULATORS CHEY3 AND CHEY6 FROM THE RHODOBACTER SPHAEROIDES CHEMOTAXIS NETWORK

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The chemotaxis signalling network of E. coli. depends on autophosphorylation of a histidine protein kinase (HPK) in response to a signal from a sensor domain, with subsequent transfer of the phosphoryl group to an aspartate on response regulator (RR) proteins that bind to the flagellar motor and alter its rotation. CheY is a 14kDa single domain RR that is conserved across motile species. It is formed by 5 alpha-helices and 5 beta-strands surrounding a conserved phosphoryl accepting aspartate residue, and once phosphorylated diffuses to the flagellar motor, binding to its FliM component to cause switching of rotational direction. The photosynthetic bacterium Rhodobacter sphaeroides has multiple chemosensory pathways formed by homologues of the E. coli chemosensory proteins. It has six homologues of the response regulator CheY with different effects on chemotaxis. Only CheY6 is able to stop the flagellar motor but either CheY3 or CheY4 is also required for chemotaxis.

In this work we have used solution-state NMR and computational methods to answer questions about the structure, dynamics and function of two of the CheY's, CheY3 and CheY6. We have used NOEs, chemical shifts and residual dipolar couplings to define the structures of CheY3 and CheY6 in solution in their inactive and active states, where phosphorylation is mimicked using BeF3-. We have investigated fast timescale backbone dynamics using the {1H}-15N heteronuclear NOE and have used CPMG relaxation dispersion experiments to detect low populations of alternative conformations. CheY6 differs from the other R. sphaeroides CheYs and E. coli CheY by the insertion of a ten-residue loop after beta-strand 5 and before the C-terminal helix. We have deleted this loop region from CheY6 in order

to determine, using in vivo and in vitro assays, if it plays a role in the unique function of CheY6 in R. sphaeroides.

P 027

LIR-DEPENDENT INTERACTIONS OF THE HUMAN ATG8 PROTEINS TO THEIR PARTNERS PROVIDE A PLATFORM FOR REGULATION OF CELLULAR SIGNALLING PATHWAYS

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Human Atg8 proteins (LC3s and GABARAPs) are small ubiquitin-like proteins involved in variety of regulatory processes in cells. Similar to ubiquitin (Ub), they undergo enzymatic conjugation to a lipid and in this form are allocated to membranes of different origin. They bind to their interaction partners - proteins - via a short motif called LIR (LC3 Interaction Region), and thus attract these proteins to the membrane. This function of the human Atg8 proteins is mainly understood in selective autophagy, where the interaction partners of Atg8 proteins - autophagy receptors - additionally are bound to the autophagic cargo via interactions to the cargo-conjugated Ub-chains. The core of LIR motif conforms to the formula Θ-X-X-Γ, where Θ is an aromatic amino acid (W/F/Y), Γ is hydrophobic (L/I/V), and X can be any amino acid – the so-called 'canonical LIR'. Additionally, acidic (or phosphorylated) groups immediately upstream of the core LIR contribute a negative charge to reinforce the interaction.

In this work, we have investigated interactions of the several atypical LIR motifs, both from real proteins as well as synthetic peptides, to the human Atg8 proteins and focused on specificity of these interactions.

We have shown recently that the two Kelch-domain containing proteins KBTBD6 and KBTBD7 are associated with the ubiquitin ligase scaffold protein CUL3, as well as with GABARAP proteins. We

discovered that KBTBD6 and KBTBD7 both contain unusual W-type LIR motif that mediate binding preferentially to GABARAP over LC3 proteins in vitro and in cells. Using ITC and NMR titration experiments, we showed that this preference is not explained in a difference in $K_D$values but rather through different driving forces of the interaction, as well as through differences in the occupancy of the two hydrophobic pockets on Atg8-proteins surface. By solving the structure of the complex between GABARAP and the LIR motif of KBTBD6, we identified a unique salt bridge that is preserved among GABARAP but not LC3 proteins. KBTBD6/7 are the first non-autophagic proteins that show a strong preference for the GABARAP subfamily and we provide compelling evidence that GABARAP proteins exhibit functions beyond autophagy via recruitment of $CUL3^{KBTBD6/7}$ to membranes to spatially regulate TIAM1 RAC1 signalling.

This work was funded by the German Cancer Consortium (DKTK), the Cluster of Excellence Frankfurt (Macromolecular Complexes), the Deutsche Forschungsgemeinschaft (BE 4685/1-1), the European Research Council (282333) and the LOEWE program of the State of Hesse.

P 030
EXPLORING RNA POLYMERASE REGULATION BY NMR SPECTROSCOPY

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RNA synthesis is a central process in all organisms, with RNA polymerase (RNAP) as key enzyme. All cellular genomes are transcribed by multisubunit, evolutionary related RNAPs that are tightly regulated by a multitude of transcription factors. Although RNAP of Escherichia coli (E. coli) has been studied extensively, only little information is available about its dynamics and transient interactions, which, however, is crucial for the complete understanding of transcription regulation in atomic detail.

Here we present initial approaches how E.coli RNAP (~390 kDa) can be studied using nuclear magnetic resonance (NMR) spectroscopy to gain insights into RNAP's dynamic behavior and its interaction with transcription factors. We developed a highly efficient procedure for the assembly of active RNAP from separately expressed subunits that allows specific labeling of the individual constituents. We recorded [^{1}H,^{13}C] correlation spectra of isoleucine, leucine, and valine methyl groups of complete RNAP and the separately labeled β' subunit within reconstituted RNAP, setting the basis for the study of structural changes that might occur upon transcription factor/ nucleic acid binding, even in multiprotein complexes like the antitermination complex. We further produced all RNAP subunits individually and established NMR experiments to determine which RNAP subunit specific regulators bind to. Thus we identified the β subunit as target for N-utilization substance (Nus) factor NusE.

Next, we determined the RNAP binding surfaces of several Nus factors by NMR spectroscopy in a conceptually simple one-experiment approach using [^{1}H,^{13}C]-labeled methyl groups of valine, leucine, and isoleucine residues within deuterated Nus factors as probes. Having verified this approach with the complex of RNAP and NusG N-terminal domain (NTD), we determined the RNAP interaction site of NusE that

overlaps with the NusE binding site for the C-terminal domain of NusG, rendering attachment of NusE to these proteins competitive and suggesting that the NusE:RNAP interaction plays a role in antitermination. Further, we determined the solution structure of NusA-NTD by high-resolution NMR spectroscopy, identified the RNAP binding site of this domain using the same approach as for NusG-NTD and present a detailed model of the NusA-NTD:RNAP complex.

Drögemüller J, Strauß M, Schweimer K, Wöhrl BM, Knauer SH, Rösch P. Exploring RNA polymerase regulation by NMR spectroscopy. Sci Rep. 2015 in press

P 033

SOLUTION NMR STUDIES OF PROTEIN COMPONENTS FROM A BACTERIAL KILLING TYPE IV SECRETION SYSTEM

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The Type IV Secretion System (T4SS) is a supramolecular complex that crosses the bacterial cell wall; it is responsible for the translocation of a variety of substrates such as proteins and protein-DNA complexes. The prototypic T4SS is composed by a dozen proteins, from which VirB7, VirB9 and VirB10 form the so-called core complex, a tetradecameric assembly that occupies the periplasmic space and crosses the outer and inner membranes. In contrast to all other T4SSs characterized to date, the T4SS from the phytopathogen Xanthomonas citri pv. citri (Xac) secretes toxins that kill other gram-negative bacteria (Souza et al. 2015). In order to characterize the structure and function of Xac's T4SS we have been studying the structure and dynamics of different protein-complexes that form the Xac-T4SS's core complex by high-resolution NMR spectroscopy. We showed previously that Xac-VirB7 contains a well-folded C-terminal globular domain ($VirB7^{CT}$) that forms an extra ring layer around the T4SS outer membrane complex (Souza et al. 2011). Here we use NMR to study the interaction between the N-terminal domain of Xac-VirB7 ($VirB7^{NT}$) and the C-terminal domain of Xac-VirB9 ($VirB9^{CT}$). $VirB7^{NT}$ is intrinsically disordered, but folds into a short β-strand that complements a β-sheet from $VirB9^{CT}$ when in complex. Disruption of intermolecular contacts between $VirB7^{NT}$ and $VirB9^{CT}$ affects the stability of the complex and impairs the T4SS function in bacterial competition assays. The ^{15}N-HSQC spectrum of $VirB9^{CT}$ in the free state is characteristic of significant flexibility on various time scales. Nevertheless, chemical shift analysis indicates that β-strands seen in the complex with $VirB7^{NT}$ are already present in the free state. NMR experiments carried out with the periplasmic N-terminal domain of VirB10 showed that it is also highly flexible in solution. These findings

suggest the presence of significant flexibility within Xac's T4SS core complex.

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P 036
130 KHZ MAS AND 1H SPIN SYSTEMS

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We shall describe recent MAS hardware developments and demonstrate reaching speeds 130 kHz. Starting already at 40 kHz, spin dynamics of a dense and viscous proton system experiences qualitative changes, enabling pulse manipulations typical for a low-viscosity matrix[1]. Further gain in MAS rate improves resolution, until inhomogeneous contribution starts to dominate at ca 100 kHz. We show that even if any further increase in spinning rate is less than linearly efficient in terms of the resolution, it is still beneficial in terms of the sensitivity. While a through-space cross-polarization generally decreases at higher speeds, through-bond transfers like INEPT may gain more than linearly and contribute critically to a feasibility of various heteronuclear correlations. In addition to experimental data, we shall present also numerical simulation and predictions to higher spinning rates. Current hardware limitation can be virtually lifted by diluting the proton system by deuteration, which serves as a validation of the computational model. This model can be expected to help with optimization of NMR strategy, from assignment[2] to characterization of molecular interactions[3].

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P 039

A STRUCTURAL MODEL OF NEUROPEPTIDE Y IN COMPLEX WITH THE Y2 G PROTEIN RECEPTOR INTEGRATING NMR AND MUTAGENESIS DATA

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G protein-coupled receptors (GPCRs) are pharmacologically highly relevant transmembrane signal transducers. Understanding their structure and function provides access to rational drug design. Here, we report on the structure and binding mode of neuropeptide Y (NPY), a 36 amino acid peptide hormone, to its GPCR subtype 2 (Y_2R) applying NMR techniques, computational modeling and targeted receptor mutagenesis. Specifically isotope-labeled NPY was synthesized by solid phase peptide synthesis, and the required milligram amounts of receptor were obtained by recombinant expression in E. coli and subsequent in vitro folding into phospholipid bicelles. Upon receptor binding, the C-terminal a-helix of NPY, which is formed in membrane environment in the absence of receptor, is unwound starting at T^{32} to make optimal contact of the C-terminal residues within the receptor binding pocket. This is concluded from significant alterations in the NMR chemical shifts recorded for the C-terminal part of NPY. In addition, signals of several hydrophobic residues in the a-helical region of NPY were broadened upon receptor binding. These experimental data were used to derive a model of the Y_2R with the docked ligand, which was verified by double-cycle mutagenesis. Accordingly, the ligand is tethered to the second extracellular loop by hydrophobic contacts, with the N-terminal part of its helix facing the solvent. The C-terminal pentapeptide of NPY inserts deeply into the transmembrane bundle, making optimal contacts to Y_2R including an interaction of NPY's amidated C-terminus with $Q^{3.32}$ in a polar cluster within transmembrane helices 2 and 3 of Y_2R.

P 042

TOWARDS AN IN-MEMBRANE SOLID STATE NMR INVESTIGATION OF THE DIVALENT CATION TRANSPORTER CORA

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Membrane proteins in lipid bilayers are one of the most exciting target for solid state NMR since they are not easily accessible by other methods traditionally used in structural biology. In the most common approach, proteins are purified with detergents and reconstituted into lipids (e.g. Liposomes) However, the use of detergent might induce structural inhomogeneity and overcoming this step would be favorable. Indeed, recent studies have demonstrated the possibility to investigate proteins in their native membrane environment. Low sensitivity, though, presents a serious challenge in this approach. The possibility to increase spinning speeds by using very small magic angle spinning (MAS) rotors is enabling the measurement of proton detected solid state NMR of very small amounts of sample. This methodology has been successfully applied to a range of proteins including membrane proteins and paramagnetic metalloproteins. The latter ones particularly benefit from fast MAS since paramagnetic effects can be detected with high sensitivity and even exploited for obtaining long range structural information.

Based on this, we are planning to apply fast MAS solid state NMR to a lipid embedded membrane protein capable of binding paramagnetic metal ions. We chose the inner membrane divalent cation transporter CorA from Escherichia coli. Initial steps aim at the measurement of well resolved spectra of isotope enriched CorA. We will present our preliminary results on the optimization of protein overexpression and membrane isolation. The presented data tackle various aspects of sample preparation for in-membrane solid state

NMR measurements. Furthermore, sensitivity based shortcomings will be discussed.

P 045

NMR STUDY OF SLURP-2: DIFFERENCES AND COMMON FEATURES WITH OTHER LY-6/UPAR PROTEINS ACTING ON NICOTINIC ACETYLCHOLINE RECEPTORS

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Several endogenous ligands of nicotinic acetylcholine receptors (nAChRs) belonging to the Ly-6/uPAR family were discovered in higher animals. These proteins share structural homology with 'three-finger' snake neurotoxins, specific inhibitors of nAChRs. Some of these endogenous ligands (Lynx1, Lynx2) are membrane-tethered via GPI-anchor and co-localize with nAChRs modulating their functions in brain. Others (SLURP-1, SLURP-2) are secreted and act as autocrine/paracrine hormones in epithelium and other tissues. Previously we successfully produced ^{15}N-labeled analogues of water-soluble domain of human Lynx1 and SLURP-1 using bacterial expression system and studied their spatial structure and dynamics by NMR.

Here using the ^{13}C,^{15}N-labeled protein, we studied the spatial structure of human SLURP-2. At concentrations above of 0.1 mM nonspecific oligomerization of SLURP-2 was observed, accompanied by significant diminishing of the spectra quality. To reduce oligomerization, 5% dioxane was added to the sample. This greatly improved the quality of the spectra and gave the opportunity to work at 1 mM concentration. According to obtained NMR data, the protein adopts typical 'three-finger' fold consisting of two antiparallel β-sheets. The first β-sheet is formed by two β-strands and involves residues from the loop I (Ile2-Gln6, His15-Cis19). The second one consists of four strands formed by the residues from the loop I (Cis7-Gly9), loop II (His25-Val33, Leu42-His48), and loop III (Val63-Cys68). The spatial structure of SLURP-2 is well defined only in the conserved β-structural core, while the tips of all three loops of the protein are disordered. The ^{15}N-relaxation data

revealed significant mobility of the unstructured SLURP-2 loops at the ps-ns time-scale.

The obtained data permit to compare structural properties of SLURP-2 with other Ly-6/uPAR proteins acting on nAChRs (human Lynx1 and SLURP-1 and snake α-neurotoxins). The main structural features of SLURP-2 (relatively short β-strands and significant conformational plasticity of all three loops) largely coincide with the previously observed ones in the SLURP-1 molecule. In contrast to that, Lynx1 and α-neurotoxins have more ordered structures with larger β-structural core. Moreover, SLURP-1 and SLURP-2 molecules have overall negative charge, contrary to positively charged Lynx1 and α-neurotoxins. The altered charge distribution together with different structural and dynamic properties could imply different mode of interaction with nAChRs.

Nevertheless, the some structural features were common for SLURP-2 and Lynx1. The both proteins have the characteristic side-chain/main-chain hydrogen bond crosslinking the first loop. In both cases formation of this bond ($N^{\delta 1}$ His4 - H^{N}His14 and $N^{\delta 1}$ His4 - H^{N} Asn15) leads to significant downfield shift of corresponding $^{1}H^{N}$ resonance (~ 11.6 ppm). In addition, the reduction in the pH value of the SLURP-2 sample from 5 to 3 resulted in the denaturation of the protein, accompanied by transitions of β-structure to the random coil conformation. Similar pH-induced denaturation was previously observed for ws-Lynx1 but not for SLURP-1 and α-neurotoxins. In this regard, it should be noted that, human Lynx1 and SLURP-2 proteins are the products of a single gene located on the chromosome 8 that arise during alternative splicing.

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P 048

ENTROPY DRIVING ENDOTHERMIC FOLDING OF POLYTOPIC MEMBRANE PROTEIN IN THE LYSOPHOSPHATIDYLGLYCEROL CONTAINING MEDIA. NMR STUDY OF KVAP VOLTAGE-SENSING DOMAIN

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Understanding of protein folding is one of the key problems in structural biology. Thermodynamic studies uncovered relationships involved in the folding of globular (water-soluble) proteins. Contrary, the folding of membrane proteins is far less analyzed and understood. Generally, helical membrane proteins are resistant to conventional chaotropic agents, but their unfolding could be achieved in anionic detergents.

Voltage-sensing domain (VSD) of KvAP channel contains four transmembrane (TM) helices (150 a.a.) and represents an autonomous folding unit. The TM bundle of VSD has relatively polar interior and is stabilized by ionic interactions. VSD preserves its native fold in the micelles of zwitterionic detergents (DPC). Here using ^{1}H,^{13}C,^{15}N NMR, we studied partially denatured VSD in the environment of anionic LPPG micelles. Solubilization in LPPG moderately changed the secondary structure, but strongly influenced tertiary structure and backbone dynamics of the domain. The paramagnetic relaxation enhancement (PRE) measured in a series of spin-labeled VSD mutants revealed the disruption of the interhelical packing, and ^{15}N-relaxation data pointed to the marked increase of ps-ns backbone mobility ($S^2 \sim 0.81 \pm 0.08$) as compared to the natively folded VSD ($S^2 \sim 0.93 \pm 0.11$). However, the observed residual long-range PRE interactions and results of translational and rotational diffusion measurements revealed the quasi-native topology and

compactness of the VSD/LPPG complex. The observed VSD structure is reminiscent of a "molten globule" state found in the folding pathway of soluble proteins. Probably, the negatively charged head groups of LPPG disrupt the salt-bridges stabilizing the native VSD fold.

Titration of the VSD/LPPG sample with DPC led to appearance of two sets of NMR signals belonging to the "native" and "molten" states of the domain. The exchange between two states was slow on the NMR timescale (K_{EX} > 100 ms). Analysis of signal intensities in the ^{15}N-HSQC spectra permitted to quantify the transition between the two states in response to change in LPPG/DPC molar ratio. It was found that unfolding of the protein by LPPG is fully reversible and obeys two-state (folded/unfolded) model with following parameters: the standard free energy of VSD folding in the absence of denaturant (LPPG) ΔG^0 ~ -8.4 kcal/mol and associated m-value ~ 14.8 kcal/mol. Analysis of temperature dependence measured at the 50-60% mole fractions of LPPG revealed increase in the population of the "native" VSD state with increase in temperature. This endothermic folding process is characterized by approximately equal positive enthalpy and entropy contributions ($\Delta H^0 \sim T\Delta S^0 \sim$ +30-40 kcal/mol at 318K). Notably, the folding of water-soluble proteins usually goes with decrease in entropy and enthalpy of the system.

Enthalpy increase upon VSD folding in the LPPG-containing media could be associated with the disruption of the energetically favorable contacts between charged VSD side chains and anionic detergent. In turn, the release of these "extra" detergent molecules in solution leads to an increase in the overall entropy of the system. The NMR data confirmed the presence of LPPG molecules tightly associated with VSD in the "molten" state. The results obtained show differences and similarities in the folding pathways of membrane and globular proteins.

P 051

NMR INVESTIGATION OF HUMAN WIP, AN INTRINSICALLY DISORDERED CYTOSKELETON–REGULATING PROTEIN.

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Intrinsically disordered proteins (IDPs) are polypeptides lacking a well-defined structure under biologically native conditions [1]. In terms of structure IDPs are a heterogeneous ensemble of rapidly interchanging conformers, which together contribute to the overall behavior in solution [2], and their existence and functionality challenge the classical structure-function paradigm. Moreover, the flexible nature of IDPs allows them to assume distinct structures upon binding to different partners and to carry out multiple biological functions [3].

WASP–interacting protein (WIP) is a multi-domain unstructured polypeptide which participates in the regulation of actin cytoskeleton dynamics in lymphocytes. The N-terminal domain of WIP binds actin, and its C-terminal chaperones the important cytoskeleton modulator WASP. Binding of WIP to WASP is crucial both to the stability and regulation of the latter [4]. Structural analysis of the C-terminal domain (residues 442-492) revealed that WASP-binding induces an increase in structural character of the binding epitopes and a rigidification of inter-epitope linker segments. Thus, weak structural propensities (<25%) at residues 448-453 and 463-468 are replaced by significant structural contributions (20-60%) throughout the central segment of the domain (residues 448-480). Particularly striking is the induced helicity for residues 448-453,458-461 and 474-480; the first of these segment is a previously unknown contributor to binding affinity.

Chemical shifts are a sensitive probe of the conformational ensemble of IDPs. To establish backbone heteronuclear couplings as a complementary descriptor of ensemble behavior a new J-modulated sequence with $^{13}C'$-readout was designed to accurately measure the ^{1}J (N^{i},C_{α}^{i}) and ^{2}J(N^{i},$C_{\alpha}^{(i-1)}$) couplings that correlate with the backbone dihedral angle Ψ and secondary structure. The accuracy

in which the couplings are measured allows small changes in the protein conformational ensemble to be detected, providing information relevant to both its structure and its biological function. This approach was applied to the unbound WIP N-terminal domain known to exhibit transient helicity at residues 30–42 and β-sheet character for residues 17-25 and 44-62 [5]. Using this method we were able to detect the effect of temperature, crowding and denaturative conditions on the structural elements of the WIP N-terminal domain. Overall our work demonstrates the importance of NMR in characterizing transient structures and dynamics of IDPs.

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P 054

PHOTOCHEMICALLY INDUCED DYNAMIC NUCLEAR POLARIZATION OBSERVED BY SOLID-STATE NMR IN A UNIFORMLY 13C-ISOTOPE LABELED PHOTOSYNTHETIC REACTION CENTER

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A sample of solubilized and quinone-depleted reaction centers (RC) from the purple bacterium Rhodobacter (R.) sphaeroides wild-type (WT) has been prepared entirely ^{13}C and ^{15}N isotope labelled at all positions of the protein as well as of the cofactors[1,2]. In this sample, the occurrence of the solid-state photo-CIDNP (photochemically induced dynamic nuclear polarization) effect has been probed by ^{13}C solid-state magic-angle spinning (MAS) NMR under illumination[3,4]. Under continuous illumination, signal intensities are modified by the three-spin mixing (TSM) mechanism[5]. Time-resolved illumination experiments reveal the occurrence of light-induced nuclear polarization on the time-scale of 100s of microseconds, initially dominated by the transient polarization of the singlet branch of the radical-pair mechanism (RPM). Upon the decay of the RPM polarization, emissive polarization caused by the three-spin mixing mechanism (TSM) is observed. The decay of TSM polarization occurs in two steps, assigned to intra- and intermolecular spin diffusion.

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P 057
SEGMENTAL ISOTOPE LABELING OF ARMADILLO REPEAT PROTEINS

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Demand for specific binding proteins in both proteomics and diagnostic applications has increased significantly over the past years. A great number of protein scaffolds has been investigated as an alternative to antibodies production, which is laborious and time-consuming. Yet, many of them were designed to bind folded proteins, while obtaining binders for unstructured proteins and peptides is still a challenge.

Armadillo repeat proteins (ArmRP) are one of the most promising peptide-binding scaffolds. These modular proteins are assembled out of tandem armadillo repeats of approximately 42 amino acids, where a single repeating unit interacts with two adjacent amino acids in the context of a target peptide. Repeats, build out of three helices each, form a right-handed superhelix surface for protein-peptide interactions. The goal is to develop an assembling scaffold platform, where binders can be created out of predefined binding units recognizing dipeptide in target sequence. For this approach the exact mode of peptide binding has to be understood. Considering the fact, that many of ArmRP cannot be crystallized because of their disordered N-cap, NMR is an essential tool for characterizing both early constructs stability as well as mode of binding of ligands.

In recent years we have focused on the characterization of ligand binding. For this purpose two methods are chosen – chemical shift mapping, also suitable when the affinity is not high, and intermolecular NOEs that define the complex with good resolution but require a low off-rate. Unfortunately resonance assignment for ArmRP is an ambiguous task since binders consist of several repeats with high sequence identity (more than 90%) resulting in severe signal overlap. For this reason our present work involves using segmental isotope labeling of individual repeats to overcome this problem and reduce

the number of obtained signals. To make it possible we use an expressed protein ligation (EPL) reaction, where two protein fragments are expressed and purified separately, and subsequently linked by native chemical ligation. The reaction follows biomolecular kinetics, where one fragment with an N-terminal MxeGyrA intein fusion is used to obtain a thioester, which then reacts with an N-terminal Cys on other fragment. This allows the investigation of partially labeled Armadillo proteins in a context of full length protein by NMR spectroscopy.

P 060

MICROSECOND MOTION MODULATES UBIQUITIN BINDING INTERFACES THROUGH AN ALLOSTERIC BACKBONE/SIDE CHAIN NETWORK

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Motion is involved in a large number of protein functions. Relaxation dispersion (RD) NMR experiments sensitively probe microsecond to millisecond motions. We conducted an in-depth high power (1,2) RD analysis of the backbone and side chain methyl groups of ubquitin. This survey showed a large number of atoms (>30) with microsecond fluctuations. These atoms are distributed throughout the structure. Strikingly, nearly all show the same exchange rate, which suggests that ubiquitin undergoes collective motion involving both the backbone and side chains. Furthermore, comparison of different methyl nuclei indicates that the nature of the side chain fluctuations is almost entirely due to changes in rotamer populations. Thus, collective microsecond backbone motion is coupled to redistribution of side chain rotamer populations through a mechanism we term "population shuffling" (2). We present a single collective mode of motion that yields a reaction coordinate corresponding to the relaxation dispersion data. The resulting model indicates that a localized conformational switch distant from the binding interface propagates changes throughout the structure. Analysis of crystal structures confirms this allosteric network and suggests that the microsecond motion modulates binding to particular interaction partners.

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P 063

SWEET STABILITY, EXPLORING THE AGGREGATION PROPENSITY OF THE SWEET PROTEIN SINGLE CHAIN MONELLIN

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An attractive model system for the study of protein aggregation is Monellin, a protein belonging to the cystatin superfamily several of whose members form amyloid fibrils that are associated with human diseases.

Monellin was originally isolated from the tropical serendipity berry (Dioscoreophyllum cumminsii) and has been studied for its intense sweetness. Folded monellin is indeed about 100000 times sweeter than sucrose on the molar basis. Monellin is naturally composed of two polypeptide chains, MNA (45aa) and MNB (50aa), linked together by non covalent interactions. In order to increase stability against thermal denaturation the A and B chain are linked together by a GF linker obtaining a single-chain monellin (MNEI). Recombinant MNEI retains sweetness and increased thermal stability. It has been shown that monellin can aggregate but very little is known on the molecular basis of the process. Since the propensity of globular proteins to aggregate is often inversely related to the stability of the native state and increased tendency for unfolding seems to promote fibrillation,in order to perform a detailed analysis of the aggregation propensities of MNEI, we have studied the dynamic and the stability of MNEI combining pH titrations and H/D exchange NMR experiments.

P 066

SOLID STATE NMR SPECTROSCOPY USED FOR CHARACTERISATION OF GRAPHENE NANORIBBONS

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In organic photovoltaics and nano-electronic applications, it is of great interest to control for example the energy band gaps of the materials in use. Graphene nanoribbons (GNRs), i.e. nano-strips of graphene, show great promise in this respect as the electronic properties depend on the width of the nanoribbons and on the edge structure. By using a "bottom-up" chemical synthesis approach, the above-mentioned parameters can be controlled. The highly inhomogeneous packing of GNRs makes solid state NMR spectroscopy to one of the preferred techniques for characterisation and NMR experimentalists can benefit from the advances over the last decade in hardware and pulse sequence development. Our experiments are often conducted under 50-60 kHz magic angle spinning conditions using a 1.3mm probe. The fast spinning regime gives a reasonable resolution of the ^{1}H signals. ^{1}H-^{1}H double-quantum single-quantum experiments are used for assigning the protons via correlations mediated by the dipolar coupling interactions. The edge can be further characterized by observing the ^{13}C signals. The magnetization on the ^{13}C nuclei has been transferred from the nearby ^{1}H nuclei by applying short mixing times and assignment rely on the ^{13}C chemical shifts.

P 069

FUNCTIONAL SIGNIFICANCE OF THE LOW POPULATION STRUCTURES OF THE INTRINSICALLY DISORDERED REGIONS (IDRS) IN PROTEINS

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The biological functions associated with intrinsically disordered regions (IDRs) in proteins have been accumulated to make the IDRs become the central focus in protein science. The IDRs are frequently found in the proteins working as hubs in signaling and metabolic networks in cells, suggesting flexible and/or plastic properties of the IDRs have essential roles in the networks. Post-translational modifications, including phosphorylation, preferentially happen to the IDRs to modify their structural properties. Some of the IDRs contain the multiple modification sites, and the IDR function changes according to the extent of the modification, which achieves the rheostatic regulation of intra-cellular reaction.

The structure and dynamics of the IDRs in protein have pivotal roles that have been hidden in the conventional structural biology, in which the structured parts are primarily focused. The comprehensive knowledge of the structure and dynamics of the IDRs in relation to their biological roles is desired to complement the less conscious side of protein science, but it still remains elusive.

We have been working on several types of proteins including IDRs to explore their structure and dynamics in relation to the functions. Each IDR has very different structural properties: one behaves as a completely random coil having no residual structure, the other has low population structure, and the other changes its dynamics and transiently folding structures according to the number of phosphorylation to the IDRs. In every target having different research focus, we used various NMR approaches in combination with the other methods, including SAXS, high-speed AFM, and the molecular dynamics simulations using all atoms or the simulations with coarse-grained model. In this presentation, I will show several aspects of the

structure and dynamics in the IDRs from our studies, with especial attentions to the functional roles of the low population structures caused by the transient folding interactions within the IDRs.[1,2]

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P 072
HISTONE H2A, H4 IN NUCLEOSOME CONDENSATION INVESTIGATED BY SOLID-STATE NMR

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In eukaryotic cells the fundamental structural unit of chromatin is the nucleosome core particle (NCP). It consists of DNA and a histone octamer, which is composed of two copies of four core histones, H2A, H2B, H3 and H4. NCP crystalline structures have been determined. In the nucleosome, each histone includes two common regions, "histone fold" and "histone tail". The histone fold is a stable formation of histones, which is well identified in the crystals. On the other hand, the N-terminal tails of the four histones and the C-terminal tail of histone H2A are flexible in NCP. The N- and C-terminal histone tails could not be determined in the crystal structures. These histone tails are responsible for gene regulations by their chemical modifications. The histone tails are known to interact with nucleosomal DNA and the acidic patches of the neighboring nucleosomes. These interactions may play critical roles in the formation of higher-order chromatin. The nucleosome aggregated by Mn^{2+} was a good model of chromosome. The local nucleosome fiber has been reported to be folded in an irregular or disordered manner without a 30-nm chromatin structure in mitotic chromosomes. Here, structures of H2A and H4 in the chromosome model were investigated by solid-state NMR and MD simulations.
Human H2A, H2B, H3 and H4 were overexpressed in Escherichia coli with N-terminal His-tags. ^{13}C, ^{15}N uniformly labeled H2A and H4 were prepared in M9 minimal medium. The His-tag was removed by thrombin treatment during the purification procedure. DNA with a 146-bp palindromic sequence derived from human a-satellite DNA for

nucleosome reconstitution was used for this study. The histone octamer was incorporated into nucleosomes by a salt-dialysis method, and the nucleosomes were purified by native polyacrylamide gel electrophoresis. Highly condensed NCP were aggregated with 12-mM $MnCl_2$.

Structural information of aggregated nucleosomes was derived from 2D ^{13}C-^{13}C correlation spectra with cross polarization (CP) and 2D ^{1}H-^{13}C correlation INEPT spectra. CP and INEPT experiments from their different mobilities, were rigid and flexible, respectively. ^{13}C-^{13}C spectra with CP suggest that the NCP aggregate is similar to that of X-ray structure in histone fold region. ^{1}H-^{13}C INEPT spectra indicate that histone tails of H2A and H4 hold flexible random coil structures. Mobile regions of histones are consisted with high RMSF regions by MD simulations of NCP. In the higher-order chromatin structure, histone tails of H2A and H4 were found to have a similar mobility to that of the nucleosome alone, suggesting that the mobilities allow the post-translational modification of histone tails, and control the various gene expression / function.

P 075
NMR SPECTROSCOPIC STUDIES OF Na+-NQR AND ITS INTERACTIONS WITH QUINONES

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The Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR) is the main sodium pump and at the same time the first enzyme of the respiratory chain of Vibrio cholerae and other pathogenic bacteria. It uses the energy gained by the electron transfer from NADH to ubiquinone to generate an electrochemical gradient across the cytoplasmic membrane by pumping sodium ions. How the six subunits of Na+-NQR, NqrA-NqrF, work together in the processes of ion pumping and substrate binding is still not fully understood.

The NqrA subdomain is the only soluble domain of this 216 kDa membrane protein complex and thus we focused our NMR investigations on function and interaction of this subunit regarding its role in electron transfer. Whereas the majority of electron transfer steps have been characterized in detail, the final step, namely the reduction of ubiquinol-8, (Q8), remains to be elucidated.

By saturation transfer difference (STD) NMR spectroscopy we could show that ubiquinone-1 (Q1) as well as the inhibitors HQNO and DBMIB bind to subunit NqrA with the quinone head group and the terminal methyl groups having closest contact to the protein. As was shown later with interligand Overhauser effects (ILOEs), Q1 and DBMIB/HQNO bind to an extended binding pocket in direct vicinity to each other. By incorporation of single point mutations we could get first hints towards the description of a possible binding pocket.

With the help of different labelling strategies (complete deuteration, ^{15}N- and ε-^{13}C-metionine-labeling) NqrA-377, a truncated construct of NqrA was used to record high quality

heteronuclear correlation spectra that revealed chemical shift perturbations upon addition of DBMIB.

P 078

CONFORMATIONAL VARIABILITY OF WILD-TYPE AND MUTANT AMYLOID PRECURSOR PROTEIN TRANSMEMBRANE FRAGMENTS

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Despite some progress in study of the molecular mechanisms of Alzheimer's disease (AD) pathogenesis initial steps of this pathogenesis are still unknown. Amyloidogenic Aβ-peptides forming plaques in the brain are products of consecutive intramembrane cleavage of Amyloid Precursor Protein (APP). More than half of familial mutations of APP predisposing to AD development occur in its transmembrane domain and juxtamembrane segment including metal-binding domain and it is thought that they affect stuctural-dynamical properties, dimerization and proteolysis of APP in the membrane.

To investigate the initial steps of Aβ-peptide formation we carried out high-resolution NMR studies of structural-dynamical properties of the APP transmembrane fragments Gln686-Lys726, Gln686-Leu720 and Gln686-Val717 corresponding to the initial steps of proroteolysis of APP by γ-secretase in membrane. The $^{13}C/^{15}N$-isotope labeled APP fragments were produced using high-performance systems of bacterial or cell-free expression and solubilized in different membrane mimetics. We showed that unlike Gln686-Lys726 fragment, containing full-length APP transmembrane domain, Gln686-Leu720 and Gln686-Val717 gradually converts from a-helical to β conformation and the shorter fragment exhibits stronger tendency to aggregation. At the same time "Australian" mutant L723P unlike more stable V717I and V717G mutants also gradually converts from a-helical to β conformation and this process accompanied by high molecular weight aggregates formation. Thereby APP transmembrane fragments are shown to be promising objects for determination of molecular mechanisms of amyloidogenesis and identifying structural

and functional determinants of APP which is necessary for understanding of AD pathogenesis.

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P 081

POSITIONAL PREFERENCES OF ACETYL ESTERASES FROM DIFFERENT CARBOHYDRATE ESTERASE FAMILIES TOWARDS ACETYLATED 4-O-METHYL GLUCURONIC ACID-SUBSTITUTED XYLO-OLIGOSACCHARIDES

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Background: Acetylation of the xylan backbone restricts the hydrolysis of plant poly- and oligosaccharides by hemicellulolytic enzyme preparations to constituent monosaccharides[1]. The positional preferences and deacetylation efficiencies of acetyl esterases from seven different carbohydrate esterase (CE) families towards different acetylated xylopyranosyl units (Xylp) - as present in 4-O-methyl-glucuronic acid (MeGlcA)-substituted xylo-oligosaccharides (AcUXOS) derived from Eucalyptus globulus - were monitored by ^{1}H NMR, using common conditions for biofuel production (pH 5.0, 50°C).

Results: Differences were observed regarding the hydrolysis of 2-O, 3-O, and 2,3-di-O acetylated Xylp and 3-O acetylated Xylp 2-O substituted with MeGlcA. The acetyl esterases tested could be categorized in three groups having activities towards (i) 2-O and 3-O acetylated Xylp, (ii) 2-O, 3-O, and 2,3-di-O acetylated Xylp, and (iii) 2-O, 3-O, and 2,3-di-O acetylated Xylp, as well as 3-O acetylated Xylp 2-O substituted with MeGlcA at the non-reducing end. A high deacetylation efficiency of up to 83% was observed for CE5 and CE1 acetyl esterases. Positional preferences were observed towards 2,3-di-O acetylated Xylp (CE1, CE5, and CE6) or 3-O acetylated Xylp (CE4).

Conclusions: Different positional preferences, deacetylation efficiencies, and initial deacetylation rates towards 2-O, 3-O, and 2,3-di-O acetylated Xylp and 3-O acetylated Xylp 2-O substituted with

MeGlcA were demonstrated for acetyl esterases from different CE families at pH 5.0 and 50°C.

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P 084

STRUCTURAL AND DYNAMICAL CHARACTERISATION OF IMMOBILISED ENZYMES USING SOID-STATE NMR

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Enzymes are macromolecular biological catalysts, responsible for regulating the rates of chemical reactions without themselves being altered in the chemical reactions. The term immobilised enzyme refers to an enzyme physically confined or localized in a certain defined region of space with retention of its catalytic activities and which can be used repeatedly and continuously for industrial applications.[1] Although immobilised enzymes have been extensively characterized by various biochemical methods, rational design of the catalytic system still remains a considerable challenge as very little is known about the state of the protein upon immobilisation. Very recently, solid-state NMR has been successfully employed to characterise an immobilised enzyme consisting of a model enzyme a-Chymotrypsin, mesoporous silica as the matrix, and (3-glycidyloxypropyl) trimethoxysilane (GOPS) as the covalent linker.[2] However, the state of the enzyme and its dynamics upon immobilisation are not well understood. The relatively large size of the Chymotrypsin (25 kDa) and the difficulty of isotopically labelling the protein in its active state make it a daunting task to gain any structural and dynamical information using solid-state NMR.

To gain atomic-level information about the structure and dynamics of immobilized enzymes, a relatively small enzyme Ribonuclease A (RNase A) was chosen as a model system and the characterisation of the isotopically labelled (^{13}C,^{15}N) protein using solid-state NMR is envisaged as a future work. RNase is a relatively low molecular weight (~13.7 kDa, 124 residues) enzyme suitable for catalysing the cleavage of single-stranded RNA and the structure of the enzyme has been solved by NMR[3] and X-ray crystallography.[4] Prior to the study of the

isotopically labelled (^{13}C,^{15}N) immobilised enzyme, we have successfully carried out the immobilisation of RNase A in the unlabelled state on epoxy functionalised silica. The silica, linker and the enzyme were then characterised by ^{1}H, ^{13}C, ^{29}Si and ^{15}N in natural abundance using solid-state NMR. **Here we report** our preliminary results for RNase A immobilized on silica support (100 Å pore size) using GOPS as the covalent linker. Our results are found to be in very good agreement with similar, previously reported studies.[2]

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P 087
TOWARDS A STRUCTURAL MODEL FOR BETA-ENDORPHIN AMYLOID FIBRILS

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Although amyloid fibrils are often associated with diseases[1], their critical role in the healthy organism is increasingly recognized. One of these functions is thought to be storage for peptide and protein hormones.[2] These hormones are stored in the dormant amyloid form in secretory granules, ready to be released quickly when required. One of the candidates for such amyloidogenic storage is the 31 amino-acid protein beta-endorphin.[2]

The solid-state spectra are well resolved. The assignment has been successfully done and reveals an almost completely rigid structure with only the first two residues unassigned. Secondary-chemical-shift analysis indicates there are three beta-sheets per monomer. After the assignment we have started to work towards the 3D structure in atomic resolution. We have acquired enough spectrally unambiguous distance restraints to yield an initial model. However, the stacking of the monomers is still an open question. Currently we are working with three possible models: flat monomer stacking, staggered monomer stacking and dimer stacking. Efforts are underway to look into this via dilution analysis and selective labeling. However, a number of questions remain difficult to answer. These difficulties and approaches how to resolve them will be discussed.

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P 090

STRUCTURAL STUDY OF COMEA, A PROTEIN INVOLVED IN DNA UPTAKE BY STREPTOCOCCUS PNEUMONIAE

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The capacity of *Streptococcus pneumonia* to turn into a virulent strain from a non virulent form was firstly described in 1928(1). This ability of acquiring competence from other bacteria has been shown in several species and it involves the sharing/acquisition of exogenous DNA. This process provides genomic variation, allows adaptation to the environment and therefore, it might be involved in anti-biotic resistance. Although the capacity of DNA uptake is well known, the molecular bases of the process are still not completely understood. Recently it has been shown that exogenous DNA recruitment is mediated by pili (2). The pili acts as DNA catcher and the endonuclease EndA converts double strand DNA to single strand promoting its internalisation.

It has been shown that DNA internalisation required the presence of the receptor ComEA, a protein anchored in the membrane pointing out thought the peptidoglycans, and able to bind DNA.

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P 093
SOLID-STATE NMR INVESTIGATION OF CALCIUM ATPASE (SERCA) REGULATION BY TRANSMEMBRANE PROTEINS

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Sarco(endo)plasmic reticulum calcium ATPase (SERCA) is a 110 kDa membrane protein found in muscle cells. It hydrolyzes ATP to transport calcium ions against a concentration gradient from cytoplasm to sarcoplasmic reticulum, a process that triggers an essential process of muscle relaxation. The biochemical activity of SERCA is regulated by at least two single-span membrane proteins: phospholamban (PLN), found in cardiomyocytes, or sarcolipin (SLN), present in fast twitch muscle. Although the recent structures obtained through X-ray crystallography offered a general view of the enzyme/regulator complexes (SERCA/SLN and SERCA/PLN), the biophysical mechanism of regulation remains elusive. We report on the combination of oriented and magic angle spinning (MAS) solid-state NMR spectroscopy in conjunction with data-driven docking and atomistic molecular dynamics simulations to gain insights into the mechanism of regulation of SERCA by the transmembrane proteins in fully hydrated lipid bilayer membranes.

To visualize protein-protein interactions within lipid membranes, we designed innovative labeling strategies based on an array of isotopic labeling schemes, paramagnetic probes, and thioalkylation/carboxymethylation of free thiols in SERCA. The restraints obtained from the experimental data were used to model the binary complexes of membrane proteins, which were subsequently refined using restrained molecular dynamics simulations in explicit lipid bilayers. The structures of the ATPase/regulator provide a detailed view of the binding interface and offer hints of the mechanism of SERCA regulation (i.e., inhibition upon binding with the regulator, and re-activation of the enzyme upon phosphorylation of

the regulator). Understanding the biophysical basis of SERCA regulation provides unprecedented opportunity for the rational design of the mutants for gene therapy. We anticipate the approach and methods used by us to be applicable for mapping protein-protein interactions in membrane protein complexes larger than 100 kDa, which are usually inaccessible to the alternative experimental methods.

P 096

STRUCTURAL INVESTIGATION OF THE C-TERMINAL INTRINSICALLY DISORDERED CYTOSOLIC FRAGMENT OF ERBB2

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Epidermal growth factor receptor (ErbB) family mainly mediates the function of cell growth and development. Aberrant expression of these receptors may lead to occurrence of malignant tumors. ErbB family consists of four receptor tyrosine kinases: ErbB1 (EGFR), ErbB2, ErbB3 and ErbB4. Ligand binding to ErbB1,3 or 4 initiates a conformational rearrangement of these receptors that results in dimerization. ErbB2 is lack of a known ligand and is constitutively active. After dimerization, transactivation of the tyrosine kinase part of the dimer moiety occurs as each receptor activates its partner by phosphorylation. ErbB2 is overexpressed in about 25% of breast cancer and is thus a potent target for anticancer therapies. The combination of anti-ErbB2 therapy with chemotherapeutic drugs shows synergistic response in controlling the disease. However, only one-third of ErbB2-positive tumors respond to trastuzumab treatment and those that do acquire resistance within three years after the initiation of treatment. New strategies are thus to be developed. One is to target the C-terminal cytoplasmic tail of the protein, in which tyrosines phosphorylation is linked to the activation of a large number of downstream signaling pathways. CtErbB2 is an intrinsically disordered, proline rich domain. We aim here to better understand the relationship between the sequence, structural and dynamic features and the function of this domain. We show here the first results of the structural studies of CtErbB2 free in solution and of its interaction with some of its effector proteins.

P 099

ACCESSING DISTANCES IN A MULTIVALENT MODEL PROTEIN USING A TAILORED, CONFORMATIONALLY UNAMBIGUOUS SPIN LABELED LIGAND

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Multivalency is a key concept in biological systems leading to targeted and strong interactions which play a role in many biological processes, among them signalling, adhesion, etc. The interplay of multiple ligand-receptor moieties has the potential to enormously increase binding affinities compared to a monovalent binding situation. Knowledge of the distances between binding sites is thus crucial for the design of high-affinity mulitvalent ligands, e.g., as potent inhibitors. An EPR based method for distance determination between ligand biniding sites with a divalent spin labeled GlcNAc ligand and the model protein wheat germ agglutinin (WGA) was presented by Braun and coworkers. [1] However, in order to apply this approach to any protein receptor also without a structural model at hand, it is desirable to determine reliable distances with monovalent ligand molecules and a spin label which allows straight forward assignment of distance information to the protein structure. Therefor, we present a newly synthesized C-glycoside of GlcNAc as monovalent ligand for WGA carrying a conformationally unambiguous spin label side chain. The ligand and its binding behavior are characterized using continuous wave EPR spectroscopy. Distance measurements with double electron-electron resonance spectroscopy performed at Q band frequency are in accordance with the X-ray crystallography structure of WGA. [2] Based on this proof of concept study with the model system WGA it is possible to apply our approach to many multivalent receptor-ligand systems.

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P 102

THE BECLIN 1 N-TERMINAL REGION: BACKBONE CHEMICAL SHIFTS ASSIGNMENTS AND INTERACTIONS WITH THE BH3 DOMAIN AND THE EVOLUTIONARY CONSERVED DOMAIN

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BECN1, a haploinsufficient tumour suppressor, was the first identified mammalian autophagy gene[1-3]. It translates into the mature 450 amino acid long Beclin 1 protein, critical in the early stages of autophagosome formation[4]. Beclin 1 consists of three well-characterised structural domains. Residues 112-123 comprise a BH3 domain which is a short conserved sequence motif important for mediating interactions between members of the Bcl-2 family in the intrinsic apoptotic pathway. Following the BH3 domain is a coiled-coil domain (residues 173-268) and the third domain (residues 269-337) constitutes the so-called evolutionary conserved domain (ECD) which is critical for Beclin 1 activity as it mediates interactions with membrane lipid. While structures of the central and C-terminal portions of Beclin 1, which cover both the coiled-coil domain and ECD, have been reported recently[5,6], little structural information is available on the entire N-terminal region of Beclin 1, comprising a third of the protein (residues 1-150). Here, we report nearly complete assignments of the backbone chemical shifts using standard triple resonance methods and, for the first time, experimentally demonstrated that the N-terminal region of Beclin 1 is intrinsically disordered. Furthermore, results of interactions of the Beclin 1 N-terminal region with either the BH3 domain or ECD as revealed by NMR titration experiments will be reported.

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P 105

STRUCTURE AND DYNAMICS OF SIGMA SUBUNIT OF RNA POLYMERASE FROM BACILLUS SUBTILIS

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RNA polymerase of gram-positive bacteria contains several unique subunits in comparison to RNA polymerase of gram-negative bacteria. Sigma subunits play a critical role in recognition of DNA promotor sequence. In order to improve our understanding of transcription mechanisms we focused on sigma A factor (SigA) from Bacillus subtilis. SigA belongs to group 1 transcriptional factors. The number of sigma factors varies in different organisms from several units to several dozens. SigA is composed of four domains, sigma1.1, sigma2, sigma3, and sigma4 that are connected by flexible linkers. Since the SigA is capable of DNA recognition without interaction with other partner, there exist a regulatory mechanism to prevent DNA binding in inappropriate times. Sigma1.1 domain is responsible for auto-regulation of SigA. We used solution state nuclear magnetic resonance to solve the structure of sigma1.1. Although sigma1.1 is relatively small in size, 9.3 kDa, the resonance frequency assignment of sigma1.1 is not a trivial task because it contains 23 glutamine or glutamic acid residues. The consequence of such amino acid composition is that the standard set of NMR experiments (2D 1H-15N HSQC, 3D HNCA, 3D HNCOCA, 3D HNCACB, 3D HNCOCACB) for sequential resonance frequency assignment can lead to ambiguous assignment. Therefore additional 3D HCCCONH experiments were aquired to obtain more complete list of carbon and hydrogen chemical shifts. Side-chain assignment was done using additional 3D HCCH-TOCSY for aromatic and aliphatic spectral regions and 3D HSQC-TOCSY. We obtained backbone assignment for all but two residues at the N-terminal end of the protein. Side-chain assignmnent of more than 90% was obtained. All experiments were performed on

700 MHz and 850 MHz spectrometers.We aquired 15N-edited 3D HSQC-NOESY experiment and 13C-edited 3D HSQC-NOESY experiments for aromatic and aliphatic spectral regions to obtain proton-proton distances from experiments based on nuclear Overhauser effect (NOE). The NOESY cross-peak assignment was done using program CANDID. Experimentally determined torsion angles, residual dipolar couplings, chemical shifts, and NOE restrains were utilized by program CYANA to calculate the structure. There is structure of one homologue protein from Thermotoga maritima available in the PDB database. However, even though the secondary structure prediction reflects a very similar pattern, our structure of sigma1.1 exhibites significant differences in comparison. The analysis of backbone internal motions was done by analyzing auto-relaxation rates R1 and R2, longitudinal and transverse cross-correlated relaxation rates, and steady-state Nuclear Overhauser enhancement. Data was obtained on 600 MHz and 850 MHz spectrometers. Aforementioned relaxation rates were used for spectral density mapping and model-free analysis.This work was supported by Czech Science Foundation, grant number GA 13-16842S.

P 108
TOOL FOR SMFT-BASED ASSIGNMENT OF RESONANCES (TSAR): APPLICATION TO INTRINSICALLY DISORDERED PROTEINS

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NMR is the primary method of studying intrinsically disordered proteins (IDPs) [1]. However, resonance assignment is in this case a more demanding task than for folded proteins. The particularly narrow chemical shift range (caused by the fast molecular motion of the protein and high incidence of sequential repeats) often makes the routinely-used 3D spectra insufficient. On the other hand, the low relaxation rates (also caused by the fast molecular motion) makes spectra of high dimensionality feasible. Such spectra can be recorded using non-uniform sampling, which provides spectra of extraordinary resolution. The data processing can be performed using sparse multidimensional Fourier transform (SMFT) [2] based on the concept of fixing some of the spectral dimensions to the frequencies known from so called basis spectrum which is acquired in advance. As a result, a set of 2D cross-sections of the multidimensional spectrum is obtained. The parallel analysis of the cross-sections of various spectra allows to achieve the resonance assignment. The TSAR program [3] automates this process.

Here we demonstrate the utility of the TSAR program for automatic assignment of resonances of IDP samples. The TSAR program, being dedicated to the analysis of 2D cross-sections, exploits all advantages of the SMFT input. Moreover, its flexibility allows to process data from any set of experiments that provide sequential connectivities (including also 13C-detected experiment). The program's performance for several high-resolution demanding IDP samples [4-8] is demonstrated. In all the cases reliable results were achieved, that

allowed automatic assignment of the majority of resonances. These applications demonstrate that TSAR is a powerful tool for the assignment of IDP's resonances.

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P 111

BROADBAND EXCITATION PULSES WITH VARIABLE RF-AMPLITUDE DEPENDENT FLIP ANGLE (RADFA)

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Using the optimal control theory-derived GRAPE-algorithm, broadband pulses with scalable effective flip angle have been studied in detail. Adjustable pulses promise applicability in fast pulsing experiments, e.g. utilizing Ernst angle excitation. Next to optimization and characterization, experimental verification is shown.

P 114

1H CHEMICAL SHIFTS IN PARAMAGNETIC CO(II) PYRAZOLYLBORATE COMPLEXES: A FIRST-PRINCIPLES STUDY

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Paramagnetic NMR (pNMR) chemical shift results by density functional theory (DFT) can be very far from the experimental values. Therefore, it is of interest to investigate the applicability of ab initio computational methods to achieve useful accuracy. Here [1] we performed the first attempt of applying ab initio wave function based quantum-chemical methods to calculate the pNMR chemical shift within the theoretical framework established in Ref. [3] and extended recently to include magnetic couplings in Ref. [4]. We applied the N-electron valence-state perturbation theory (NEVPT2) on CoII systems, where the active space of the underlying state-averaged CASSCF wave function consists of seven electrons in the five metal 3d orbitals. These complexes have S = 3/2 electronic ground state consisting of two doublets separated by zero-field splitting (ZFS). To calculate the hyperfine coupling tensor A, DFT (both PBE and the hybrid PBE0) were used with the SVP and TZVP basis sets, while the g- and ZFS (D) tensors were calculated using CASSCF and NEVPT2 [5] with the same basis sets (TZVP was used in a locally dense fashion). These results were combined to obtain the total chemical shift values. The quantum chemistry packages used for chemical shift were ORCA and GAUSSIAN, while the molecular geometries were optimised by Turbomole. The results obtained from these calculations are in a good agreement with the experimental results [6], in some cases suggesting a reassignment of the experimental signals. The accuracy of this mixed ab initio/DFT approach is very promising for further applications to demanding pNMR problems involving transition elements.

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P 117
INFORMATION CONTENT OF DISTANCE RESTRAINTS FOR PROTEIN NMR STRUCTURE CALCULATION

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Nuclear magnetic resonance (NMR) protein structure determination relies primarily on distance restraints derived from NOESY cross peaks. Up to now a concise quantification of their information content, such as resolution in X-ray crystallography, has been lacking. Accordingly, in publications reporting NMR structures it is common practice to give an overview of the input data that were used for the structure calculation in a table. Restraints are usually reported by the total number, and further classified as intraresidual, sequential, medium-range and long-range.

A first approach to quantify the information content of a distance restraint data set in a more precise way was the NOE completeness (Doreleijers et al. 1999) that is evaluated against the expected NOEs based on the available structure. Another approach called QUEEN (Nabuurs et al. 2003) was based on ideas derived from Shannon's information theory.

Our new information content measure implemented in the CYANA software package is mostly based on probability theory. It relies on a small number of fundamental assumptions and is designed to overcome shortcomings of the earlier approaches. The information content is defined as the negative logarithm of the probability to fulfill the restraints by a set of random structures. This measure considers how much each restraint restricts the accessible conformation space and how redundant it is with other restraints. The restriction of the accessible conformation space is calculated relative to a given set of structures, which are typically random but may also embody prior

structural knowledge. The information content is defined without user-adjustable parameters, fast and straightforward to calculate prior to the structure calculation, and correlates strongly with the precision of the resulting structure.

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P 120
CONFORMATIONAL MOBILITY IN MONOLAYER-PROTECTED NANOPARTICLES: FROM TORSIONAL FREE ENERGY PROFILES TO NMR RELAXATION

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We present an integrated computational approach that proved to be a self-consistent route to inspect and interpret the conformational mobility of single alkyl chains in gold nanoparticles (AuNP) passivated with a monolayer of decanethiols.

From the experimental point of view, the overall dynamics of an alkyl chain can be indirectly accessed by nuclear magnetic resonance spectroscopy (NMR). In particular, 13C T1 and T2 relaxation times are modulated by the complex, global plus internal, dynamics of the system. However, the synergistic complex interplay of the molecular details in affecting the relaxation process makes it necessary to employ an appropriate modeling to quantitatively bridge between the single-molecule description and the bulk response. To this aim, we employ a stochastic description of the relevant coordinates, with respect to the NMR observables, of the system. Here, such coordinates are the global tumbling of the AuNP and the probe alkyl chain torsion angles. The remaining degrees of freedom constitute a thermal bath, i.e. they contribute with a fluctuation-dissipation effect to the dynamics of the relevant coordinates. Two ingredients are required to fully parameterize the stochastic equation: the potential of mean force acting on the selected coordinates, and the friction, which describes how the bath opposes to a change of the system configuration. We setup a multiscale modeling scheme in which the system is treated at different levels of detail based on the different molecular properties entering the stochastic equation that need to be evaluated. In particular, a united-atom description of the whole

molecular system is employed to build-up the free energy profiles along the alkyl chain torsion angles, while hydrodynamics modeling is employed to model the generalized friction (rotational and conformational). Then, stochastic, Langevin dynamics simulations are carried out to evaluate the spectral densities that enter in the calculation of NMR relaxation data.

Our results show a good agreement with the trend of relaxation times as function of the distance of the 13C carbon atom from the surface of the AuNP giving the possibility to rationalized the experimental observables in terms of local mobility. In particular, it emerges that the mobility of the single chains resembles that of an ideal chain made of connected n-butane-like bonds.
Since no free parameters have been employed, the proposed computational protocol, tailored to the study of the dynamics of flexible coatings of AuNP's, showed to be predictive when employing ad hoc theoretical tools suited for the problem. Specially, the employment of a novel strategy to calculate free energies (based on Jarzynski's equality [1]) recently introduced by some of us [2,3] turned out to be efficient to construct torsion free energy profiles which may display barriers even of several kB*T units. Our belief is that the proposed protocol, coupled to the information that can be obtained from NMR experiments, may become of particular help in the design of functionalized nanomaterials.

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P 123

EXPERIMENTAL PROTECTION OF STATES IN 1D AND 2D SUBSPACES ON AN NMR QUANTUM INFORMATION PROCESSOR

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In recent work, we experimentally demonstrated the freezing of evolution of quantum states in one- and two dimensional subspaces of two qubits, on an NMR quantum information processor. State evolution was frozen and leakage of the state from its subspace to an orthogonal subspace was successfully prevented using super-Zeno sequences [1,2]. The super-Zeno scheme hinges on a sequence of unitary pulses punctuated by pre-selected asymmetric time periods and has been worked out numerically for N <= 6. We demonstrate the efficacy of the scheme by preserving different types of states, including separable and maximally entangled states of known two-dimensional subspaces of two qubits on an NMR quantum information processor.The change in the experimental density matrices was tracked by carrying out full state tomography at several time points. We use the fidelity measure for the two-dimensional case, as qualitative indicators to estimate the resemblance of the density matrix at a later time to the initially prepared density matrix. For entangled states, an additional entanglement parameter is constructed to quantify the residual entanglement in the state over time. State fidelities, the leakage parameter and the entanglement parameter are plotted as a function of time, to quantify the performance of the super-Zeno sequence. A stronger result extending the above argument, has been derived analytically and has been categorized under the umbrella of dynamical decoupling schemes[3]. Dynamical decoupling schemes have been shown to be efficient in suppressing one- and two-axis decoherence. We also discuss the extension of the super-Zeno schemes to more general dynamical decoupling sequences that use layering to achieve state protection in one and two-dimensional subspaces.

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P 126
"IN CELL" SOLID-STATE NMR OF BIOLOGICAL MEMBRANES

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Biological membranes are essential components of any living cell. They dictate the shape of the cells, protect them from the environment and are at the heart of numerous biological processes ranging from molecular transport to enzymatic activities. Two of the major components of procaryotic membranes are the lipids which have more of a structural role and the proteins that are mostly responsible for those biological processes. In fact these two components are intimately linked, and a lot of membrane proteins need lipids to accomplish their function. Due to the heterogeneity of biological membranes (from a proteic and lipidic stand point) an "in vivo" or "in cell" approach would allow to better understand the relationship between this two components or at least to refine some information extracted from "in vitro" studies.

For now several decades, solid-state NMR has been extensively used for the study of native cell membranes, whether by ^{31}P, ^{1}H, ^{13}C or ^{2}H (Jacobs and Oldfield 1980). These "in-cell" or "in membrana" approaches have allowed to better understand the dynamic and structure of these membranes under various physical and physiological conditions. Conversely, the use of solid-state NMR to study membrane protein structures in their native environment is quite new (Fu et al. 2011, Renault et al. 2012) and remain a major challenge due to the inherent issues of membrane proteins (expression level, folding, stability,...).

Here we will discuss our current work on "in-cell" solid-state NMR of biological membranes. We will present first some developments we have made in ^{2}H MAS solid-state NMR of biological membranes, which

allowed us to characterize quickly, thus avoiding sample degradation, the dynamical properties of membranes. We will then focus on the characterization of a membrane protein, the b subunit of the F1F0 ATP synthase, in its native environment, using the C43λDE3 mutant of E. coli (Miroux et al. 1996 and Arechaga et al. 2000).

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P 129
INITIAL EXPERIENCE IN PEDIATRIC IMAGING WITH A HOMEBUILT XENON-129 HYPERPOLARIZER

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Despite decades of forward progress, the clinical translation of hyperpolarized-gas magnetic resonance imaging (MRI) remains unrealized due in part to intellectual property rights and slow commercialization and regulatory-approval efforts. While hyperpolarized ^{3}He is the preferred gas for pulmonary imaging (especially in pediatrics), the worldwide shortage of ^{3}He has reignited interest in hyperpolarized ^{129}Xe MRI. In progress toward this transition, a few groups have reported 'open-source' ^{129}Xe polarizers that can produce up to 0.5 L STP of 50% polarized gas. We present preliminary results on a scaled-up version of this open-source design of from our Center's homebuilt ^{129}Xe hyperpolarizer for human pediatric pulmonary imaging applications, constructed for less than €120k. Our overall goal is to contribute meaningfully toward expanding accessibility of hyperpolarized gases to speed clinical translation of the techniques.

The polarizer (approximate dimensions: 1.6 m length x 0.8 m width x 1.5 m height) features a custom-designed laser diode array capable of delivering 200 W power with a spectral width ≈ 0.19 nm full-width half-maximum centered at 794.7 nm. The narrow linewidth, which minimizes photons outside of the Rb D_1 line in this low-pressure (2 atm) regime, is achieved via five separate, individually thermoelectrically-cooled volume Bragg gratings and allows some tunability of the laser wavelength. The fully-enclosed module provides homogenous illumination of a 6-cm diameter x 31-cm length cell, providing 2 L STP of hyperpolarized gas at high polarization. A Rb reservoir outside of the main cylindrical cell body ensures that resonant light is not directly incident on the surface of the bulk Rb, reducing clustering that can reduce nuclear spin polarization.

The polarizer's lower chassis is spaced for standard rack-mountable components, which include the laser water chiller and power supply, B_0 coil power supply, cell temperature control, gas handling manifold, vacuum pump, and NMR spectrometer. The polarizer's four 60-cm diameter electromagnetic coils (spaced per the Barker arrangement, with 208 and 92 wraps for the outer and inner coils, respectively) provide a homogenous field of 53 Gauss (< 0.2% variation over the length of the cell), corresponding to a ^{129}Xe NMR frequency of 62.5 kHz. Xenon polarization is monitored via a 2.5-cm diameter surface coil and home-built NMR spectrometer; a separate polarization measurement system (Polarean 2881, Polarean, Inc.) provided calibrated measurement of the xenon polarization.

In situ ^{129}Xe polarization near 90% with a 50:50 N_2:Xe mixture at 2 atm has been achieved at modest temperatures (Rb reservoir ~125 °C) with a T_1 exceeding 2 hours; these polarizations exceed previously-reported values. Preliminary human imaging at 3T (Philips Achieva) demonstrates the utility of the device in a translational, clinical setting, in both adult and pediatric patients.

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SHEDDING LIGHT ON AGEING OF N–DOPED TITANIA PHOTOCATALYST

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Nowadays, titanium dioxide or titania attracts deep scientific interest as a promising nanomaterial for photovoltaic, water photospliting and air purification. Unfortunately, the widespread use of titania-based photocatalysts is restricted by a heavy requirement of operation using artificial UV illumination. It was shown by Asahi that the N-doping of titania seems to be a facile approach to overcome that drawback. Since than, many studies on N-TiO_2 has been published but only a limited number of papers have been devoted to explore a storage stability and operation durability of such material under different conditions, which is important in the context of practical application of the photocatalyst.

In present work we have provided a detailed analysis of N-dopant behaviour in N-TiO_2 storaged under different temperatures. Investigated samples were obtained by the method of thermally assisted reactions in aqueous sprays (TARAS) using urea as a nitrogen dopant precursor. We examined the sample obtained almost two years ago at 1% of urea concentration in the initial hydrolyzing solution annealed at 1000 ◦C (denoted TON1-S) and two as-prepared samples obtained at different concentration of urea in the initial hydrolyzing solution: 0.5% (denoted TON05) and 1% (denoted TON1) with the same calcination temperature. To monitor the temperature impact on storage stability a portion of TON1 sample was stored at 80 ◦C for totally 5 weeks (denoted TON1-T).

EPR spectra were detected by the standard Bruker EPR spectrometer ELEXSYS-500 (X-band, sensitivity of about $5 \cdot 10^{10}$ spin/G, modulation frequency 100 kHz). Computer fitting of the spectra were obtained using the EasySpin MATLAB toolbox. UV-vis absorption spectra were

obtained by means of the UV/Vis spectrometer Lambda35 (Perkin-Elmer, USA) operated in a diffusion reflectance mode in the range of 190-1100 nm.

During the work it was found that the EPR spectrum of the sample TON1-S shows a strong depletion of the nitrogen radicals signal with time. Exponential fit of this dependence gives an extinction coefficient R_0(20 °C)=-0.008 day^{-1}. A signal intensity reduction, for the first glance, can be attributed to the exhausting of N species when leaking out from the sample, which would mean an impairment of the samples photocatalytic properties. However, UV/vis spectroscopy data do not show any significant nitrogen concentration reduction in the structures. Therefore, the depletion mentioned is due to the internal transformation processes. A similar situation was observed in the case of short term ageing at slightly evaluated temperature (80 °C, sample TON1-T): the paramagnetic nitrogen concentration in the sample shows almost five-fold decrease during the experiment time (extinction coefficient R_0(80 °C)=-0.1day^{-1}). We assume the interstitial nitrogen atom reduction is most likely to be responsible for the observed EPR signal depletion.

It should be noted that the observed ageing of N-TiO_2 samples has not led to any significant impairment in their photocatalytic activity, which is an advantage, since even long-term temperature storage of such structures has no effect on their functional properties.

The experiments were performed using the facilities of the Collective Use Center at MSU. The authors are grateful to A. Tarasov, who has kindly prepared the investigated samples.

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11B MAS NMR INVESTIGATION OF SUPERSTRUCTURAL UNITS IN CRYSTALLINE LITHIUM BORATES

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The phase diagram of the Li_2O-B_2O_3 system is extremely rich in crystalline structures. Even though it has been explored over the last five decades, some structures have been only resolved very recently[1-2] The wide range of accessible compositions opens the possibility to follow closely the evolution of network topologies upon the addition of lithium oxide into pure B_2O_3. Among the different crystalline structures, LiB_3O_5, $LiBO_2$, $Li_6B_4O_9$, $Li_4B_2O_5$ and Li_3BO_3 are of particular interest since they are composed of one unique superstructural unit, corresponding to a particular arrangement of the two constitutive units, i.e. triangular BO_3 and tetrahedral BO_4 units. Each of these superstructural units gives information on the medium range order, which is of primary interest in the understanding of glasses and melts. Determining the spectroscopic signature of these superstructural UNITS would help to disentangle the complex problem of the structure of glasses.

^{11}B NMR has been widely used in order to characterize and quantify the proportion of four-fold coordinated boron in borate-derived materials. In the present study, we will use ultrahigh-field NMR spectroscopy to obtain precise NMR parameters for the three-coordinate borate units in crystalline lithium borates. Multiple-quantum MAS will provide unprecedented resolution of distinct BO3 units and improve the measurement of highly accurate chemical shifts and quadrupolar tensor components.

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IN SITU MAGNETIC RESONANCE IMAGING STUDY OF γ-ALUMINA PELLET IMPREGNATION

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In petroleum refining, γ-alumina is used as a solid support for hydrodesulfurization: a process of removing sulfur nuclei from fossil fuels. Following alumina extrusion, the obtained pellets are impregnated with catalysts, such as the salts of Mo or W, promoted by Ni or Co. They make the base of a trickle-bed reactor, through which petroleum or natural gas will pass, allowing the catalysts to prompt desulfurization of the molecules. The ever decreasing norms for the sulfur presence in gasoline require better and more efficient catalysis, encouraging extensive research of the materials and the factors influencing the process.

The penetration of ions into the center of an alumina extrudate can be followed with Magnetic Resonance Imaging (MRI) because their presence shortens the ^{1}H relaxation times, introducing a contrast into the image. Here, the alumina extrudates, obtained via industrial extrusion, contain traces of paramagnetic elements, precluding the use of spin-echo based pulse sequences. Hence, a solid-state MRI method, Single Point Imaging (SPI), was adapted to observe the impregnation of γ-alumina pellets with aqueous metal salt solutions. Rather than encoding a slice selection and phase gradients and acquiring a line of points at once, SPI uses simultaneous gradient encoding in all three directions and an acquisition of a single voxel, allowing the acquisition of fast-relaxing signal components.

Investigating alumina with different porosities, as well as varying the composition and concentration of the impregnating solutions permits to evaluate the parameters required for optimization of the process.

P 147
MOBILITY OF WATER MOLECULES IN POLYCRYSTALLINENATROLITE AND SECOND MOMENT OF 1H NMR SPECTRUM

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The dynamics of water molecules in natrolite ($Na_2Al_2Si_3O_{10}\cdot 2H_2O$) have been studied by NMR in [1-3]. From temperature measurements of the spin-relaxation times (T_1 and $T_{1\rho}$) of ^1H nuclei in natrolite it was concluded that in a temperature interval 330 K ¸ 450 K takes place the reorientation of water molecules around their pseudo-axes of second order symmetry (180^0 flip motion), and in an interval 450 K ¸ 540 K the availability of reorientation of water molecules around the one hydrogen bond has been assumed [1]. In the subsequent it has been established, that this second type of water molecules mobility is connected with diffusion of water molecules in the channels of natrolite and the 180^o flip motions take place simultaneously with diffusion along the c-axis [2,3].

The theoretical calculations of T_1 and $T_{1\rho}$ on the basis of different dynamical models require calculations of the second moments of NMR spectra of a range, both rigid lattice, and the lattice with molecular mobility [4]. In the case of monocrystalline samples these theoretical calculations can be carried out rather simply [5]. However in the case of polycrystalline samples the calculations of the second moment become complicated. In this communication on the example of polycrystalline natrolite the algorithm of the calculation of the second moment of NMR spectrum is considered. The obtained results were used for the interpretation of experimental data obtained in natrolite.

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P 153
ANOMALOUS TEMPERATURE DEPENDENCES OF LINEWIDTHS OF 57FE NMR IN MAGNETITE ABOVE THE VERWEY TRANSITION

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Magnetite Fe_3O_4 is a typical mixed valence iron oxide. This ferrimagnet has inverse spinel structure, in which the iron cations occupy two different types of sites: tetrahedral A and octahedral B positions. The A sites contain Fe^{3+} ions, whereas the average valence of the B site iron ions is 2.5+. When the temperature falls below ~120 K, the Verwey phase transition occurs, during which the cubic lattice of magnetite transforms in a monoclinic one and electrical conductivity drops by two orders of magnitude. The mechanism of the Verwey transition, as well as a charge ordering and related properties of magnetite has been drawing attention of researchers for several decades. Beside many experiments carried out on pure magnetite, an important direction of study concerns impact of intentionally introduced point defects on its electronic and magnetic structure.

Application of NMR in the research of magnetically ordered materials yields valuable information on electronic and magnetic structure of the material as the resonating nuclei serve as local probes of hyperfine field. In the case of magnetite, the ^{57}Fe NMR has been successfully employed for studies of both stoichiometric and defect-containing samples in many experiments. This work focuses on temperature evolution of NMR signal frequencies and linewidths measured above the Verwey transition. Single crystal samples of stoichiometric composition, as well as with various types and concentrations of substitution defects were investigated. Resonance signals originating from both the A and B sites were studied. The linewidths decrease with increasing temperature, which is behaviour anomalous for magnetic iron oxides. The data are analyzed in the context of electronic ordering and electrical conductivity mechanism of the high-temperature phase of magnetite.

P 156
MICRO AND NANO PATTERNABLE PARAMAGNETIC CARBON

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We have developed a micro and nano patternable carbonmaterial by pyrolyzing carbon-rich polymer precursors that exhibits strongroom-temperature paramagnetism and a superparamagnetic-like magnetic phase. This material offers tremendous MEMS and NEMS design fabrication capabilities with design flexibility owing to the fact that the starting materials used inthis process are common polymers such as photoresists.

The main principle of our inexpensive and scalable fabrication technique is to tune the fraction of the unpaired electrons in the ensuing carbon during its pyrolysis. Carbonization of a polymer via pyrolysisen compasses bond-dissociation and reconstruction.[1] By simply optimizing the thermodynamics and kinetics of this process one can obtain carbon with pre-defined properties.[2] We utilized this principle to produce our paramagnetic carbon [spin population: (3.97 ± 0.8) × 10^{17}/mg], which is extensively characterized for its properties and microstructure employing Electron Paramagnetic Resonance (EPR) spectroscopy, High Resolution Transmission Electron Microscopy (HR-TEM), Raman spectroscopy and magnetization studies. Micro-device fabrication capabilities of this material starting from a common photoresistSU-8 are also demonstrated.

There is an emerging consensus on the existence of magnetismin sp and sp^2 carbon materials, which is attributed to the presence of unpaired electrons at the exposed graphitic edges.[3] However, there is no convincing explanation of the mechanismof magnetic behavior

in elemental carbon that can be supported with reliable experimental data. Therefore, in addition to those who envision fascinating engineering applications using magnetic carbon in MEMS and lab-on-a-chipdevices, our novel material is also expected to be of great value toresearchers investigating the mechanism of carbon's magnetism.

The outcome of this work is expected to have an impact on various state-of-the-art research areas because (i) carbon's controversial magnetism interests both theoreticians and experimentalists, (ii) a robust and highly reproducible micro and nano patternable magnetic material is being introduced to the microfabrication community at an affordable cost, (iii) this material is chemically inert, which is an additional advantage for biological applications and (iv) the magnetic properties of this material are very stable, even at elevated temperatures, which makes it perfectly suitable for spintronics andmagnetic MEMS.

A brief review of the state-of-the-art, details of our fabrication methodology and its optimization for controlling the fraction of unpaired electrons, and the mechanism of magnetic properties in this material will be presented in the poster.

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POLYMERIC PROTON CONDUCTOR BASED ON MICROCRYSTALLINE CELLULOSE FUNCTIONALIZED BY IMIDAZOLE MOLECULES: THERMAL AND ELECTRICAL PROPERTIES

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The aim of the present study was the synthesis and characterization of a new biodegradable proton conductor (Cell-Im) based on microcrystalline cellulose (Cell) as the host polymer functionalized by imidazole molecules (Im) as dopant. The combination of selected natural polymers with heterocyclic molecules containing nitrogen atoms is the strategy which allows to find new, biodegradable and flexible proton conducting materials, which can be used in the temperature range above 100 ^{O}C under anhydrous conditions.

The polymer protonic conductors are important materials to apply for proton exchange membrane fuel cell, battery and sensor applications as solid electrolytes. A newly synthesized polymeric material, Cell-Im, is inexpensive to obtain, non-hazardous, environmentally friendly and can be considered as a solid electrolyte for application in electrochemical devices.

The prepared Cell-Im powder was analyzed by elementary analysis, differential scanning calorimetry (DSC), thermogravimetry analysis (TGA), and Fourier transform infrared spectroscopy (FTIR). To investigate the temperature behavior of the electrical property of the samples, the electrical impedance spectroscopy (EIS) measurements were performed. The equivalent measurements were also conducted for the powder sample of the pure cellulose in order to compare its properties with those obtained for the Cell-Im composite.

The obtained results have shown that the sample of Cell-Im under anhydrous conditions at 160 ^{O}C exhibits close to four orders of magnitude higher electric conductivity (up to approximately 2 x 10^{-4} S/m) than that of the pure cellulose. The thermal stability of the Cell-Im was confirmed in the temperature range from 110 to about 150 ^{O}C.

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IN-SITU CHARACTERIZATION OF POLYMERS BY COMPACT NMR

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Magnetic resonance is well known in the fields of chemical analysis and medical imaging, where typical instruments use superconducting magnets that generate high magnetic fields. However, the development of high-field NMR is limited by the cost and complexity of the equipment. During the last years considerable progress has been achieved with compact NMR machines employing permanent magnets, which provide sufficient sensitivity and robustness for nondestructive characterization in applications outdoors and on the factory floor [1]. Among the different applications in material science and chemical engineering, compact NMR machines promise novel applications for polymer industries where the quality of raw and intermediate need to be monitored during production in the chemical plants and after production during use of the product in the field.

This presentation focuses on different quantitative NMR methods for analysis of polymer materials. In particular examples of applications will be shown where open and closed low-field magnets were used to study thermally induced crystallization and the thermal degradation of polymers exposed to elevated temperatures in the presence of air. Moreover, solvent-induced crystallization was investigated for comparison with temperature-induced crystallization. Such investigations are relevant in application areas such as fluid transport in polymer pipes, coating technology, and paint applications including the restoration of painted artwork where paint layers and bulk polymers may be exposed to solvents.

The sensitivity of a compact NMR spectrometer capable of measuring high-resolution ^{1}H NMR spectra of polymer solutions was shown to be good enough to quantitatively monitor the wax content in solvents

from polymer pellets and determine the wax content as an indicator for the quality of the polymerization process during polymer production. Furthermore, the NMR-MOUSE (Mobile Universal Surface Explorer) was used to study the structural heterogeneity in polymer materials by scanning the sensitive slice across the sample volume [2]. By using an NMR-MOUSE with a short dead time semi-crystalline polymers such as crosslined polyethylene (PEX) can be studied accurately without significant signal loss from the crystalline domains. Moreover, the NMR-MOUSE was applied on polymer-modified asphalt (PMA) to estimate the polymer fraction and study the structural changes in different layers. PMA is a well-established product for improving the effectiveness of asphalt pavements and increasing fatigue resistance [3].

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SOLID-STATE NMR FOR THE INVESTIGATION OF BIOLOGICAL HYBRID MATERIALS

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Biominerals are ubiquitous in nature and can be found in almost every living organism. They are usually highly organized organic-inorganic hybrid materials with exceptional mechanical properties.[1] Biominerals based on amorphous silica occur mainly in plants, marine sponges and diatoms. The latter are unicellular eukaryotes which build porous, intricately patterned cell walls from silica. The cell walls contain organic molecules such as proteins, polyamines and polysaccharides. They are believed to control the silica precipitation and to act as a template for the biomineral. The mechanisms underlying this process are a matter of ongoing research. Their better understanding might lead to the development of novel synthetic hybrid materials.[1-3]

We describe how solid-state NMR can be used to study the silica formation processes in diatom cell walls. Triple-resonance NMR methods allow for the detection of polarization transfer between different heteronuclei, such as 13C - 29Si or 15N - 29Si. These techniques selectively highlight the interactions at the interface between the organic molecules and the silica. They thus give better insight into the interface structure. Furthermore, CP-REDOR experiments allow for an estimation of the distances between certain molecules at the interface. For all these experiments, high magnetic fields and isotope labelling of the samples are usually indispensable to gain sufficient signal intensity.[4]

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SOLID-STATE NMR STUDY OF CATION EFFECTS ON THE PHOTODIMERIZATION OF CINNAMATE SALTS

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Nanoparticles undergoing light-induced transformations (e. g., photodimerization) between structural phases with different optical properties are key components of optical gate and memory elements, thus can play an important role in miniaturization of digital technology. For example, upon exposure to UV light of $\lambda > 260$ nm in the solid state, the double bonds of two neighboring cinnamic acid molecules dimerize to form a cyclobutane ring; reversibly, the cyclobutane ring can be photocleaved upon exposure to UV light of $\lambda < 260$ nm [1, 2]. Therefore, this group of molecules has potential to be used as switching segments for instance in shape-memory polymers or embedded in porous materials [3-6]. Furthermore, cinnamic acid and its derivatives are small and relatively simple molecules, therefore they are also good model compounds to improve the understanding of solid state reactions in general. For the [2+2] photocycloaddition to occur in the solid state favorable crystal packing is very important. Different cations as counterions influence the arrangement of the anions and the reacting double bonds (e. g., distance and parallelism). Thus, the objective of this work is to investigate the influence of different alkali cations on the photoreaction, including characterization of reactant and product forms, studying the kinetics of the photoreaction and to identify spectroscopic changes due to cation replacement by solid-state NMR. In this study, four cinnamate salts (potassium 3-chloro-trans-cinnamate, potassium 3-bromo-trans-cinnamate, sodium 3-chloro-trans-cinnamate, ammonium 3-chloro-trans-cinnamate) were prepared and irradiated under UV for several hours and characterized by ^{13}C solid-state NMR. Distinction between the reactants and their photoproducts, notably identification of phase changes and the presence of crystallographic inequivalences and disorder are studied. The results highlight that potassium as

counterion showed better crystal packing and photoreaction than sodium and ammonium cations. We also report on the kinetics of photoreaction, especially of the potassium 3-bromo-trans-cinnamate salt.

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THERMAL AGING OF POLYAMIDE-12 BY 1H SOLID-STATE NMR

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Polyamide-12 (PA12) is a semi-crystalline polymer with improved impact resistance and lower moisture absorbance than many other commercially available polyamides. Thus, PA12 is used today in a variety of demanding applications including fuel lines, connectors, seals, conveyor belt, etc. In many of these applications, the macroscopic properties deteriorate under the impact of temperature. Thus, to improve the material performance and to develop reliable aging models which can predict the properties of a polymer at a given time, the molecular origin of the aging mechanism needs to be understood.

This study compares for the first time the thermal aging at elevated temperature up to 100 days of stabilized and non-stabilized PA12 samples. A detailed morphological study is done by a combination of ^{1}H solid-state NMR, Fourier-Transform Infrared (FTIR), and Differential Scanning Calorimetry (DSC). Based on simple ^{1}H FID measurements, three phases with different mobility could be distinguished for both kinds of PA12 samples: a rigid phase, a semi-rigid phase, and a mobile phase. Their amounts and the corresponding chain dynamics change for both samples with the exposure time. However, the non-stabilized sample shows the most prominent changes. The detected changes can be explained in terms of annealing and thermo-oxidation. The interpretation of the NMR results is supported by the DSC and FTIR data.

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HOMODECOUPLED 1,1- and 1,n-ADEQUATE NMR EXPERIMENTS: APPLICATION TO THE STRUCTURAL ELUCIDATION OF PROTON-DEFICIENT NATURAL PRODUCTS

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Pure shift NMR methods have recently been the subject of intense research focus. By collapsing homonuclear proton-proton couplings, resolution and experimental sensitivity both increase. Cryptospirolepine is the most structurally complex alkaloid discovered thus far from any Cryptolepis. Characterization of several degradants of the original sample a decade later called the validity of the originally proposed structure in question. We now wish to report the development of improved homodecoupled variants of 1,1- and 1,n-ADEQUATE (HD-ADEQUATE) and the utilization of these techniques in resolving long-standing structural questions associated with crytospirolepine. In addition, we evaluate the combination of NUS and homonuclear decoupling for the acquisition of both $^{1}J_{CC}$ and $^{n}J_{CC}$ homonuclear coupling constants in related J-modulated ADEQUATE experiments.

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EXTENDING LONG-RANGE HETERONUCLEAR NMR CONNECTIVITIES BY MODIFIED HSQMBC EXPERIMENTS

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The detection of long-range heteronuclear correlations associated with J(CH) coupling values smaller than 1-2 Hz is a challenge in the structural analysis of small molecules and natural products. LR-HSQMBC, HSQMBC-COSY and HSQMBC-TOCSY pulse schemes are evaluated as complementary NMR methods to standard HMBC/HSQMBC experiments. The re-optimization of the interpulse delay and the incorporation of an additional J(HH) transfer step in the HSQMBC pulse scheme can favor the sensitive observation of traditionally missing or very weak correlations and, in addition, facilitates the detection of a significant number of still longer-range connectivities to both protonated and non-protonated carbons under optimal sensitivity conditions. A comparative ^{1}H-^{13}C study is performed using strychnine as a model compound and several examples are also provided including ^{1}H-^{15}N applications.

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MULTIPLICITY-EDITING IN LONG-RANGE HETERONUCLEAR CORRELATION EXPERIMENTS: APPLICATION TO NATURAL PRODUCTS

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Even C/CH_2 and odd CH/CH_3 carbon-multiplicity information can be directly distinguished from the relative positive/negative phase of cross-peaks in a novel ME(Multiplicity-Edited)-selHSQMBC experiment. The method can be extended by a TOCSY propagation step, and is also fully compatible for the simultaneous and precise determination of long-range heteronuclear coupling constants. In addition, broadband homonuclear decoupling techniques can also be incorporated to enhance sensitivity and signal resolution by effective collapse of J(HH) multiplets. Strychnine, taxol, staurosporine, and sungucine are utilized as model compounds to demonstrate the usefulness of these techniques.

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THE IMPACT OF SUGARS ON ASTRINGENCY AS VIEWED BY NMR

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Astringency is governed by the interaction between polyphenols present in beverage such as wine and saliva proteins belonging from the Proline Rich family. It has been recently demonstrated that the interaction mode depends on the colloidal state in which the polyphenols are (1, 2). Below their Critical Micellar (or Aggregation) Concentration(3), polyphenols fix the proline rich saliva peptide in a specific way and with an affinity depending on the chemical nature of the polyphenols. Above, they interact in a non specific way leading to the precipitation of the polyphenols-protein complex. So that, one may wonder whether the finding of commonalities between the physico-chemical phenomena observed and the sensory descriptors used to describe the mouth feel sensation of astringency are relevant or not .

Astringent sensations are also influenced by other components present in beverage, notably the sugars content . In fact, the ripening of the fruit has been associated to the release of soluble fragments of pectin as the cellular structure of the fruit softens up. For this reason, the implication of polysaccharides in the sensation of astringency is commonly accepted.

In this work, we try to bring our contribution about this subject by working on models that can give some answers at a molecular level : were used for this study a define synthetic peptide representative of the proline Rich proteins repeat unit, a model of polyphenol such as epigalocatechin gallate and different sugars, from the simplest one, glucose to the more complex Arabic gum. These choices were done due to the content in sugars commonly found in wine in which two categories can bedistinguished, simple sugars or polysaccharides. After the fermentation step, simple sugars such as hexoses reach a concentration of 1 g/L in dry wine when polysaccharides can attain

a concentration up to 300 mg/L. Liquid state and HRMAS NMR spectroscopy were used to follow the interactions processes and permit to determine that Sugars were able to sequester polyphenols decreasing its active concentration susceptible to interact with PRP.

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NOVEL SULFANYL PORPHYRAZINES AS POTENTIAL BUILDING BLOCKS FOR BIOMEDICINE AND NANOTECHNOLOGY – NMR AND PHYSICOCHEMICAL STUDIES

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Porphyrazines (Pzs) are synthetic analogues of naturally occuring porphyrins. They consist of four pyrrole rings linked together with azamethine groups in place of methine bridges present in porphyrins [1]. Pzs may be modified in their periphery by various substituents. Among them, the sulfanyl groups are known to enrich them with interesting physico-chemical properties. Pzs revealed potential towards nanotechnology and medicine, especially as photosensitizers for photodynamic therapy of cancer and various noncancerous diseases [2,3].

Herein, we present novel Pzs with peripheral isophthaloxyalkylsulfanyl substituents. The sulfanyl porphyrazines were purified using chromatography and characterized by NMR, UV-Vis spectroscopy, and MALDI MS spectroscopy. Moreover, they were subjected to photophysical and singlet oxygen generation studies. The obtained sulfanyl Pzs may be considered as model compounds for the series of their hyperbranched dendrimeric derivatives with well-defined particle size and shape of potential applications in biomedicine.

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SOLID-STATE NMR INVESTIGATIONS OF NSAID DRIVEN LARGE OLIGOMERIC ASSEMBLIES OF THE ALZHEIMER'S DISEASE PEPTIDE AMYLOID-BETA

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Aggregates formed by the Alzheimer's peptide amyloid-β (Aβ) contribute significantly to neurodegeneration in Alzheimer's disease patients. In particular, small soluble oligomers constitute the toxic entity of Aβ species [1]. The non-steroidal anti-inflammatory drug (NSAID) sulindac sulfide has been proven to be a modulator of Aβ populations [2,3]. By employing solution-state NMR spectroscopy as well as biochemical assays, we studied the effect of the NSAID on Aβ. We find that sulindac sulfide reduces monomeric soluble Aβ populations and drives the formation of large non-toxic oligomeric complexes. Conventional MAS solid-state NMR spectra, as well as ^{13}C-^{19}F and ^{13}C-^{15}N TEDOR measurements are evaluated to elucidate structural details of the resulting Aβ-NSAID co-assemblies.

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UNUSUALLY HIGH ROTATIONAL BARRIERS IN SUBSTITUTED 5-NITROSOPYRIMIDINE DERIVATIVES

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We succeeded in preparation of a series of substituted 5-nitrosopyrimidine derivatives with strong intramolecular hydrogen bonds which might work as purine mimics and theoretically target two different metabolic pathways. Two strong intramolecular hydrogen bonds lead to two stable conformers differing only in a geometry of nitroso group. These two rotamers were observable as two sets of signals in NMR spectra. The relative concentration was estimated from 1H NMR signals integration. In some cases, it was possible to separate both conformers from each other at room temperature. The chemical structure of both separated rotamers in solid state was confirmed by NMR spectroscopy as well as by X-ray analysis. Thanks to the rotamer isolation, we could measure kinetics of rotamer interconversion and determine rotational barriers to be higher than 20 kcal/mol. Having the clear evidence of the structure of both separated rotamers and kinetics of their interconversion, we considered this behavior as a special case of atropisomerism. We compared atropisomers with our rotamers and the most obvious difference is their mode of stabilization. Contrary to atropisomers, which are stabilized by sterically hindered rotation, our rotamers are isolable thanks to the strong intramolecular hydrogen bonds. This significant difference led us to make a distinction from relatively common atropisomerism. We suggested a term 'planamerism' and defined planamers as small aromatic molecule rotamers with planar conjugated moiety that are isolable as chemical species. In this work, we prepared a new series of 5-nitrosopyrimidine derivatives with strong intramolecular hydrogen bonds with variety of substituents in position 2 on pyrimidine skeleton. It was found that the more electron-donating substituents is (e.g. NH2

or NH-CH3), the higher the rotational barrier around C5-NO bond is. The presence of nitroso group has significant influence on the chemical shifts in the whole molecule. For instance, nitrogen atom from 2-**N**H-CH3 group is approximately 20 ppm lower in the case of 5-nitroso derivative than without NO. Furthermore, we found that the C2-NH bond has partially double-bond character, which indicates to a "push-pull" system. Experimental data were complemented by DFT calculations.

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EXTENSIONS AND LIMITS OF THE ASAP-HSQC

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Previously we introduced the ASAP-HSQC as a fast method for the detection of heteronuclear single quantum coherence spectra of small molecules at natural abundance. Here we are exploring the limits of the experiment by combining the ASAP approach with other time saving techniques. This allows the acquisition of HSQC spectra in a couple of seconds. Further developments of the experiment will be presented, which include for example a multiplicity edited version of the sequence.

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SPARSE PRINCIPAL COMPONENT ANALYSIS ADAPTED TO NMR METABOLOMICS

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In NMR metabolomics, sets of spectra of a mixture of metabolites, e.g. blood plasma or urine, from various individuals are analyzed. Peak intensities corresponding to various compounds vary from spectrum to spectrum, which is associated with changes in the composition of a mixture. These variations are usually too complicated to be recognized and explained by sight. Thus, various techniques have been suggested to identify varying peaks of spectra, group them according to the compounds they refer to, find correlations between the changes of concentrations and divide the samples into particular groups. These groups will represent deviations of metabolites possibly connected to illnesses or other factors.

One of the techniques used for this aim is Principal Component Analysis (PCA). Mathematically, a data matrix X of dimensions (n x p) of n spectra of metabolites, each consisting of p spectral points, has to be analyzed (usually p>>n). PCA searches for a directions of highest variance in the data and constitues a new basis for such a representation of X that: 1) requires less dimensionality, 2) easily allows to divide the set of samples into meaningful categories. Instead of p dimensions used for initial data representation, only few axes in a suitable basis are sufficient to explain the majority of the variance of spectral points from sample to sample. Such axes are called principal components. They correspond to the eigenvectors of the covariance matrix of data X.

If only a small part of p variables contribute to the meaningful variance of data, which is the case with NMR spectra, it is convenient to use sparse PCA [1]. It looks for principal component vectors with

mostly zero entries, which can approximate the eigenvector decomposition of X well enough. Various algorithms were proposed for sparse PCA realization outside the NMR context [2].

Here, we discuss another modification of sparse PCA designed especially for NMR spectra. It takes the assumption of Lorentzian shape of spectral peaks. The idea is based on one proposed in [3], where the authors introduce iterative thresholding on components into a certain variation of PCA (QR algorithm). We suggest substituting subtraction performed by thresholding at each iteration with placing Lorentzian peaks of adjustable widths above a threshold. Thus the components will better fit for the shape of spectra, providing a more accurate approximation and an easily interpretable result.

We discuss the features of the proposed method on the example of both simulated spectra and a real dataset from a medical study.

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ASYNCHRONOUS THROUGH-BOND HOMONUCLEAR ISOTROPIC MIXING: APPLICATION TO CARBON-CARBON TRANSFER IN PERDEUTERATED PROTEINS UNDER MAS

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Nowadays, efficient homonuclear mixing scheme, which can be employed under rapidly increasing MAS rates is required. We introduce an isotropic homonuclear 13C-13C through-bond mixing sequence, MOCCA-XY16 in application for the solid state NMR. This strategy performs well without rotor synchronization and significantly reduces the load for the used hardware. This method was applied on the deuterated the SH3 domain of a-spectrin and further tested for hydrophobin with fast and ultra-fast magic-angle spinning rates in the absence of proton decoupling. We compare current method with the different mixing schemes, which were previously reported. Current mixing sequence is particularly useful for ultra-fast spinning rates and high external magnetic fields. Presented pulse sequences can also be applied to fully protonated samples.

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METABOLOMIC PROFILING OF THE PARKINSON'S DISEASE RELATED CATP-6 GENE IN CAENORHABDITIS ELEGANS

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In this study we investigated the metabolomic composition of whole worm extracts from C. elegans wildtype and the Parkinson's disease (PD) associated mutant strains catp-6 ok3473 and catp-6 tm3190, using a µ-NMR based approach. The human ortholog of catp-6 is the human PARK9 gene, which encodes a lysosomal P-type transmembrane cation transporting ATPase. It is estimated that the degradation function of lysosomes is impaired in catp-6, resulting in the accumulation of cytotoxic alpha-synuclein and consecutive neurodegeneration [1]. This disease is known as Kufor-Rakeb syndrome with an early juvenile-onset and subacute Parkinsonism often accompanied by dementia and other neurological signs.

Worm extracts were prepared with a focused ultrasonicator in polar aqueous methanol solution and vacuum concentrated to individual worm pellets. One- and two-dimensional NMR experiments (1H, JRES, TOCSY) were performed with a commercial high resolution magic angle spinning (HRMAS) probe (25 µL sample volume) on a Bruker Avance III 500 MHz system (11.7 Tesla), in order to identify metabolomic alterations. 1H NMR spectra were used for statistical analysis [2] and data based metabolite assignment with the Chenomx NMR Suite 8.2. Phase and baseline corrected spectra were referenced to TSP, normalized to peak sum intensities, and pareto scaled. Advanced bucketing with the Bruker AMIX software resulted in a matrix of 378 chemical shifts. Analysis of variance ANOVA ($p < 0.5$) scores served as criteria to identify significant features.

Assignment of statistically relevant peaks resulted in the identification of 20 altered metabolites. Seven compounds (ATP, betaine,

glutamate, glutamine, lactate, succinate and trehalose) were found at higher ratios in wildtype spectra and 13 metabolites were identified to be significantly increased in catp-6 (alanine, AMP, choline, formate, fumarate, GTP, hypoxanthine, isoleucine, leucine, phenylalanine, tryptophan, tyrosine and valine).

Previous studies on catp-6 mutants identified strong morphological and functional changes in their mitochondria [1]. Our results of altered TCA metabolites (fumarate, succinate), branched-chain amino acids (leucine, isoleucine and valine) and nucleotides (AMP, ATP and GTP) appear to support these findings. Formic acid is known to inhibit the mitochondrial respiratory chain complex IV [3] and high hypoxanthine in catp-6 indicates an increased nucleotide salvage pathway. Alterations in the remaining metabolites may be the result of the recently discovered activation of AMPK and inhibition of mTOR pathways [1] together with a catabolic response to recover energy production.

These results of catp-6 defective C. elegans demonstrate how its human ortholog PARK9 might contribute to Parkinsonism and the Kufor-Rakeb syndrome. The use of µ-NMR for metabolomic profiling of worm extracts proved to be a suitable and versatile tool. Future studies should thus investigate other PARK genes in the same way to obtain a more complete impression of the bigger PD picture.

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NMR SPIN CHROMATOGRAPHY IN LIPID RESEARCH

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Selective one dimensional (1D) total correlation spectroscopy (TOCSY) nuclear magnetic resonance (NMR) experiment has become an important NMR technique for establishing 1H-1H connectivity via scalar coupling in small- and medium-sized molecules. By optimizing the magnetization transfer parameters, excitation frequency and mixing time the proton network of a selected molecule can be revealed thus allowing a spin chromatographic separation.

The technique has been applied in the lipid fraction of two common dairy products, milk and cheese. The experimental parameters were optimized allowing the spin chromatographic separation and quantification of selected analytes i.e. 18:2 conjugated linoleic acids, caproleic acid, glycerol moieties of 1,2 DG and 1, and 2, MG, even in strongly overlapped regions of the corresponding 1D 1H spectrum.

The proposed spin chromatography method can become a technique/procedure of primary interest in lipid analysis and metabolomics since it compares favorably with 2D TOCSY experiment and diffusion ordered spectroscopy (DOSY) due to increased digital resolution, absence of dynamic range issues and decreased experimental time [1,2].

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NMR-BASED LIPIDOMIC ANALYSIS OF REDBLOOD CELL MEMBRANES FOR THE IDENTIFICATION OF BIOMARKERS OF THE PRESENCE AND PROGRESSION OF ISCHEMIC HEARTDISEASE

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Background: Alterations in the composition of red blood cell membranes have been regarded as an important contributor to the initiation and progression of atherosclerosis leading to Ischemic Heart Disease (IHD). In the present study, we investigated the ability of NMR-based lipidomic analysis of red blood cell membranes to identify novel lipid biomarkers monitoring IHD disease progression.

Methods: Whole blood samples from 85 men with IHD [36 with one (mild), 35 with two (moderate) and14 with triple (severe) vessel disease)], and 18 men with normal coronary arteries (NCA) age- and conventional lipid parameters-matched and all angiographically documented, were collected after an overnight fast. The total lipid content of the membranes of isolated red blood cells was extracted according to a standard procedure and pattern recognition analysis was applied on the ^{1}H NMR lipidomic data recorded on a Bruker DRX-500 Spectrometer.

Results: The NMR-based lipidomic analysis showed that patients with severe IHD presented statistically significant different lipid profile of the membranes of red blood cells from those recorded from NCA patients. The alterations occurring in fatty acid pattern, mainly in saturated and unsaturated fatty acids, as well as in cholesterol and

phospholipid (phosphatidylcholine and sphingomyelin) content were able to distinguish patients with severe IHD from those with NCA. Moreover, the lipidomic analysis showed a trend for separation of patients at different disease stages (mild, moderate and severe) from those with NCA.

Conclusions: NMR-based lipidomic analysis reveals significant alterations in lipid composition of red blood cell membranes that possibly influence their shape, fluidity and biological functions. These lipid alterations could constitute novel biomarkers for the early evaluation of the presence and progression of IHD.

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RESIDUAL DIPOLAR COUPLING-ACCELERATED MOLECULAR DYNAMICS FOR STRUCTURAL ELUCIDATION OF SMALL MOLECULES WITH INCREASING FLEXIBILITY

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Time averaged molecular dynamics simulations with orientational constraints (MDOC) [1] can be performed based on residual dipolar couplings (RDCs). The tensorial properties are computed as time averages over the MD trajectory and pseudo forces are derived after comparison with experimental values which allow fast sampling of the global rotation and internal dynamics. The full tensorial calculation performed allows improved characterisation of the orientation of the system. This contrasts to other approaches for RDC analysis, where an alignment tensor is calculated for a sterically fixed orientational model, and makes the approach in principle very suitable for the structural analysis of molecules.

The MDOC protocol is implemented in the program COSMOS [2]. We demonstrate here the applicability of this methodology for a set of organic molecules with different degrees of flexibility: from rigid models to flexible compounds using $^1D_{CH}$ couplings as constraints. The run is monitored with two parameters such as a quality factor for the correspondence of experimental (imported) and calculated data and the overall temperature of the MD simulation. The quality measure for the RDCs is defined as the summed squared difference of the experimental and calculated RDC divided by the respective error. High quality measure and low overall temperature during the simulation serve as an indication of configurational assignment being correct. Here, we present our results obtained within the evaluation

process for a number of test molecules with increasing flexibility: norcamphor, staurosporine and spiroindene and oidiolactone B.

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BIOCHEMICAL EFFECTS OF RESVERATROL IN RAT URINE BY NMR- AND MS-BASED METABOLOMICS ANALYSIS.

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Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring non-flavonoid polyphenol abundant in peanuts, grapes, and other foods widely consumed as part of the Mediterranean diet [1]. It has been suggested to have many different health-promoting effects including antioxidant, anti-inflammatory, antitumor, anti-platelet aggregation, cardioprotective, neuroprotective properties, aging-delay effects as well as prevent ischaemic injuries [2].

Its non-invasive and ease of multiple collections make urine a popular biological fluid for analytical studies. Generally, NMR spectra of urine contain thousands of signals derived from hundreds of endogenous molecules present in this fluid with complex biochemical nature. The combined use of MS and NMR data in multivariate statistical analysis can benefit from the complementarity of the two approaches [3-5].

In continuation of our previous work on in vivo studies of phytochemicals such as Curcumin [6] we evaluated the effect of daily intragastrically administered RES (100 mg/kg of body weight) to 12 healthy rats (6 males, 6 females). Treatment was started on day 7 and carried out until day 36 and urine was collected over the time-period of 50 days with the interval of days 1, 7, 15, 22, 29, 36 and 50. Urine collection stopped at day 50 (two weeks after the end of treatment) to evaluate the time and treatment effect of RES.

Twenty four hour urine samples were analyzed by ^{1}H-NMR and UPLC-QTOF/MS and both sets of data were independently studied both by untargeted and by targeted multivariate statistical methods.

Urine samples collected from the treated animals were mainly characterized by the presence of resveratrol and its metabolites, i.e., resveratrol glucuronide, resveratrol sulfate, dihydroresveratrol, and dihydroresveratrol glucuronide. These metabolites were identified both in the ^{1}H-NMR and in the MS spectra and excluded from the statistical analysis.

Several biochemical markers were found in different concentrations in the treated group. These differences included increased urinary concentrations of hippuric acid, TMAO, and glycine, as well as decreased levels of taurine, allantoin, creatinine, 2-oxoglutarate, succinate and citrate. Changes within the same group of animals also occurred with time, and were recognized by the statistical analysis.

The effect of time and of treatment will be fully discussed and interpreted in terms of the effect that resveratrol might exert on biosynthetic pathways involved in oxidative energy metabolism.

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IN-SITU NMR METABOLOMICS ON A CHIP AS A TOOL FOR DEVELOPING OLIGONUCLEOTIDE GENE THERAPIES

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This work aims at providing a convenient microfluidic NMR platform to support the development and optimisation of oligonucleotides for gene silencing. The manipulation of gene expression by target-specific oligonucleotides holds promise for developing novel therapeutic approaches. Due to the marked difference between the energy metabolism of primary and cancer cells, modulation of metabolic pathways may provide opportunities to develop highly specific anti-cancer drugs. In the present work, an oligonucleotide that hinders the expression of glucose phosphate isomerase (GPI) has been developed. GPI plays an important primary role in the glycolytic pathway. In a secondary role, it is also implicated in the process of metastasis.

The development of target-specific oligonucleotides is currently a laborious process requiring a large number of cell cultures to separately test different candidate structures. This is usually done at the scale of well plates, which require substantial amounts of each oligonucleotide candidate to be synthesised. The knock-down effect of each candidate is then judged by quantitative PCR.

By contrast, we have recently demonstrated that it is possible to directly observe the metabolism of cells in µl-sized lab-on-a-chip systems. The main objective of the present work is to replace or complement the conventional approach to oligonucleotide development with a microfluidic NMR platform.

Our setup is based on expendable microfluidic chips that support a culture of 5000–10000 adherent cells in a 5 µl of perfusion medium. These chips are incubated at physiological conditions, and can be periodically inserted into a dedicated transmission-line NMR probe in order to obtain time-resolved data on the exa-metabolome. This system achieves 1H sensitivity of 10 nmol ·s 1/2 and resolution of better than 0.01 ppm, thus allowing direct quantification of the effects of candidate oligonucleotides on the metabolic rates. In turn, this enables efficient testing of oligonucleotide candidates and transfection strategies.

P 222
FRAGMENT-BASED APPROACH IN DRUG DISCOVERY

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Lead identification is an essential step in drug discovery process. Two main approaches are established for early stage drug development – fragment-based drug design (FBDD) used in academia and high-throughput screening (HTS). FBDD relies on weakly binding small organic molecules (Rule of Three compliant) that are elaborated (chemically modified) into more potent compounds. Huge advantage of FBDD in comparison with HTS is that only fraction (hundreds) of molecules are necessary to be screened against molecular target to sample even larger proportion of chemical space.[1]

We use two types of ligand observed NMR experiments - Saturation Transfer Difference (STD) and Water-Ligand Observed via Gradient SpectroscopY (WaterLOGSY) - to discover potential fragment hits. Studied biological targets include integrase binding domain (LEDGF/p75) involved in transformation of hematopoietic cells to leukemic, ubiquitin E2 variant (UEV) domain, the part of ESCRT-I complex and serine/threonine-protein kinase 6 (Aurora A kinase) implicated with important cell processes (meiosis & mitosis). The fragment-based approach will be demonstrated on some practical examples.

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REDOX- AND MEMBRANE MIMETIC-INDUCED CONFORMATIONAL AND DYNAMIC CHANGES IN THE N-TERMINAL REGION OF MYCOBACTERIAL PROTEIN KINASE G (PKNG)

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Mycobacterium tuberculosis protein kinase G (PknG) is a eukaryotic-like ser/thr kinase mediating intracellular survival of mycobacteria by blocking phagosome-lysosome fusion within host macrophages [1]. The protein has three folded domains, an N-terminal rubredoxin-like metal binding motif (RD, ~74-147), the catalytic ser/thr kinase domain (~147-390) and a C-terminal tetratricopeptide repeat domain (TPRD) (~391-750), which generally plays an important role for protein-protein-interactions [2]. The N-terminal 73 residues are intrinsically disordered (NORS) and harbour the only site (T63) phosphorylated in vivo [3,4]. Deletions or mutations in the NORS or the redox-sensitive RD significantly decrease the survival function of bacteria. It has been shown that PknG is more active in the presence of an oxidizing agent [4]. Here, we present the initial NMR characterization of the N-terminal regions and their assignment as well as of the redox-regulated un- and refolding of the RD and its ability to interact with membrane mimetics. Based on the presented data, controlled unfolding of the RD by oxidization may regulate the kinase activity. The detected membrane mimetic interactions may play a role for PknG localization.

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1H NMR BASED METABOLOMICS PROFILING AS POTENTIAL DIAGNOSTIC TOOL FOR IRRITABLE BOWEL SYNDROME

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Irritable Bowel Syndrome (IBS) is a chronic disease with affecting 20% of human population. The reasons of these changes are not fully defined because of their great complexity. However is regarded that IBS is related to stress and environmental factors. Diagnosis of this disease is mainly based on various clinical symptoms and performing of the colonoscopy. Because of invasiveness and lack of information, colonoscopy it is not preferred, however there aren't any methods of verification with are fully standardized as diagnostic tools for IBS. Therefore, the additional support in the diagnostics of IBS is required and application of metabolomic studies of faeces samples can be very helpful.

In our studies based on the use of ^{1}H NMR spectroscopy, we measured 72 samples of chloroform extract of faeces. The samples belonged to the two groups: patients IBS containing subgroups (depending on symptoms, n = 50) and control (n=22). The application of chemometrics methods allowed to create models for discrimination healthy subjects from patients regardless subclasses of disease. This finding can be very informative for medical doctors in early diagnostics of potential IBS patients. However it is essential to conduct research on a larger number of samples.

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LAB-ON-A-CHIP PERFUSION SYSTEMS WITH GAS EXCHANGE FOR IN-SITU NMR METABOLOMICS OF MICROFLUIDIC CELL CULTURES

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A novel approach to microfluidic cell culture that allows in-situ and non-destructive metabolomic studies by NMR spectroscopy is presented. Microfluidic culture of cells and tissue samples holds great promise for medical diagnostics, drug development, and drug safety testing, since it provides very precise control of the extracellular microenvironment.

As a versatile and non-destructive analysis technique NMR spectroscopy is extremely convenient for real time monitoring of living cells. However, integration of NMR with microfluidic lab-on-a-chip systems poses a number of technical challenges. The materials and design of the microfluidic chips are usually driven by biocompatibility, fluidic func- tionality, and micro-fabrication concerns. In-situ NMR spectroscopy with satisfactory sensitivity and resolution poses additional constraints in terms of magnetic susceptibility (to restrict line broadening) and background NMR signals from the chip materials.

In this contribution we present an NMR friendly, compact and integrated lab-on-a-chip device that allows cell culture at the 1 µl-scale under physiological conditions. The chip design is based on poly(methoxy methacrylate) (PMMA), using poly(dimethyl siloxane) membranes for gas exchange. These chips allow cell culture under normoxic conditions, and can directly be inserted into a specialised micro-NMR transmission line probe.

After seeding with cells, the chips are incubated in a 4% CO2 atmosphere at physio- logical temperature. Perfusion and gas exchange are driven by a piezoelectric peristaltic pump integrated in the chip.

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METABOLOMICS ANALYSIS OF BIOFILM DEVELOPMENT IN ASPERGILLUS FUMIGATUS AND QUORUM SENSING MECHANISMS BASED ON ARACHIDONIC ACID.

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The development of drug resistance mechanisms among many microorganisms is a biological phenomenon, though they can effectively counter the medical associations and exerting a negative impact on the health and life of humans and animals. During last decades, these mechanisms are constantly evolving and therefore invasive infections can be caused by more broad spectrum of drug-resistant strains. The mechanisms of drug resistance concern not only bacteria. In recent time, a major problem is occurring among the population many diseases caused by a variety of opportunistic fungal strains (Candida spp, Aspergillus, Fusarium, Geotrichum), particularly in immunocompromised patients. One of them is found world-wide filamentous fungi of Aspergillus fumigatus (approximately 65% of infections).

In our studies we used ^{1}H NMR-based metabolomics approach to analysis metabolic changes during biofilm development of Aspergillus fumigatus. Moreover, in this study was investigated by the effect of arachidonic acid on the growth and metabolism of mold of the A. fumigatus species, both planktonic and biofilm form. Finally, we demonstrated the main differences in metabolism after stimulating of arachidonic acid in Aspergillus fumigatus.

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DEGRADATION OF ATRAZINE BY FENTON SYSTEM STUDIED WITH ESR, NMR AND QUANTUM CHEMISTRY CALCULATIONS

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Atrazine is one of the most widely used herbicides in the United States and in most major agricultural nations outside of the European Union. It is known to be a persistent compound, although its half-life can differ extremely depending on external factors. Traces of atrazine are found in surface waters and groundwaters. Recent studies have reported potential toxicity and xenoestrogenic activity of atrazine, especially toward aquatic organisms, thus suggesting that the presence of this compound (being one of endocrine-disrupting compounds (EDCs)) can be treated as an environmental hazard. In order to degrade the xenoestrogens to potentially less toxic compounds a number of advanced oxidation processes has been proposed as an attractive approach to the treatment of polluted waters. Among these, the process utilizing Fenton reaction is one of the most often used methods.

In this work, the path of atrazine degradation by Fenton reaction has been studied with the aid of DFT calculations, spin trapping ESR, ^{1}H and ^{13}C NMR, and molecular docking. Total energy analysis of the reaction pathway was performed by DFT calculations at different levels and basis sets (B3LYP/6-31G(d,p), MO6X/6-311G+(d,p)) in gas phase and in water. The DFT calculations revealed the formation of six atrazine-derived radicals during the degradation of atrazine to HA (hydroxyatrazine), DEA (deethylatrazine) and DIA (deisopropylatrazine) metabolites. To verify these results, various spin traps, namely 4-hydroxy-5,5-dimethyl-2-trifluoromethylpyrroline-1-oxide (FDMPO), (a -4-Pyridyl-1-oxide)-N-tert-butylnitrone (POBN) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO), were used to study the

formation of radicals during the degradation of atrazine by Fenton reaction with ESR spectroscopy. The obtained ESR spectra were analysed with the help of simulations using the EasySpin package for Matlab.

DFT calculations of NMR chemical shifts of atrazine and its metabolites were in good agreement with experimental results. Additionally, the atrazine as well as its metabolites were evaluated based on their affinity to bind to estrogen receptor. The calculations revealed that all studied atrazine metabolites have lower binding affinity than the parent molecule.

P 240
DEVELOPMENT OF NMR IN HIGH PULSED MAGNETIC FIELDS AT THE LNCMI-T

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Nuclear magnetic resonance (NMR) is a very powerful technique to study the magnetic, electronic and structural properties of matter. As sensitivity and resolution of NMR rises considerably with magnetic field strength, there was during decades a strong trend towards higher fields in commercial NMR magnets. Magnetic fields higher than 45 T can currently only be produced by pulsed-field magnets which offer an upper field limit of more than 100 T [1], over timescales of milliseconds. Evidently, new applications and further development of NMR in high fields, which can only be generated using pulsed magnets, will bring scientific profit in condensed matter physics and other areas.

Since 2009 development work aiming on the implementation of NMR experiments in pulsed high-field magnets is performed at LNCMI. A pulsed NMR spectrometer managed by a computer has been constructed. The spectrometer operates in the frequency range from 250 MHz to 1 GHz. In addition, a cryogenic tunable probe optimized for NMR experiments in pulsed fields was developed. The probe operates in the 1.3–300 K temperature range. In order to reduce a noise level an in-situ cryogenic pre-amplifier setup has been developed and successfully tested.

An effort has been made to achieve a better resolution and sensitivity by constructing a new homogeneous pulsed magnet capable of generating field pulses up to 55 T. Our recent investigations of the field homogeneity in this new magnet showed homogeneity of 10 ppm across a reasonable sample volume of several at 13 T and 30 ppm at 47 T, which are the best values ever observed for this type of magnets.

This progress allows measuring spectra with a better resolution and using samples of a larger size, hence giving an increased signal-to-noise ratio.

Recently we successfully performed ^{11}V-NMR experiments on an $LiCuVO_4$ single crystal in pulsed magnetic fields up to 50 T, and compared with results obtained in static magnetic fields [2]. These results prove the efficacy of NMR on real physical system in pulsed magnets.

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REAL-TIME INVESTIGATIONS OF LI- AND NA-ION BATTERIES BY AUTOMATIC TUNING MATCHING CYCLER (ATMC) IN SITU NMR SPECTROSCOPY

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Li-ion batteries (LIBs) are the most desirable form of energy storage but an increasing demand of Li commodity chemicals combined with geographically-constrained reserves might drive up Li prices in the future. Due to the high abundance, low costs and very suitable redox potential, Na-ion batteries (NIBs) should open new avenues of research as complementary alternatives to LIBs. The shift from Li to Na batteries has to be accompanied with a deeper understanding of the chemical reactions involving the multiple cell components in these newer systems. The application of a non-invasive analysis tool that can follow the reactions in operando is therefore highly desired.

We are developing and exploring the use of a new Automatic Tuning Matching Cycler (ATMC) in situ NMR probe system – to track the formation of intermediate phases and investigate electrolyte decomposition during cycling of LIBs and NIBs. The application of in situ NMR[1]comes with challenges. Significantly different shifts of the multi-component samples, changing sample conditions during electrochemical cycling, signal broadening due to paramagnetism and bulk magnetic susceptibility effects as well as interferences of the NMR and external battery cycler (EBC) circuit impair the experiments. The ATMC in situ NMR approach addresses many of these difficulties allowing an "on-the-fly" adjustment of the NMR circuit during the measurement. Moreover, new sample holder and NMR-EBC-connection designs benefit the real-time experiments regarding battery compression, filling factor, and shielding.

We applied ATMC in situ NMR on $LiFePO_4$ (LFP) and $Na_3V_2(PO_4)_2F_3$ cathodes as well as Li and Na metal anodes. Frequency step experiments enabled the detection of the strongly

paramagnetic broadened ^{7}Li NMR signal of LFP. In situ ^{7}Li NMR reveals significant changes of the line shape in agreement with the structural changes due to delithiation in which a complete overlap of the LFP with the Li metal and electrolyte signals still limits the analysis. ^{31}P in situ NMR overcomes the problem and shows full separation of the electrolyte and cathode NMR signal. Cell design and synchronisation of NMR-EBC-connection are currently optimised for high rate cycling of LFP to track intermediate and metastable solid solution phases recently described[2]. $Na_3V_2(PO_4)_2F_3$ is reported as a cathode in NIBs with rapid Na motion being at least partially responsible for a good rate performance[3]. Complex ex situ ^{31}P NMR signals indicate the high sensitivity on the structural changes due to Na extraction. ATMC in situ ^{31}P on $Na_3V_2(PO_4)_2F_3$ offers a next step of insights into the mechanism of Na extraction from the cathode when used in a NIB. Furthermore, we applied ATMC in situ ^{23}Na NMR on symmetrical Na—Na cells. An "on-the-fly" adjustment of the NMR carrier frequency while measuring the Na metal or Na electrolyte signal allow a quantification of the on-resonant species. Changes of the Na metal line shape indicate the formation Na microstructures. Additionally, an in situ quantification of the Na-ion consumption from the electrolyte is possible.

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SPIDYAN - A MATLAB LIBRARY FOR SIMULATING ULTRA-WIDE BAND EPR

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While short rectangular microwave pulses in pulse electron paramagnetic resonance (EPR) spectroscopy can cover bandwidths of about 100 MHz, spectral widths of paramagnetic transition metal complexes usually exceed 1 GHz. This limited excitation bandwidth significantly restricts the application of pulse EPR sequences on such compounds. With the recent advent of arbitrary waveform generators (AWG) with sampling rates in the GS/s range it has become possible to extend the excitation bandwidth up to and beyond 500 MHz, into the ultra-wide band (UWB) regime [1].

AWGs allow for excitation with frequency-swept pulses, which provide effective flip angles between 0 and π. While these passage pulses are well known in NMR spectroscopy, their effects are not yet fully understood in EPR. In need of a package for simulation of frequency swept pulses the open-source SPIn DYnamic ANalysis (SPIDYAN) library was developed, which runs in the MATLAB environment. If relaxation can be neglected, SPIDYAN uses propagators to evolve spin density matrices in Hilbert space. For simulations explicitly considering relaxation effects, the program solves the quantum mechanical master equation in Liouville space [2]. SPIDYAN provides routines to simulate bandwidth limiting effects of resonators on pulse EPR experiments.

By exciting electron spin echoes with frequency swept pulses on a home-built UWB EPR spectrometer [3], based on an AWG with a sampling rate of up to 12 GS/s, we measured electron spin echo envelope modulation (ESEEM) spectra on single crystals of γ-irradiated malonic acid and Cu2+ in TiO2 (rutile). By recording the entire echo it is possible to correlate the ESEEM spectrum to the EPR spectrum [4], resulting in two-dimensional spin echo correlation spectra, which provide a higher resolution.

We were able to reproduce the experimental EPR/ESEEM correlation spectra with SPIDYAN.

By extending detection of the echo to hyper fine sublevel correlation (HYSCORE) experiments, we were able to correlate the two-dimensional HYSCORE to the EPR dimension, creating a novel three-dimensional spectrum.

With this work we show that SPIDYAN can be used to simulate and investigate the effect of frequency-swept pulses on commonly used EPR experiments. Such simulations can be used to predict effects, which are specific to chirp pulses. In future, SPIDYAN could become a tool for optimizing new UWB methods and for aiding spectral analysis in EPR.

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A CMOS-BASED USB CAMERA SYSTEM TO DELIVER REAL-TIME MONITORING OF SUB-MILLIMETER SAMPLES DURING MR-BASED INVESTIGATIONS

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State of the Art

The combination of magnetic resonance (MR)-based investigations, as a non-invasive and non-destructive technique, and automated tracking of biological sample movement (e.g., free moving microorganisms) provides a unique opportunity to link chemically specific spectroscopic information to morphological and behavioural data from a complete organism under normal physiological conditions in vivo.

Electromagnetic compatibility of the electronic components inside MR systems is a main challenge for investigations. Because regular image acquisition systems are incompatible with strong magnetic fields, the combination of MR-based investigation with imaging systems requires a unique interdisciplinary approach. Therefore, the custom-made design of a modular CMOS-based USB imaging platform for MR-based investigation is needed to facilitate high image resolution and real-time monitoring of sub-millimeter samples.

System hardware and software design

The platform is composed of commercially available functional parts, including an OV7670 CMOS-based camera module, a Teensy 3.1 control board, two multiplexers (74LCX157), and a UM232H serial-to-USB data transfer board. The Teensy 3.1 was used for setting the OV7670's registers, thereby configuring the camera module parameters (e.g., window size of the collected image, working mode,

etc.), via an SCCB interface. The Teensy 3.1 additionally triggered the UM232H and controlled the multiplexers. The UM232H was used to transfer the high-speed image acquisition of the CMOS sensor via USB bus to a laptop. The image sensor has a pixel size of 3.6 µm × 3.6 µm. The operating system of the image acquisition system includes three software packages: a bootloader for the Teensy 3.1, a SCCB interface to configure the OV7670, and a MatLab program to initiate the UM232H programming and facilitate the image acquisition.

Experimental results

The functionality of the image acquisition system and the data output to an external device (e.g., laptop) was successfully proven in the 11.7 Tesla magnetic field and in the presence of applied 500 MHz RF pulse sequence. The custom made MR compatible setup allowed the single frame recording of the quarter common intermediate format (QCIF) and and video graphics array (VGA) sized colour image. For higher frame rates, setting of the corresponding registers of the OV7670 must be made.

Conclusions and Outlook

The proposed configuration resulted in the high-performance, compact (33 mm × 90 mm × 30 mm), low-cost (< 70 Euro), and low-power image processing system, as required for many applications. The current work is focused on reducing the processing time of the image data required for real-time monitoring of sub-millimeter samples.

The system is already a useful tool for imaging inside the magnet and to study the behaviour of freely moving sub-millimeter objects inside the MR system. In conjunction with the MR spectroscopy and a spatial correlation between the two data sets (i.e. the location and the chemical composition), metabolites (i.e. intermediate products of metabolism) within the metabolic pathway could be identified and quantified in-vivo.

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QUANTUM GATES WITH HIGH FIDELITY IN SOLIDS AT ROOM TEMPERATURE

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The science of quantum control is at the heart of modern physics. Various applications of quantum control have emerged and we witness great development in recent years, such as quantum computations [1, 2, 3]. Spin systems, consist of electrons and nuclei, are among the most promising physical systems, in which quantum coherence can be generated and manipulated. However, the loss of quantum coherence is inevitable due to the couplings between the quantum systems and their environment. The performance of the quantum gates is also limited by the noise from the environment. Thus it is important to protect quantum coherence / gate in the presence of the environmental noise.My presentation will mainly focus on our recent experimental study of quantum control over electron / nuclear spins in solids. Novel quantum control methods have been developed to suppress the noise during the quantum gates. The high-fidelity gate have been demonstrated in NV centres at room temperature[4, 5]. Our experimental implementation of precise quantum gates marks an important step towards realistic fault-tolerant quantum computation.

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NMR CHEMOSENSING WITH MONOLAYER-PROTECTED NANOPARTICLES

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Several chemosensing methods already exist to analyze environmental or biological samples, but they all exhibit a main drawback: the signal is generated by a property of the receptor and does not provide any information on the identity of the analyte. Consequently, false positives and interferences may occur. To overcome this problem, in previous works we have developed a NMR chemosensing procedure based on the combined use of the NOE-pumping experiment and monolayer-protected nanoparticles as receptors. Limits of detection (LOD) between 0.5 and 2.5 mM were achieved [1, 2] on standard instruments. Indeed, when a higher sensitivity is required, NMR diffusometry appears as better strategy provided that strong interactions are at play between the analyte and the nanoparticles [3].

Nanoparticles passivated with triazocyclononane (TACN) moieties and including Zn^{2+} have been tested as receptors, based on reports by Prins and coworkers [4] that these species strongly bind phosphate ions and -to a lesser extent - carboxylate ions. Preliminary results indicate that, when the nanoparticles are mixed with phosphate-bearing analytes, the application of a diffusion filter allows the separation of the analyte signals from those of the other unbound molecules in the sample. The methodology was optimized using a test mixture including diphenyl phosphate, carboxylic acid as well as two "negative controls", arbutin and tyramine HCl. It was found that decreasing the pH increases the sensitivity of the method, as well as the selectivity for compounds bearing a phosphate moiety. More insight into the nanoparticles selectivity was provided by DOSY (diffusion-ordered spectroscopy) experiments.

This procedure was firstly used to follow the dephosphorylation of riboflavin-5'-phosphate, based on the evidence that the phosphorylated compound strongly interacts with TACN-

Zn^{2+} nanoparticles, while riboflavin does not. The same methodology may also be useful in a quality control process of a pharmaceutical industry for both the identification and the quantification of an active principle. As an example we analysed Bentelan®, a drug containing betamethasone sodium phosphate together with some excipients (sodium citrate, sodium bicarbonate, polyvinylpyrrolidone and sodium benzoate). Signals of the active principle were clearly detected also in the presence of a large excess of overlapping interferents.

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SEQUENTIAL ASSIGNMENT OF RNAS VIA PHOSPHODIESTER BACKBONE: 1H-31P CORRELATION WITH HIGH RESOLUTION 4D NMR

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High-resolution 1H-31P NMR correlation has long been desired to achieve unambiguous sequential assignment of RNAs. In spite of favourable H-P (3J) couplings, severe overlap of backbone 1H and 31P resonances make this correlation scheme quite difficult. We propose a novel through-bond, non-uniformly sampled, H4'/C4' selective, 4D HPCH NMR experiment which provides sequential connectivities in 13C-labeled RNAs via phosphodiester backbone. For obtaining reasonable resolution, non-uniform sampling is employed in indirect evolution of 1H (t1), 31P(t2) and 13C(t3) chemical shifts. To reduce sensitivity losses, multiple quantum coherences are preserved. Shaped inversion pulses are used for selective coherence transfer and prevent 13C-13C couplings evolution. The performance of the experiment was tested on a 34-nt hairpin RNA consisting of two A-RNA form stems, one adenine bulge, an asymmetric internal loop and a GAAA terminal loop [1] and a 14-nt RNA hairpin capped by cUUCGg tetraloop. The proposed experiment complements the set of recently reported high dimensional experiments [2],[3],[4] for sequential assignments in RNAs.

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POSTTRANSLATIONAL MODIFICATIONS OF INTACT PROTEINS DETECTED BY NMR SPECTRSOCOPY

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Posttranslational modifications (PTMs) are an integral part of the majority of proteins and fulfill essential functions. The characterization of structure and function of these essential parts can be very challenging especially for glycans. Mass spectrometry combined with other techniques is the paramount approach used so far to analyze PTMs. However, complicated sample preparations, inability to detect certain modifications, inability to identify linkage types and thus chemical structure, are serious drawbacks of this methodology. The few applications of NMR spectroscopy to detect posttranslational modifications so far suffered from the limitation to small proteins, low sensitivity and severe chemical shift degeneracies.

A direct, robust and simple NMR method for the detection and identification of PTMs in proteins will be presented [1]. The new twist of the method is denaturing the investigated proteins before two-dimensional NMR spectra are acquired. Under denaturing conditions even large proteins give sharp signals and thus the size limit of biomolecular NMR is circumvented. In addition spectra are significantly simplified due to random coil chemical shifts that are common knowledge. Measurements in D_2O together with cryo-probe technology are sensitive enough to detect ^{1}H-^{13}C correlations at natural ^{13}C abundance so that isotope labeling is not required. The strength of the method is that it can be applied to natural proteins of which the molecular weight does not limit the application. Another advantage is that the obtained information is complementary to other techniques like mass spectrometry: it provides insights into chemical structure of the modification for example monosaccharide types and linkage types. The method can directly detect

modifications on intact proteins without sophisticated sample preparation. Possible applications range from diagnostics of proteins derived from native organisms to quality control of biotechnologically produced therapeutic proteins. A combined application of this NMR approach together with mass spectrometry will be extremely powerful and a major impact on proteomics and glycomics is expected.

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NANOSCALE MAGNETIC RESONANCE BY SINGLE ELECTRON SPIN SENSOR

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Current spin-magnetic resonance spectrometers are based on the principle of ensemble detection and the test object is an ensemble sample containing billions of identical spins. However, at room temperature NMR/ESR at nano-scale is still a huge challenge. To achieve the scientific goal, we choose single spins in solids based on NV defect center in diamond - (NV) as the sensitivity magnetic probe. The single NV spin can be easily visualized, polarized and detected with a confocal microscope. Ultra-long spin coherence time for such qubits, even at room temperature, enables it is ultra-sensitivity to external magnetic noise with characteristic frequency. Instead of traditional detection manner, weak magnetic signals generated by the nano-scale spin system is mapped to coherent state phase, so as to realize high sensitivity signal detection.

We designed and constructed the S-band Optical Detected Magnetic Resonance spectrometers to meet the requirement of the quantum manipulation on single NV spin at room temperature. By the quantum interferometer of NV sensor on the home-built setup, we have experimentally realized atomic-scale structure analysis of single nuclear-spin clusters [1] and succeed to detect a single electron spin[2] in diamond. Collaborates with scientists from Germany and America, we succeed in detection of $(5nm)^3$ hydrogen nuclear spin sample using shallow NV probes. It's a "first step toward molecular-scale magnetic resonance imaging".

These results indicate that, central spin decoherence under dynamical decoupling control is a useful probe for nuclear magnetic resonance single-molecule spectroscopy and structure analysis. It

provides a new method for nano-scale science, including physics, life science and chemistry.

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MAGNETIC FIELD STABILITY OF HIGH TEMPERATURE SUPERCONDUCTING (HTS) MAGNETSHIGH RESOLUTION NMR WITH A DRIVEN-MODE MAGNET

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If we use a low temperature superconducting (LTS) outer coil and an HTS inner coil, it is possible to exceed a magnetic field of 23.5 Tesla (1 GHz in 1H Larmor frequency); this magnet is called here a LTS/HTS NMR magnet. Thus, the HTS is a key technology for the next generation super high field NMRs operated far beyond 1 GHz. High current density and high critical temperature also enables us to develop a compact size table top NMR magnet operated at 40 K to 77 K.

A shortcoming for an HTS coil is residual resistance of the conductor and improbability of persistent joint between conductors, preventing the persistent current sufficient for the NMR operation. Thus, the LTS/HTS magnet must be driven by an external DC power supply, causing magnetic field fluctuations, resulting in temporal NMR peak shifts and degradation of the NMR spectrum.

Our aim here is to acquire basic method to achieve high resolution NMR spectra using a driven-mode magnet. For this purpose, we have investigated the performance of both a 500 MHz LTS/HTS NMR magnet [M. Takahashi, Rev. Sci. Instrum., 83, 105110 (2012)] and a 1020 MHz LTS/HTS NMR magnet [K. Hashi, JMR in press (2015)], driven by DC current supplies.

There are two kinds of temporal magnetic field instability caused by an external DC current supply. The first one is slow fluctuations (<0.1 Hz) over hours and days. The second is 50 Hz / 60 Hz ripples coming from AC power ripples.

We have already reported that the former should be less than +-1.5 ppm for the 2H lock operation, absolutely necessary for high resolution NMR measurement [Y. Yanagisawa, JMR 102, 329-337(2008)]. This condition is not easily satisfied for some type of power supplies, which are sensitive to the temperature change over the current supply; based on the NMR measurement for a driven mode LTS/HTS NMR magnet, a small temperature change easily causes magnetic field fluctuations >1 ppm, in the case of air cooled DC power supply.

The 50 Hz / 60 Hz ripples coming from AC power supply is another serious problem. They appear on NMR spectra as sidebands at 50 Hz / 60 Hz from a main peak. The spectrum modulation is evaluated based on the magnet voltage measured during the current ramp up to 10A, about 5% of the operation current. Based on the measurement, we can estimate the magnetic field ripples in ppm against rated current and predict the influence on NMR spectra. In this regard, a method to reduce 50 Hz / 60 Hz ripples will be discussed.

The relation between the performance of an external power supply and the quality of NMR spectrum will be also shown in this presentation, which is important for the development of high resolution NMR systems using a driven-mode magnet.

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RECENT INSIGHTS INTO OVERHAUSER DNP WITH OPTICALLY ACTIVE FUNCTIONALIZED NITROXIDES

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The efficiency of dynamic nuclear polarization (DNP) as a tool to improve NMR critically depends on a choice of a paramagnetic polarizer and its initial polarization. Thus, the DNP effect can be substantially enhanced if the electron spins, used to polarize the nuclei, are far from thermal equilibrium. Such a condition can be attained with optically excited triplet states[1]. One of the ways to use the high polarization of triplet states, applicable in liquids and therefore potentially useful for liquid state DNP, is linking of a stable radical to the optically active dye[2]. However, in the context of the Overhauser DNP, it is not clear whether any functionalization of the radical might attenuate or even destroy its DNP activity. The difficulty arises from a complexity of the Overhauser mechanism, efficiency of which is a subject of a system specific polarizer/target motional behavior.

To explore this avenue, we have synthesized a series of functionalized dye-nitroxide derivatives and performed their low field (0.35 T / 9.7 GHz) DNP studies in toluene. To interpret the DNP enhancements in terms of the Overhauser mechanism, the Overhauser parameters were independently evaluated for each polarizer derivative. Particularly, the effective saturation factors were accurately determined using Polarization Recovery PELDOR (PR-PELDOR)[3-6], which was extended and applied for a three-line ^{14}N-nitroxide based EPR system. We will present our recent results and discuss the method for an unambiguous analysis of the saturation data. The potential of the dye-nitroxide polarizers for liquid state DNP will be discussed as well.

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DISTANCES AND ORIENTATIONS WITH PELDOR/DEER AT LOW AND HIGH FIELDS/FREQUENCIES

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The low field (0.3 T/9 GHz) pulsedelectron-electron double resonance (PELDOR/DEER) is a widely used technique fordistance measurements on biomacromolecules.If applied at higher fields and frequencies (35, 95 and 263 GHz), the method is affectedby orientation selectivity. This feature is usually considered as a nuisance thatprevents an accurate distance measurement. However, in many cases,high-frequency PELDOR delivers information inaccessible at low fields. Forexample, if distances are known, high-frequency PELDOR permits to correlate themutual orientation of paramagnetic centres[1,2]. Furthermore, distance measurements with metal centres arefacilitated[3]. Besides, the absolute sensitivity at highfields/frequencies is increased andallows performing experiments on limited sample volumes.

However, at high frequencies, thelimited available power and increased microwave losses render excitation pulseslonger and therefore the signal weaker. This, combined with a broader EPRspectrum of anisotropic centres, predicts weaker modulation depthsthan those observed in X-band. Furthermore, the "out-of-phase" signalobservable at high fields[4,5] must be considered and adequatelyanalysed.

Here, we present comparative low and high-frequency (35, 95 and 263 GHz) PELDOR studies on model RNA systems containing rigid[6]and flexible nitroxide spin labels and discuss the advantages and possible bottlenecksof the high field measurements. We show that at high fields/frequencies aconsiderable PELDOR modulation can be

detected and orientation selectivity issignificant, which is particularly important for orientation selective studies.

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P 276
OPTIMAL CONTROL MEETS AVERAGE HAMILTONIAN THEORY

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For many years, effective Hamiltonian theory [1] has been the only tool for analysis and design of new solid state NMR experiments. Although very successful, it quickly becomes prohibitively complicated when one wants to address larger spin systems, multiple internal interactions with dispersed strengths, and diverse instrumental errors. As an alternative, optimal control theory (OC) methods [2] have been developed for experiment design within the field of liquid-state NMR, solid-state NMR and magnetic resonance imaging. OC offers a powerful optimization strategy that can efficiently handle hundreds-to-thousands of variables to exploit the full degree of experimental freedom. However, the solutions very often look as random sequences and provide limited insight how they actually work. Our recent experience with new improved optimization protocols [3] suggests this is going to change.

Recent studies of the Nielsen group (University of Aarhus, Denmark) document the very important cross-fertilization potential of optimal control with respect to conventional analytical design based on average Hamiltonian theory. For example, the novel dipolar recoupling experiment RESPIRATION-CP [4] was strongly inspired by numerical optimizations. Beside robustness, it is also better compensated for spread of interaction strength imposed by orientational distribution in solid powders, beyond the generally accepted limit of γ-encoded sequences. My own optimizations of heteronuclear dipolar recoupling for polarization transfer reveal another patterns that can be directly translated into conventional pulses and understood analytically, again breaking the γ-encoding barrier. I will also draw a parallel between powder efficiency of OC sequences and composite recoupling introduced by Nielsen in 2006 [5]. I believe we will see much more examples of such "well-behaved" OC sequences in the near future.

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P 279
SOLID-STATE 33S NMR OF ORGANOSULFUR COMPOUNDS

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Field-swept and frequency-swept types of solid-state ^{33}S NMR spectra of organosulfur compounds, such as [^{33}S]-diphenyl disulfide, are presented. The field-swept solid-state ^{33}S NMR spectra were acquired at Larmor frequencies of approximately 16 - 20 MHz and using a superconducting magnet at between 7.00 and 0.50 T, while the frequency-swept ^{33}S NMR spectra were obtained at extremely low magnetic fields, e.g., less than 0.10 T. The analytical methods were developed for extracting ^{33}S NMR parameters from the two types of solid-state ^{33}S NMR spectra in which both Zeeman and quadrupole interactions were dominant. The relationship between frequency-swept and field-swept solid-state NMR spectra of half-integer quadrupole nuclei is discussed. The quadrupole coupling constant, C_Q, for the present organosulfur compounds were found to be more than 40 MHz, whose NMR spectra are hardly measured by a conventional NMR method. I also briefly discuss the relation between ^{33}S NMR parameters and the dihedral angles in disulfide bonds on the basis of quantum chemical calculations and the experimental results. The implication of the present work is that there is no limitation of the magnitudes of C_Q values, which implies that all half-integer quadrupole nuclei in the periodic table can be potentially measured.

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EASY AND UNAMBIGUOUS SEQUENTIAL RESONANCE ASSIGNMENTS OF INTRINSICALLY DISORDERED PROTEINS BY CORRELATING MULTIPLE CONTIGUOUS RESIDUES IN HIGHLY RESOLVED 3D SPECTRA

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Sequential resonance assignment strategies are typically based on matching one or two chemical shifts of adjacent residues. However, resonance overlap often leads to ambiguity in resonance assignments in particular for intrinsically disordered proteins. We investigated the potential of establishing connectivity through the three-bond couplings between sequentially adjoining backbone carbonyl carbon nuclei, combined with semi-constant time chemical shift evolution, for resonance assignments of small folded and larger unfolded proteins. Extended sequential connectivity strongly lifts chemical shift degeneracy of the backbone nuclei in disordered proteins. We show here that 3D (H)N(COCO)NH and (HN)CO(CO)NH experiments with relaxation-optimized multiple pulse mixing correlate up to seven adjacent backbone amide nitrogen or carbonyl carbon nuclei, respectively, and connections across proline residues are also obtained straightforwardly. Multiple, recurrent long-range correlations with ultra-high resolution allow backbone $^{1}H^{N}$, $^{15}N^{H}$, and $^{13}C'$ resonance assignments to be completed from a single pair of 3D experiments.

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AN EFFICIENT APPROACH TO 6D HNCO(NCA)CONH

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Intrinsically disordered proteins due to their low chemical shifts dispersion are difficult target for NMR studies. The resonance assignment is usually performed using 4D and 5D experiments [1-2] which are needed to resolve overlapping signals. Approaches using even more dimensions are believed to be very insensitive, and thus leading to unreasonably long measurement times. What is more, performance of experiments exploiting amide protons detection is additionally hampered by chemical exchange of well exposed to the solvent amide protons. As a remedy several direct carbon detection experiments were proposed [3-5].

In this study we present that optimized 6D HNCO(NCA)CONH experiment can be successfully acquired in about 13 hours using 800 MHz spectrometer with Room Temperature probe (288 K sample temperature was set to suppress amide protons exchange). Such experiment allow to obtain nearly all possible correlation signals using standard 1 mM a-Synuclein sample leading to a nearly full assignment of all nonproline/pre–proline residues. Two dimensions (H and N) were co evolved to obtain projection to 5D space. As a result, presented experiment can be processed as a normal 5D experiment.

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LIQUID-STATE PARAMAGNETIC RELAXATION FROM FIRST PRINCIPLES

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Diamagnetic liquid-state, NMR relaxation is typically well-described by the Redfield relaxation theory [1]. The Redfield theory is a good approximation in the regime where the magnitude of the interaction Hamiltonian is weak as compared to the average Zeeman term and the time scale of relaxation is much greater than the time scale of molecular fluctuations contributing significantly to relaxation. However, in paramagnetic systems the former condition is usually not met whereas, sometimes the latter is. We simulate paramagnetic relaxation from first principles by sampling a molecular dynamics trajectory with quantum-chemical calculations, and producing a time series of instantaneous parameters of the spin Hamiltonian that is, in turn, used to numerically solve the Liouville-von Neumann equation for the time evolution of the spin density matrix. We demonstrate the approach by simulating the electron and nuclear spin relaxation in an aqueous solution of the Ni(2+) ion [2,3]. The spin-lattice (T1) and spin-spin (T2) relaxation rates are extracted from the simulations of the time dependence of the longitudinal and transverse magnetization, respectively. Good agreement with the available experimental data is obtained by our method.

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NUCLEAR MODULATIONS OF COPPER THROUGH ULTRA-WIDEBAND CHIRP ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

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Pulse electron paramagnetic resonance (EPR) spectroscopy is a very versatile tool to separate and correlate different interactions in a system with unpaired electron spins. Still, the application remains restricted today, since the spectral excitation range is relatively limited. While short rectangular microwave (mw) pulses can cover a bandwidth of about 100 MHz, EPR spectra of paramagnetic transition metal complexes easily exceed 1 GHz at usual magnetic fields.

A way to overcome this drawback is to sweep the mw frequency while applying pulses and keep the magnetic field constant. Such 'chirp' pulses are known in NMR for many years [1], but only recently became accessible for EPR with the advent of fast arbitrary waveform generators (AWG).

Based on such an AWG (12 GSa/s), a new an ultra-wideband (UWB) EPR spectrometer was realized at X-band (9-10 GHz) in our laboratory [2] ('UWB' is used for a frequency range exceeding 500 MHz). By exciting electron spin echoes with UWB chirp pulses, EPR spectra ranging over 800 MHz could be covered. Nuclear modulations on top of these echoes were recorded in a second electron spin echo envelope modulation (ESEEM) dimension, where frequencies up to 300 MHz were observed for a single crystal of bispicolinate Cu(II) complex. Simulations confirmed that these high frequencies stem from hyperfine-coupled copper nuclei. Since the full echo is digitized, ESEEM peaks appear resolved at their corresponding EPR frequencies in a two-dimensional spin echo correlation spectrum [3]. For the hyperfine sublevel correlation (HYSCORE) experiment, this leads to a novel three-dimensional EPR-resolved HYSCORE spectrum containing copper nuclear frequencies besides the usual proton frequencies.

The current limitation for these UWB experiments is the bandwidth of

the mw resonator. An optimally adapted sweep rate of the chirp pulse helps to provide a uniform excitation over the whole frequency range [2,4].

In EPR text books, ESEEM is listed as the method of choice to study small hyperfine interactions of ligand nuclei to their paramagnetic centers. For nuclei with larger hyperfine or quadrupole couplings, electron nuclear double resonance (ENDOR) is suggested. With this work, we show that nuclear modulations of paramagnetic metals at the center of a complex can be detected by ultra-wideband chirp ESEEM spectroscopy. In future, this could become a new tool to study the structure of metalloproteins.

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ESR -SPECTROSCOPY METHOD FOR STUDIING OF MOLECULAR MOBILITY OF NANOCELLULOSE GELS

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Due to developing nanotechnologies, great interest is attracted to amorphous-crystalline materials, particularly, natural polymers based on cellulose as a source of nanocellulose.Molecular mobility of cellulose nanoparticles in hydrogel isolated from macrocrystalline cellulose was investigated using paramagnetic probe method. It was estimated from correlation time t (seconds) and radical rotation frequency n = (t)$^{-1}$.

Water (starting) solution of gel and water-alcohol (with 25% C_2H_5OH) solution of gel were studied. Concentration of nitroxyl radicals, $(CH_3)_4C_5H_5(OH)NO^{\cdot}$, (NR) was ~3´10^{16} spin/sample. The EPR spectra of solid solutions at 77 K showed that NR is a triplet with the anisotropic HFI constant A_{ii} = 3.85–3.9 mT, g-factor=2.0045, and a 1:1:1 integral intensity ratio. Correlation time t was ³ 10^{-7} s at 77 K. The triplet shape of the spectrum was evidence of complete dissolution of NR and the formation of true solution in both media. The triplet of nitroxyl radicals retained in the water matrix at 77-265 K and in the water-alcohol solution at 77-180 K. Thus, the temperature of rotation of radicals was by 85^0 lower in the presence of alcohol than in water. Provided free rotation of radicals of PMP at 300 K, fully isotropic triplets with a_{iso}=1.7 mT, g=2.0045, and a 1:1:1 intensity ratio were recorded in solution. Narrow lines of the isotropic triplets (DH_i = 0.15–0.22 mT for water and water-alcohol systems of gel) justified high hydrophobicity and a weak interaction of radicals with the environment. Correlation time t was estimated from the EPR spectra registered at different temperatures. Activation energy of rotation of radicals (E) and a pre-exponential factor n_0 (s^{-1}) were estimated from the derived dependence. The pre-exponential factor is frequency of rotational vibrations of a particle about the equilibrium position. E = 11.2 kcal/mol for water gel and E=5.2 kcal/mol if alcohol was added, the pre-

exponential factor was $n_0 = 7´10^{18}$ s^{-1} for water and $n_0 = 6´10^{14}$ s^{-1} for water-alcohol matrices. E @ 10.4 - 5.6 kcal/mol, the pre-exponential factor was n_0 » $3.9´10^{18}$ -$1.2´10^{14}$ $s^{-1}$for pure water and water-alcohol solution (without nanocellulose). The high pre-exponential factor, namely, 10^{18} s^{-1} for water and the water system of hydrogel can be interpreted in terms of "water compensation effect". The effect consists in that water molecules are squeezed out from the nearest environment of the active center to provide the rearrangement and the disorder in water structure, and, consequently, the growth of entropy of the system. Therefore, compensation effect provides a smoother temperature dependence of active centers than it would be with the participation of the activation energy without the entropy factor. In terms of thermodynamics, effect consists in that essential changes in enthalpy and entropy compensate each other, and the changes in free energy are relatively small.

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EPR SPECTROSCOPY OF MANGANESE-DOPED PEROVSKITE METAL FORMATE FRAMEWORK

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Lately, a novel type of porous materials called coordination polymers or metal-organic frameworks (MOFs) emerged and immediately attracted attention of the scientific community [1]. These crystalline compounds are unique due to the highly porous structures which can be utilized for gas adsorption related applications. Some of MOF materials contain paramagnetic transition-metal ions, resulting in peculiar magnetic properties of these compounds. In addition, the organic part in some coordination polymers consists of polar molecules, which below a certain phase transition temperature order into a ferroelectric phase [2].

Recently a promising MOF $[(CH_3)_2NH_2][Zn(HCOO)_3]$ with perovskite-type architecture and inherent ferroelectricity was synthesized [3]. It is believed that the ferroelectric phase in this material is due to the ordering of $(CH_3)_2NH_2^+$ ions, but, however, the precise phase transition mechanism is still obscured.

In this work we investigate the $[(CH_3)_2NH_2][Zn(HCOO)_3]$ MOF doped with 0.05 mol% paramagnetic Mn^{2+} ions using the continuous-wave (CW) and pulsed EPR methods. The temperature dependent X-band CW and field-sweep as well as Q-band CW EPR spectra reveal that the local Mn^{2+} ion-probes are indeed sensitive to the local structural changes occurring at the phase transition point. Spectral simulations were used to obtain the **g**, hyperfine **A** and fine-structure **D** tensors and temperature dependence of their components allowing to further characterize the observed phase transition and the MOF

structure. Following the temperature dependence of the axial zero-field splitting parameter D, it was concluded that the phase transition into the ferroelectric phase is of the first order.

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NEW STRATEGY IN PARAMAGNETIC TAGGING PROTEINS AND APPLICATION IN STRUCTURAL BIOLOGY

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Site-specific tagging proteins with paramagnetic metal ions have received great interests in structural biology for NMR and EPR spectroscopy. The main issue in paramagnetic tagging protein relies in the chemoselectivity and chemoreactivity of paramagnetic tag with respect to the protein, of which thiol chemistry has been mostly applied. In addition, the chemical stability of tether between protein and paramagnetic tag, the affinity of paramagnetic tag with paramagnetic metal ion, and the size of the paramagnetic tag should also be considered in structural biology by using paramagnetic NMR spectroscopy. Herein, we will discuss a new strategy in site-specific labeling of proteins with a very rigid, short and stable paramagnetic tag via the reaction of phenylsulfonated pyridine derivative and protein thiol. The high performance of this tagging method has been demonstrated in a number of proteins.

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ORTHOGONAL SPIN LABELING OF PROTEINS USING CLICK CHEMISTRY FOR IN VITRO AND IN VIVO APPLICATIONS

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Site-directed spin labeling (SDSL) EPR spectroscopy is a powerful tool to study the structure of biomolecules in vitro. However, standard cysteine-based labeling rendered impractical for in vivo applications due to the large number of native cysteines and high reduction of commonly employed nitroxide spin labels in the intracellular environment.

As an alternative coupling strategy, Click Chemistry[1] offers major benefits by providing a fast and highly selective, biocompatible reaction between azide and alkyne groups. Here we establish click chemistry as a tool to target unnatural amino acids (UAAs)[2] using azide- and alkyne- functionalized spin labels. eGFP was chosen as a model protein for optimization of reactions. The effective labeling with nitroxides was achieved using the copper-catalyzed azide-alkyne cycloaddition (CuAAC)[3] yielding labeling efficiencies of about 100% within short incubation times. We show first EPR experiments carried out in E.coli cytoplasm and cells and DEER experiments with new synthesized spin labels.

In the future we want to apply stable Gd(III)-DOTA complexes and their derivates as spin labels in order to overcome the reduction problem. The toxicity and incorporation into cells of Gd(III)-DOTA

derivates were determined for E.coli strains. So far Gd(III)-azido-DOTA was successfully labeled to eGFP.

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AB-INITIO CALCULATIONS AND EPR STUDY OF GAMMA-IRRADIATED 2-CHLORO-3',4'-DIHYDROXYACETOPHENONE POWDER

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Gamma-irradiated powders of 2-Chloro-3',4'-dihydroxyacetophenone (2C34DHA) were investigated using the EPR techniques. EPR spectra of powder sample were recorded between 123 and 390 K temperatures. The EPR spectra were found to be slightly temperature dependent. Taking into consideration the chemical structure and experimental spectra of irradiated powders of 2-Chloro-3',4'-dihydroxyacetophenone we assumed that one paramagnetic species was formed by the abstraction of H atom from the OH fragment of molecule so the unpaired electron was localized on benzene ring. The molecular structure of 2-Chloro-3',4'-dihydroxyacetophenone molecule was investigated with X-ray technique. Then seven possible radicals were modeled using the DFT methods and B3LYP/6-311++ G(d,p) basis set. EPR parameters were calculated the same method and basis set. The calculated hyperfine-coupling constants and g-value were used as initial values for simulations. The experimental and simulated spectra were well matched for the modeled radical R2 model radical. Thus we identified the radical type produced in gamma irradiated 2C34DHA.

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13C-TMDOTA, A SOLID-STATE NMR THERMOMETER FOR HYDRATED LIPID BILAYER MEMBRANS

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For biological membrane systems, temperature is one of the most important parameters. Therefore, the in situ temperature of membrane preparations has to be measured accurately. In solid-state NMR, the sample temperature is usually controlled by a variable temperature (VT) gas flow. Magic angle spinning (MAS) and high power ^{1}H decoupling are adopted to attain high resolution and high sensitivity. These routine procedures generate a significant amount of heat, causing a temperature difference between a sample inside a rotor and VT gas. To measure the temperature accurately, we examined a ^{13}C-labeled paramagnetic lanthanide complex, ^{13}C-TmDOTA, as an internal thermometer that shows temperature-dependent change of $\delta^{13}C$.

To evaluate the utility of ^{13}C-TmDOTA as an internal thermometer for hydrated lipid membrane preparations, DMPC was dispersed in ^{13}C-TmDOTA solution to form multi-lamellar vesicles (MLVs), and subjected to solid state NMR and differential scanning calorimetry measurements. The results showed that the addition of the Tm complex did not significantly influence the NMR properties of DMPC bilayers, such as phase transition temperature, MLVs formation, and T_1relaxation time.

Then we evaluate the ^{13}C-TmDOTA as a thermometer by measuring ^{13}C spectra at different temperatures, and found that the $\delta^{13}C$ showed linear dependence on sample temperature with slope ca. 1.2 ppm/°C. Additionally, we successfully estimated the temperature rise caused by MAS and ^{1}H decoupling, which would be discussed in the poster session.

P 312
OPTIMISING SPIN PROPERTIES OF PARAMAGNETIC QUANTUM DOTS

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Advantages of colloidal quantum dots (QDs) over nanostructures formed by lithographic processing or by self-assembly include flexibility in the control of QD size, shape, chemical composition and environment [1]. This allows fine tuning of the electronic, optical and magnetic properties of colloidal QDs for a range of applications from biomedical imaging to quantum information processing.

The incorporation of single Mn^{2+} spins into colloidal PbS QDs [2] has led to the observation of Rabi oscillations and long electron spin coherence times (T_M ~ 1 μs at 5 K) [3]. Recent studies have shown that the dispersion of the QDs in a glassy matrix leads to considerable improvements of the spin coherence time (T_M ~ 10 μs at 5 K) which was found to persist up to the matrix melting point (T_M ~ 1 μs at T ~ 230 K) [4]. We envisage that by embedding the QDs in a solid state matrix, Mn spins in colloidal QDs may present the potential for room temperature molecular spin qubits.

We fabricate three dimensional microstructures of photosensitive material by two photon polymerization. This 3D printing or additive manufacturing technique allows microcavities to be produced from hybrid polymers (e.g. Ormocers) with a resolution of ~100 nm. The nanocrystals can be incorporated by soaking of the printed structures in the QD solution. The incorporation of the QDs in an optimized microcavity, could provide rigid matrix up to room temperature and

would also enhance the QD optical properties. By exploiting the interactions between the excitons and the Mn spins the optical properties could be utilized for the initialization and readout of the spin qubits [5] in photo-EPR studies.

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P 315
NMR RELAXATION AND DIFFUSION INVESTIGATIONS OF MOLECULAR DYNAMICS IN BULK AND CONFINED SUPERCOOLED IONIC LIQUIDS

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Low melting organic salts commonly referred to as ionic liquids (ILs) reveal different properties from molecular liquids mainly due to their ionic character [1]. Their high chemical, thermal and electrochemical stability makes them suitable to be used as electrolytes in electrochemical devices. Therefore from both, the fundamental research and applications point of view, there is much interest in the dynamical properties of IL under confinement in porous media, where the molecular transport is predominantly affected by the geometrical restrictions and wall interactions. In this context, reorientation and translational dynamics can be suitably studied by NMR, in particular using field cycling NMR relaxometry [2].

In this contribution, the calorimetric and spin-lattice relaxation (T_1) behaviour of several imidazolium based ionic liquids with different anions, in bulk as well as confined in porous media, is presented. We have varied the sidechain length of the 1-alkyl-3-methyl imidazolium cation by studying ethyl, butyl and hexyl units (Xmim), whereas the commonly used bis(trifluoromethylsulfonyl)imide anion (Tf2N) has been compared to Bromide anions. The degree of supercooling varied substantially between the different sidechain lengths, and T_1 relaxation times were measured at temperatures that cover a wide range from ambient conditions down to close to the glass transition of the corresponding IL. The dynamics of the cation is selectively monitored by ^{1}H relaxometry; when Tf2N was used as an anion, the latter's dynamics could be followed by ^{19}F relaxometry.

The relaxation experiments reveal pronounced changes in the dynamics, relative to the bulk, of both the cation and anions when the ionic liquids are under confinement. Rotational and translational

correlation times could be obtained reflecting different dependences upon the temperature. The different ions show super-Arrhenius and heterogenous dynamics in bulk [3], while Arrhenius dynamics is found under confinement, as a consequence of the physical restriction to the correlation length. Diffusion coefficients obtained from the relaxation experiments follow Vogel-Fulcher-Tammann (VFT) temperature dependence above a dynamic transition temperature $T_d \approx 1.28\ T_g$, below which a further deviation was observed.

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AROMATICITY-SPECIFIC INTERACTION OF FLUID MOLECULES WITH ASPHALTENE AGGREGATES DISSOLVED IN CRUDE OIL BY MEANS OF DNP AND NMR RELAXOMETRY

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The relation of NMR relaxation times and diffusion coefficients with overall viscosity and molecular size in a typical fluid composition has been established for bulk oils [1-3]. A major obstacle for the task of compositional analysis remains, however, the widely unknown role of molecular shape and chemistry, most importantly aromaticity, on the NMR relaxation behaviour. This is particularly important in asphaltene-containing oils where the relaxation of solvent molecules, so-called maltenes, is significantly affected by interactions with radical-containing asphaltenes [4]. However, aromatic and aliphatic maltenes are expected to interact differently with asphaltene aggregates [5], and size-dependent residence time variations within the porous aggregates become important [6]. While fundamental studies have attempted to provide a molecular dynamics description of relaxation times that take advantage of data obtained at variable magnetic fields [7], they still suffer from a lack of distinction of dynamics between molecules of various architecture, and from the generally broad relaxation times distribution in natural oils.

In this study, we have applied ^{19}F containing tracer molecules at low concentrations to natural oils of different asphaltene content [8], and investigated the tracer's relaxation time ratio T_1/T_2 and the field dependence of relaxation times, $T_1(w)$. This strategy has the advantage of specifically determining the behaviour of different tracers, where molecular weight and aromaticity are considered as variables. One main finding of this study is the significant increase of T_1/T_2 for aromatics in the presence of asphaltenes compared to alkanes [9]. The results are interpreted in terms of selective maltene-asphaltene interaction based on frequency dependent relaxation

results. The strong contrast of relaxation times allows for a simplified quantification of either asphaltene concentration or maltene aromaticity in crude oils. The role of asphaltene has further been quantified by concentration-dependent measurements of protonated and fluorinated test molecules in a deuterated solvent, and of non-aggregating polycyclic radical and neutral molecules mimicking asphaltene, respectively. First results of the enhancement in dynamic nuclear polarization (DNP) experiments, making use of microwave saturation of the naturally occurring radicals in oils, confirm the significant difference in interaction strength of asphaltenes with aromatic and aliphatic molecules.

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P 321
EVOLUTION OF MOLECULAR TRANSLATIONAL DYNAMICS IN COMPLEX-FLUIDS BY MGSE SPECTROSCOPY

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Translational dynamics of liquid solutions is more complex than dynamics of individual components alone. Latter is often described as Brownian diffusion, but the model fails if molecules form clusters with strong short-lived bonds. Knowledge of translational dynamics is important to understand mechanism of glycerol/water in maintaining the structure of biological macromolecules and its cryoprotective role in living beings. New experimental insight into molecular interactions in this system is achieved by MGSE spectroscopy, a method allowing direct observation of the molecular velocity autocorrelation spectrum (VAS).

Originally, the Modulated Gradient Spin Echo method [1] was realized with a combination of CPMG RF-train and the interspersed gradient pulses or waveforms. The upper frequency of this technique is limited by the gradient coil induction to about 1 kHz [2]. By applying the CPMG train simultaneously with a steady gradient field, the upper frequency is lifted to a few tens of kHz depending on the available strength of magnetic field gradient [3]. The method also enables to track the evolution of spectrum during the applied RF/gradient sequence [4]. MGSE excels among other methods by providing instant insight into different modes of molecular translation and by tracking the evolution of spectrum during molecular translation providing information on the interaction with circumventing molecules.

The measurements of glycerol/water solutions in the frequency range from 100 Hz to 3 kHz show that VAS of water molecules exhibits features not described by any known model. A 3D plot of spectrum vs time and frequency reveals the evolution of the spectrum from the excitation to the transition into equilibria during the interval of 100 ms. For low

glycerol concentrations, the initially dominating spectrum typical for the unbounded water changes during the time interval, which depends on the concentration. Change in spectrum can be attributed to frequent contacts of water molecule with a glycerol molecule. Same spectrum appears immediately at the concentration with equal proportion of both kind of molecules. Model of harmonically coupled Brownian particles gives the spectrum that fits well to the experimental data. The parameters of the model are structural relaxation time of water-glycerol bond and the mean self-diffusion coefficient of the compound.

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MICRO-RHEOLOGY BY NMR: STUDY OF NON-NEWTONIAN PROPERTIES OF FLUIDS BY MGSE SPECTROSCOPY

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Micro-rheology is a technique used to measure properties of fluids via the micro-tracer flow in complex fluids, while the passive micro-rheology uses the thermally driven diffusion of tracers to obtain the viscosity, shear thinning or thickening and other visco-elastic properties of media. Method is based on the generalized Stokes–Einstein relation given by the fluctuation-dissipation theorem, by which the response of a system in thermodynamic equilibrium to a small applied force is the same as its response to a spontaneous fluctuation. A key quantity is the power spectrum of the velocity autocorrelation function (VAS), which is in linear proportion to the spectrum of particles mobility according to Kubo[1]. The modulated gradient spin echo (MGSE) is a tool of magnetic resonance providing directly the VAS of molecular translation by the fast modulation of phase of spin bearing particles in the inhomogeneous magnetic field.

At first, the notion of MGSE[2] was realized by the combined sequence of CPMG radiofrequency (RF)train and the interspersed gradient pulses in order to measure VAS of liquid flow and restricted diffusion in porous media. However, the gradient coil induction limits the frequency range of method below 1 kHz[3]. With a better understanding of spin evolution under the simultaneous application of RF pulses and gradient fields, the MGSE technique was developed that pushes the upper frequency limit in the range of a few tents of kHz and even more, if available sufficiently strong gradients[4]. The extended frequency range of MGSE method made it interesting for the studies in micro-rheology.

The viscosity spectrum of fluids was measured on the Bruker 100 MHz NMR system with variable gradient to 6 T/m to cover the frequency range of 100 Hz-3 kHz, and on the Magritek NMR mouse with the fixed gradient field of 20T/m and fixed frequency range of 1-10 kHz. The results of measurements show that very ordinary liquids such as water and ethanol have a non-Newtonian properties with the change of viscosity for almost 40% in the range between 100 Hz to 1 kHz, while the viscosity spectrum of glycerol changes for more than 250% in the range between 2 to 10 kHz. Method was used also to study rheological properties of polymers[4] and some mixtures of liquids.

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P 330
MOLECULAR DYNAMICS IN SELECTED POLYCYCLIC AROMATIC COMPOUNDS MONITORED BY MEASUREMENT OF SELECTED STRUCTURAL PARAMETERS

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The polycyclic aromatic compounds attract scientific attention namely due to their conjugated π-π aromatic systems which promise many interesting and unusual optical and electronic properties. Some of these compounds were shown to be effective in the development of materials useful e.g. in molecular-based electronics.[1]

The electronic structure of these compounds is studied mainly by theoretical approaches.[2] The theoretical results can be compared and also correlated to some experimentally accessible parameters. The experimental data reflecting current electron distribution between given atoms can be obtained either by detail X-ray structure analysis and/or by NMR spectroscopy. The results can be mutually compared and reveal the key differences in the electronic structures adopted by these molecules in the liquid and solid state. The DFT calculations can explain intra and intermolecular effects leading to different structures in solid and liquid state.

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SYNTHESIS AND CHARACTERIZATION OF STAR POLYMERS WITH VARIOUS ARCHITECTURES AS A MODEL SYSTEMS FOR DRUG AND NUCLEIC ACIDS DELIVERY

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In the times of the harmful effects of the external environment on our bodies, many genetic diseases appear which results from our way of life and the influence of environment. Therefore, it becomes extremely important to discover and to synthesize the new materials that could prevent these diseases as well as to study the effects of such nanoparticles on compounds of natural origin - biomolecules. The method which involves the nanoparticles as modern vectors for transfection is known as gene therapy. Atom transfer radical polymerization (ATRP), one of the most robust controlled radical polymerization (CRP) techniques, is used to prepare polymers with diverse architectures (e.g., nanogels and star polymers) enabling innovative functionalities to be introduced. We want to report an efficient, biocompatible polymeric carriers for nucleic acids delivery using PEG-based star polymers with a cationic and degradable core. Four different architectures are considered including: multi-arms stars with biodegradable, and cationic core; 4-arms with biodegradable, cationic arms; linear polymers which are equivalent with other architectures in terms of molecular weight and chemical composition. Their structural characteristics were characterized by various methods such as NMR, NMR diffusion, DLS, DSC, microscopic techniques such as SEM, TEM). The biocompatibility was revealed on porcine skin fibroblast NT14 cell line. Methodology involved WST-1 proliferation assay and bioimaging INCell Analyzer 2000 system. The investigation

shows, that there is no cytotoxic effect in analyzed range of polymer concentrations in biological schemes. These results lead to the conclusion, that star polymers represent high biocompatibility profile, which enable their use in biomedicine. DLS and NMR measurements were used to determine the sizes of star polymers in solution, and the behavior of the polymers in the solution was characterized by determining the diffusion coefficients. Thanks to extensive research, the cationic, biodegradable star-shaped polymers were obtained and their physical and chemical properties were investigated. Combined results of carried out measurements have allowed us to select the most suitable and promising candidates as nonviral vectors for gene therapy.

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SOLVENT-GELATOR INTERACTON IN MOLECULAR GELS STUDIED THROUGH THE SOLVENT DYNAMICS BY FFC NMR RELAXOMETRY

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The supramolecular gels based on low molecular mass gelators (LMMGs) are the subject of an ever increasing number of studies, which improved understanding of the self-organization of gelator molecules into a three-dimensional network structure, the gelation phenomenon, and the dependence of the thermal stability and of gel morphology on the gelation [1]. However, some issues, e.g., concerning the interactions of solvent molecules with gelator aggregates during and after the gel formation, the role of solvent after the gel formation, and the influence of the gel matrix on the solvent dynamics, are still open for discussion. Usually, to quantify solvent effects, the different solvent parameters are employed. Based on such an approach some correlation between the solvent and properties of the gel can be established but they are valid only for gels formed by particular class of LMMGs. Moreover, the direct evidence of the solvent-gelator interaction is not possible. We proposed a different approach: the study of dynamic processes of confined solvents. If the interaction of solvent-solid surface (gelator aggregates which formed the matrix) occurs then the solvent molecules will be adsorbed on the pore surface and consequently their dynamical parameters such as the correlation time and the diffusion coefficient will be altered by this interaction.

The gels formed by ethylene glycol (EG) and 1,3-propanediol (PG) with different concentrations of 4,6,4',6'-O-terephthylidene-bis(methyl a-D-glucopyranoside) (**1**) were the subject of studies [2,3]. The spin-lattice relaxation times, T_1, of solvents were measured with the aid of field-cycling NMR relaxometry. The observed low-frequency dispersions of the T_1 of confined EG and PG solvents were interpreted as the result of solvent-pore surface interaction occurring in the

studied molecular gels. It was found that in a low-frequency range a dominating NMR relaxation mechanism is reorientations mediated by translational displacements (RMTD). This dynamic process allows us to explain a very long correlation time of the order of 10^{-5} s calculated for confined EG molecules and even longer one for PG. To the best of our knowledge this is also a first evidence of RMTD relaxation mechanism in gels composed of LMOGs with organic solvents.

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P 339
INVERSE LAPLACE TRANSFORM WITH NOT NECESSARY SPARSE REGULARIZATION

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Inverse Laplace Transform (ILT) is excessively used both in NMR Diffusometry and Relaxometry.
However, the methods suffer from numerical instability of the ILT. To circumvent this problem many regularization methods were proposed like: Maximum Entropy [1], CONTIN [2], ITAMeD[3], TRAiN[4].
In this poster we discuss the advantages of the sparse regularization for ILT together with it generalization for the cases where sparse model is not the proper choice (e.g. Diffusometry of polydisperse samples).

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THE USE OF 1H-HRMAS NMR FOR DESCRIBING THE MECHANISM OF ENANTIODISCRIMINATION OF MDMA ENANTIOMERS BY IMMOBILIZED POLYSACCHARIDE-BASED CHIRAL PHASE

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Studies about the toxicological effects of each single enantiomers of 3,4-methylenedioxymethamphetamine (MDMA), always found as a mixture, takes an important role, and their full separation in semi preparative conditions should be efficient. The separation of chiral secondary amines, such as MDMA, in such condition may not be an easy task. However, the development of new chiral stationary phases (CSP) based on immobilized polysaccharides has been solving this problem properly. A step toward the comprehension of the mechanism involved in the interaction between these chiral analytes with CSP is an interesting subject. Although theoretical approaches can be helpful to understand these interactions, experimental data are always preferable once it reflect the realistic condition. In this context high resolution ^{1}H NMR spectroscopy under magic angle spinning conditions (HR-MAS) has been successfully used to differentiate the interactions of each enantiomer with CSP according to the formation of an enantiomer-CSP diastereoisomeric complex. The MDMA was kindly provided by Prof. Regina Moreau (University of São Paulo-Faculty of Pharmaceutical Sciences) with authorization of Federal Police of Brazil. Both chiral stationary phase (amylose 3-chlorophenylcarbamate-based) and CHIRALPAK ID column were supplied by Chiral Technologies Inc. (subsidiary of Daicel Corporation). The separation of MDMA enantiomers was done by

using a CHIRALPAK-ID chromatographic column, over stacked injections under mass overload conditions and both were obtained with very high enantiomeric purity. The order of elution was determined by circular dicroism, where S-(+) is firstly eluted . After separation, both enantiomers were studied under a suspended-state by ^{1}H HR-MAS NMR, where measurements of longitudinal relaxation times (T_1) and STD experiments were carried out. These suspensions were prepared by mixing the enantiomer solution and the CSP used for packing the column. T_1 experiments were also performed for solutions. The difference between T_1 values, DT_1, obtained for each MDMA enantiomers in solutions and in CSP suspensions was bigger for R-(-) enantiomer, in agreement with the chromatographic data. Aromatic hydrogens also exhibited large variation of their T_1 values. The STD experiments permitted to obtain the epitope map for both enantiomers, and the STD factor was, as expected, bigger for aromatic hydrogens of R-(-) enantiomer, confirming the T_1 and chromatographic data. These results led us to propose a pi-pi stacking interaction between the aromatic moiety of MDMA and the aromatic group of the chiral selector (3-chlorophenyl group). Although the HR-MAS conditions and the intrinsic heterogeneity of the sample do not favor the determination of the on-off equilibrium constants, STD titration experiments could be performed for the suspension containing the R-(-) enantiomer and its dissociation constant could be estimated (K_D=7.4x10^{12} ± 1.6 mg mL^{-1}). As the sample consists on a suspension under high spinning speed (4 kHz); the diffusion of the species is highly favored, weakening the interaction and resulting in the obtained high value of K_D. The same procedure failed for S-(+), due to the weaker interaction between this enantiomer and the CSP here used, as previously confirmed by HPLC. We acknowledge FAPESP, CAPES, CNPq, FAI-UFSCar.

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PROTEIN DYNAMICS IN ANTICANCER THERAPEUTICS, THE FIRST ENOE COMPLEX STRUCTURE

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HDM2 is a natural binder of the tumor suppressor protein p53 and overexpression of HDM2 inhibits the p53 activity. p53 plays a key role in the regulation of many cellular mechanisms such as apoptosis or DNA repair [1]. Therefore, inhibitors of the interaction between HDM2 and p53 are promising anticancer therapeutics. Nutlin-3a is such an inhibitor of HDM2 with nanomolar affinity. Here we present for the first time an exact Nuclear Overhauser Effect (eNOE) structure of a complex, of HDM2 and Nutlin-3a.

The NOE is the most important measurement for structural determination by solution-state NMR. Vögeli et al. developed a new method for the measurement of exact distances with sub angstrom accuracy [2]. To derive distance restraints from NOEs, spin diffusion is a major source of error. Consequently, the eNORA program developed by Orts et al. corrects for spin diffusion using a full matrix approach leading to very accurate intra- and inter-molecular distance restraints [3]. Furthermore, since the eNOE is a time averaged observable, it does not only contain information about the structure, but also about the dynamics. Therefore, the use of eNOEs allows for the determination of multiple-state 3D structures at atomic resolution [4].

In this study we will apply these new methodologies to the HDM2-Nutlin-3a complex. The binding site of HDM2 is very dynamic [5]. Therefore, a complete analysis of its structural landscape can support drug design and can help to improve the understanding of very dynamic protein-protein interactions.

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SURFACE INTERACTION AND DIFFUSION OF SPIN PROBES INSIDE TAILOR-MADE MESOPOROUS ORGANOSILICA MATERIALS

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Porous materials are used for many purposes like gas adsorption and separation, drug delivery, catalysis or liquid chromatography. In all cases the interaction of molecular species with the surface and its transport inside the porous material is of essential importance for the application. Undoubtedly the interaction of guest molecules with the pore surface will influence its mobility. Until now, there is only limited insight into how the chemical nature of the surface inside a porous material influences the interaction with guest molecules on a molecular level and how this interplay can be precisely adjusted. In this context materials are needed which can act as model systems for systematic investigations and differ in only one parameter like the surface functional groups. Further a method is needed which is sensitive for the above mentioned processes and dynamic of the guest molecules.

Here we used temperature dependent electron spin resonance (ESR) spectroscopy of solute nitroxides with different functional groups as model probe molecules for liquid chromatography processes. Systematic investigations have been enabled using different periodic mesoporous organosilica materials (PMOs) prepared from bridging polysilesquioxane precursors $X_3Si\text{-}R\text{-}SiX_3$ (with X a hydrolysable group) containing a bridging phenyl entity, which is substituted in 3-position by different organic moieties [1]. In that way it is possible to systematically vary the surface interaction of the host material with the spin probe while other material characteristics remain unchanged [2]. Using cw-ESR we've been able to probe the molecular interactions of the nitroxide within different functional organosilica materials. One focus was given on diastereotopic interactions using (R)-/(S)-3-carboxy-PROXYL as chiral probe molecule and PMOs synthesized from different amino acid precursors. It is found that mobility of the nitroxide is controlled by the chemical nature of the

surface functional groups and further stereoselectivity of the desired material in combination with the nitroxide. Now diffusion inside the mesoporous materials was studied to probe the surface interaction on greater length scale. Therefore we used a combination of different magnetic resonance methods to monitor diffusion from molecular scale over mesoscale up to the macroscale. Molecular scale diffusion coefficients have been calculated considering spin exchange occurring from the diffusive collision of radicals, and have been compared to MAS PFG NMR (sensitive for meso-scale) and EPR-imaging (sensitive for macroscale diffusion). Our results show, that the choice of surface bound functional groups influence diffusion much stronger than pore-size [3].

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P 351
NMR RELAXATION STUDY OF HALOALKANE DEHALOGENASE DHAA

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Haloalkane dehalogenases (HLDs, EC 3.8.1.5) are bacterial enzymes cleaving a carbon-halogen bond in halogenated hydrocarbons. HLDs are potentially important bio-catalysts with both bioremediation and industrial applications. DhaA is a 33.5 kDa member of the HLD-II subfamily discovered during the 1980s. DhaA structurally belongs to the alpha/beta-hydrolase superfamily. DhaA is composed of the main domain made of an eight-stranded parallel beta-sheet which is flanked by alpha-helices and serves as a scaffold for the catalytic residues and the helical cap domain. The catalytic residues of DhaA constitute a catalytic triad Asp-His-Glu and a pair of halide-stabilizing residues Asn-Trp. Preferable substrates of DhaA are 1,2-dibromoethane and 1,3-dibromopropane.

The complete deuteration of side chains was applied to improve the signal-to-noise ratio in the NMR spectra by suppressing spin diffusion and by decreasing the relaxation rates of 15N spins. Triple resonance experiments utilizing transverse relaxation-optimized spectroscopy (TROSY) were performed. R1, R2 and 15N-{1H} steady-state NOE experiments were carried on the Bruker 850 MHz US2 spectrometer and they were processed for description of motions on the fast time scale. Spectral density mapping and Model-free analysis were used for this purpose.

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DNP ENHANCED SOLID-STATE NMR FOR SURFACES, MATERIALS AND PHARMACEUTICALS

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High field dynamic nuclear polarization (DNP) spectroscopy has emerged as a technique to improve the sensitivity of solid-state NMR spectroscopy by several orders of magnitude. We will show how DNP enhanced solid-state NMR can improve structural characterization of surfaces and inorganic materials[1] and "ordinary" organic crystalline solids such as pharmaceuticals.[2] Notably, many solids and materials can be remotely polarized by the transport of DNP enhanced ^{1}H polarization by ^{1}H spin diffusion.[3] We will also present an overview of our efforts to develop improved biradical polarizing agents.[4] Finally, we will also demonstrate some simple strategies for obtaining proton DNP enhancements that approach the theoretical limit of 658.[5]

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31P NMR HYPERSENSITIVITY ACHIEVED BY USING REVERSIBLE CHEMICAL INTERACTION WITH PARAHYDROGEN IN ULTRA-LOW MAGNETIC FIELD

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SABRE (Signal Amplification by Reversible Exchange) is one of methods which can be applied to create hyperpolarization of nuclear spins by using parahydrogen [1]. It is based on reversible chemical exchange of pH_2 and free ligand molecules with bound ligands of a metal complex. Commonly SABRE was used to hyperpolarize molecules which were coordinated with metal center exclusively by nitrogen atoms. Recently, this approach was successfully applied for hyperpolarizing such nuclei as ^{1}H, ^{13}C and ^{15}N. At the same time there is a large number of ligands coordinating to metal through phosphorus atom. In particular, it is known that dynamic equilibrium of unbound ligand molecules (PPh_3 and H_2) with neutral iridium complex $Ir(H)_2(PPh_3)Cl$ takes place [2], but SABRE was not studied for this system.

In the present work we show for the first time that a substantial hyperpolarization of ^{31}P nuclei of free and bound PPh_3 molecules can be created by reversible chemical interaction of pH_2 and PPh_3 with iridium complex $Ir(H)_2(PPh_3)Cl$ in ultra-low magnetic field [3]. A 260-fold signal enhancement was achieved. The hyperpolarized PPh_3 obtained by this approach was successfully utilized for increasing the sensitivity in ^{31}P NMR imaging experiments. It was shown that polarization transfer has temperature dependence, and the largest enhancement factor (260-fold) observed at 80°C. Kinetic parameters were also measured for temperature range from 60 to 80°C. Theoretical simulations taking into account exchange of $H_2$and PPh_3 reproduced well the observed polarization effects.

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INVESTIGATION OF HYDROGENATION OF TRIPLE AND DOUBLE CARBON-CARBON BONDS OVER IMMOBILIZED IRIDIUM COMPLEX BY USING PHIP

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Parahydrogen-induced polarization (PHIP) is a powerful tool, which opens new opportunities of application of NMR spectroscopy for investigation of hydrogenation reactions. On the one hand, this is due to the fact that PHIP leads to the significantly increased sensitivity of NMR technique (for modern NMR devices up to 10^4 times) [1]. Boosting sensitivity is very important when application of conventional NMR is very difficult or impossible to use due to a low concentration of NMR-active substance, as it often takes place for intermediates of hydrogenation reaction. It also should be noted that characteristic shape of PHIP signals allows to identify hydrogen atoms originating from parahydrogen molecule. This in turn allows to determine the path of addition of hydrogen atoms to a substrate molecule. For these reasons, PHIP is actively used for investigations of both homogeneous hydrogenation mechanism with transition metal complexes and heterogeneous hydrogenation mechanism with supported metal catalysts.

In the present work, we investigated the mechanism of hydrogenation of unsaturated organic molecules on immobilized iridium complex Ir(COD)/P-SiO_2 by using PHIP. Important feature and advantage of such catalysts is combining the advantages of homogeneous catalysts (high extents of pairwise addition) and heterogeneous catalysts (the ease of separation of catalyst from a reaction mixture). Complex Ir(COD)/P-SiO_2 was prepared from $[Ir(COD)Cl]_2$ by covalent grafting on phosphine-modified silica gel. It was found that gas-phase hydrogenation of triple carbon-carbon bond (propyne) leads to a significant enhancement of NMR signals (200-250 times) corresponding to protons of vinyl fragment under PASADENA conditions. In the hydrogenation of double corbon-carbon bond

(propene), only a weak PHIP signal enhancement was observed. In liquid-phase hydrogeantions, PHIP was also detected with substrates containing triple bond (phenylacetylene, 3-butyn-2-ol). At the same time, for the substrates such as styrene, 3-buten-2-ol, acrolein and acrylamide, PHIP was observed only in hydrogenation of acrylamide. Investigation of Ir(COD)/P-SiO_2 in deuteration of styrene shows the generation of HD. HD was also observed when it interacted with D_2 in solution without a substrate. Taking this into account, we assume that intramolecular exchange of hydride ligands of dihydrogen complex with hydrogen atoms of coordinated substrate or phenyl groups of immobilized complex is a characteristic feature for Ir(COD)/P-SiO_2 [2, 3]. These processes can lead to the loss of pairwise addition, and PHIP is not possible to observe. Probably, in the case of hydrogenation of substrates with double corbon-carbon bond, the rate of exchange processes is higher in comparison the rate of the hydrogenation reaction. At the same time, observation of PHIP indicates that in the hydrogenation of alkynes and acrylamide, the influence of these processes is not significant.

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P 366
NUCLEAR SPIN POLARIZATION ALIVE FOR HOURS: A RATIONAL DESIGN.

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Long-lived nuclear spin states (LLS) are configurations of coupled magnetic nuclei exhibiting extended relaxation times, often exceeding the magnetization relaxation time T_1 by an order of magnitude[1]. Currently nuclear spin orders with a lifetime exceeding one hour have been achieved for noble gases. Recently a molecular agent 1,2,3,4,5,6,8-Heptakis(methoxy-d_3)-7-((propan-2-yl-d_7)oxy)-naphthalene (compound $^{13}C_2$-**I** in Fig. 1) containing two ^{13}C isotopic labels has been investigated. This agent, based on a $^{13}C_2$-naphthalene core, has been designed to support very long-lived nuclear singlet order in solution. The singlet decay time constant T_S exceeds 1 hour when dissolved at room-temperature (20 C) in acetone-d_6, in a magnetic field of about 0.4 T. Most importantly, once $^{13}C_2$-**I** is hyperpolarized by dissolution-DNP[2] it can be used for the transport and storage of nuclear hyperpolarization under ambient conditions over an extended period of time. The methodology developed is likely to soon express other long-lived molecular systems with even longer lifetimes.

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ACCESSING LONG LIVED SINGLET STATES BY COUPLING TO DEUTERIUM

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Long lived states (LLS) with hyperpolarization can explore slow chemical or metabolic processes. Here we explore the use of pairs of deuterium atoms to provide access to LLS[1]. A surprising possibility given the quadrupolar nature of ^2H.

A big challenge of hyperpolarized magnetic resonance is the fast signal decay of the hyperpolarized material after injection into the system under study. A potential solution to this problem is the usage of Long Lived States (LLS) to store hyperpolarization on timescales much longer than T_1 (the normal signal decay time constant). In particular, singlet states on pairs of spin ½ particles often exhibit significantly longer lifetimes (T_S) than T_1. Even at high magnetic fields if the spins are chemically equivalent. However, in fully equivalent two-spin systems it is difficult to access the LLS and convert it into a detectable magnetization. Therefore, we have designed chemically equivalent systems, where the magnetic equivalence is broken by coupling to other nearby spins.[1,2,3] The first demonstrations involved coupling ^{13}C pairs to protons, and we were able to extend T_1 times of 12 s seconds to T_S times of 300 s (5 min).[2,3] (For this demonstration we used $^{13}C_2$-diphenyl-acetylene). Coupling $^{13}C_2$ pairs to protons has the slight disadvantage that the protons themselves will induce some relaxation of the LLS. Therefore, we were able to achieve even further lifetime extensions by replacing protons with deuterium nuclei. In these systems ($^{13}C_2$-D_{10}-diphenylacetylene) we couple the $^{13}C_2$ pair in the acetylene bond to deuterons on the phenyl ring drastically reducing relaxation and allowing for hyperpolarization lifetime constants of above 8 min.

In addition, we demonstrate that we can access long lived states on pairs of protons in a comparable manner. We use deuterium atoms as a "handle" to load and unload the desired long lived states in trans-ethylene-D_2. Trans-ethylene-D_2 forms a four spin system with two chemically equivalent spin pairs (two ^1H and two ^2H atoms). On this system we performed life-time measurements using the "M2S" (magnetization-to-singlet)[4] and "S2M" (singlet-to-magnetization) [4] pulse sequences and found a lifetime of up to 120 s (2 min) on the protons. We will be presenting the full quantum mechanical details of these intriguing spin systems and discuss potential applications.

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P 372
SPECIFIC PROTEIN ENHANCEMENT AND IMPROVED RESOLUTION VIA LOCALIZED DNP

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Dynamic Nuclear Polarization (DNP) has become a powerful tool for enhancing signal in solid-state NMR. In general the usage of an optimized concentration of soluble biradical (e.g. TOTAPOL or AMUPol), has become the most frequently used technique in biological DNP studies[1,2,3]. A statistically distributed electron spin reservoir is, however, accompanied by two potential drawbacks: (i) it will homogenously enhance the whole sample including target protein and background (buffer, lipids, etc.) and (ii) it will lead to line broadening effects induced by radicals that are too close to the target protein.

Notably, a few studies have already described the placement of the radical in a more defined position, providing the opportunity to obtain localized information[4,5,6]. In our study we aim to contribute to this new field of localized DNP by presenting a simple but widely applicable approach of placing the radical in a well-defined position in respect to the target protein. Our approach is based on chemical modification of the commonly used TOTAPOL biradical, which allows its targeted ligation to a desired position (e.g. a cysteine residue of the target protein or of an interaction partner) using common ligation procedures.

We demonstrate feasibility and applicability of our approach by using the anti-apoptotic 21 kDa Bcl-x_L protein as well as its interaction partner the 2 kDa Bak peptide. In this setup we covalently attach the modified TOTAPOL (mTOTAPOL) to unlabelled Bak peptide with the aim to specifically enhance the isotope labelled Bcl-x_L protein. We show that: (i) the chemical modifications of mTOTAPOL do not decrease its DNP enhancement capabilities, (ii) the radical-attached Bak peptide still strongly binds to Bcl-x_L, (iii) our setup allows specific polarization enhancement of the target protein and (iv) spectral resolution of the target protein is significantly improved as compared to the conventional setup.

While the improved line width will be generally beneficial for a broad range of DNP applications, we anticipate that the specificity introduced by our approach will facilitate the investigation of target proteins in large backgrounds such as crude cell lysates or (membrane) proteins in whole cell preparations.

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POLYMERIZATION PRODUCTS FORMATION STUDIED BY PHIP

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Parahydrogen-induced polarization (PHIP) has been successfully utilized for NMR/MRI sensitivity boosting. At the same time, there are many examples of chemical reaction mechanistic studies performed using PHIP. The high NMR signal enhancements were utilized to detect key intermediates and side-products of hydrogenation processes [1]. An example of PHIP-enhanced NMR signals observation in hydroformylation reaction is also present [2]. Meanwhile, polymerization reaction is one of the most important industrial processes, which mechanism often debated.

Herein, we used PHIP for detection of polymer products formed during the hydrogenation of acetylene. Selective hydrogenation of traces of acetylene is the basic approach for production of polymer-grade ethylene from the products of thermal cracking of petrochemicals. Polymerization processes always accompany the hydrogenation of acetylene performed using conventional heterogeneous industrial catalysts, and lead to deactivation of the hydrogenation catalysts. The minimization of polymer production in this case is an important practical problem, which can be solved by elucidating the mechanism of this process. In this respect, PHIP can expose the formation of polymers present in low concentration and can provide information about pairwise route of H_2 addition in the course of the polymerization. In the experiments, we used Pd heterogeneous catalyst capable of hydrogenating acetylene to ethylene selectively. In addition to production of ethylene, which is the important substance in terms of production of nuclear spin isomers of polyatomic molecules [3], the reaction produced polarized oligomeric C4

products: 1-butene, 2-butene and 1,3-butadiene. Smaller amounts of heavier polymeric polarized products were also detected. The observed nuclear polarization and considerable signal enhancements demonstrated the presence of pairwise route of H_2 addition in the course of the polymerization as well as the possibility of PHIP for polymerization reaction products in principal. To the best of our knowledge, this is the first example of using PHIP for polymer formation studies.

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METAL-FREE CATALYSIS FOR PARAHYDROGEN-INDUCED POLARIZATION

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Catalysts play a key role in production of parahydrogen-induced polarization (PHIP). Until recently, only metal-containing hydrogenation catalysts have been utilized for producing substantial NMR signal enhancements by means of PHIP. As a result, PHIP has been successfully utilized for mechanistic studies and NMR/MRI sensitivity boosting in homogeneous and heterogeneous processes that involve H_2 activation mediated by metal complexes and nanoparticles. Meanwhile, pairs of various sterically separated ('frustrated') Lewis acids and Lewis bases (FLPs) were reported to split dihydrogen. We have found that molecular tweezers, i.e., unimolecular FLPs containing functional centers connected by a chelating molecular link, are capable to produce PHIP of tweezers-captured parahydrogen molecules [1]. In principle, dihydrogen molecules captured by the tweezers and other FLPs can be transferred to other organic substrates providing metal-free catalytic hydrogenation.

Despite progress in FLPs chemistry and applications, a detailed mechanism of H_2 splitting by FLPs is still debated. Herein, we present a systematic study of PHIP effects observed for series of ansa-aminoborane compounds differing in structure. In particular, new path of hydrogen activation mediated by dimeric structures was found using PHIP for the ansa-amoniborane with the smallest boryl site in addition to the reported path [2]. Chemical exchange, quantum exchange and H_2 splitting processes were examined experimentally

and theoretically by DFT analysis. The influence of aminoborane steric hindrance on PHIP effects were elucidated from variable temperature experiments.

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