

**XX. WORKSHOP OF
BIOPHYSICAL CHEMISTS AND
ELECTROCHEMISTS**

BOOK OF ABSTRACTS

17TH AND 18TH SEPTEMBER 2020

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The organizers thank a lot to all this year's sponsors for the support, which enabled to organize this traditional conference: Metrohm Czech Republic s.r.o., Institute of Biophysics of the Czech Academy of Sciences in Brno, ANAMET s.r.o., Anton Paar Czech Republic s.r.o., Eppendorf Czech & Slovakia s.r.o., HELAGO – CZ, s.r.o., CHROMSPEC spol. s r.o., MERCI, s.r.o. and Czech Chemical Society, subdivision Brno.



An introductory word...

As you know, due to the coronavirus pandemic, this year's jubilee, 20th Workshop of Biophysical Chemists and Electrochemists was postponed from the summer term to the autumn one. The coronavirus situation of the last few days is not entirely clear and it is necessary from the point of view of your protection to observe stricter hygienic recommendations and to maintain a disinfected environment for the whole two days of the conference. Together, we will certainly be able to do it and protect ourselves from COVID-19 (using masks, keeping a distance, frequent disinfection of hands, especially before eating, etc.). Although the course of the conference is influenced by this situation, we wish you the conference filled with many interesting presentations, both in the form of lectures or posters. We wish students and young scientists competing in the Youth Section successful presentations that can be finalized by winning both Awards (the Metrohm Award and the Emil Paleček Award).

According to the attached program you can see the schedule of plenary lectures, invited lectures as well as oral presentations of young scientists. During the conference, we will remember also the 90th birthday anniversary of Prof. Emil Paleček, the famous Czech electrochemist who worked in Brno and devoted his career to the electrochemistry of biologically important substances, especially of nucleic acids. The conference will also include presentations of posters and companies which financially support the event. Special thanks go to two main sponsors, the Metrohm and the Biophysical Institute which financially participates in the Emil Paleček Award. All participants are invited to the Poster Session followed by the gala dinner on Thursday afternoon. On Friday, the conference will be concluded with the announcement of three winners of the “Young Scientists’ Session”, one winner of Emil Paleček Award, and one winner of the Poster Session.

We are confident that our 20th Workshop of Biophysical Chemists and Electrochemists (WOBICE) will be memorable for its scientific quality, also thanks to your contributions. We do hope that you will welcome the opportunity to present and discuss your scientific results.

Welcome to Brno (Masaryk University) and enjoy this conference!

Don't forget to protect yourself from COVID-19!

Libuše Trnková

Motto: “The love of science is the love of truth, because honesty is a fundamental virtue of scientists.”

Ludwig Feuerbach

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ELECTROCHEMISTRY FOR SINGLET FISSION

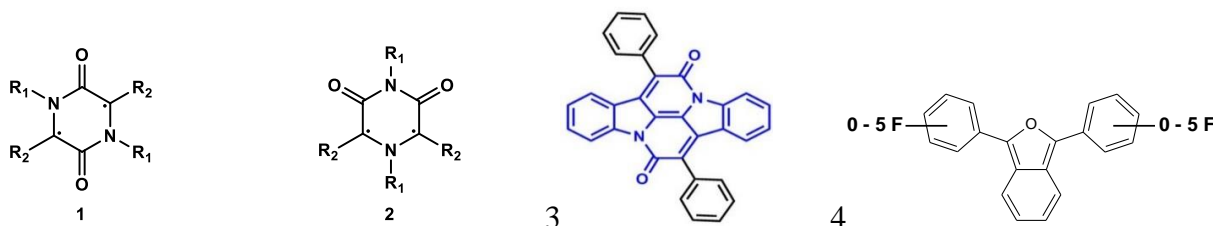
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Singlet fission is a photophysical process where the excited singlet state can share its excitation energy with a neighbouring ground-state chromophore and both are converted into two triplet states [1,2]. This is possible only when the triplet energy of an organic chromophore is equal or even lower than a half of its singlet excitation energy. Singlet fission does not require change of spin of any from the molecules. In this way from one excited molecule two electrons can be separated, hence the effectivity of the transformation of light to electricity can be higher. This is the reason for increasing interest in molecules offering this property.

For successful singlet fission, the promising molecules should form so called biradicaloids [3]. Suitable available compounds are e.g. substituted 2,5- and 2,6-diketopiperazines (**1,2**) [4], substances derived from indigo – e.g. Cibalackrot (**3**) [5] or derivatives of 1,3-diphenyl-isobenzofuran (DPIBF, **4**) [6].



Recently, electrochemical investigations of newly synthesized series of fluorinated DPIBF derivatives¹ were performed⁶ where the influence of number and position of fluorine atoms in the molecule on the first oxidation and first reduction potentials was followed and the respective mechanism suggested. Besides standard steady-state, as well as dynamic electrochemical techniques were used, together with *in situ* UV-vis and EPR spectrometry.

When the derivatives of DPIBF have 1 to 4 fluorine atoms (in one case even 6), their oxidation (two-electron, irreversible) as well as reduction (one-electron, reversible) patterns are in principle analogous to non-fluorinated parent DPIBF. It appeared that a) the fluorine atoms in *p*-position has no big influence on potential values; b) fluorine atom in *m*-position shifts oxidation potential by about 50–60 mV more positively; c) two fluorine atoms in *o*-position have steric influence (twisting the phenyl ring out of the plane). Redox mechanisms of DPIBFs with 5 and 10 fluorine atoms differ substantially from the rest of the series.

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The grant 19-22806S (GAČR) and the institutional support RVO 61388955 are acknowledged.

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BIOSENSORS - WHERE ARE THE LIMITS

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The ability to detect very low concentrations of analytes is of fundamental importance. In many critical situations, even few molecules of toxins can be harmful, individual pathogenic microbes initiate infectious disease, and traces of cancer markers indicate beginning of malignant transformation. From the suitable approaches, single-molecule immunochemical sensors and assays provide the detection of individual analyte molecules reaching the ultimate sensitivity in analytical chemistry [1]. Immunosensors are being developed to enhance the sensing capabilities, lower limits of detection and provide point-of-care mode of use with short analysis times. The promising approaches recently initiated in our laboratory include digital affinity sensing - single-molecule assays (or better single-analyte to include also viruses, bacteria and cells), combination of optical and electrochemical transduction (electrochemiluminescence, photoelectrochemical conversion), and inorganic nanocrystals for either luminescent labelling (quantum dots, photon up-conversion nanoparticles) or catalytic (Prussian blue) signal enhancement [2]. Furthermore, advanced nanotools based on scanning probe microscopies allowed to touch and interface individual cells using nanomechanical biosensors, in combination with microelectrode arrays and fluorescence [3]. This provides detailed information on the physiological state of patients-derived cells, allows to model disease progress at the cellular level and optimize medication leading to personalized medicine. In conclusion, the combination of biosensors with new tools and materials resulting from nanobiotechnology studies allowed to direct this field to new horizons.

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SPECTROSCOPY APPROACH TO ELECTROCHEMISTRY

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Spectroscopy and electrochemistry are related in several ways. Spectroscopy of unstable species is studied in electrochemical cells, electronic spectroscopy is used for estimating redox potentials in the excited state. In my presentation, I will focus on photoelectron spectroscopy, allowing in principle direct determination of redox potentials. Oxidation and ionization are the same processes and measuring the binding energies of solvated molecules, therefore, bear electrochemical information. Photoelectron spectroscopy was for a long time effectively restricted to molecules in the gas phase and for solid surfaces. The recent development in liquid photoemission spectroscopy (PES) opens an alternative way towards estimating redox potentials. The PES provides however non-equilibrium quantities, vertical ionization energies while redox potentials represent equilibrium quantities. The missing link can be provided by quantum chemical calculations or other theoretical considerations.

I will describe the history and present status of photoemission spectroscopy in the liquid environment [1], with a particular emphasis on modeling ionization and redox properties of various molecules in the hydrated environment [2], e.g. nucleic acids [3,4]. I will also discuss the potential pathways towards single-ion solvation energies and absolute redox potentials.

ACKNOWLEDGEMENT

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RELATING IN-MEMBRANE OLIGOMERIZATION NUMBERS OF PROTEINS TO THEIR FUNCTION

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Oligomerization of proteins is often related to their function. However, many completely opposite cases have been reported in literature where formation of protein clusters is rather indicative of their biological disfunction. As an example may serve a fibroblast growth factor 2 (FGF2) or well-known proapoptotic protein Bax, that oligomerize on the membrane into functional membrane pores. Researchers have used all kinds of biophysical approaches to characterize such in-membrane aggregates and intuitively connected the obtained oligomerization numbers with the formation of a functional pore. The question, however, is, whether this intuitive connection indeed holds.

We address this question by investigating membrane translocation of FGF2, a protein that has many biological functions from wound healing, cell differentiation to functioning as a signaling molecule in tumor-induced angiogenesis. Based on mainly biochemical assays, it has been suggested that FGF2 oligomerizes into permeable pores at cellular plasma membranes from which it is released into the extracellular space. To study the process of oligomerization in detail, we determined the size of FGF2 oligomers by measuring the brightness of individually diffusing FGF2 oligomers, and correlated the size of these oligomers to membrane permeability. Moreover, we monitored the oligomeric state of FGF2 on the same lipid vesicle over time, which allowed for correlating the formation of pores to FGF2 oligomerization. We show that solely determining oligomerization numbers of proteins gives very limited information on the formation of pores. On the contrary, the statistics of our data implies that some published in-membrane aggregation numbers might not be physiologically relevant.

RECENT STUDIES REVEAL NEW RELATIONSHIPS BETWEEN THE STRUCTURE AND ELECTROCHEMICAL BEHAVIOR OF NUCLEIC ACIDS

(in memory of Professor Emil Paleček on the occasion of his 90th birthday)

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Nucleic acids are electrochemically active [1]. Traditionally, adenine (A) and cytosine (C) have been reported to undergo irreversible electrochemical reduction and yield (usually a collective) cathodic signal at mercury or silver amalgam electrodes. Guanine (G) reduction on the mercury electrodes has been observed indirectly via re-oxidation of the guanine reduction product 7,8-dihydroguanine (GH₂). All four canonical DNA bases, uracil (U) and 5-methylcytosine (5-mC) give anodic responses upon their oxidation at carbon electrodes. Electrochemical signals of DNA are influenced by its structure via differences of the nucleobase accessibility in structured, base-paired (e.g., duplex) DNA segments *versus* those located in unstructured regions [1]. Thus, the DNA secondary structure has been considered as the primary factor influencing the behavior of DNA at the electrodes. Our recent observations suggest this scheme to be incomplete and/or outdated in several aspects:

(a) Not only the mercury (or amalgam) electrodes are useful to observe nucleobase reduction in the cathodic region. Basal plane pyrolytic graphite electrode (PGE) has been successfully applied to detect both oxidation and reduction of the canonical DNA bases, U and 5-mC in synthetic oligonucleotides [2] and in nucleosides [3]. Notably, unlike with the mercury electrodes, with the PGE we observed specific reduction signals of G, U and T. It has been also possible to detect products of the primary irreversible reductions/oxidations of most of the bases via their oxidation/reduction, respectively.

(b) In contrast to analogous effects exhibited by proteins [4], electrocatalytic properties of unmodified nucleic acids have been ignored for decades. Recently it has been shown [5] that under suitable conditions, both RNA and DNA produce signals analogous to the “peak H” of proteins, which can be utilized for sensitive detection of the nucleic acids as well as for monitoring of structural changes. Moreover, based on the catalytic reactions, hydrogen-deuterium exchange in DNA has been for the first time monitored using electrochemical methods [6]. More recently, A and C (or, more precisely, their reduction products) have been demonstrated to be responsible for the CHE by DNA or RNA.

(c) Our earlier observations have suggested that reduction of G to GH₂ includes a chemical step, probably reduction of the G moiety by electrochemically generated nascent hydrogen. This was in accord e.g., with the positive effect of CHE in the presence of platinum complexes [7]. Recently we have observed [8] a strong positive effects of A and/or C residues (i.e., bases exhibiting the CHE) on the G reduction. In the absence of A and C (such as in oligonucleotides composed of G+T only), the G reduction was depressed, as indicated by a negligible peak due

to GH₂ oxidation. These observations suggest that not only secondary structure, but also base composition (and/or sequence) of DNA strongly influences DNA electrochemical responses via mutual influence of individual bases on relevant electrode processes.

(d) Homo-oligonucleotides exhibit specific interfacial behavior at the mercury electrode surface [9]. Particularly homopyrimidine stretches are specific in this respect, since they undergo 2D condensation processes at the negatively charged surface. On the other hand, homopurine blocks do not tend to formation of the condensed films but show a behavior which is qualitatively similar to that of random-sequence DNA. Besides their characteristic surface activity, facilitated reduction of C residues in homo-C blocks has been observed, depending on the block length.

Taken together, our observations suggest electrochemical and surface processes to be more complex than generally accepted until recently. This extends possibilities of nucleic acid electrochemistry. On the other hand, the above-mentioned phenomena should be carefully considered particularly in DNA structural studies.

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PHOTON-UPCONVERSION SCANNER FOR MULTIPLEXED IMAGING

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In analytical chemistry, multiplexed assays facilitate parallel detection of analytes. The multiplexing reduces the consumption of sample, reagents, and other resources and can be applied for medical diagnostics, the studies of biomolecule interactions, single-cell and single-molecule assays, environmental monitoring, pathogen detection, multiparameter chemical and biological assays and screening of chemical libraries. Probably the most convenient approaches towards multiplexing utilize fluorescence reporters – organic fluorophores and quantum dots are the most prominent examples [1-3].

Nowadays, photon-upconversion nanoparticles are of increased research interest because of their unique spectral properties [4]. Photon-upconversion nanoparticles are lanthanide-doped nanocrystals that emit visible photon-upconversion luminescence under near-infrared excitation. The advantages of photon-upconversion include multiple and narrow emission bands, negligible autofluorescence, and high stability. However, a wider use of photon-upconversion for multiplexing was prevented by spectral overlaps between different types of nanoparticles. Just recently, we have overcome this limitation by introducing a non-negative least squares algorithm [5]. When several types of nanoparticles are presented, the algorithm performs deconvolution of their emission and estimate the relative concentration of each nanoparticle type. These relative concentrations are subsequently interpreted as multiplexed detection channels.

Previously, we utilized this approach for barcoding droplets in droplet microfluidics [5]. Here, we step further and apply this principle on photon-upconversion scanning of planar surfaces. This scanning technique has the potential for use in multiplexed immunochemical assays with photon-upconversion labeling and documenting gel electrophoresis of photon-upconversion nanomaterials [6]. The laboratory-made scanner consists of an epiluminescence laser scanning head, which is attached to an XY-moving stage [6]. A CCD array spectroscopy is used for recording an emission spectrum for each point of the scanned surface.

The multiplexed imaging was proved on three types of nanoparticles containing either Er^{3+} , Ho^{3+} , or Tm^{3+} as activator ions (see Figure). The dispersions of these nanoparticles were dispensed on a microtiter plate and scanned by a laser scanner. The relative concentrations of each nanoparticle were estimated and imaged as red (Er^{3+}), green (Ho^{3+}) and blue (Tm^{3+}) color.

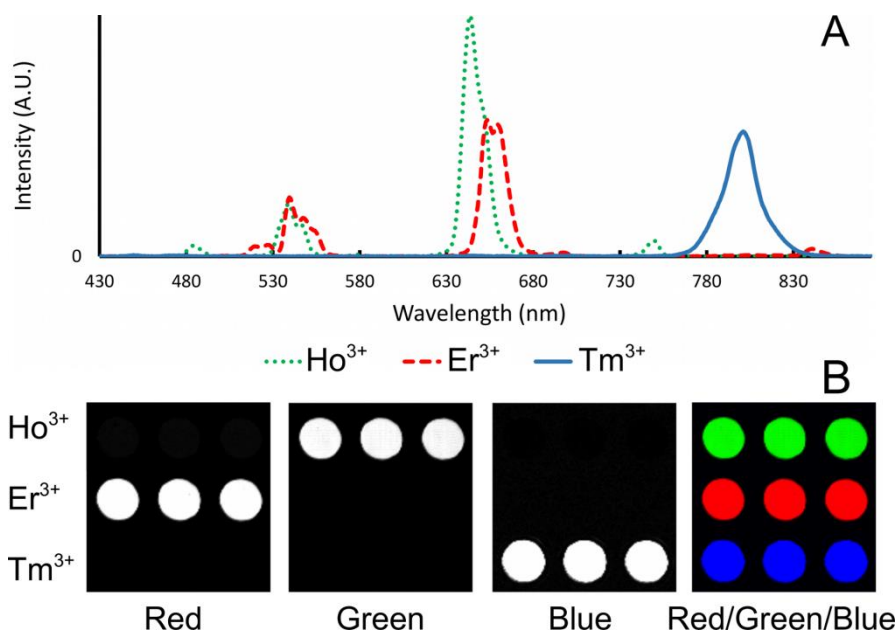


Figure: (A) Emission spectra of photon-upconversion nanoparticles. Photon-upconversion nanoparticles were dispersed in water. These dispersions were loaded on 96 well microtiter plate and emission spectra were recorded (100 μL per well, integration time 1 s, 980 nm excitation, concentrations 10 mg mL^{-1} , 0.25 mg mL^{-1} and 0.13 mg mL^{-1} for nanoparticles doped with Er^{3+} , Ho^{3+} and Tm^{3+} , respectively). (B) Scanning of photon-upconversion nanoparticle dispersions on a microtiter plate. Each dispersion was dispensed in three replicates (100 μL per well) and the plate was scanned with a spatial resolution of 200 μm . The photon-upconversion emission spectrum was recorded at each point with 10 ms integration time (980 nm excitation). By the non-negative least squares algorithm, the relative concentration of each photon-upconversion nanoparticle type was estimated independently. These relative concentrations were subsequently used as independent detection channels and imaged as red (Er^{3+}), green (Ho^{3+}), and blue (Tm^{3+}) colors in the scanned image.

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COMBINATION OF BIOPHYSICAL TECHNIQUES FOR PROTEIN CHARACTERIZATION – A CASE STUDY OF *ASPERGILLUS FUMIGATUS* LECTIN

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Studying proteins requires a combination of various approaches. As we try to describe the protein behaviour in detail, we start to understand that we need to determine basic protein characteristics properly. These include protein sequence, molar mass, conformational state, short- and long-term stability, temperature stability, activity, etc. It is also important to evaluate the obtained data with respect to each other and not independently. The more information we need to get, the higher is the demand on material and time resources. However, the quick development of biophysical techniques in last decades enables more precise and information-rich analysis with lower sample consumption. Since the instrumentation is relatively costly and requires good expertise, many institutes gather biophysical instruments in dedicated core facilities. This is also true for Central European Institute of Technology and its Core facility Biomolecular Interactions and Crystallization. Here, the complex protein analysis can be performed in one place.

As a case study, we have chosen the lectin AFL from the opportunistic pathogenic mold *Aspergillus fumigatus*. This sugar-binding protein was shown to bind fucosylated oligosaccharides with moderately high affinity and therefore it was proposed to assist the mold to invade human lungs and other tissues [1]. As such, it could be a promising target for diagnostics and/or drug development. We performed a series of basic characterization experiments that include a check of temperature stability using differential scanning calorimetry (DSC) and differential scanning fluorimetry (nanoDSF), pH stability using circular dichroism spectroscopy (CD) and analytical ultracentrifugation (AUC) and homogeneity check using the dynamic light scattering (DLS) plate reader. Since the protein is not an enzyme, its “activity” is determined by binding to its sugar ligand. For that, we combined isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), microscale thermophoresis (MST) and bio-layer interferometry (BLI) binding assays. Some of these techniques are high-throughput, hence we performed also a buffer screening using an in-house developed 48-condition buffer screen. Our study demonstrates not only the broadness of biophysical techniques that can be combined in protein analysis but also the possibility to perform a complex study with a relatively low amount of sample needed.

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CHALLENGES IN STRUCTURAL AND INTERACTION PROPERTIES OF INTRINSICALLY DISORDERED PROTEINS

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Intrinsically disordered proteins (IDPs) lack a stable 3D structure. Their significance is increasingly recognized after proteomic analysis showing that about one tenth of all eukaryotic proteins are IDPs and more of one third contain considerably long intrinsically disordered protein regions (IDPRs). The missing stable 3D structure prevents using crystallographic and cryoEM methodologies.

Here, we present the approach combining the solution protein NMR and computation simulations producing structural ensembles at atomistic resolution. In the case of NMR of IDPs, signal overlaps cause problems that can be overcome by collecting spectra of higher dimensionality, feasible thanks to slower relaxation rates of IDPs. Another problem in the combined approach is the observation that the biomolecular force field parameters optimized for globular proteins fail to properly reproduce properties of IDPs. Here, we present an application of force field parameters recommended for IDPs in molecular dynamics (MD) simulations of two hybrid proteins containing IDPRs in about their half sequence and one complete IDP in its full length. The obtained MD trajectories were used to predict measurable parameters, including radii of gyration of the proteins and chemical shifts, residual dipolar couplings, paramagnetic relaxation enhancement, and NMR relaxation data of individual residues. The predicted quantities were compared with the corresponding experimental data. The results showed that the NMR relaxation parameters, rarely used for benchmarking, are particularly sensitive to the choice of force-field parameters, especially those defining the water model. Additional analysis revealed only one particular force field capable of retaining the transient helical motif observed in NMR experiments.

Solution NMR proved also to be useful for recognition of interaction epitopes and the corresponding binding affinities for both IDPs as well as hybrid proteins. Multiple binding epitopes represent another layer of complexity in the interpretation of such interaction studies.

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ION-SPECIFIC EFFECT ON PROTEINS PROBED BY SURFACE WATER

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Water dynamics in protein solutions indirectly provide information about the **protein surface** [1]. This surface can be modified by binding the ligands, such as ions of simple salts added to aqueous protein solution [2, 3]. In the present study we measured relaxation of water molecules (spin-lattice relaxation rate, R_1 , measured by NMR relaxometry) in hen egg-white lysozyme (LZM) and bovine serum albumin (BSA) solutions. Surprisingly, an addition of the same type of salt has an opposite effect on LZM compared to the BSA solutions (Fig. 1). In order to explain observed differences between the two globular proteins, we proposed a model in which protein surface roughness as given by the fractal dimension was included as a parameter. The model calculations suggested that a given salt may modify the surface of different protein species differently, what is reflected in the reversal of the liophilic series.

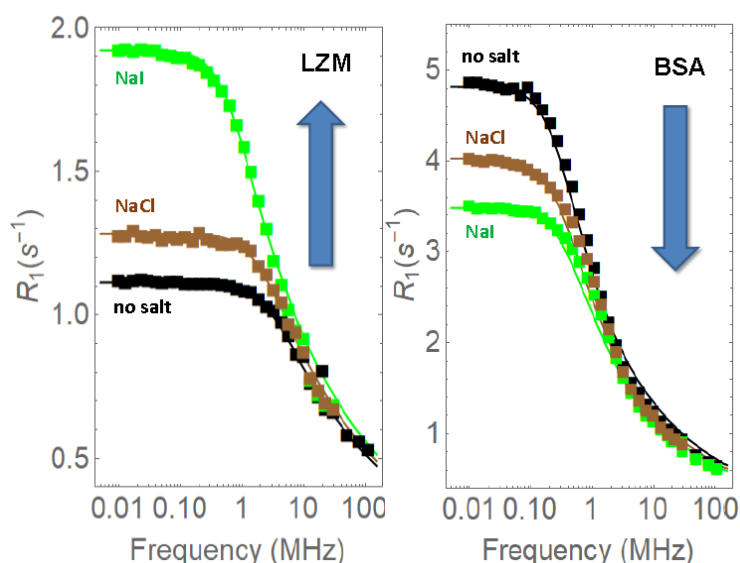


Figure: Water ^1H R_1 profiles for LZM (left) and BSA (right) solutions. Salt addition causes an increase of the relaxation rate for LZM and decrease for BSA (arrows). Symbols represent measurements while the continuous lines represent model

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REDOX PROPERTIES OF GERMANIUM-BASED PHOTOINITIATORS

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Diacylgermanes such as Ivocerin[®] are used for dental applications serving as non-toxic photoinitiators for radical polymerization. The corresponding photo-induced reaction mechanisms are well established [1].

Irradiation with light in the visible region leads to a homolytic C-Ge bond cleavage resulting in radical formation. The absorption spectra of the photoinitiators depend on the electron donating/withdrawing character of substituents at the aromatic moieties [2-3]. Because of penetration depth, it is desirable that the candidate compounds would undergo the cleavage process using as long wavelengths as possible. Push-pull effects, however, also indicate a substantial effect on the redox properties of the acylgermanes monitored by means of electrochemical methods. The first electron reduction of the parent compounds yields an anion radical. Thus, electrochemical investigation of acylgermanes presents a convenient and independent tool in searching for another promising photoinitiators.

In this context, we present our results obtained by DC-polarography and cyclic voltammetry in aprotic media (dry DMF) across a series of mono-, di-, tri- and tetraacylgermanes, di- and tri-nuclear polyacylgermanes as well as analogical silanes and stannanes, respectively [4]. The observed trends in their first reduction potentials will be discussed connected with the substitution pattern at the central atom.

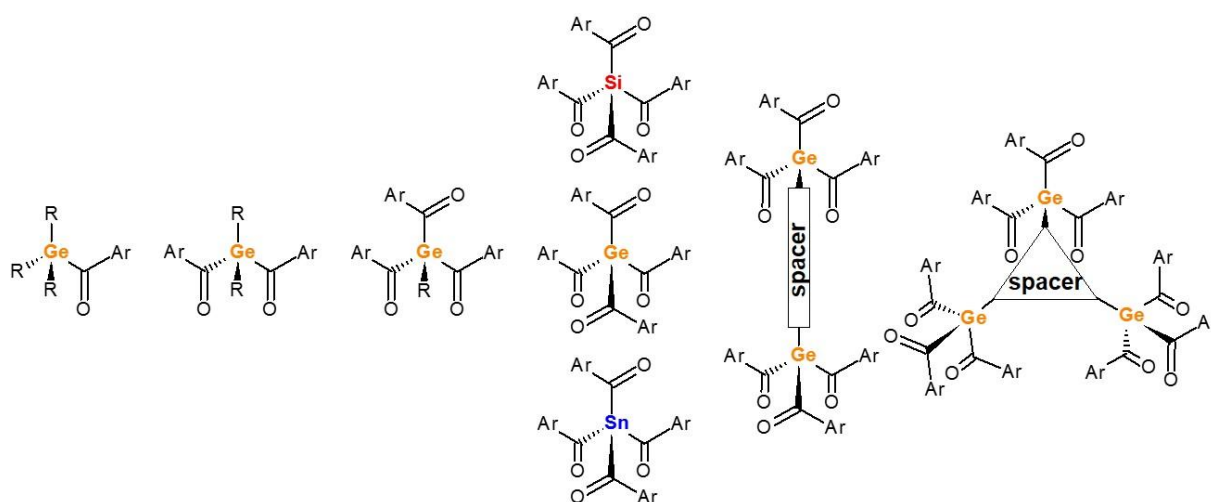


Figure: Structures of investigated compound classes (Ar=aryl, R=alkyl).

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MECHANICAL PROPERTIES OF BIO-SAMPLES EXPLORED BY ATOMIC FORCE MICROSCOPY

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Atomic force microscopy (AFM) is a high-resolution imaging technique; however, also a sensitive tool able to study mechanical properties of samples in a variety of sizes, i.e., from nanostructures, biomolecules to the individual cells and tissue slices. Moreover, such measurements can be performed in a laboratory atmosphere (with dry samples) but also under near-physiological conditions – i.e., in buffered solutions and elevated temperature (mostly 37 °C). Micromechanical transducers (cantilevers) are often used to map surface stiffness distribution, adhesion forces, and viscoelastic parameters of a variety of different surfaces – starting from solid surfaces such as nanostructured surfaces (electrodes, pillars, nanotubes), through the analysis of single molecules and their complexes, the range of sample may finish with the less stiff samples such as hydrogels and living cells.

Use of AFM microscopy to measure structural and mechanical properties on various sample sizes will be presented – starting from chromatin-protein complexes, cardiomyocytes biomechanics up to stiffness measurements on living plants.

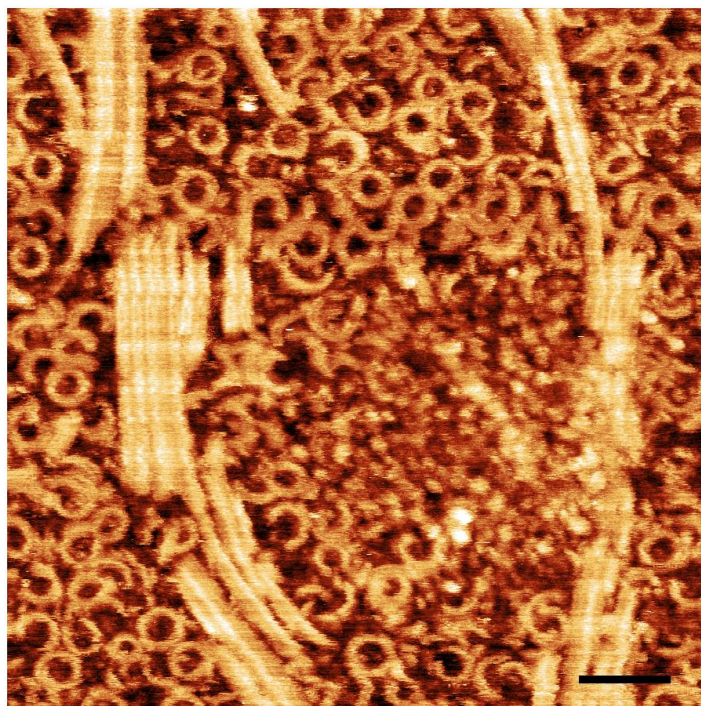


Figure: Structure of native microtubulin captured under physiological conditions. The scale-bar is equal to 100 nm.

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USE OF G-QUADRUPLEX LIGAND AS ELECTROCHEMICAL PROBE IN DNA STRUCTURE RECOGNITION

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G-quadruplex (G4) is a type of alternative secondary structure of DNA present in guanine rich regions of the genome. G4 play a role in key biological processes in cells and their stabilisation by interactions with small molecular ligands can be potentially used in cancer treatment [1]. In this work, the basic electrochemical properties of the selected G4 ligand *meso*-5,10,15,20-tetrakis(4-(*N*-methyl-pyridinium-4-yl)phenyl)porphyrinato copper(II) (Cu-TMPyPP) were determined by the means of cyclic voltammetry at hanging mercury drop electrode (HMDE). The same method was further used to monitor the interaction of Cu-TMPyPP with G4 forming oligodeoxynucleotides (ODNs) as well as single- and double-stranded ODNs. As a result of the interaction with DNA the reduction peak of Cu-TMPyPP undergoes a significant shift towards more negative potentials allowing the discrimination between free and bound form of the ligand. However, the observed shift is not structurally or sequentially specific for the individual ODNs. Subsequently, Cu-TMPyPP was used as electrochemical probe for the characterisation of ODNs adsorbed on the HMDE surface. After the application of increasing number of potential cycles from 0 V to -1.85 V to the adsorbed G4-forming ODNs and subsequent interaction with Cu-TMPyPP, the observed cathodic signal was gradually shifted to the potential corresponding to the unbound ligand. This effect is the result of desorption of the weakly adsorbed G4 structures due to the highly negative potentials present during the cycling, which create free electrode surface accessible for the reduction of Cu-TMPyPP. This behaviour is not observed for more strongly adsorbed linear ODNs, with the exception of homothymine 30-mer, which likely interacts with Hg^{II} atoms to form a coiled structure with electrostatic properties similar to those of G4.

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CATALYTIC HYDROGEN EVOLUTION REACTION OF AVIDIN AND NEUTRAVIDIN. INFLUENCE OF PROTEIN MODIFICATION AND GLYCOSYLATION.

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The first paper about the electrochemical analysis of proteins was published with J. Heyrovsky and J. Babička. Their experiments were performed with albumin in the presence of ammonium ions, using the direct current (dc) polarography. Albumin yielded the so-called "presodium wave" due to catalytic evolution of hydrogen reaction [1]. Later M. Tomschik et al used constant current chronopotentiometry (CPS) instead of polarography for the study of peptides and they observed well-developed CPS peak H (nominated peak H because of its High sensitivity, Hydrogen evolution and Heyrovsky) [2]. Amino acid residues with labile protons such as cysteine, histidine, arginine and lysine are involved in catalytic reaction responsible for peak H [3]. This peak is sensitive to changes in the protein structure and low protein quantities can be analysed by CPS peak H [4].

In this work, we compared unmodified avidin with neutravidin having similar size and structure at the charged interface to better understand the role of charged amino acid residues and glycan presence in the electrochemical behaviour of proteins. Neutravidin is the product of avidin deglycosylation and neutralisation. Neutralization is done by modification of some positively charged lysine residues without losing biotin-binding affinity. We analysed avidin and neutravidin by label-free CPS analysis and alternating current voltammetry (ACV) combined with a mercury electrode. We found a significant difference in CPS responses of these two species due to the different amounts of catalytically active free amino groups of lysine and arginine residues in their structure. We acetylated both avidin and neutravidin to modify all charged residues and to find the impact of glycan on the electrochemical behaviour of this protein.

Acetylation of free amino groups suppressed differences between CPS responses of avidin and neutravidin. The glycan presence in acetylated avidin resulted in about 30% higher the CPS peak H in comparison to acetylated neutravidin. The effect of the glycan presence in protein was further evaluated using neutravidin and its complexes with biotinylated oligosaccharides [5].

We suggest that the presence, size and composition of the glycans influenced the CPS signal due to differences in orientation at a charged surface.

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DEPOSITION OF DRIED COLLOIDAL SUSPENSION VIA NANO-ELECTROSPRAY

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The controlled deposition of nanoparticles on the surface of a substrate can significantly modify its properties and adjust it for a target application. The change in wettability, conductivity, or biological compatibility belongs to typical examples. For most applications, it is beneficial to treat the surface equally to reach the system of high robustness. Unfortunately, the straightforward dripping or deposition of wet colloids (e.g. inject printing) often result in non-homogenous distribution of nanoparticles known as the coffee-ring effect. [1]

Electrospray is a relatively well-established tool for the deposition of nanoparticles. In this work, we use nanoelectrospray (nES) operating at flow rates down to tens of nanoliters per minute. As a result, the formed aerosol consists of very small droplets quickly drying during the flight towards a counter-electrode. Therefore, we obtained deposits of increased homogeneous distribution without an undesirable coffee-ring effect. Moreover, the character of the aerosol plume is only a few millimeters wide, so it allows good control over the deposit localization.

For the demonstration of the potential of nES system, silver nanoparticles were chosen. The silver colloid was synthesized according to Lee-Meisel protocol (reduction and stabilization via sodium citrate) achieving the nanoparticles of an approximate diameter of 70 nm and 0.1 mg/mL theoretical concentration of silver. No colloid treatment was performed prior to nES treatment.

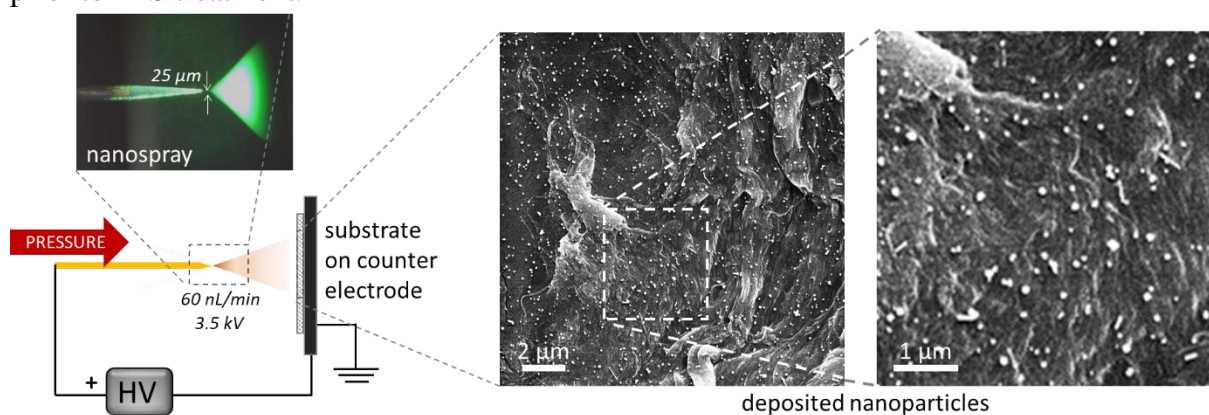


Figure: The schematic illustration of instrumentation for even distribution of silver nanoparticles. As a substrate, a common office paper was used. The deposit of nanoparticles was characterized by SEM.

For the robust deposition of a silver colloid, we designed and fabricated simple yet very effective instrumentation. The instrumentation consists of a pressurized chamber with a

container carrying a colloid with an immersed platinum electrode as well as silica-fused capillary. The other end of the capillary was ground into a sharp tip and acted as an emitter, while the electrode defined the spray voltage for stable nES performance. [2] The capillary tip was aimed against a flat counter electrode with the attached substrate (see Figure). The flow rate was regulated via nitrogen pressure generated inside the container.

The character of the produced aerosol is highly dependent on experimental parameters. Applied voltage, flow rate, solvent composition, and the inner diameter of an emitter are widely-accepted as the most crucial factors. At improperly optimized conditions, large droplets, spray instabilities, or discharges could occur, all negatively affecting the deposit profile. Using a 27 cm long capillary with the inner diameter of 25 μm , we determined the flow rate of 60 nL/min at 3.5 kV as the most robust settings for achieving fine droplets.

Scanning electron microscopy (SEM) was used for the characterization of created deposits and did not reveal any significant effects of nES-processing on the shape of the nanoparticles. As the most crucial factor for the deposition of dry colloid, the tip-substrate distance was recognized. The complete removal of water was achieved only at distances larger than 2 mm. Notably, the tip-substrate distance regulated also the area of the treated surface. The surface density of nanoparticles can be therefore controlled with both, the tip-substrate distance as well as the time of the deposition.

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HOW PHOSPHORYLATION IMPACTS 14-3-3 ζ DIMERIZATION

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The 14-3-3 proteins represent a large group of dimeric proteins. Specifically, the 14-3-3 family consists of 7 isoforms, that can create many homo- and heterodimeric forms, not even accounting for the possibility of changing the oligomerization properties by posttranslational modifications such as phosphorylation. The functions of 14-3-3 are very often dependent on its oligomeric state. Therefore, the parameters of oligomerization are very interesting in order to correctly understand the regulation and behavior of 14-3-3 itself.

In our study, we focused on the zeta isoform (most abundant isoform in human brain, also forming most stable dimers) and its phosphorylated form. Using standard biophysical methods we have only seen that the K_d is lower than 1 μ M. Therefore, we designed very sensitive fluorescence based methods to allow for study of such tightly bound dimers. Using these methods, we determined the dissociation constant, as well as kinetic parameters of the oligomerization process.

In order to determine the effect of phosphorylation on Ser58, which the literature is ambiguous about, we used our fluorescence assays. The serine S58 is located at the dimeric interface and therefore its phosphorylation affects the dimerization process. We succeeded in determination of both kinetic and equilibrium parameters of the interaction between non-phosphorylated and phosphorylated 14-3-3 ζ protein.

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HOW THE SERIES RESISTANCE INFLUENCE THE TRANSCONDUCTANCE OF THE OECT PLANAR STRUCTURE

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The organic electrochemical transistor (OECT) plays an important role in modern bioelectronics. Its use in study of living cells physiology keeps this device very attractive and at the forefront of various organic bioelectronic devices [1]. In a case of some bioelectronic applications, namely in sensing, the high gain of the OECT is needed. The key parameter which describes high gain in OECT is transconductance. Since the transconductance describes the modulation of the gate voltage to the drain current [2,3], a strong dependence on channel parameters is known [3,4]. To develop the OECT with high transconductance, an optimal electrical properties of the semiconductor and the length and width of the channel must be found. However, for the sample with high ratio of width and thickness to channel length (Wd/L), the dependence of transconductance on channel parameters was shown to be nonlinear [5]. Kaphle et al. shown that the contact resistance plays the significant role in a case of highly doped organic semiconductors [6]. This means that the extracted OECT parameters are burdened by contact resistance error. To compensate for this effect the corrected values of transconductance, i.e. intrinsic transconductance, can be calculated [7].

To prepare high transconductance OECT devices, the effect of the thickness of organic semiconductor and electrode system and the effect of busbars on the OECT transconductance were studied in this contribution. The electrode systems with variable thickness were prepared by chemical vapor deposition and the semiconductor films with variable thickness were prepared by spin-coating. As a semiconductor film, the poly(3,4-ethylene dioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) was used. By varying the geometric parameters of the electrode system and the channel, the OECT serial resistance was decreased and the transconductance value 63 mS was achieved. By lowering the serial resistance, the peak transconductance was increased and the difference between the peak transconductance and intrinsic transconductance was lowered, see Figure. In the Figure the lines represents calculated theoretical dependences for given contact resistance based on Chou model⁷. As can be seen from the Figure 1, our measured data for different electrodes thickness well represents the model. Due to additionally lowered serial resistance with the help of silver-plated electrodes, the transconductance was further increased.

We showed that the OECTs can be limited by their series resistance. With proper analysis based on the voltage drop division on resistors in series it is possible to (i) design an arrangement that will not be burdened by this effect when analyzing new materials, and (ii) optimize the OECT layout to obtain the maximum transconductance and thus the sensitivity of the OECT sensor.

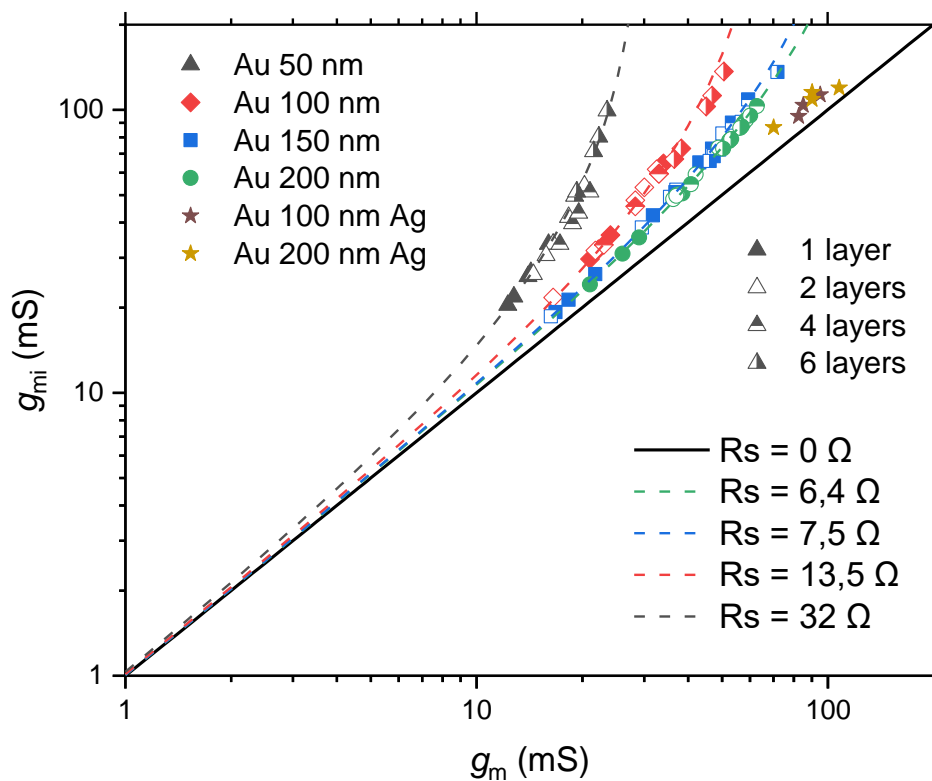


Figure: The dependence of intrinsic transconductance on the peak transconductance. The lines represent calculated theoretical dependences for given contact resistance.

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ELECTROCHEMICAL LAMP-BASED ASSAY FOR SCREENING OF HPV16/HPV18 INFECTION IN CERVICAL SAMPLES

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Cervical cancer is caused by a persistent infection with high risk human papillomavirus (HPV) strains. HPV testing has become an important complement to standard cytological screening since untreated long-term infection can be detected before morphological changes appear on cervix. However, standard methods are often laborious, expensive and time-consuming. Here, we propose new detection technique for HPV16 and HPV 18, the two most widespread oncogenic HPV subtypes. This determination is based on loop-mediated amplification (LAMP) [1], coupled with hybridization on magnetic beads and electrochemical readout [2]. Our method was validated on clinical samples and the presence of HPV16 and HPV18 was confirmed using techniques routinely used in clinical laboratories.

Concretely, to demonstrate usefulness of the method, we screened 61 cervical samples for both of these subtypes and compared our results with standard PCR and with two commercially available tests – COBAS (Roche) and INNO-LiPA (Fujirebio). The best concordance was obtained with PCR reaction; we reached high significance (p-value <0.0001) and positive and negative predictive values over 90% [3].

These results demonstrate that our electrochemical assay may serve as an interesting tool for rapid and inexpensive screening of oncogenic HPVs.

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PHOTON-UPCONVERSION NANOPARTICLES AS A BACKGROUND-FREE LABEL FOR THE DETECTION OF BIOMARKERS AND BACTERIA

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Due to their high specificity, immunochemical assays are widely used for the detection of various analytes within complex matrices. The most commonly used immunoanalytical method – enzyme-linked immunosorbent assay (ELISA) – relies on signal generation by enzymes, typically horseradish peroxidase. The enzymes provide highly sensitive detection because a single enzyme molecule can transform numerous molecules of the substrate. However, enzymes also suffer several disadvantages, most notably low stability. Recent progress in nanotechnology has provided various nanomaterials, which can be used as labels in immunoassays [1].

In particular, photon-upconversion nanoparticles (UCNP) are useful as alternative detection labels because their anti-Stokes luminescence can be excited by the NIR laser and detected in the Vis region without optical background interference. However, the as-synthesized UCNPs are unstable in high ionic strength buffers, lack suitable functional groups, and are prone to nonspecific interactions in complex samples. Hence, their surface needs to be modified for successful biological applications. The most common methods include silanization or modification by ligands that can coordinate to the lanthanide ions on the UCNP surface [2].

We have introduced a novel approach for the conjugation of biomolecules with UCNPs based on heterobifunctional poly(ethylene glycol) (PEG) linkers bearing neridronate and alkyne or maleimide. The alkyne groups were used to conjugate the UCNPs with streptavidin and anti-human serum albumin antibody *via* copper-catalyzed click chemistry. The alternative modification approach consisted of a mild reduction of the disulfide bonds of the antibody and conjugation *via* maleimide. The nanoconjugates were used as labels for an upconversion-linked immunosorbent assay (ULISA) for the detection of human serum albumin (HSA), which is a marker of kidney dysfunction. The streptavidin-based labels achieved the LOD of 0.17 ng/mL for the target HSA (Figure 1A), which was superior compared to a fluorescence immunoassay (LOD 0.59 ng/mL) or an enzyme-linked immunoassay (LOD 0.56 ng/mL). The optimized assay was also employed for the analysis of real samples of spiked urine (Figure 1B), demonstrating the practical potential of the method [3].

The conjugates of UCNPs are also suitable for the detection of bacteria. We have developed an ULISA assay for *Melissococcus plutonius*, the causative agent of honeybee disease European foulbrood. The assay provided an LOD of 340 CFU/mL and was successfully employed in the analysis of real samples of bees, larvae, and bottom hive debris [4]. Due to the high reliability

and relatively simple detection scheme, the ULISA assays can pave the way for a new generation of immunoassays with a strong potential for commercialization.

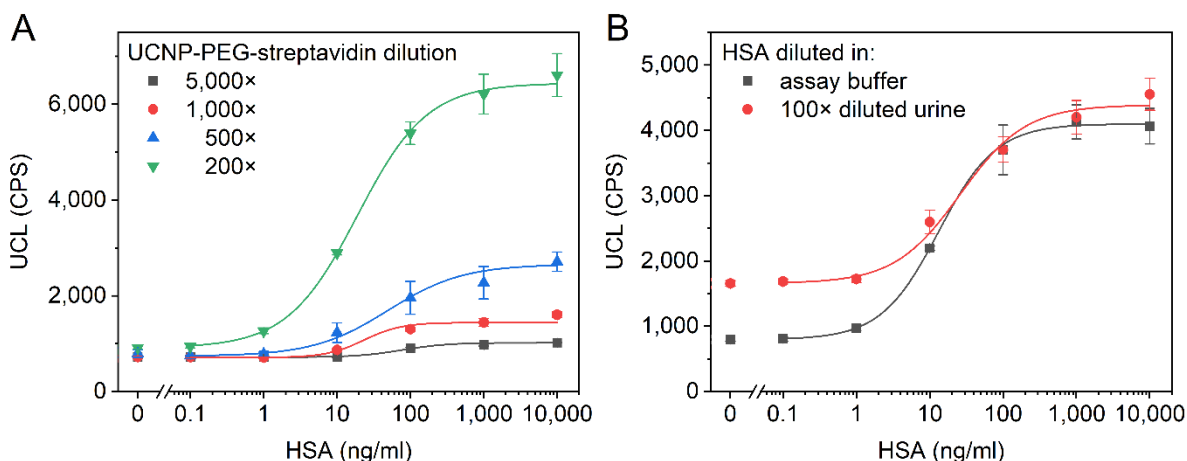


Figure: ULISA for the detection of HSA using UCNP-PEG-streptavidin label. (A) Detection in assay buffer with varying label concentration; (B) detection in spiked 100× diluted urine with 200× diluted label.

ACKNOWLEDGEMENT

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FUNCTIONALIZED CHIRAL BAMBUSURILS: SYNTHESIS AND HOST-GUEST INTERACTIONS WITH CHIRAL CARBOXYLATES

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Bambusurils are a group of macrocyclic compounds that features notable anion recognition properties [1]. Bambusurils can bind inorganic anions as well as carboxylates, sulfonates or organic phosphates [2-4]. Recently we started investigating enantioselective binding of chiral carboxylates by chiral bambusuril derivatives [5]. Hereby we report synthesis and supramolecular behavior of two new representatives of chiral bambusuril macrocycles bearing ester groups that are suitable for further modifications [6]. Supramolecular properties of the new macrocycles were studied by means of NMR. New bambusurils exhibit enantioselective binding of chiral carboxylate anions. Comparison of the new macrocycle with a previously reported one shows that introduction of the substituents has significant impact on the enantioselectivity. The results also indicate that selectivity towards structurally different carboxylates is governed by bulkiness of the substituents that surround bambusuril portals. Lastly, we would like to show the application of the previously reported chiral bambusuril as chiral solvating agent.

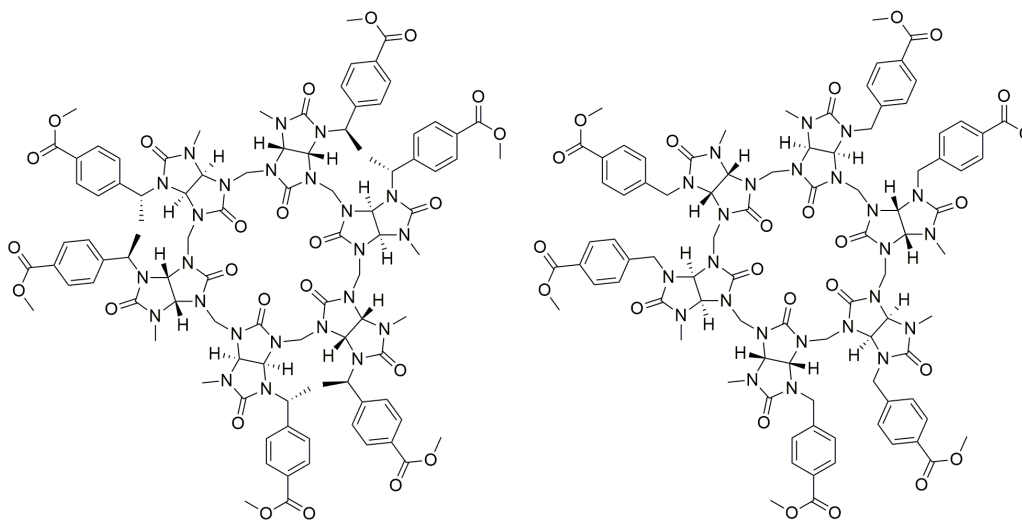


Figure: Structures of new chiral bambusuril macrocycles.

ACKNOWLEDGEMENT

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AdTSV DPV DETERMINATION OF INTERACTION BETWEEN CdTe QUANTUM DOTS AND OLIGONUCLEOTIDES SPECIFIC FOR THE AFRICAN SWINE FEVER VIRUS

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INTRODUCTION

African swine fever virus (ASFV) is a virus, with high morbidity and mortality, which is infectious for pigs and causes serious socioeconomic impact [1]. Migration and travelling supports transfer of not only ASFV, but the whole spectrum of bacterial and viral pathogens [2]. Thus, the instruments for reliable and fast detection of ASFV are necessary. Therefore, the cornerstone of design of biosensor for rapid analysis of ASFV was suggested. The AdTSV DPV was used for studying of oligonucleotides KING R and KING F, which are complementary to viral DNA and their interaction with CdTe quantum dots as well.

MATERIAL AND METHODS

Electrochemical measurements were performed with AUTOLAB Analyser connected to VA-Stand 663, using a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm². The reference electrode was an Ag/AgCl/3M KCl electrode and the auxiliary electrode was a graphite electrode. The supporting electrolyte containing 0.2 M acetate buffer (pH 5.0) was used. Two independent methods for measuring a.) Oligonucleotides and b.) Cadmium were used. AdTS DPV parameters were as follows: method a.) an initial potential of 0 V, an end potential -1.7 V, a modulation time 0.05 s, a time interval 1 s, a step -5 mV/s, a modulation amplitude of 25 mV, E_{ads} = 0 V.; method b.) an initial potential of -1.7 V, an end potential 0.05 V, a modulation time 0.05 s, a time interval 0.5 s, a step 50 mV/s, a modulation amplitude of 25 mV, E_{ads} = -1.2 V.

RESULTS AND DISCUSSION

Additionally, the PCR fragment (250 bp) containing the DNA complementary to KING R and KING F was analysed. The calibration curves from 0.4 to 1.7 µg.ml⁻¹ for KING F and from 0.1 to 0.6 µg.ml⁻¹ for KING R and from 1 to 10 µg.ml⁻¹ were measured. When the intensities of signals for oligonucleotides and PCR fragment are compared, the best sensitivity was calculated for PCR fragment (50 nA/amol molecules/min) then KING R (25 nA/amol molecules/min) and finally KING F (14 nA/amol molecules/min). When only ODNs were accumulated on Hg electrode, the signals of cytosine and adenine (CA signal) in potential about -1.3 V (Hg against Ag/AgCl/3 M KCl) were 33 ± 8 nA for KING F and 22 ± 3 nA for KING R (3 minutes; 1.0 µg.ml⁻¹ KING F and 0.3 µg.ml⁻¹ KING R; 10 measurements). For interaction ODNs and CdTe,

CA signals were detected as follows: KING F/QDs 15 ± 8 nA, KING R/QDs 16 ± 5 nA and cadmium ions signals were detected as follows: KING F/QDs 61 ± 15 nA and KING R/QDs 89 ± 18 nA. Successful verification of interaction was proved by measurement of signal of cadmium ion and lower signals of oligonucleotides in interaction complex because sensitivity of detection of nucleotides was reduced with bound CdTe QDs.

Table: Parameters of regression equations KING F and KING R ODN

OLIGONUCLEOTIDE	b	a	R ²	r	Deviation(x,y)	RSD			Range	Coeff		Coeff.		QC coeff
						(%)	LOD	LOQ		T	P	K1 T	P	
KING F	40.54	1.91	0.9886	0.9943	2.57	6.5	0.28	0.92	0.92-1.75	1.18	*	26.34	***	6.5
KING R	132.6	2.31	0.9699	0.9849	8.21	11.2	0.15	0.49	0.49-0.60	0.84	*	16.07	***	11.49

b = intercept, a = slope, R² = determination coefficient, r = correlation coefficient, RSD = relative standard deviation, LOD = limit of detection, LOQ = limit of quantification, coeff = coefficient, P = probability level (* 0.05; *** 0.001)

CONCLUSION

It was found out, that oligonucleotides interact with CdTe QDs and thus they can be used as oligonucleotides probes for detection real ASFV DNA. Further experiments are necessary for characterization of PCR fragment, which represents viral DNA [3].

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ELECTROCHEMICAL ANALYSIS OF NUCLEIC ACIDS COUPLED WITH ISOTHERMAL AMPLIFICATION TECHNIQUES FOR CANCER DIAGNOSTICS

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Sensitive analysis of low amounts of DNA or RNA tumor biomarkers, including viral oncogenes, microRNAs or long non-coding RNAs (lncRNAs), usually requires an enzymatic pre-amplification step, most often PCR. Despite its versatility, PCR has several drawbacks, such as a need for thermal cycler, high temperatures, longer reaction times or susceptibility for false positive results. Isothermal amplification techniques (IATs) offer simpler protocols and instrumentation, along with shorter reaction times, by utilizing special polymerases that amplify DNA or RNA template under constant, usually mild temperature, using various ingenious strategies [1]. Most common IATs include e.g. loop-mediated amplification (LAMP), nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA), recombinase polymerase amplification (RPA) or strand displacement amplification (SDA).

When coupled with an electrochemical end-point detection platforms, excellent sensitivity and selectivity can be achieved [2]; moreover, electrochemistry offers parallel measurement of samples and miniaturization of the whole system in a rapid and inexpensive manner. It is thus not surprising that electrochemical detection of nucleic acids pre-amplified with various IATs has been a subject of many studies oriented on cancer diagnostics. Here, basic principles of chosen IATs will be described; then, our results that utilized several IATs for detection of human papillomavirus (HPV) DNA or mRNAs, associated with cervical carcinogenesis [3-4], will be presented.

ACKNOWLEDGEMENT

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AUTOMATION OF ARGENTOMETRIC TITRATIONS

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Argentometry as one of the oldest volumetric methods is based on a precipitation of insoluble silver salts in which the volumetric solution of silver nitrate is used to determine the concentration of halides (*e.g.* chlorides, bromides and iodides) and pseudohalides. Equivalence point can be determined either visually using a chemical indicator or recording of the real-time electrode potential during the course of titration when the observed potential difference of the working and reference electrodes is measured. In argentometry, the silver electrode is usually used as the working electrode and calomel electrode as the reference one, the potential dependence on volume is called a titration curve, and the equivalence point is the inflection point of this curve. This point can be assessed using various mathematical approaches, *e.g.* the first/second derivative and/or Gran transformation of the titration curve. Once the equivalence point is determined, the concentration of halide(s)/pseudohalide(s) in the sample can be calculated. Automation of argentometric titration enables a faster and more precise analysis of samples containing these analytes.

In this work, the automation of titration using LabVIEW and Python software tools was realized. These two tools were chosen because students could get experience with them in practical laboratory courses. LabVIEW (National Instruments, USA) is a programming environment, which offers endless possibilities for the control of various analytical devices [1-3]. In our laboratory, a program software running under LabVIEW environment was written to drive and control automatic argentometric titration carried out by in house-made titrator. This program software is able to record the volume and the potential in real-time, and then to evaluate the equivalence point of titration curve and to calculate the amount of halide(s) in the sample. Figure shows the titrator and a window of the program made in LabView.

Python is a general-purpose programming language created by Guido van Rossum in 1991 [4]. The software written in Python for control of the titrator exhibits similar functions as that one running in LabView environment.

It has been demonstrated that the automatization of argentometric titration using a home-made titrator driven by software running in LabVIEW or Python is possible. This option significantly increases lab work efficiency and provides a higher quality of results. Thus, it leads to possibility to analyze more samples in shorter time.

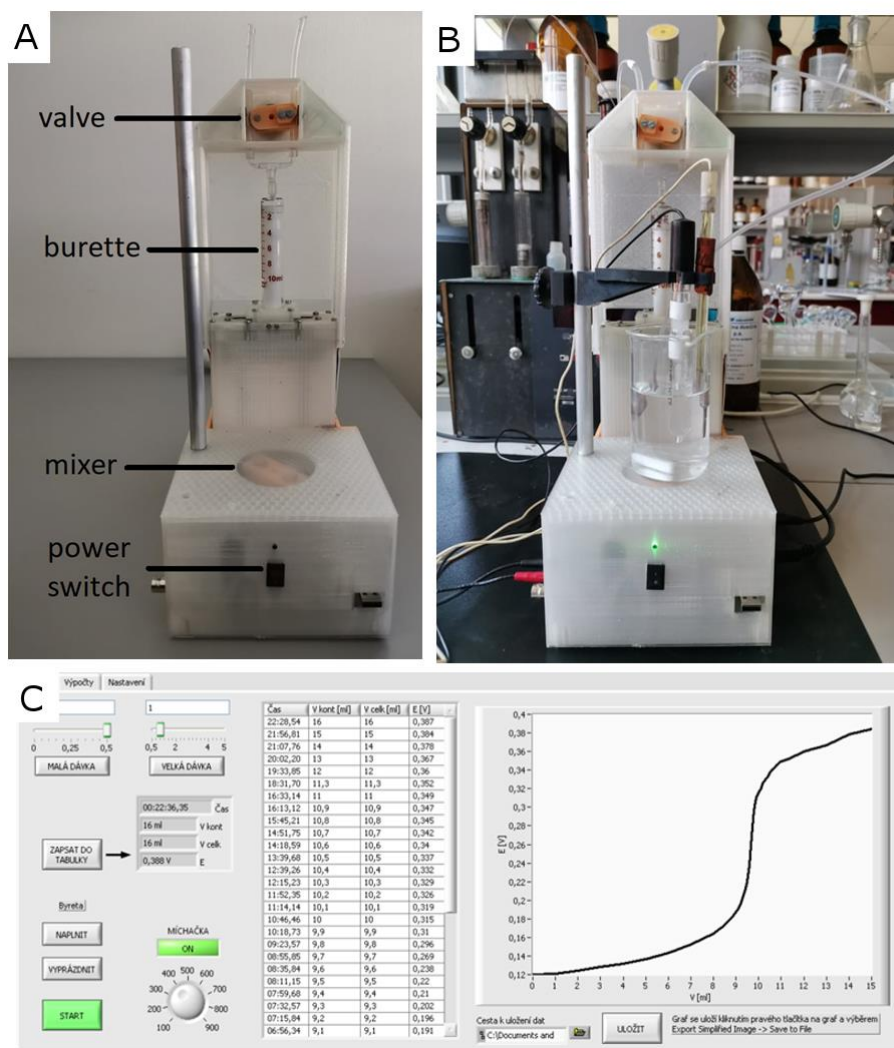


Figure: A) Home-made titrator, B) Titrator during titration of chloride ions by silver nitrate, C) Window of the program created in LabView.

ACKNOWLEDGEMENT

The work has been supported by the project MUNI/A/1424/2019.

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INFLUENCE OF ETHANOL ON THE ITP DETERMINATION OF ORGANIC ACIDS IN BEVERAGES

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Isotachopheresis (ITP) is an electrophoretic technique that enables the separation and determination of ionic species (anions or cations). It provides information about the qualitative and quantitative composition of the sample [1,2]. The sample is introduced between the leading (LE) and terminating (TE) electrolyte while the LE has higher mobility of ions and the TE lower mobility than ions present in the sample. When an electric current is passing through such system, ionic chemical compounds present in the sample are separated due to different migration speed leading to establishment of distinct zones in equilibrated mode. The first zone contains the ionic species with the highest effective mobility, on contrary the last zone is consisting of those ions having the lowest effective mobility [2,3].

In this work, the ITP was applied for the analysis of alcoholic and non-alcoholic beer-based beverages. The analytes of interest were sulfate, malate, succinate, citrate, and phosphate ions because the content of majority of organic and some inorganic acids in beer is important marker of its quality [4]. Therefore, the model mixtures of selected acids in aqueous solution were analyzed by ITP. As the presence of an ethanol in the beer is supposed, also the model mixtures with various additions of ethanol were analyzed by ITP. In Figure, one can see ITP record of model mixture consisted of sulfate, malate, succinate, citrate, and phosphate ions (concentrations 0.1 mM) without and in presence of ethanol. It was observed that the presence of ethanol has some impact on ITP analysis because the ITP zones become shorter for studied acids at the higher concentration of the ethanol. As consequence of these findings, the quantification of the acids in beer should be carried out by suitable approach.

The ITP analyses were carried out on electrophoretic analyzer EA 102 (Villa Labeco, Slovakia) with a conductivity detector. The length of the column was 180 mm, and diameter of PTFE capillary 0.3 mm. The analyses were performed in a two-step mode with 70 μ A/30 μ A current. The LE composition was 10 mM HCl + 5,5 mM BIS-TRIS propane, pH = 6,6; and the TE consisted of 10 mM *hexanoic acid* (pH = 7.2).

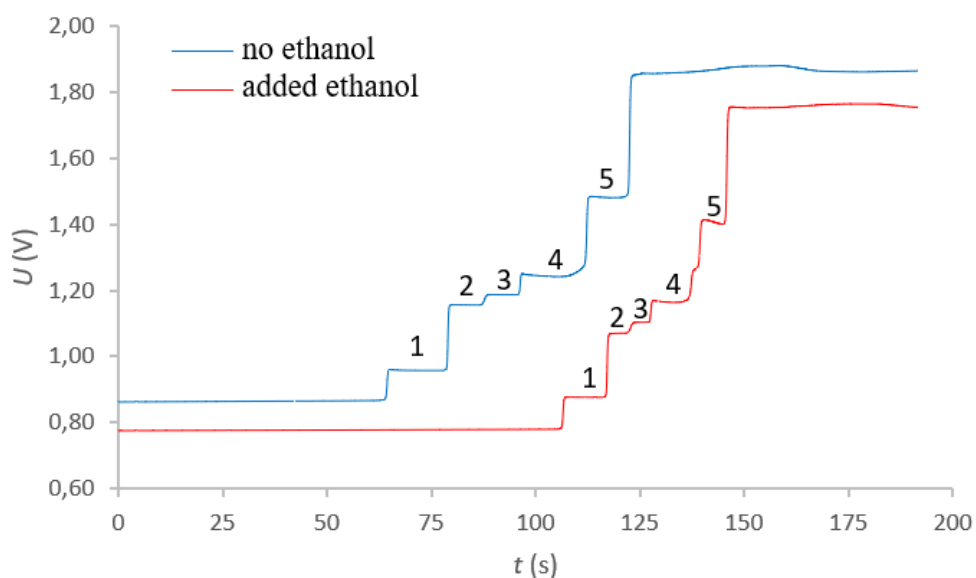


Figure: ITP records of model mixture of acids without ethanol and with added 0.1% ethanol.
1 – sulfate, 2 – malate, 3 – succinate, 4 – citrate, 5 – phosphate

ACKNOWLEDGEMENT

The work has been supported by the project MUNI/A/1424/2019.

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**ANTIPROLIFERATIVE, DNA BINDING, AND CLEAVAGE
PROPERTIES OF DINUCLEAR Co(III) COMPLEXES CONTAINING
THE BIOACTIVE QUINIZARIN LIGAND**

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The negative properties such as side effects and acquired resistance of platinum-based anticancer drugs used in clinical applications have forced search for novel transitional metal-based compounds exhibiting cytostatic activities. Over the last years, it has been discovered that cobalt complexes are exhibiting anticancer properties and in-depth analyses of their mode of action have been conducted. Here, we present antiproliferative activity against human cancer cells of the dinuclear Co(III) complexes bearing the quinizarin ligand and tris(2-aminoethyl)amine (tren, compound 1) or tris(2-pyridylmethyl)amine (tpa, compound 2) co-ligands. It is essential to understand mechanism of biological action of these compounds, their association with DNA in the cells, DNA binding in cell-free media, and DNA cleavage capability. The obtained results show that both complexes interact with DNA in tumor cells. The mechanism of antiproliferative action of 1 differs significantly from that of 2. The antiproliferative effect of 1 is connected with its ability to intercalate into DNA and subsequently to inhibit activities of DNA processing enzymes. The antiproliferative effect of 2 seems to be connected with the ability of 2 to form radicals, and consequently to cleave DNA.

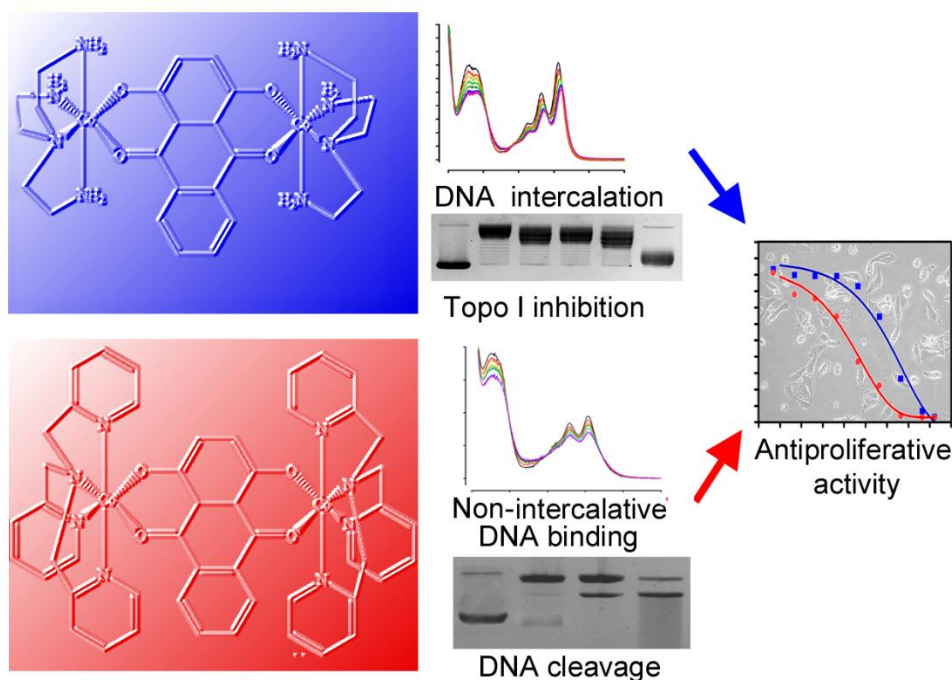


Figure: Dinuclear Co(III) complexes containing the bioactive quinizarin ligand exhibit antiproliferative activity based on distinct mechanism

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BYPASS OF THE Pt-DNA ADDUCT BY DNA POLYMERASES AND THERMODYNAMIC ASPECTS OF THIS PROCESS

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Platinum-based coordination compounds are widely used anticancer chemotherapeutics. Their design depends on understanding the details of molecular and biochemical mechanisms associated with the biological effects of the existing agents [1]. DNA adducts of antitumor platinum drugs arrest several key cellular functions, including DNA replication and cause programmed cell death [2-4]. On the other hand, cells also try to recover the halted DNA replication and rely on activities of translesion DNA polymerases (TLS pols), which are capable of bypassing the adducts. TLS pols are less strict than most of the replicative DNA polymerases and can more easily accommodate damaged bases and incorporate an incorrect nucleotide – induce mutation [5].

In the present work, we investigated the bypass of the site-specific adduct of AMD (Figure A) catalyzed by four TLS pols which differ in their processivity and fidelity employing “running and standing start” assays [6]: Klenow fragment from DNA polymerase I (KF^{exo-}), DNA polymerase η (pol η), DNA pol kappa (pol κ) and DNA polymerase iota (pol ι). We also examined kinetic parameters for the nucleotide insertion and extension during TLS by KF⁻ and pol η .

The effects of the lesion formed in DNA by AMD on DNA polymerases activity may also be a consequence of the energetic impact of the lesion on the process of TLS [7-9]. In our presented work, we used microscale thermophoresis (MST) to measure the thermodynamic changes associated with translesion synthesis across single, site-specific adducts formed in DNA by AMD as well.

In the presence of all four dNTPs, the AMD adduct was very potent in inhibiting the TLS activity of all investigated DNA polymerases. On the other hand, pol η was able to incorporate the incorrect dGTP opposite the platinated G with slightly higher efficiency than opposite the G in the non-modified control template. This observation was confirmed by MST, where thermodynamic parameters showed higher stability of platinated G~G mismatch than non-modified one. Pol η was more error-prone polymerase than KF⁻.

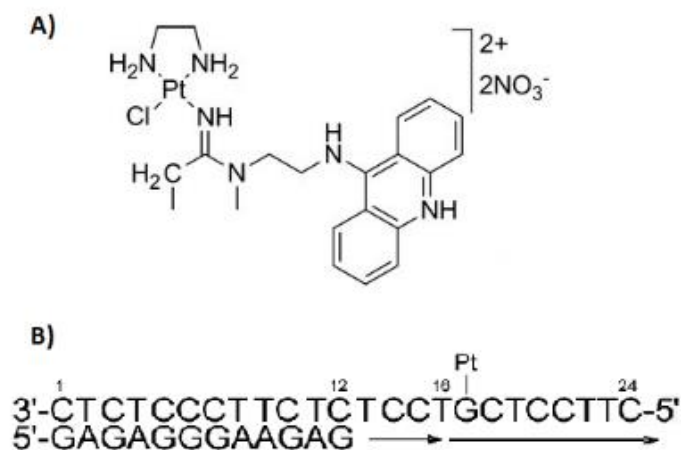


Figure: A) Structure of the investigated AMD complex $[PtCl(en)(L)](NO_3)_2$ (en=ethane-1,2-diamine, L=*N*-[2-(acridin-9-ylamino)ethyl]-*N*-methylpropionamide]) B) Analysed DNA sequence

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S. AUREUS METALLOTHIONEIN ANALYSIS AFTER ITS EXPOSITION TO SILVER AND CADMIUM IONS

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INTRODUCTION

Metallothioneins (MTs) belong to a group of intracellular, cysteine-rich proteins with molecular weight from 6 to 10 kDa [1]. Cysteine sulfhydryl groups participate in covalent bonding with heavy metals. MTs occur throughout the animal kingdom, including prokaryotes. In eukaryotes, MTs are relatively intensively studied and described, but in many prokaryotes we do not have more detailed information. It is known that electrochemical methods are very suitable for monitoring the level of thiol groups [2]. Catalytic reactions of hydrogen deposition from the basic electrolyte are very sensitive to SH groups, as described by Brdicka and Heyrovsky [3,4]. The aim of our work was to study the effect of cadmium and selenium ions on *S. aureus* content of MT.

MATERIAL AND METHODS

240 µl of the bacterial suspension was pipetted into each well of the microtiter plate. 10 µl (from 1500 to 0 µM); of the metal ions was added into the well. The positive control was the 20% NaClO, negative control was the LB medium. After 12 hours the sample was removed to the 2 ml plastic tube and was washed twice by the sterile ultrapure water (18 MΩ) and centrifuged (14,000 g, 5 min, 4 °C). Pelet was homogenized in 500 µl of the ultrapure water by the ultrasound (1 min, 2 kJ). The concentration of the proteins was measured (Pyrogallol method). Measurements were performed on 743 VA Trace analyser connected to VA-Stand 663, using a standard cell with three electrodes. The Brdicka supporting electrolyte containing 1 mM Co(NH₃)₆Cl₃ and 1 M ammonia buffer (pH 9.6) was used; surface-active agent was not

added. AdTS DPV Brdicka reaction parameters were as follows: an initial potential of -0.35 V, an end potential -1.95 V, a modulation time 0.057 s, a time interval 0.2 s, a step potential of 1.05 mV/s, a modulation amplitude of 250 mV, $E_{\text{ads}} = 0$ V.

RESULTS AND DISCUSSION

The catalytic signals of MT have already been studied in our previous studies [5,6]. The growth inhibition of *S. aureus* was observed in microtiter plate. Cadmium nitrate and cadmium acetate ($1500; 750; 375; 187.5; 93.7$ μM) served as the source of the cadmium ions and sodium selenite of the selenium ions ($1500; 750; 375; 187.5; 93.7; 46.8; 23.4; 11.7; 5.8; 2.9$ μM). Then, the sample was denaturated (99 $^{\circ}\text{C}$, 25 min), centrifuged and MT was determined. The toxicity of cadmium ions in the form of acetate was slightly higher than that of nitrate. This is related to the better bioavailability of acetate. In case of selenite application, we found that bacteria were able to reduce selenite to selenium. Thus, the toxicity of selenite has been greatly reduced and may be related to enzymes involved in the reduction of arsenate [7]. From the analyzed data, we found that slope of signals 2 and 3 in the AUC zones. The signal was -0.007 (RSD 7.1% , $n = 10$) for cadmium nitrate and -0.0061 (RSD 11.1% , $n = 10$) for cadmium acetate. A significant increase in the AUC signal was observed for selenium and the slope was found to be $+0.0153$ (RSD 19.9% , $n = 5$).

CONCLUSION

We found that the monitored signals are strongly related to the applied metal and its concentration. These facts have already been proven in a number of works, but systematic study is still lacking evidence. More detailed information on the expression and levels of selected thiols is needed to be investigated further.

ACKNOWLEDGEMENT

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DETERMINATION OF NITROGEN IN BONES USING KJELDAHL METHOD

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Nitrogen determination in bone remains is a useful method for establishing of relative chronology of burials in archaeological [1,2], forensic [3] or paleontological [4,5] context. Degradation of bone organic matter can be also simulated with higher temperature treatment [6,7]. In this work, some experiments with thermal treatment of animal bone were made:

1. Bone sample was heated in drying box (80 °C).
2. Bone sample was heated in distilled water (80 °C and 100 °C).

After cryogenic milling (SPEX 6775 Freezer/Mill), nitrogen content in the powdered samples was determined using Kjeldahl method in **Pro-Nitro M Kjeldahl Steam Distillation unit**. Distillate from the device was collected in 2.5% (m/m) of boric acid with Tashiro indicator. After the distillation, titration of obtained ammonia was made with 0.1045M solution of sulphuric acid. Nitrogen content in non-treated bone was determined 4.59 % (m/m). In case of the samples treated with distilled water, approximately exponential decrease of nitrogen content with time was observed (Figure). On the other hand, no changes of nitrogen content were found in the samples heated in drying box.

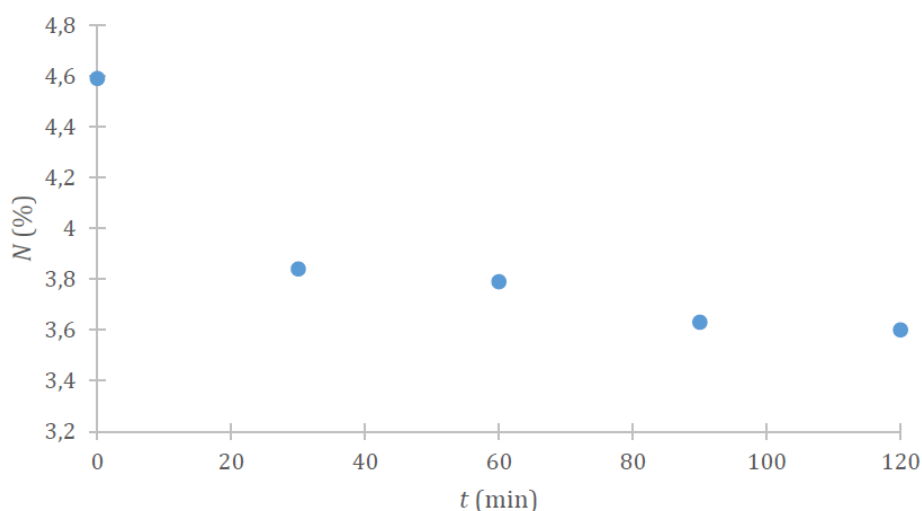


Figure: Decrease of nitrogen content in bone samples treated in distilled water (100°C).

ACKNOWLEDGEMENT

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PHOSPHOMIMICKING MUTANTS: WHY TO BE CAREFUL

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Stability of protein is the ability to maintain its structure and thus function under stress. This stress can be caused by alternations of surroundings such as temperature or pH change, addition of denaturant, presence of salts, etc. [1]. Many methods to assess protein stability were developed, including nanoDSF, which measures changes in the intrinsic fluorescence of tryptophan during protein unfolding, to determine the melting temperature [2].

Phosphomimicking mutants are a tool commonly used to study the properties of phosphorylated proteins [3]. Here we compared thermodynamic stability of such mutant and phosphorylated form of 14-3-3 ζ protein. This particular protein plays a role in regulation of many cellular functions [4]. The phosphorylation of S58 located at the dimer interface is one of the factors affecting interaction of 14-3-3 ζ with its partners [5].

The results have shown, that phosphomimicking mutant S58E cannot be considered a sufficient replacement for phosphorylated 14-3-3 ζ at S58. Not only does their stability differ in various conditions, with overall better stability of S58E, but their intrinsic fluorescence behaves differently as well.

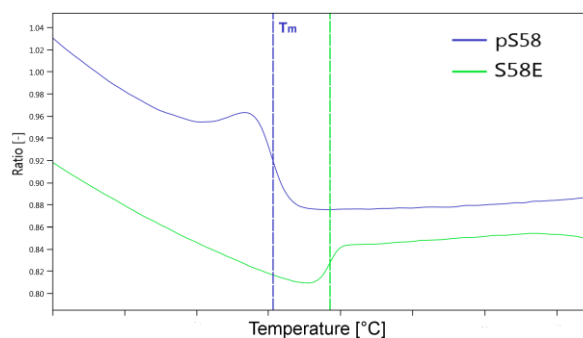


Figure: Example of dissimilarities in protein thermal stability between S58E and pS58 14-3-3 ζ in 20 mM TRIS, pH = 8, and 200 mM NaCl

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OPTIMIZATION OF EXPRESSION AND PURIFICATION OF FRAGMENT FROM TAU PROTEIN

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Tau protein is abundant in the central nervous system, where it stabilizes microtubules by binding to the interface between tubulin sub-units [1,2]. Hyperphosphorylation of Tau leads to aggregation of protein and disruption of normal function of the neurons. These disorders can cause neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease [3,4]. This work is focused on a short fragment from Tau protein, which has a key role in microtubule binding [2].

Our goal was to measure Tau fragment with NMR spectroscopy, therefore an efficient way to express isotopically labelled protein was needed. The optimal solution for preparation of labelled proteins is M9 minimal media. The downside of this media is lower overall growth of the bacteria. We present an optimized expression of Tau fragment in M9 minimal media.

In our experiments, the classical approach for protein purification with fast protein liquid chromatography did not result in sufficient yield, so alternative methods had to be employed. We have explored the possibility of salting out and heat treatment methods. In comparison with traditional methods, they showed promising results.

ACKNOWLEDGEMENT

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ELECTROCHEMICAL ANALYSIS OF ZINC CONTENT IN THE URINE AND ITS RELATION TO PATIENTS WITH PROSTATE CANCER

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INTRODUCTION

Metallomics deals with the relationship of metals to metabolic processes in living organisms. There are a number of studies that focus on the relationship between metal content and various diseases, including cancer [1]. Various analytical approaches are used for the analysis of metal ions. AAS is the method of choice, whereas ICP and ICP/MS are used for special analysis. However, electrochemical methods are also suitable especially for the detection of the level of zinc in the blood [2]. The aim of our work was to monitor the levels of zinc ions present in the urine.

MATERIAL AND METHODS

Electrochemical measurements were performed with AUTOLAB Analyser connected to VA-Stand 663, using a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE). The reference electrode was an Ag/AgCl/3M KCl electrode and the auxiliary electrode was a graphite electrode. The supporting electrolyte containing 0.2 M acetate buffer (pH 5.0) was used. AdTS DPV parameters were as follows: an initial potential of -1.2 V, an end potential 0.05 V, a modulation time 0.05 s, a time interval 0.5 s, a step 50 mV/s, a modulation amplitude of 25 mV, $E_{ads} = -1.2$ V.

RESULTS AND DISCUSSION

Urine is a relatively complex biological matrix that contains low-molecular as well as high-molecular substances. In addition, it varies considerably depending on food intake and composition. The electrochemical analysis was performed in the most suitable environment of

0.2 M acetic buffer (pH 5.0). Under these conditions, a calibration dependence was prepared in the culture water matrix ($r = 0.9916$, RSD 13.3 %, LOD 61 nM, LOQ 183 nM). We also tested the effect of a urine matrix of 100–1,200 μL on the electrochemical signal. We found that the average signal value of zinc ions was 22 ± 2.2 nA; RSD 10.2% and all analyzes were in the range of 1 SD, $n = 6$. However, with an increasing amount of sample, considerable foaming was observed, which negatively affected the analysis. Therefore, a 200- μL sample was selected for further analysis. Subsequently, calibration dependence (0–1485 nM) was performed. The calibration curve was linear ($r = 0.9953$, QC 5.0, LOD 190 nM, LOQ 627 nM, RSD 5.5 %, $n = 6$). To monitor the reproducibility of the method, zinc ions (1 μM) was added to the mixed urine and analyzed by a control chart (signal mean was $12,483 \pm 1771$ nA, RSD 14.1%, 5 independent analyzes). The obtained values ranged from 1 to 2 SD. To verify the method and its sensitivity to zinc ions, a simple test of the application of a food supplement containing 30 mg of Zn in zinc gluconate was performed. The dynamics of excretion of zinc ions into the urine is shown in Fig. 1. A maximum increase in zinc ion excretion was observed in the proband 60 min after application (104 μM). In other samples, the zinc level gradually decreased, ranging between 10 and 25 μM . We found that in patients with prostate cancer ($n = 40$) the mean value of zinc content in the urine was 31 μM

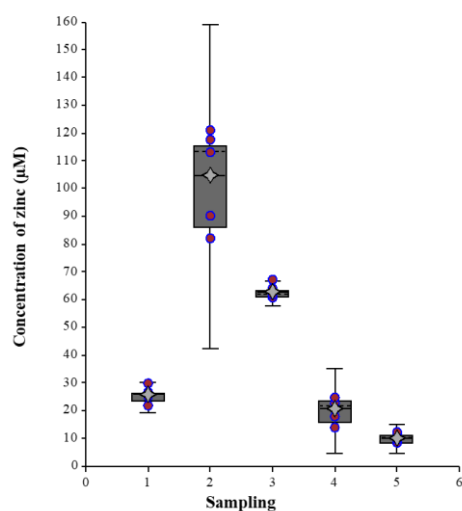


Figure 1: Zinc excretion after *p.o.* application of 30 mg zinc gluconate. The samples were processed immediately after their collection. Each sample was analyzed in 6 replicates.

CONCLUSION

The study was performed to monitor the content of zinc ions in the urine. Zinc ions concentration was also closely monitored in patients with prostate cancer and the association between urinary zinc levels and the course of the disease was investigated.

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SELECTION OF NANOPARTICLES FOR GENE AND PROTEIN DELIVERY AND QUANTIFICATION OF PLASMID/PROTEIN SURFACE ADSORPTION

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INTRODUCTION

The surface charge of liposomes is an important parameter affecting electrostatic interactions between liposomes and surrounding molecules, particles and surfaces. The liposomes are traditionally synthesized by thin-lipid film generating consistent particle size, with positive zeta potential and high pDNA encapsulation efficiency. However the effectivity of pDNA adsorption is determined not only by surface properties of liposomes, however also by surrounding solution and temperature. Liposomes-pDNA complexes can be a safe and non-invasive approach to transfect endothelial and muscle cells, with high potential in medicine.

METHODS

Step 1 (Liposomes preparation and transfection complex preparation) Liposomes were prepared by hydration of phospholipid film method composed of N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP) and 1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine (DOPE) [1]. The suspension was extruded through polycarbonate filters (pore size of 100 nm). Resultant liposomes were mixed with plasmid pcDNA4/TO-bio-myc-NES-EGFP to form lipid-DNA complexes. Conjugation was tested under different physical conditions and ratio of primary components /details in Results/ and analysed by gel electrophoresis. Step 2 (Cell culture preparation). Mouse microvascular cells were precultivated in confocal culture wells. Step 3 (Transfection step) The complex liposome-pDNA was added to cell culture (12 hours). Additionally, the cell sample was exposed to effects of the electromagnetic stimulus generated by magnetic coil (peak of the magnetic field was of 4 mT, pulse width of 340 μ s, with rectangular shape [2], timing 3x45min during 12h cultivation). Intracellular GFP expression was quantified 3 days after plasmid addition.

RESULTS

The electrophoresis results have shown optimal timing and temperature for plasmid-liposome conjugation (Fig.1). Confocal microphoto found that cationic lipids were introduced in 63 ± 12 % of endothelial cells, or 78 ± 15 % after application of coil stimulus. The GFP positivity was detected in 39 % of cells after 3 days.

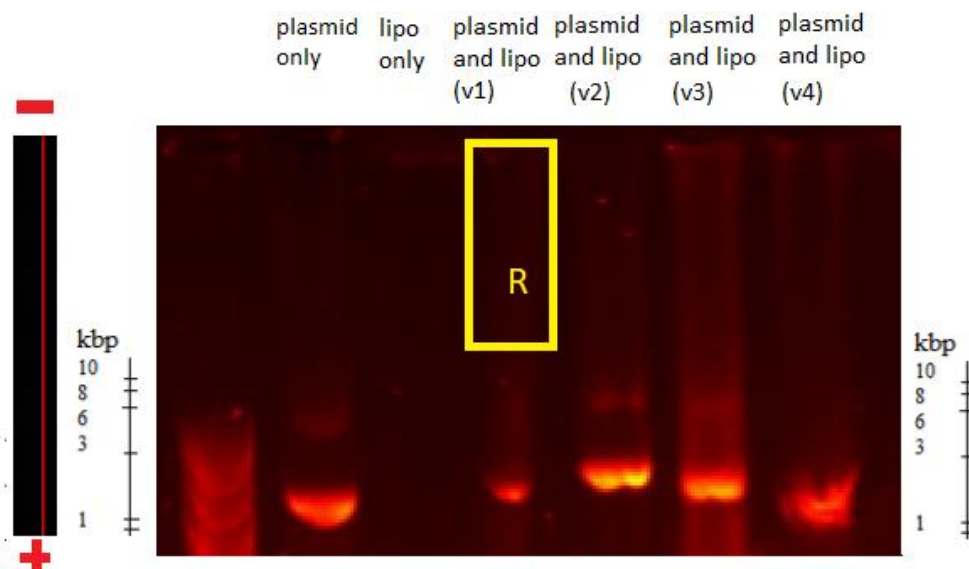


Figure 1: Electrophoresis results - bands of DNA (plasmid) detected on different samples after plasmid-liposomes conjugation step. R= region of complexes. Variant V1 to Variant V4 were different in used ratio of components and conjugation temperature (V1 = optimal, ratio pDNA 800 ng: liposomes 3000 ng, t = 37°C, time 20min)

CONCLUSION

Our investigation have two basic outputs. The first, optimal condition for specific plasmid pcDNA4/TO-bio-myc-NES-EGFP conjugation with liposomes was found and the final complex was shown as usable transfection agents on cells. The second, the gel electrophoresis-based techniques may be suitable for characterization of creation of liposomal-plasmid complex and their stability.

ACKNOWLEDGEMENT

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ELECTROCHEMICAL STUDY OF ALBUMIN IN A BUFFERED ENVIRONMENT AND IN A BIOLOGICAL URINARY SAMPLE

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INTRODUCTION

Albuminuria is an important feature of chronic kidney disease and is commonly detected as a measure of urinary albumin to creatinine ratio (ACR) in humans [1] and in animals [2]. In addition, the association of albuminuria with diabetes [3,4] and with the diagnosis of schistosomiasis [5] has been described. Brdicka and Heyrovsky in the Nature journal the use of polarography in the diagnosis [6-8]. The biological matrix utilised in this study was urine. Experimentally, albumin levels were analyzed using various methods such as spectrophotometry. The aim of this work was to study the electrochemical effect of albumin in urine.

MATERIAL AND METHODS

The chemicals used for the analysis were purchased from Merck and Greiner in analytical grade. Electrochemical measurements were performed with AUTOLAB Analyser connected to VA-Stand 663, using a standard cell with three electrodes. The Brdicka supporting electrolyte containing 1 mM $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ and 1 M ammonia buffer (pH 9.6) was used; surface-active agent was not added. AdTS DPV Brdicka reaction parameters were as follows: an initial potential of -0.35 V, an end potential -1.95 V, a modulation time 0.057 s, a time interval 0.2 s, a step potential of 1.05 mV/s, a modulation amplitude of 250 mV, $E_{\text{ads}} = 0$ V. A Mindray analyzer (total protein, creatinine, uric acid, and urea) was used for chemical analysis of urine.

The samples were analyzed immediately after collection. All analyzed data were transferred to the laboratory database QINSLAB and statistically evaluated.

RESULTS AND DISCUSSION

In our work, we used Brdička's reaction to monitor urinary albumin levels. We studied the behavior of albumin (0 – 3.800 ng / mL) in a buffered medium and in a urine sample. We observed typical Cat 1, Cat2, and RS2Co complex signals. We analyzed individual MT curves using mathematical AUC evaluation. The obtained voltammograms were evaluated by AUC in 4 zones: -0.6 to -1.0 V; -1.0 to -1.3 V; -1.3 to -1.6 V; and -1.6 to -1.9 V. The best signals were shown in the 2nd and 3rd zones. The RSD AUC was around 17% in zone 2 and 28% in zone 3. In the case of the sum of zone 2 + 3, the RSD was 21.4%. The calibration dependence was compiled from the AUC of the zone 2 + 3 results. When interpolating the values with the square root, a linear dependence was obtained ($r = 0.9955$). The typical calibration dependence was $LOD = 159.2$ ng / mL, $LOQ = 358.8$ ng / mL, and $RSD = 6.4\%$.

CONCLUSION

A study was performed to monitor the behavior of albumin in a urine sample. We have introduced new ways of evaluating the obtained voltammograms. The RSD of these signals ranged between 10 - 20%.

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APPLICATION OF THE ELIMINATION PRINCIPLE IN SPECTRAL EXPERIMENTS

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The elimination principle is based on the fact that certain parts of the overall experimental result show different dependencies on one of the set experimental parameters. The principle of elimination was developed and widely applied in electrochemistry (Elimination Polarography - EP and Elimination Voltammetry with Linear Scan - EVLS) in order to study electrode processes and create sophisticated platform for electroanalytical approaches [1-13]. While the chosen experimental parameter in EP is time and the elimination procedure uses linear combinations of time derivatives and integrals, in EVLS, the changing parameter is the polarization rate and elimination is the solution of the elimination function, expressed as linear combinations of total voltammetric currents measured at different scan rates [1,4,6,7].

The concept of a linear combination gave rise to the use of the elimination principle in spectral experiments as well. The basic attributes of the elimination principle are also preserved in spectral applications, i.e.: (i) in general n different parts of the experimental result can be eliminated by a linear combination of $n+1$ results, (ii) one experimental result is chosen as reference and other records registered with changed parameter they are related to it (normalized values), (iii) elimination coefficients or increment of values in appropriate linear combinations - functions play a significant role in the elimination procedure and (iv) the solution of functions provides other, very important and often hidden information.

The elimination principle was applied to a spectral system, in which both a monomer and a dimer are present in equilibrium. Based on normalized spectra measured at three different analyte concentrations, where the concentration dependence of absorbance or molar ellipticity is linear (UV-Vis or CD spectra), theories for the calculation of the dimerization constant were theoretically derived. In addition, the proportion of spectra increments with reference, half and twice the analyte concentration gave the possibility to calculate the ratio R_{ES} (ratio), which should be independent of the wavelength monomer-dimer in the case of wavelength λ . The possible dependence of R_{ES} on λ may be proof that the studied system is more complicated and does not correspond to the model for which the theoretical relations were derived.

The theoretical apparatus of elimination spectroscopy is now tested on known calibration system (Rhodamine 6G) [14-16], since we assume its application to a group of biologically important molecules, such as purines and pyrimidines, or their higher structures.

ACKNOWLEDGEMENT

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OXIDATION SIGNALS OF INSULIN ON SCREEN-PRINTED ELECTRODES

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Direct and efficient electro-oxidation of insulin is important for the development of fast and sensitive electrochemical detectors for this hormone [1,2]. However, the study of the oxidation processes of insulin on conventional graphite electrodes is limited by the very slow electron transfer kinetics. To overcome this shortcoming, it is possible to modify the electrode surface with a suitable catalyst, which reduces the value of the polarization overvoltage and the oxidation signals are measurable in the potential window area of the treated electrode. There are a number of modifying agents, ranging from various nanoparticles and nanocomposites to metal and polymer layers [3-6]. To realize our idea to catalyze the direct oxidation process of insulin (Figure 1), we chose two types of surface modifications of the graphite electrode, namely the modified screen-printed carbon electrodes (SPCEs) with bismuth oxide [7,8] or carbon nanofibers [9]. Using both types of SPCEs, the linear sweep voltammetric (LSV) signals of recombinant human insulin were recorded.

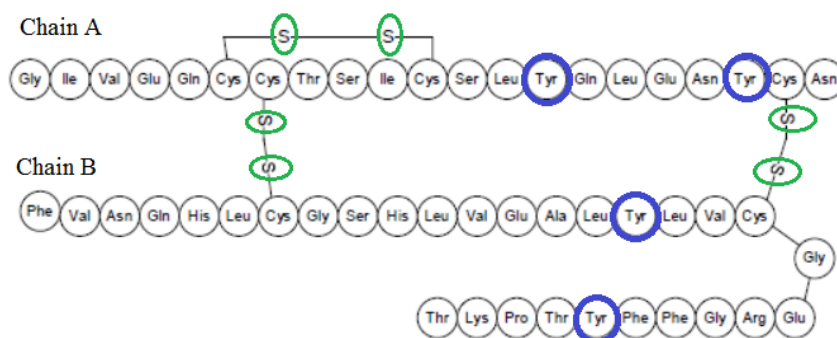


Figure 1: Primary structure of human insulin (empirical formula: $C_{257}H_{383}N_{65}O_{77}S_6$; molecular weight: 5808, 51 amino acids); the predictive redox active sites in the insulin molecule – tyrosines and disulfide bridges are marked in blue and green, respectively.

The electroanalytical parameters, such as the potential position (E_p) of the oxidation peak and its height (I_p) (Figure 2), were investigated as a function of bulk and surface insulin concentration. The limit detection (LOD) was determined to be 1.5 μM , the limit of quantification (LOQ) was 4.2 μM . The range of concentrations where the voltammetric oxidation signals were directly proportional to the insulin concentration was from 0 to 10 μM .

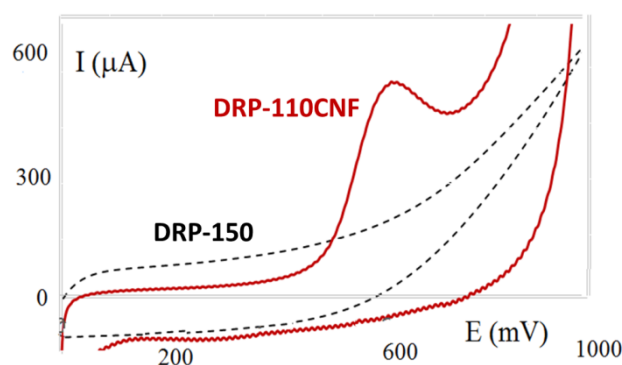


Figure 2: Linear sweep voltammograms of recombinant human insulin ($30 \mu\text{M}$) in PBS buffer (pH 7.4), on non-modified SCPE (DRP-150; black) and carbon nanofiber-modified screen-printed graphite electrode (DRP-110CNF; red); scan rate: 100mV/s ; potential step: 2 mV ; time of accumulation $t_a = 0 \text{ s}$; room temperature.

This research is the starting point for the development of a nanostructured non-enzymatic insulin sensor with the highest possible sensitivity, which will be ensured not only by the optimal experimental conditions but also by suitable electroanalytical methods. In order to understand the catalytic effect of nanoparticles modifying the surface of an electrochemical sensor, our further research will focus on the study of the morphology of electrode surfaces in relation to the kinetics of electron transfer in the oxidation process of insulin.

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COMPARISON OF CONCENTRATION MEASUREMENTS USING NEW MULTI-ANGLE DLS AND NANOPARTICLE TRACKING FROM MALVERN PANALYTICAL

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New Multi Angle Dynamic Light Scattering (MADLS) technology combines autocorrelation functions and scattering intensities predicted by Mie theory for three different angles (back, side and front) into a single particle size distribution with greater confidence in accuracy of the results and improved resolution. By subtraction of background scattering intensity, it is newly possible to measure concentration of particles. The new concentration measurements are unique in speed (5-10 minutes) and precision-effort ratio especially for particle sizes below 40 nanometers of weakly scattering particles (biomolecules, etc). This is the range in which Nanoparticle Tracking Analysis (NTA) cannot compete. In the case of strongly scattering particles, the lower limit of detection is 10 nm for NTA. MADLS concentration works best in the general particle size range from 0,5 nm to 500 nm.

The concentration range of the two technologies overlaps and the concentration results are in good agreement, as shown in Figure on the example of liposomes. MADLS can provide concentration results quicker, with less sample prep and in greater concentration range compared to NTA, the advantage of which lies in the ability to distinguish particles by fluorescent labeling and measure at lower concentrations.

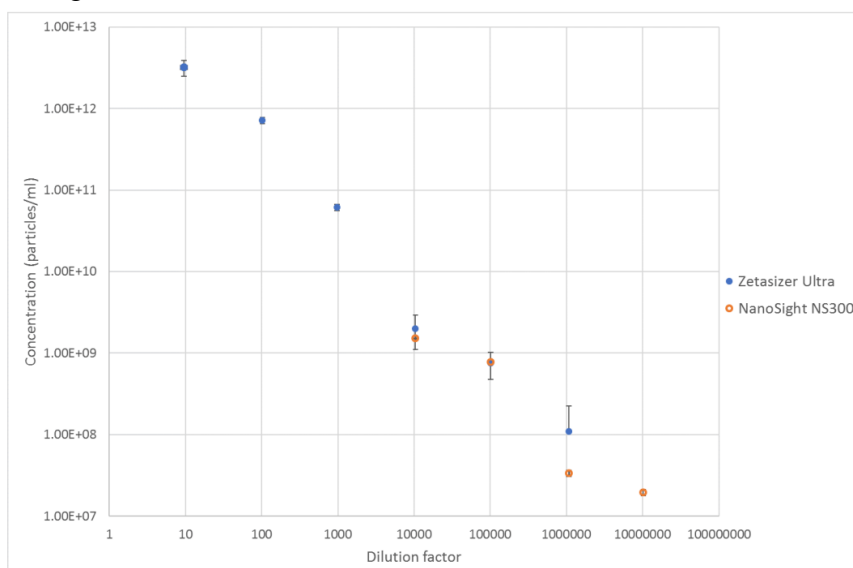


Figure: Concentration results from measurements of liposomes on a Zetasizer Ultra and Nanosight NS300.

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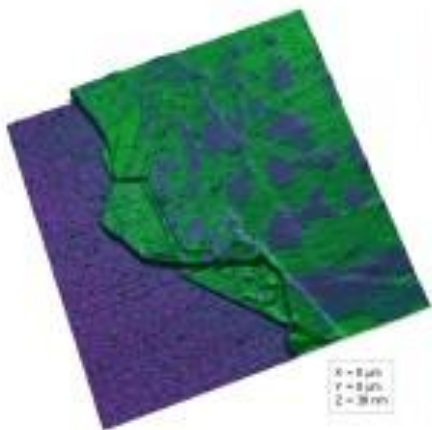
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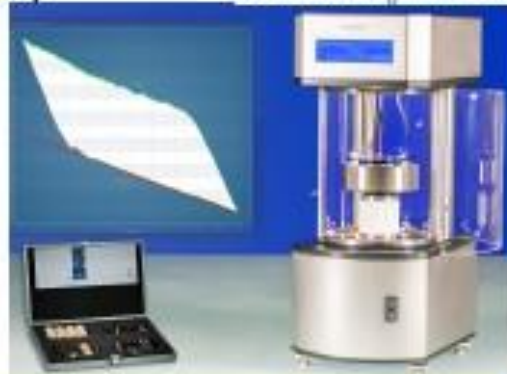
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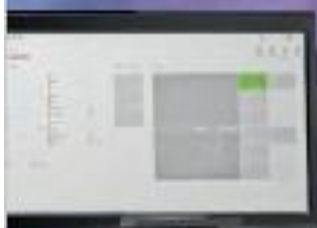
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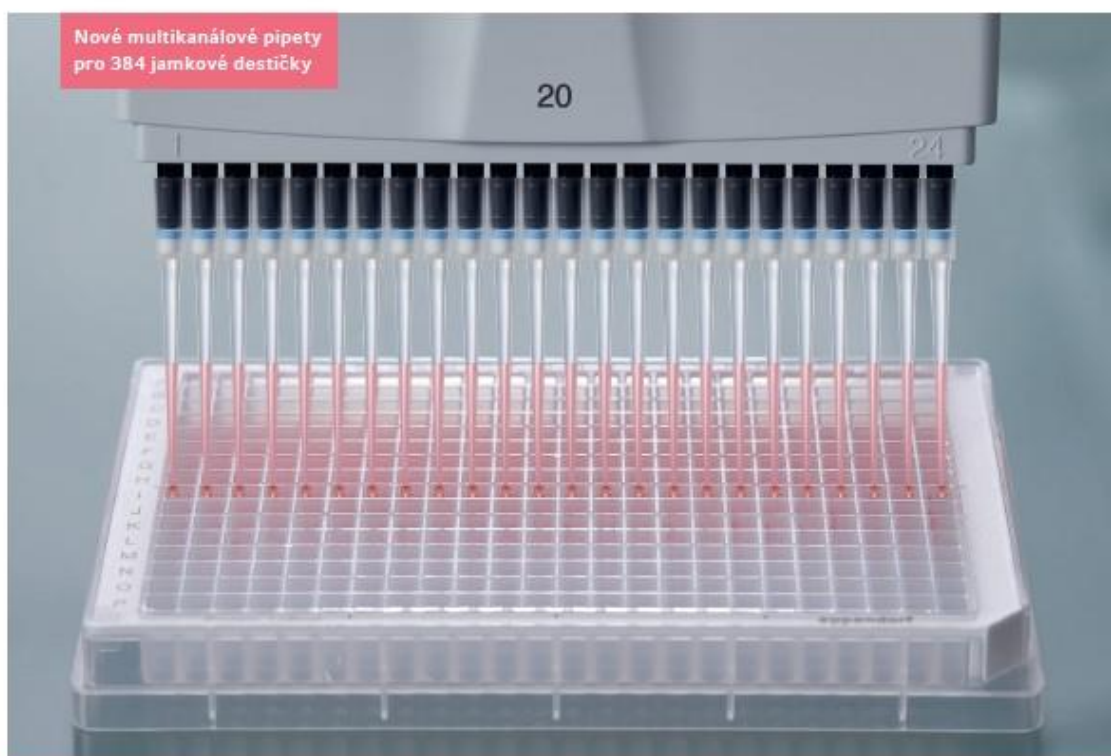
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