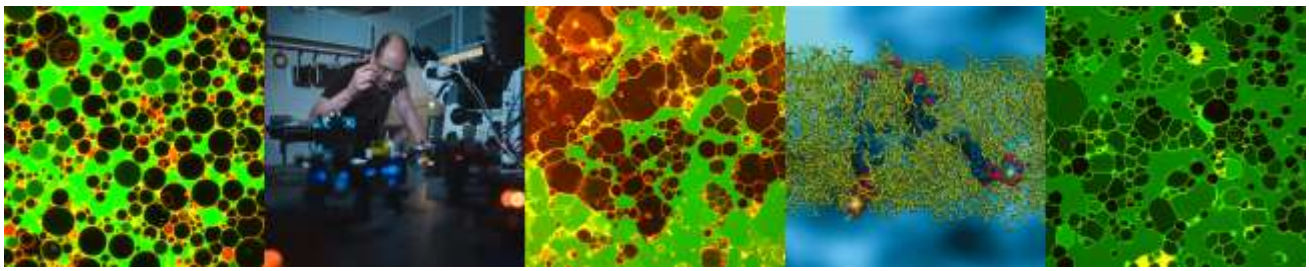
J. Heyrovský Institute of Physical Chemistry

23rd Joint Meeting of Biophysical Chemists and Electrochemists

J. Heyrovský Institute, Prague – Brdička hall

1. – 2. 11. 2023



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Acknowledgments

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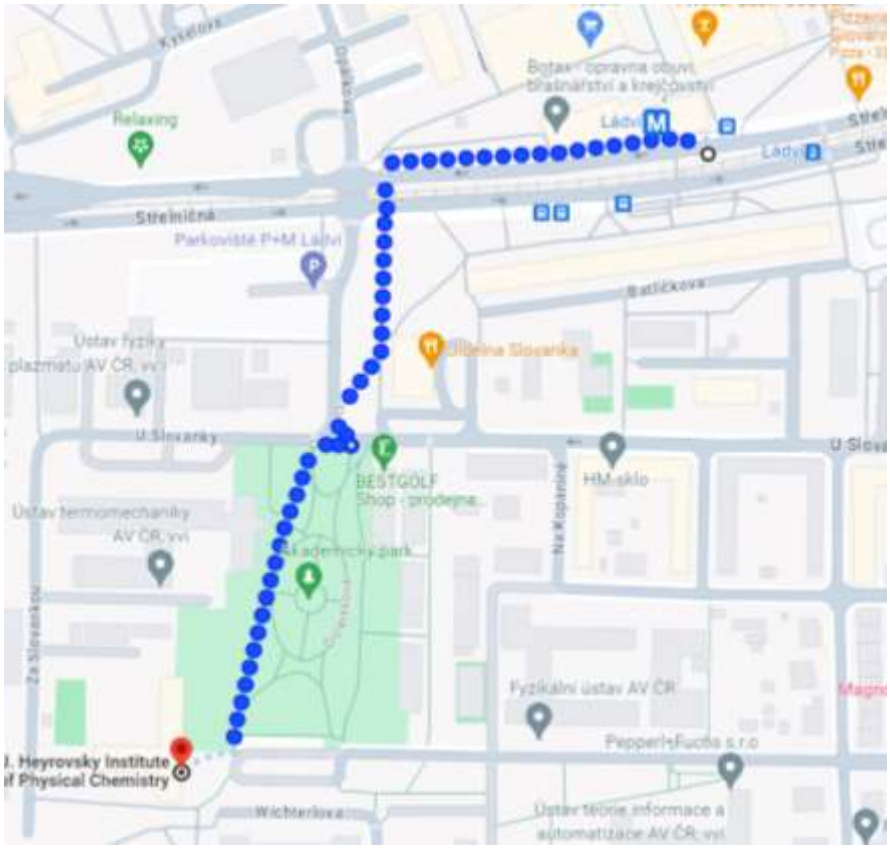

Main organizers:

Radek Šachl, Jiří Ludvík (JH IPC)

Co-organizers:

Libuše Trnková, Iveta Třísková (MU)

Jan Sýkora, Alan Liška (JH IPC)

Program Overview

Wednesday 1tst of November, 2023

08:55 **Conference opening**

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Chairman – Radek Šachl

9:30 – 9:45 **Talk 1** **Robert Vácha**
9:45 – 10:00 **Talk 2** **Štěpán Timr**
10:00 – 10:15 **Talk 3** **Martin Srnec**

10:15 – 10:45 Coffee break 1

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Chairman – Miroslav Fojta

10:45 – 11:00 **Talk 4** **Karolína Schwarzová**
11:00 – 11:15 **Talk 5** **Michal Zelenský**
11:15 – 11:30 **Talk 6** **Anton Lytvynenko**
11:30 – 11:45 **Talk 7** **Libuše Trnková**
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19:30 – 21:00 **POSTER SESSION WITH WINE AND BEER**

Thursday 2nd of November, 2023

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(Jiří Ludvík, Radek Šachl)

Scientific program - Wednesday 1tst of November, 2023

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09:00-9:30 **PLENARY LECTURE**

Jiří Homola Plasmonic biosensors and their applications

SESSION 1 THEORY AND SIMULATION

Chairman – Radek Šachl

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9:45 – 10:00	**Štěpán Timr**	Molecular simulations of liver phosphofructokinase 1 provide insight into the role of its C-terminal tail
10:00 – 10:15	**Martin Srnec**	Off-diagonal thermodynamics and its effect on reactivity

10:15 – 10:45 Coffee break 1

SESSION 2 ELECTRODES AND METHODS

Chairman – Miroslav Fojta

10:45 – 11:00	**Karolína Schwarzová**	Coupled dehydration-electrochemical oxidation of primary bile acids
11:00 – 11:15	**Michal Zelenský***	Characterization of laser treated polycrystalline BDD electrodes with various sp2 /sp3 carbon ratio
11:15 – 11:30	**Anton Lytvynenko**	Metal/polyaniline composite electrodes for electrochemical detection of aliphatic alcohols
11:30 – 11:45	**Libuše Trnková**	Recent development and application of elimination voltammetry with linear scan
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12:00 – 13:30 LUNCH

SESSION 3 SCATTERING MICROSCOPY AND OTHER METHODS

Chairman – Josef Lazar

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Chairman – Jiří Ludvík

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I. Kopal**	Unveiling the Behaviour of 4-Aminobenzenethiol Using a Combination of Interferometric Scattering Microscopy and Raman Spectroscopy
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V. Smeliková**	Creation of Molecule-metal Surface Complexes and their Effect on SERS spectra in the Systems of Amphetamine based Drugs and Colloidal Nanoparticles
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D. Šťastný**	Probing local protein/peptide microenvironment by GP-FRET
K. Umar**	High resolution near infrared imaging with DNA-PAINT
Vandana**	Investigating Functional and Dysfunctional Oligomeric States of Membrane-Associated Protein Oligomers Forming Membrane Pores on Giant Lipid Vesicles

18:30 – 21:00 Dinner + POSTER SESSION

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* student lecture eligible for Emil Paleček Award

** student flash talk eligible for the award of the Czech Biophysical Association

Scientific program - Thursday 2nd of November, 2023

09:00-9:30 **PLENARY LECTURE**

Miroslav Fojta: DNA structure at electrodes: possible challenges and ways to overcome them

SESSION 6 BIOPHYSICS AND ELECTROCHEMISTRY

Chairman – Ludmila Šimková

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10:00 – 10:15	**Jiří Ehlich**	Characterization of Electrochemical Reactions on PtIr Neuromodulation Electrodes

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SESSION 7 RNA AND DNA DAMAGE

Chairman – Petra Riegerová

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11:00 – 11:15	**Monika Hermanová**	Complexes of DNA oligonucleotides and DNA components with mercury ions
11:15 – 11:30	**Niklas Hansen***	A versatile DNA-based platform for investigating interactions between plasmonic nanoparticles and fluorescent nanodiamonds
11:30 – 11:45	**Oleg Lunov**	Mechanical regulation of hepatic tumor cell functions
11:45 – 12:00	**Eric Glowacki**	The Faraday Scalpel: on-demand manipulation of oxygen, reactive oxygen species, and pH in biological environments

12:00 – 13:30 LUNCH

SESSION 8 CHEMISTRY IN BIOPHYSICS

Chairman – Jan Sýkora

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* student lecture eligible for Emil Paleček Award

Talk Abstracts - Wednesday

Plasmonic biosensors and their applications

Jiří Homola

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In this lecture, we review the principles of label-free plasmonic biosensors and discuss the state of the art in this field. We highlight the main challenges in developing plasmonic biosensors for bioanalytical applications and present selected advances in plasmonic biosensor research that tackle such challenges. In particular, these include advances in plasmonic nanostructures, sensor instrumentation, transport of target molecules in microfluidic systems, functional coatings, and assays for the detection of biomolecular analytes in complex biological media. Furthermore, we present several examples of applications of plasmonic biosensors in medicine, especially in the diagnostics of oncohematological diseases.

Counterintuitive Binding of Phosphorylated DEP Domain to Negatively Charged Membranes

Francesco L. Falginella[1,2], Martina Drabinová[1], Vítězslav Bryja[3,4], and Robert Vácha[1,2,5]*

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[3]*Department of Experimental Biology, Faculty of Science, Masaryk University, Brno 62500, Czech Republic*
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To accomplish its role of signaling hub in all Wnt signaling pathways, Dishevelled (DVL) protein needs to dynamically relocalize to the inner leaflet of the cellular plasma membrane (PM). Combined experimental and computational evidence showed that the binding of DVL to the PM is mainly driven by the electrostatic attraction between a stretch of positively charged amino acids located on the C-terminal DEP domain of DVL and anionic phospholipid species, with a striking preference for phosphatidic acid (PA). Here, by means of computational simulations and QCM-D experiments, we demonstrate that four recently identified phosphorylation sites on DEP domain, alter the electrostatic potential of the membrane binding interface, but do not prevent the recruitment to anionic membranes. On the contrary, the phosphorylated residues are involved in hydrogen bond and ion-mediated interactions with the lipid headgroup of PA. Our results suggest that the effect of phosphorylation on protein-membrane association could be counterintuitive and sensitive to changes in the local environment including specific lipids, salts, and pH.

Molecular simulations of liver phosphofructokinase 1 provide insight into the role of its C-terminal tail

Timr Š.[1], Cooper M. P.[2], Webb B. A.[2], Lynch E. M.[3], Kollman J. M.[3]

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Phosphofructokinase 1 is a highly regulated enzyme that—by committing glucose to breakdown—serves as a "gatekeeper" of glycolysis. In cells, the liver isoform of phosphofructokinase 1 (PFKL) was previously shown to form dynamic assemblies [1], which were suggested to play an important role in the regulation of glycolysis [2]. However, the precise mechanisms and function of such spatial organization of glycolytic enzymes remain unknown.

To better understand the mechanisms of the allosteric regulation of PFKL and the significance of its dynamic assemblies, we performed extensive all-atom molecular dynamics simulations of PFKL in different conformational and oligomerization states. In particular, we focused on the role of the partially disordered C-terminal tail, the truncation of which was found to decrease the inhibition of the enzyme by ATP in experimental assays. Our simulations showed that the removal of the C-terminal tail destabilized the inactive state of PFKL. At the same time, the removal induced structural changes that occurred near the active site and that went in the direction of the active state.

Thus, providing a rationale for the experimental observation, our simulations highlight the role of the C-terminal tail in the stabilization of the inactive state. These mechanistic insights will be important for the construction of a multi-scale model describing the formation and function of PFKL assemblies.

References

B.A. Webb, A.M. Dosey, T. Wittmann, J.M. Kollman, D.L. Barber, *J. Cell Biol.* **216** (2017) 2305.
G.G. Fuller, J.K. Kim, *J. Cell Sci*. **134** (2021).

Off-diagonal thermodynamics and its effect on reactivity

Martin Srnec,[1] Mauricio Maldonado-Domínguez,[1] Daniel Bím,[1] Zuzanna Wojdyla[1]

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We formulated an original and unique theoretical framework aiming at the prediction of C-H bond activation reactivity.[1,2,3] In its current form, it features two thermodynamic factors that we named asynchronicity and frustration that together modulate coupled proton-electron transfer reactivity. Only after addition of these two factors to the classical well-documented effect known as linear free energy relationship a complete thermodynamic basis for the control of reactivity/selectivity is formed. In principle, each of the two factors and their combination enable changing the preference of which C-H-bond is likely to be activated that would be otherwise driven by LFER, which favors the weakest C-H bonds in molecules. To demonstrate the power of the approach, we will show and discuss H-atom abstraction reactivity of several transition-metal complexes and organic radicals. Finally, we also discuss the generalization of the approach to reactions with radical group transfer.

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Coupled dehydration-electrochemical oxidation of primary bile acids

Schwarzová-Pecková K.[1], Jelšíková K.[1], Skopalová J.[2], Kočovský P.[3], Veselý J.[3]

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Bile acids are formed in the liver as the end products of cholesterol catabolism. They play vital roles in mammals; they emulsify cholesterol, dietary lipids, fat-soluble vitamins, possess antimicrobial properties, are important regulatory molecules and in fact act as hormones [1]. Their structure is characterized by the presence of hydroxyl group on the steroid core and a side chain on C17 bearing the carboxylic group. The molecules possess an amphiphilic character, because they are curved thanks to the *cis* junction of the A and B ring of the steroidal nucleus, thus have hydrophilic concave α–face bearing hydroxyl groups and the carboxylic moiety, and hydrophobic convex β–face with alkyl groups [2].

The detection of bile acids is rather complicated due to the absence of chromophores and fluorophores in their structure and low volatility, thus derivatization, advanced chromatographic techniques relying on MS detection or enzymatic assays are needed [3, 4]. Recently, we introduced an electrochemical method for their detection relying on acid-induced dehydration in acetonitrile, which leads to products oxidizable at bare electrode materials within their potential window and absorbing in UV spectra [5, 6]. The research was motivated by the Lieberman-Burchard dehydration reaction of cholesterol, which is a base of classical Abell-Kendall method for its colorimetric detection [7]. This reaction is carried out in the mixture of sulfuric acid, acetic anhydride, and acetic acid and leads to introduction of doubles bonds in the steroid core by repeated sulfonations/sulfonic group eliminations [8].

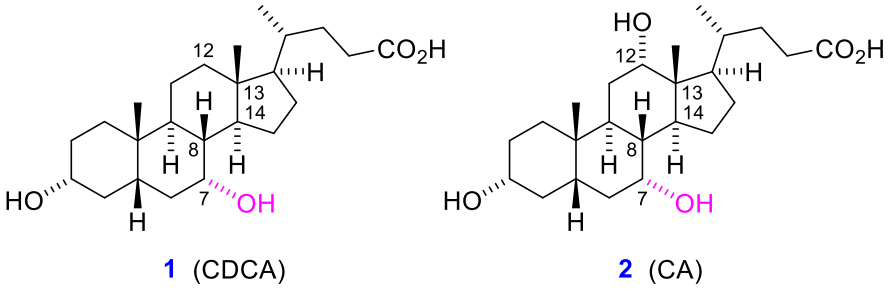

Fig. 1. Chemical structures of chenodeoxycholic acid (1, CDCA) and cholic acid (2, CA).

Our results show that bile acids with non-saturated steroid core and possessing an axial 7α-hydroxyl group favouring the dehydration step due to antiperiplanar position to the hydrogen atom at C(8) (*i.e.*, cholic (CA), chenodeoxycholic acid (CDCA), for structures see Fig. 1) provide a well-developed voltammetric signal at *ca* +1.2 V (*vs*. Ag/$AgNO_3$ in acetonitrile) in 0.1 mol L^{-1} $HClO_4$ in acetonitrile on platinum and carbon-based electrodes (glassy carbon, boron doped diamond). These oxidation signals are present upon water addition and pH change of the solution after the dehydration step. Our research has revealed that this coupled dehydration-electrochemical oxidation of CDCA leads to rearrangement of the steroid core induced by dehydration and formation of a C13 spirocentered steroid with Δ16 double bond (dehydration step), which is subsequently oxidized to a spirocyclic C15 enone (electrochemical step). This main reaction pathway was proposed by extensive GC-MS, LC-MS, ^{1}H NMR, ^{13}C NMR, and electrochemical analysis of reaction mixtures obtained by dehydration and oxidation of CDCA and comparison with independently synthetized standards of the main reaction products. Rigorous characterization of this simple reaction lays the groundwork for development of

simple analytical methods for detection of primary bile acids and their conjugates as will be demonstrated on HPLC with electrochemical and UV detection.

Acknowledgement

The research was carried out within the framework of Specific University Research (SVV 260690). Financial support from the Grant Agency of the Charles University (project GAUK 362621) and the Czech Science Foundation (project 19-11268S) is gratefully acknowledged.

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Characterization of laser treated polycrystalline BDD electrodes with various sp^2 /sp^3 carbon ratio*

Zelenský M. [1], Hrabovský J. [2,3], Zukerstein M. [2], Schwarzová-Pecková K. [1], Bulgakova N.M. [2]

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Michal Zelenský (michal.zelensky@natur.cuni.cz)

Boron doped diamond (BDD) electrodes nowadays represent one of the most popular electrode materials for electroanalysis. Its extraordinary properties include low capacitive current, wide potential window and biocompatibility [1]. Laser irradiation of the BDD surface leads to the conversion of carbon hybridization from sp^3 to sp^2 with the possibility to accurately control the sp^2/sp^3 carbon ratio and the lateral distribution of sp^2 carbon. This technique opens a way to precisely tailor the electrode surface for specific applications [2].

In this work three types of polycrystalline BDD electrodes were irradiated by fs IR laser: as-grown undoped diamond electrodes (deposited at B/C in gas phase 0 ppm), as-grown BDD electrodes (B/C ratio 500 ppm) and chem-mechanically polished [3] BDD electrodes (B/C ratio 500, 1000 and 2000 ppm). By IR laser irradiation the BDD electrodes with different sp^2/sp^3 carbon ratios were prepared: 0; 0,1; 1; 5; 10; 25; 50 a 100 %.

To prepare the BDD electrodes with accurate lateral distribution of sp^2 carbon first the optimization of laser fluence was carried out. By Raman spectroscopy and confocal microscopy, the optimized laser fluence was identified when majority of the light energy was absorbed in BDD film leading to the most efficient conversion of sp^3 to sp^2 carbon. For electrochemical characterization of the BDD electrodes the inner-sphere ($[Fe(CN)_6]^{3-/4-}$) and outer-sphere ($[Ru(NH_3)_6]^{3+/2+}$) redox probes were used. Cyclic voltammetry and electrochemical impedance spectroscopy determined that the electrochemical parameters (ΔE_p, R_{ct} and Y^0) are greatly affected when sp^2/sp^3 carbon ratio is ≥25%.

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Metal/polyaniline composite electrodes for electrochemical detection of aliphatic alcohols

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Electroanalytical chemistry opens wide possibilities for development of cheap and portable sensors for detection of various analytes. This is especially important for medical applications, where such devices can allow for constant monitoring of biomarkers of interest in hospitals as well as long-term self-monitoring for outpatients. Beyond the medical applications, similar sensors can be employed for other kinds of screening outside the laboratory, such as discovering forbidden substances in food, assessment of water or fuel quality *etc.*

Aliphatic alcohols constitute especial challenge for electroanalysis in case they don't contain other redox-active groups. A number of organic substances which are basically aliphatic alcohols have significant importance for human health, food production and safety and other branches of industry. This includes simple molecules like methyl, ethyl, propyl, and cetyl alcohols as well as more complicated glycerol, menthol, inositol or cholesterol. Enzyme-based electrochemical alcohol sensors exist [1], but low stability and high price of enzymes constitute an inevitable drawback. In our group, direct detection of sterols on boron-doped diamond electrodes was reported recently [2] in acetonitrile, but the required high potentials and poorly shaped oxidation peaks impaired the detection. Aliphatic alcohols can be also quite easily oxidized in alkaline aqueous medium on electrodes made from some noble metals such as Au, Pt or Pd [3] (typical oxidation potentials are around 0 V vs Ag/AgCl reference electrode). To the best of our knowledge, this option was only sporadically considered for electroanalytical applications so far. Moreover, prospects of utilization of the noble metal nanostructures and semipermeable polymeric electrode coatings for improvement of sensitivity and selectivity of the detection remain obscure.

The work aimed at elucidation of possibilities of electroanalytical detection of aliphatic alcohols on the example of 1- and 2-propanol on Au and Pt electrodes with and without electrochemically formed composite coatings on the base of polyaniline (PANI) and nanoparticles of Au and Pd.

PANI films were electrochemically synthesized on the surfaces of 2 mm Au, Pt and glassy carbon (GC) disk electrodes from aniline solution in 1M H_2SO_4 via two procedures (modified from the ones reported elsewhere, *e.g.* [4]). Procedure 1 consisted of application of +0.9 V constant potential until selected charge (10–40 mC) passed through the cell. Procedure 2 included performing 15 consequent cyclic voltammetry (CV) scans within −0.1 to +1.0 V.

Pd and Au particles were deposited on neat and PANI-modified electrodes via cathodic reduction employing modified procedures reported previously [4]. The targeted electrode kept under +0.8 V constant potential was immersed in a solution of $K[AuCl_4]$ or $K_2[PdCl_4]$ in aqueous H_2SO_4. In approach 1, the excess of the metal salt was washed out by 10-fold excess of aqueous H_2SO_4 (it was therefore expected that only the complex anions retained by positively charged pernigraniline salt form of PANI underwent the reduction on the further stage). In approach 2, the washing out was avoided. After that, 5 CV cycles between +0.8 V and -0.2 V were performed to achieve the reduction.

On neat Pt and Au, both 1-propanol and 2-propanol in 1M NaOH are oxidized at *ca.* −0.27 V; and +0.17 V, respectively, with an order of magnitude higher peak currents on Au. Peak current *vs.* 1-propanol concentration dependence was highly linear in the case of Au electrode (Fig. 1), but the linearity was significantly poorer in the case of Pt, which can be caused by more significant Pt surface fouling by the oxidation byproducts (*e.g.* CO). PANI film deposition did not improve the performance of the electrodes by itself, but affected the deposition of metal nanoparticles. In particular, PANI-covered Pt electrode with deposited Au nanoparticles exhibited

significantly higher sensitivity than the neat Pt one and allowed to reach the limit of detection of 0.2 mmol/L, the lowest one observed within this study.

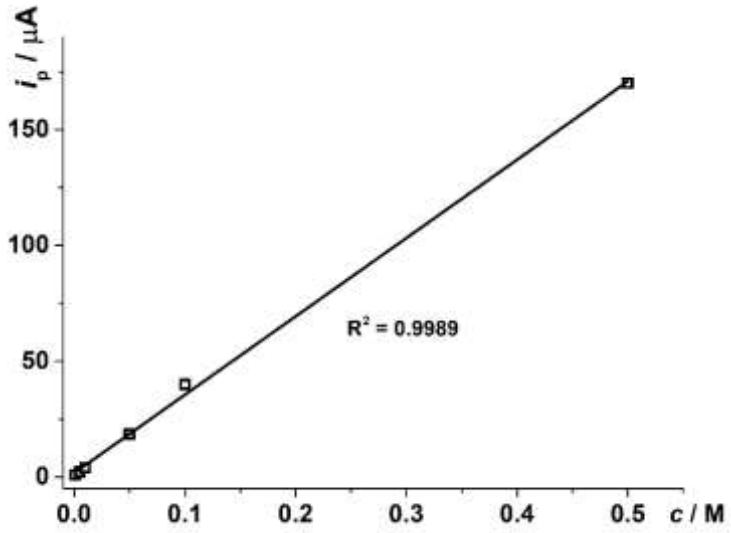

Fig. 1. Calibration curve for detection of 1-propanol (0.001–0.5 M) on neat Au electrode in 1M NaOH.

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Recent development and application of elimination voltammetry with linear scan

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Linear sweep voltammetry (LSV) or cyclic voltammetry (CV) are widely used potentiostatic methods that help reveal and specify the redox and transport properties of electrochemical systems [1-3]. Both voltammetric techniques are the experimental basis for software data processing of elimination voltammetry with linear scanning (EVLS), which can eliminate some selected partial voltammetric currents and preserve others. Compared to differential pulse voltammetry, EVLS can eliminate not only the charging current but any kind of sub-current for which the value of the polarization rate exponent is known. For example, for the diffusion (I_d), charging (I_c), and kinetic current (I_k) components, this exponent corresponds to 1/2, 1, and 0, respectively. The process of eliminating or preserving the selected component of the particular current is based on the basic assumption that the total voltammetric current corresponds to the sum of all partial currents with different dependence on the scan rate. The most important idea of EVLS is the principle of normalization, where one scan rate acts as a reference scan and the corresponding total current measured at that scan rate is the reference current I_{ref}. The other total currents are normalized to this reference current. For simplicity and also for a lower error rate, the most used ratio is 2 (whole number 2), that is, one scan rate is half and the other is double the selected reference scan. The coefficients of the elimination function $f(I)$ are calculated according to the assumption of which current component we intend to remove and which to keep. In the case of an adsorbed analyte, the EVLS function, which eliminates the capacitive and kinetic current components while preserving the diffusion current, provided a special peak-counterpeak signal that was theoretically confirmed [4-6]. Various EVLS functions have been successfully applied for the determination of organic and inorganic substances on various electrodes, including the study of their electrode processes [5-7].

We are currently focusing on the use of EVLS to study the electrical double layer and the influence of the composition and type of electrolyte on its structure and function. Basically, we take advantage of the fact that EVLS was derived primarily for a reversible electrode system. Thus, if we use a reversible, diffusion-controlled probe such as $[Fe(CN)_6]^{3-/4-}$, then according to the EVLS criteria in the case of the elimination of the diffusion current component, the kinetic and charging current components should be zero. This assumption is not always fulfilled, and our research has shown that in addition to the study of the electrical double layer using electrochemical impedance spectroscopy (EIS), our EVLS can also be used to study the morphology or chemical composition of the electrode surface. Careful investigation of the charge transfer across the electrode/electrolyte interface using three EVLS functions E4, E5, and E6 showed a lot of interesting findings; the most important of which was the course of the EVLS function E6 (maintenance of the capacitive component of the current) in the form of a droplet depression [8].

The mathematical expression of used EVLS functions with the fact that the current I is the reference voltammetric current, $I_{1/2}$ and I_2 are one-half and double of the reference current, respectively, correspond to these equations:

$E4\ f(I) = -11.6570I_{1/2} + 17.4850I - 5.8284I_2$,which eliminates simultaneously charging and kinetic currents while retaining the diffusion current,

$E5\ f(I) = 6.8284I_{1/2} - 8.2426I + 2.4142I_2$, which eliminates simultaneously charging and diffusion currents while retaining the kinetic current, and

$E6\ f(I) = 4.8284I_{1/2} - 8.2426I + 3.4142I_2$, which eliminates simultaneously the kinetic and diffusion currents while retaining the charging current.

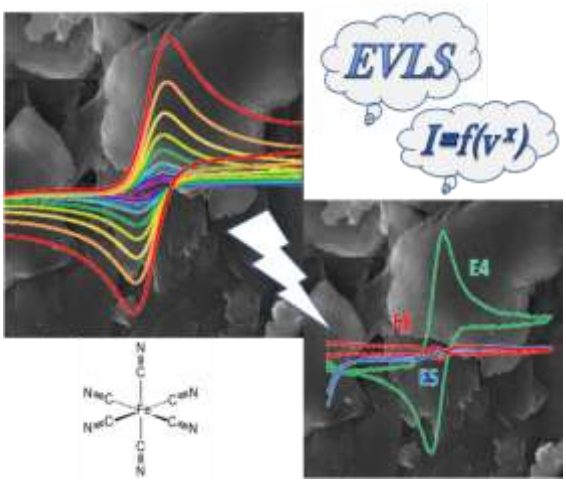

Fig. 1 Cyclic and elimination voltammograms (E4, E5, and E6) of 1mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl on Tombow polymer pencil graphite electrode (pPeGE).

The proposed elimination voltammetric model promotes an understanding of the electrochemical processes, influenced especially by changes in the electrical double layer and opens a perspective of further research to facilitate a rational clarification and optimization of electrode processes [8].

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Electrochemical dual detection platform for analysis of *BRAF* V600E point mutation in clinical samples using isothermal amplification techniques *

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DNA point mutations in oncogenes or tumor suppressor genes serve as potential cancer biomarkers. One of these mutations is V600E in BRAF gene, reported to be a prognostic and predictive biomarker for melanoma, colorectal cancer or lung cancer [1]. The gene sequencing is considered to be a gold standard method for detection of this mutation, however, it is relatively expensive, time consuming and requires highly skilled expert for data analysis [2]. Here, we describe an alternative novel electrochemical assay for the detection of BRAF V600E coupled to rolling circle amplification (RCA) and LNA capture probes [2]. This is a first dual detection system that employed two padlock probes that are complementary to either wild type target (wt) or V600E mutation sequence (mut), leading to highly selective rolling circle amplification of either wild type or mutant target, respectively. The second level of selectivity stemmed from an application of magnetic beads modified with wt or mut LNA capture probes, and final readout was performed on the screen printed carbon electrode. This dual system was successfully applied to analysis of V600E mutation status of cancer cell lines as well as eight cancer patients with melanoma or colorectal cancer. To our knowledge, this is the first isothermal RCA-based electrochemical system for analysis of BRAF V600E mutation status in dual format for analysis of cancer cell lines and tumor tissues. This work was supported by AZV NU21-08-00078.

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Designing the interface of nanoprobes operating in biological environments

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The use of nanoparticles in diagnostics, imaging, and therapeutics has revolutionized these fields with new properties not available with small molecules. Nanoparticle interface provide possibilities for polyvalent and independent attachment of different molecules serving as recognition/targeting structures, optical probes, spin probes or catalysts. However, nanoparticles operating in biological environments require precise control of multiple factors related to surface chemistry. To avoid for example aggregation, off-target interactions, and protein corona formation, appropriate interface design is essential. This talk will present general nanoparticle design strategies and specific examples including nanodiamonds, virus-like particles, and lipid nanoparticles.

Label-free interferometric scattering microscopy and spectroscopy with single-molecule sensitivity

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Interferometric scattering microscopy is a breakthrough in ultrasensitive, label-free microscopy that enables fast and sensitive detection of light scattered by weakly scattering objects, such as single bio-molecules (1). Our research focuses on discerning subtle fluctuations in the scattering signal to describe biomolecular interactions and processes hidden deep within the subdiffractional volume of the probe beam. We demonstrate how the fluctuation in the scattering amplitude can be associated with conformation changes taking place at the level of a single, or a few unlabeled biomolecules and open new possibilities for the next generation of super-resolution microscopy techniques (2).

To achieve this, we combine ultrasensitive microscopy with a novel, highly precise, and fast phase detection method, allowing us to perform quantitative phase imaging of scattering objects and understand their three-dimensional dynamics at high speeds (3,4). Our work demonstrates 3D mapping of microtubule networks and real-time 3D localization of unlabeled single proteins or proteins labeled with small metallic nanoparticles at a microsecond temporal resolution. For instance, we reveal the complex trajectory of protein diffusion on the surface of microtubules through the 3D trajectories of microtubule-associated proteins (5). Furthermore, we establish that the interferometric detection of the elastic scattering signal vastly improves the performance and reliability of ultrasensitive Raman spectroscopy enabling unparalleled single-molecule sensitivity.

In summary, our work showcases the potential of interferometric scattering microscopy for studying biomolecular interactions and processes at the single biomolecule level, with high sensitivity and speed. This technique allows for understanding the dynamics of biological matter and opens new avenues in label-free super-resolution microscopy.

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Application of scattering methods for studying structure and dynamics on the nanoscale

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Scattering of radiation (light, X-rays, neutrons) has been proven useful for studying morphology and dynamics of nanostructured materials [1]. In this communication, I will demonstrate application of several scattering methods – static and dynamic light scattering, small-angle X-ray and neutron scattering (SAXS, SANS) and neutron spin echo spectroscopy (NSE) – for revealing structure of coacervate emulsion formed by electrostatic complexation of comb copolymer with polyanionic backbone (poly[methacrylic acid-*stat*-poly(ethylene glycol) methyl ether methacrylate], PMAA-PEGMA) and cationic surfactant (*N*-dodecylpyridinum chloride, DPCl) [2,3]. As the emulsion structure exhibits two characteristic length scales differing by 3 orders of magnitude (micrometer-sized coacervate droplets dispersed in water and nm-sized densely packed DPCl micelles aggregated on PMAA backbones), combination of light scattering with SAXS/SANS is necessary to cover the large range of scattering vector magnitudes. Moreover, as the overall scattering of the emulsion is the sum of the scattering from the aqueous phase and the polymer-rich coacervate phase (droplets), dynamic methods such as NSE appeared to be useful for distinguishing between these two scattering contributions as they differ in the rate of translational diffusion.

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Exploring the Impact of High-Intensity Pulsed Electric Field on Cytoskeletal Proteins: from Simulations to Experiments

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Short (us-ns) duration intense pulsed electric field (PEF) represents a unique tool to modulate the function of biological systems with potential applications in bionanotechnology and biomedical therapies. The target of PEF we explore in our work are the cellular fiber microtubules and its subunit, protein tubulin, due to their exceptionally high electric charge and dipole [1].

Our approach involves three stages: computational simulations (in silico), experiments on isolated proteins (in vitro), and investigations within living cells (in vivo). First, we use molecular dynamics simulations to examine the effects of nanosecond PEFs on tubulin and microtubules [1, 2]. These simulations help us understand how nanosecond PEFs can influence tubulin conformation and control microtubule assembly in vitro [3]. Next, we demonstrate how chip microfabrication technology and advanced microscopy enable us to observe the effects of nanosecond PEFs on the cytoskeletal network in living cells [4, 5]. We showed that intense short electric pulses can influence the structure of the tubulin hence modifying its function.

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Nanofluidic Scattering Microscopy for label-free single-molecule detection of biomolecules in free motion

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Understanding of chemical and biological processes at the single molecule level is essential for different areas such as molecular biology, biochemistry or medicine. Label-free characterization aims to complement single-molecule fluorescence microscopy, in cases where the labelling with a fluorophore may perturb the chemical or structural properties of the studied biomolecule. An alternative approach, interferometric scattering microscopy (iSCAT), has allowed to resolve individual biomolecules in a label-free manner. However, iSCAT requires attaching biomolecules to a surface, possibly influencing the properties and reactivity of such molecules. Recently, the ground-breaking method of Nanofluidic Scattering Microscopy (NSM) has been developed [1]. The NSM enables the label-free imaging of individual biomolecules diffusing inside a nanofluidic channel. It can in real-time observe biomolecules with size ranging from tens of kDa up to units of MDa in physiologically relevant conditions without the need for immobilization or labelling. For each detected biomolecule, NSM can determine its hydrodynamic radius from the measured diffusivity and molecular weight from the measured optical contrast, thus distinguishing between its conformational states. This presentation will discuss the innovative approaches aimed at pushing the boundaries of NSM capabilities. Emphasis will be placed on harnessing alternative optical configurations of the experimental setup, alongside employing cutting-edge data processing techniques. These enhancements pave the way for groundbreaking applications in biomolecular research, enabling the scrutiny of biomolecular interactions at the single-molecule level, all in a label-free and tether-free format.

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Dynamic second harmonic imaging of divalent cation translocation through giant vesicle membranes

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The interaction of divalent cations with lipid membranes is crucial for the structure and proper function of cell membranes. In order to understand molecular level mechanisms behind those interactions, the role of interfacial water cannot be omitted. A recent improvement in imaging throughput has resulted in the construction of a second harmonic (SH) imaging device that can non-resonantly and dynamically image interfacial water molecules[1,2]. Here, we use this label-free approach to investigate the mechanism of divalent cation transport through lipid membranes using giant unilamellar vesicles (GUVs) in aqueous solution. By SH imaging interfacial water, we create spatiotemporal images from which structural heterogeneity, binding constants and translocation times can be retrieved. We observe binding heterogeneity, which implies that local chemical structure is relevant for ion transport. The comparison of surface potential values and translocation rates for several divalent ions (Cu^{2+}, Ca^{2+}, Ba^{2+}, and Mg^{2+}) points towards a new ion permeation mechanism[3-5].

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Copper(II) Azamacrocyclic Complexes for Medical Applications

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Complexes of macrocyclic ligands show high thermodynamic stability and kinetic inertness [1-4]. Cyclam (1,4,8,11-tetraazacyclotetradecane) and its derivatives are suitable for complexation of the first raw transition metal ions. Properties of these complexes can be modified and tuned by introduction of suitable pendant arms at nitrogen atoms of the macrocycle [5]. Cyclam complexes are investigated as redox sensors, redox catalysts and for simulation of redox-active centres of redox enzymes. They can be exploited in electronics as well as in medicine.

Nuclear medicine is dealing with both diagnostics and therapy. The diagnostic goal of imaging is to get information about condition of a tissue, eventual cancer detection. The objective of therapy is disease treatment and medication. The theranostic approach combines diagnostics and therapeutics, both operating simultaneously or sequentially, enabling switching between them. This modern medical approach requires radionuclides with different properties and the choice of appropriate isotope is critical. For imaging, radionuclides with minimal interaction with the organism are required (photon emission or β^+), while therapy requires deposition of radiation energy in the tissue (β^- or α emission). Last but not least, availability, easiness and safety of radioisotope and/or radiopharmaceutical preparation and production are crucial and play an important role in medical applications. [6-8]

Copper ions are essential in living organism – as catalytic cofactors of enzymes, as structural components of functional proteins, play fundamental role in cell replication and growth [8].

Medical application of radioactive copper provides new challenges. Copper has 32 isotopes, five radionuclids ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu and ^{67}Cu are promising radiotheranostics. The ^{60}Cu, ^{61}Cu, ^{62}Cu radioisotopes providing β^+emission (positron) are suitable for diagnostics, primarily for PET (Positron Emission Tomography). ^{64}Cu decay yields principally β^+ and β^- particles and Auger electrons. The mentioned combination of emitted particles predetermines theranostic application of ^{64}Cu. It is suitable for imaging using PET as well as for therapeutic applications as radiopharmaceuticals – radioisotopes bound to biological molecules targeting specific part of the body. ^{67}Cu represents combination of β^- and γ emissions and is exploited for imaging using SPECT (Single-Photon Emission Computed Tomography) and for radioimmunotherapy – radioactive isotopes bound to monoclonal antibodies targeting tumor cells. [6-8] Stable isotope ^{63}Cu, ^{65}Cu complexes investigated in our study serve especially as “model molecules” for further research with radionuclides.

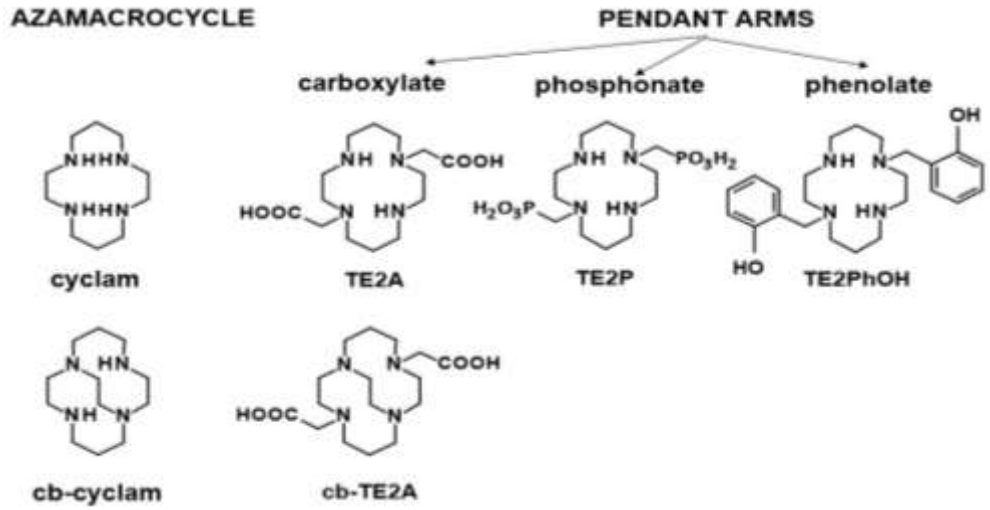

Fig. 1. Studied ligands

Tetraazamacrocyclic cyclen and cyclam ligands with pendant arms have been intensively studied as kinetically inert and stable chelators for metallopharmaceuticals [7, 8]. In order to reveal also reduction behaviour of the cyclam complexes and to evaluate the abilities of peripheral groups, series of the cyclam or cross-bridged cyclam ligands bearing carboxylate, phosphonate or phenolate groups were synthesized (Fig. 1) and electrochemically investigated. Reduction properties of complexes (3.10^{-3} M) were studied at mercury electrodes in aqueous solutions of Britton-Robinson buffer in the whole pH scale by the means of polarography and cyclic voltammetry at hanging mercury drop electrode.

The ligand itself is electrochemically inactive. The introduced electrochemically active metal ion (copper) represents the main redox centre of the complex.

Influence of azamacrocycle modification on central divalent metal ions, reduction potentials, kinetic and thermal stability, molecular geometry, reduction mechanism, isomerisation and coordination, as well as effect of pH on all studied features were revealed and discussed.

The reduction of the complex is significantly shifted to the more negative potentials comparing to the free Cu(II) ion due to its stabilization by electron donation of the ligand. Coordinated Cu(II) is irreversibly reduced to Cu(0) and the complex is decomposed to amalgamated copper and ligand. After Cu(0) electrochemical in-situ re-oxidation to Cu(II), copper can be re-complexed with the ligand present in excess.

Complexes of cyclam derivatives form several isomers differing in mutual orientation of substituents on cyclam nitrogen atoms (isomers I–V, Fig. 2) and in mutual orientation of the two coordinated pendant arms or additional two ligands coordinated in vacant position of the coordination polyhedron – *cis* or *trans* [5]. Electrochemical reduction revealed, that the studied copper complexes can adopt three different forms – isomers I, III and V depending on pH and temperature. Isomerisation can be accelerated by increasing the temperature resulting in the most stable hexacoordinated isomer III. Cyclam bridging blocks isomerisation

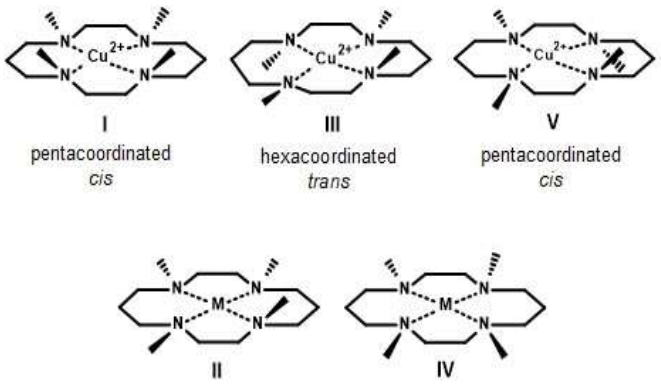

Fig. 2. Schematic representation of cyclam complexes isomers.

Measurements of reduction response in the whole pH scale revealed significant pH dependence of reduction potential as well as of cathodic current. Simultaneously, influence of the acid-base equilibrium on the rate of complexation, type of formed complex and reversibility of its reduction has been proved.

Acknowledgements
This research has been supported by the Czech Science Foundation project (GAČR) GA21-23261S and institutional support RVO 61388955.

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Copper complexes of phenolate-bridged dimeric cyclam ligand *

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Dinuclear complexes of the first-row transition metal ions are widely studied in applications ranging from cytotoxic activity[1] to anion sensing probes[2]. Some of these applications employ coordinating pendant arm, but not much is known about electrochemical, spectral, and acid-base properties of such compounds. Some activity of these dinuclear complexes stems from their ability to coordinate small molecules between the metal ions. Our work was intended to study possibility of this mode of coordination with cyclam based dinuclear ligands.

Three cyclam-based ligands with phenolic coordinating pendant arm were prepared (fig. 1) and characterized, two of those prepared ligands are dinuclear (and their copper(II) complexes were also prepared and characterized), third was prepared mainly for comparison in electrochemical study and its copper(II) complex was prepared only *in situ* in CV cell.

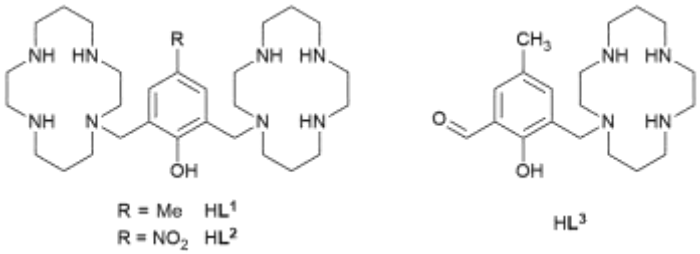

Figure 1: Structures of prepared ligands

Potentiometric and UV-Vis studies of $\mathbf{L^1}$ and $\mathbf{L^2}$ were used to ascertain deprotonation of phenolic moieties. Those can be compared with UV-Vis data from titrations of prepared copper(II) complexes: $[Cu_2\mathbf{L^1}]^{4+}$, $[Cu\mathbf{L^1}]^{2+}$ and $[Cu_2\mathbf{L^2}]^{4+}$ in which we can observe great change of pK_a of phenolic hydroxyl group for $[Cu\mathbf{L^1}]^{2+}$ and $[Cu_2\mathbf{L^2}]^{4+}$ when compared with their parent ligands, but only small change between $[Cu_2\mathbf{L^1}]^{4+}$ and $[Cu\mathbf{L^1}]^{2+}$, implying that coordination of second copper(II) cation isn't as significant. This is further corroborated by the fact that in no crystal structure that was obtained was this mode of coordination observed, although all obtained structures have the hydroxyl group of phenolic pendant arm protonated. To observe coordination situation in basic conditions, polarography and cyclic voltammetry were used to study ligands and complexes, revealing that the copper(II) cations act largely independently and they are reduced to elemental copper through 2 electron irreversible reduction. Comparison of these reduction between $[Cu_2\mathbf{L^1}]^{4+}$ and $[Cu\mathbf{L^1}]^{2+}$ ¨show only insignificant differences even at high pH, further confirming that there isn't any remarkable interaction of copper(II) cations. During electrochemical studies, unexpected instability of pendant arm was observed. Combination of CV studies of $\mathbf{L^3}$ and $[Cu\mathbf{L^3}]^{2+}$ and HPLC-MS has shown that ligands and complexes undergo break between pendant arm and cyclam resulting in various decomposition products that include pendant arms bearing aldehyde or hydroxylic group.

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* student lecture eligible for Emil Paleček Award

Combining electro- and photochemistry: acyl compounds of the 14th group elements for medical use

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Acylgermanes are challenging, innovative class of compounds with attractive application possibilities in photochemically induced polymerizations. Moreover, due to their low toxicity, they are frequently used in human medicine for white dental fillings (Ivocerin®, the corresponding photo-induced reaction mechanisms are well established [1]).

Upon visible light irradiation, C-Ge bond is cleaved and radicals are formed. The absorption spectra of the photoinitiators depend on the electron donating/withdrawing character of substituents at the aromatic moieties [2][3]. 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. Therefore, electrochemical research of acylgermanes is an independent powerful tool of electronic structure investigation, important for tuning the properties of novel compounds [4].

Despite numerous publications and even patents already published in the field of acylgermane based photoinitiators, several problems are still not resolved. Mainly:

(a) high price of germanium - analogical derivatives containing Si or Sn are being synthetized and investigated. While the acylstannanes are expected to have similar photochemical properties to acylgermanes (Norrish type I cleavage), acylsilanes should differ (Norrish type II rearrangement).

(b) irradiation wavelength - the lower energy (longer wavelength), the larger penetration depth, therefore the more perfect curing of the polymerization mixture. The new proposed photoinitiators are tailored with respect to inductive and mesomeric effects of peripheral substituents to achieve the absorption band in the visible region instead of UV.

Because of strong connection between the photochemical and electrochemical point of view (the electron is the same in both methods), except from the descriptive molecular electrochemistry (DC-polarography and cyclic voltammetry across a series of mono-, di-, tri- and tetraacylgermanes, di- and tri-nuclear polyacylgermanes, analogical silanes and stannanes as well as other related compounds - halides, enolates etc.), combinations with photochemical generation step (photo-electrochemistry) as well as with spectroscopically followed electrochemical generation (spectroelectrochemistry) will be discussed.

Acknowledgements

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Salt effects on N-isopropylacrylamide in aqueous solutions

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In this work, we investigate salt-specific effects on N-isopropylacrylamide (NiPAM) by means of densimetry and vapor pressure osmometry, employing family of sodium and guanidinium salts from low NiPAM concentration up to the solubility limit. Employing Kirkwood-Buff (KB) theory, complete set of effective pair interactions, KB-integrals, at any composition are determined, serving as a bridge to well calibrated molecular dynamics (MD) simulation. All atom MD simulations were used in direct analogy to the experiments and volumetric properties determined. For the first time, in-silico vapor pressure osmometry experiment was performed and excess osmolality evaluated from ternary solution structure. Finally, we establish a strong correlation between salt effect on NiPAM hydration and the salting-out ability of studied salts.

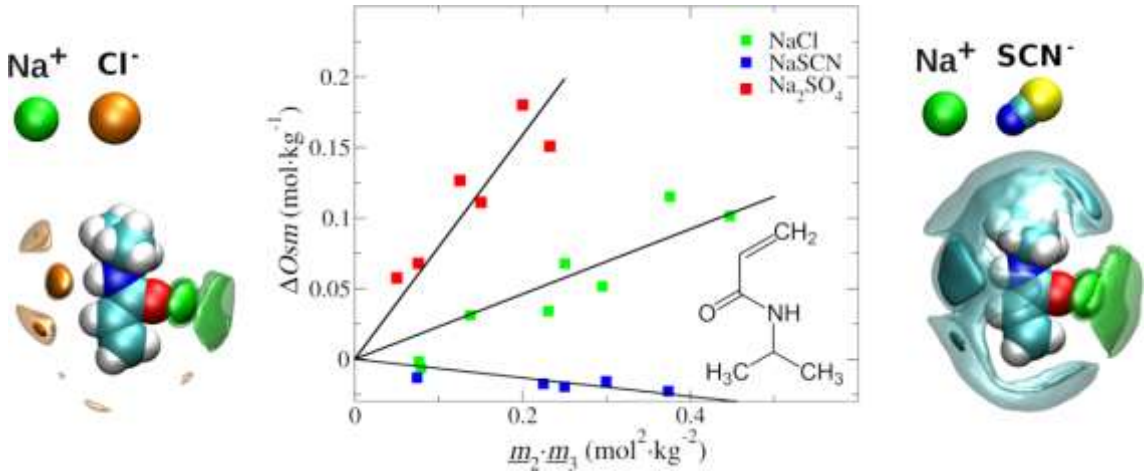

Figure 1: Spatially resolved affinity of salts to N-isopropylacrylamide molecule (left - sodium chloride (NaCl), right sodium thiocyanate (NaSCN)). The highly site specific binding of Na^+ to carbonyl group and Cl^- to amide group (N-H) leads to the net depletion of NaCl salt. In contrast the unspecific affinity of SCN^- around the nonpolar parts of the molecule strengthens the binding of Na^+ and results in net enrichment of NaSCN. The depletion of Na2SO4 and NaCl and enrichment of NaSCN are quantified by the slope of excess osmolality (ΔOsm) measured in vapor pressure osmometry.

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Talk Abstracts – Thursday

DNA structure at electrodes: possible challenges and ways to overcome them

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Electrochemical methods have proven to be excellent analytical tools for the studies of nucleic acids, including characterization of their structure - for example, monitoring the denaturation of double-stranded DNA in solution and on electrodes, detection of breaks and other damage, or conformational changes associated with the presence of superhelical coiling [1]. In general, we can state that the structure of DNA can be electrochemically studied with high reliability based on the measurement of the bases' own reduction, oxidation or tensammetric signals, if we work with relatively long DNA molecules with an average nucleotide sequence and our goal is to distinguish between double-stranded and single-stranded DNA. However, when studying alternative DNA structures, such as guanine [2] or cytosine quadruplexes, other factors come into play, such as the asymmetry in the nucleotide composition of individual chains affecting their interactions with the electrode surface, repetitive nature of the investigated molecules and dependence of the electrochemical signals of certain bases (e.g. guanine) on the presence of other bases. Because of these phenomena, it is not always easy to decide whether a given structural arrangement detected by an independent method in solution also exists in the adsorbed state on the electrode surface. To overcome these difficulties, alternative indirect methods can be used, e.g. the use of electroactive probes to evaluate the compactness of adsorbed DNA layers on electrodes [3] or a method based on monitoring the differences in the rate of DNA hybridization on a graphite electrode under conditions of stabilization and destabilization of the coiled secondary structure.

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Electron-Poor Acridones and Acridiniums as Super Photooxidants in Molecular Photoelectrochemistry by Unusual Mechanisms

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Photoredox catalysis is a powerful tool in organic synthesis and new catalytic systems that enable challenging transformations are desired. Novel acridinium and acridone catalysts were found to catalyze model *N*-arylation of pyrazoles (Figure 1). Redox and spectroscopic properties of new catalysts were studied and compared to known acridinium catalysts[1,2] to shed light on their mechanism of action.

Firstly, cyclic voltammetry and spectroelectrochemistry of all catalysts were measured to determine the oxidation and reduction potentials and the characteristic absorption spectra of their reduced and oxidized forms. The catalysts were also studied in the presence of substrates and upon irradiation (400 nm) to detect changes in spectra induced by the reaction.

Subsequently, the reactivity of catalyst's excited state was studied by Stern-Volmer fluorescence quenching with reagents. Acridinium catalysts were efficiently reductively quenched with arenes. Interestingly, no quenching was observed for acridone catalyst. Instead, the transformation of acridone to acridinium induced by the combination of acidic conditions and applied potential was detected. We conclude that acridone is a pre-catalyst which forms the catalytically active acridinium species in situ[3]. Remarkably, this finding solves the problem of limited photochemical and redox stability of acridinium catalysts.

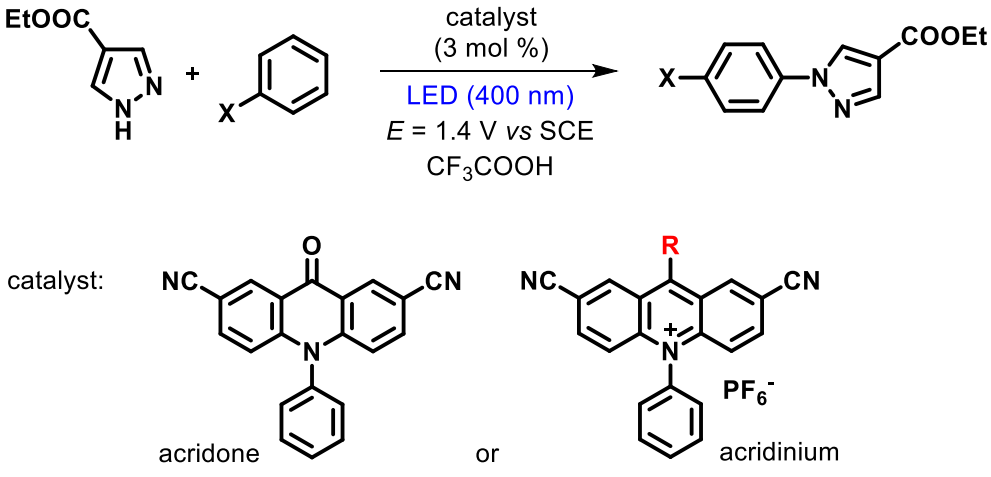

Figure 1: Model catalytic reaction

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Changes of ICT State Dynamics of 8'-apo-β-Carotenal Induced by Bulk Electrolysis

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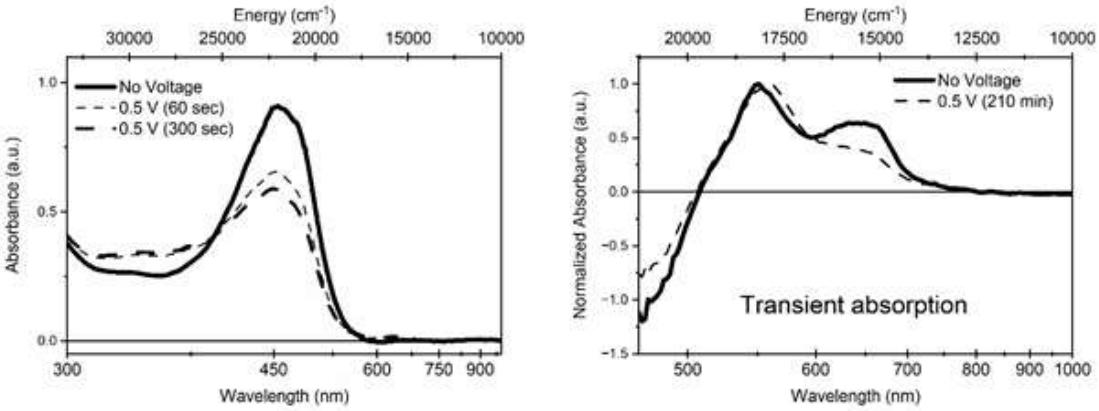
The intramolecular charge-transfer (ICT) state is a typical excited state of carotenoids that contain carbonyl group in their conjugation. It becomes pronounced only in the polar environment and is strongly coupled to the S_1 state, forming a new electronic state, usually denoted S_1/ICT state. If present, it leads to decrease of the S_1 lifetime compared to nonpolar environments. For example, the S_1 state of carotenoid 8'-apo-β-carotenal has a lifetime of 25 ps in non-polar solvent *n*-hexane, but is reduced to 8 ps in polar solvents such as methanol or acetonitrile. However, the influence of applied voltage on ICT has not yet been investigated. Therefore, this work examines the influence of applied external voltage on the excited-state properties of 8'-apo-β-carotenal in acetonitrile by steady-state and ultrafast time-resolved absorption spectroscopy. The steady-state measurements showed that although the magnitude of the S_0-S_2 absorption bands decreased with applied voltage, their spectral positions and shape remain nearly the same. Comparison of pump-probe time-resolved measurements shows that the magnitude of the ICT band decreases during bulk electrolysis. The decrease of magnitude of ICT band is also accompanied with a prolongation of the S_1/ICT state lifetime from 8 ps to 13 ps. Furthermore, turning off the applied voltage resulted in returning to no-voltage data within about 30 min. We have obtained satisfactory results to demonstrate that it is possible to tune the ICT state properties of carotenoids by applying an external voltage.



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Mapping of Electrochemical Reactions on PtIr Neuromodulation Electrodes.

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Electrical neuromodulation is integral to various implantable bioelectronics devices, including deep brain stimulators, peripheral nerve stimulation devices, spinal cord stimulators, and retinal prosthetics. The fundamental process behind electrical stimulation is charge injection from an electrode into the surrounding physiological electrolyte. Ensuring the safety and reliability of this electrochemical process is paramount.
Research in this field has extensively examined the mechanisms of charge exchange at the electrode/electrolyte interface, categorizing them as capacitive, pseudo-capacitive, and faradaic. Capacitive charge injection primarily involves the charging and discharging of electrolytic double-layers, with no net charge transfer to solution species. The pseudo-capacitive mechanism, or pseudo-faradaic, involves redox reactions of the electrode material itself, potentially leading to highly reversible charge transfer. The faradaic category encompasses charge transfer to solution species through a redox process, with reversibility dependent on factors like activation barriers and diffusion rates.
Neurostimulation electrodes function by injecting current into a physiological medium, creating electric fields that modulate nearby excitable cell membrane potentials. To ensure the safety of this process, any charge artificially injected into the physiological environment must be subsequently removed to prevent electrode polarization or adverse electrochemical changes in the biological surroundings.
Our study focuses on platinum iridium electrodes, the most commonly used electrode material in clinical neuromodulation devices. Specifically, we aim to map the faradic reactions likely occurring during charge injection in neurostimulation. We have chosen to quantify water electrolysis and the resulting pH changes. Amperometric microsensors were employed to directly measure local variations in oxygen, hydrogen, and pH concentrations above the electrodes under investigation.
In addition electrolysis, we have mapped oxygen reduction reactions, as previously studied [1]. We monitored oxygen depletion in the electrode vicinity using amperometric microsensors during the application of cathodic pulses. Furthermore, we quantified the accumulation of hydrogen peroxide during oxygen reduction. Finally, we explored the oxidation of chloride ions and characterized the resulting chlorine reactive species.
Understanding the precise nature of faradic reactions during neurostimulation on platinum iridium electrodes is crucial for enhancing the safety and efficacy of implantable bioelectronics devices. Our findings contribute valuable insights into the electrochemical processes involved, paving the way for further improvements in neuromodulation technology and the development of safer and more effective devices for patients.

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RNA modifications and repair of damaged genome

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The DNA damage response is mediated by both DNA repair proteins and epigenetic markers. Chemical modifications in mRNAs, tRNAs, rRNAs, and non-coding RNAs stabilize these nucleic acids; thus, distinct types of RNA could play a role in genome response to the stress factors, such as radiation background or pollutants. We showed that epigenetic marks in RNA, including N^6- or N^8- methyladenosine (m^6A, m^8A) and N^4-acetylcytosine (ac^4C) seem to be markers of either DNA damage repair or RNA damage and repair. We study the role of several RNA modifications in the repair of the damaged genome. For example, we observed that N^8-methyladenosine (m^8A), a mark of the epitranscriptome, binds to phosphorylated histone H2AX (γH2AX, a marker of DNA damage) and is abundant in RNAs accumulated at UV-damaged chromatin. Significantly, the PARP inhibitor, olaparib, prevented m^8A RNA as well as XRCC1 accumulation at UV-induced DNA lesions. PARP-dependent recruitment to UV-damaged chromatin we also observed in the case of m^6A and ac^4C in RNA. This nuclear process was additionally accompanied by radiation-induced depletion of 2,2,7-methylguanosine (m_3G/TMG) and N^1-methyladenosine (m^1A) in RNA. Based on these results, we suggested a model of non-canonical $m^6A/m^8A/ac^4C$ RNA-mediated DNA damage response. This process is PARP-dependent and associated with the function of BER (the Base Excision Repair) proteins, including XRCC1. Moreover, the later phases of DNA damage response are accompanied by active DNA demethylation. Taken together, it is evident that damage in the genome induces changes in only DNA sequences but also in epigenetic marks of DNA, RNA, and histones.

Complexes of DNA oligonucleotides and DNA components with mercury ions

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In addition to the natural way of nucleobases pairing using hydrogen bonds [1], the possibility of forming base pairs through transition metal ions has also been described, with the best described type being the formation of complexes of thymine with mercury. In this type of base pair, the mercury atom is bound between the nitrogen atoms in position 3 of both thymine residues [2]. This interaction and subsequent formation of secondary DNA structures is widely used in construction of sensors for mercury detection [3]. The ability of nucleic acids components and DNA oligonucleotides to form complexes with mercury ions can also be monitored using electrochemical methods and a hanging mercury drop electrode (HMDE), which was used in this work. Taking advantage of this approach, the interaction with mercury was studied - in addition to natural DNA components - also with modified bases, namely thiopyrimidines, which are pyrimidine bases where an oxygen atom has been replaced by a sulfur atom. The obtained results show the ability to form complexes with mercury both for the already described thymine, when the most pronounced signals were observed for thymine in the form of homooligonucleotide dT_{30}, and for guanine in the form of nucleoside triphosphate. As expected, the signals of base complexes with mercury were also observed for selected thiopyrimidines.

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A versatile DNA-based platform for investigating interactions between plasmonic nanoparticles and fluorescent nanodiamonds*

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Here, we introduce a highly versatile and easy-to-prepare platform for assembling plasmonic nanoparticles and fluorescent nitrogen-vacancy center-containing nanodiamonds in defined distances to each other. This approach enables us to investigate changes in emission behavior of the NV-centers while in close proximity to the plasmonic nanoparticles, as well as modulations of the emitted light. To control positioning and distances of this heterogeneous particle assembly, we use DNA nanotechnology. More precisely, the DNA origami technique enables us to place different particles with nanometer precision. The simple synthesis of these nanostructures can be scaled up easily and provides reliable yields of the desired assemblies.

We present different case studies to demonstrate the versatility of our platform. We are able to assemble fluorescent nanodiamonds and gold nanoparticles with variable distance and particle number. By doing so, we were able to engineer the position of the plasmon resonance that suits preferred applications and needs.

The choice of using fluorescent nanodiamonds comes from the fact that they exhibit a broad absorption and emission spectrum with a large Stoke's shift and are resistant to photobleaching. These properties make them the ideal candidate to probe plasmonic light modulations. This phenomenon was recently observed in super-resolution microscopy and is expressed through an unexpected spatial shift of the detected signal when a fluorophore was found in proximity of a plasmonic particle. To elucidate the mechanism behind this phenomenon, NV center-containing nanodiamonds act as robust model systems.

Further, we believe that our approach has great potential in diamond color center research and applications such as spintronics and magnetometry.

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* student lecture eligible for Emil Paleček Award

Mechanical regulation of hepatic tumor cell functions

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Emerging evidence suggests the significance of the physical and mechanical properties within the tissue microenvironment and the resulting forces that impact cellular behavior. Among these properties, extracellular matrix (ECM) stiffness emerges as a universal signal governing cell functions such as proliferation, differentiation, and cell death. ECM stiffness plays a pivotal role in regulating the normal function of tissues and contributes to the unusual behavior observed in diseased cells. For instance, metastatic cancer cells tend to migrate to organs characterized by a softer microenvironment. However, it remains unclear whether and how the mechanical attributes of the local tissue influence these cells' response to treatment.

To address this question, we designed soft 3D collagen scaffolds that mimic a pathological mechanical environment. This allowed us to decipher how liver tumor cells adapt their metabolic activities in response to physical cues from the cellular microenvironment. Our findings reveal that the soft 3D microenvironment enhances glycolysis in both HepG2 and Alexander cells. Moreover, both cell lines adjust their mitochondrial activities and functions when grown in this soft 3D context. We have also identified the YAP-mTOR axis as a downstream effector of mechanotransduction within 3D cellular cultures. Importantly, we have demonstrated that cellular mechanics, which are influenced by the physical constraints of the 3D collagen scaffolds, exert a profound impact on cellular proliferation in a YAP–mTOR-mediated manner. Functionally, this YAP–mTOR connection plays a pivotal role in mediating cellular plasticity in hepatic tumor cell lines. These findings expand our understanding of the role of YAP–mTOR-driven mechanotransduction in controlling hepatic tumor cellular responses within the physical confines of 3D cultures.

Furthermore, our research suggests a tentative mechanism that coordinates the rewiring of signaling pathways with cytoplasmic restructuring during cell growth within 3D microenvironments. Additionally, cells cultured in the soft 3D microenvironment exhibit marked mitochondrial depolarization, a downregulation of mitochondrially encoded cytochrome c oxidase I, and a slower proliferation rate when compared to cells grown in stiff monolayer cultures.

In summary, our data reveal a connection between liver tumor glycolysis and mechanical cues. We propose that soft 3D collagen scaffolds could serve as a valuable model for future studies aimed at understanding the mechanically regulated cellular functions of various liver (and potentially other tissue) tumor cells. This research holds promise for advancing our knowledge of cancer treatment and tissue engineering.

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The Faraday Scalpel: on-demand manipulation of oxygen, reactive oxygen species, and pH in biological environments

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Oxygen is the fundamental electron acceptor in metabolism. As a byproduct of aerobic respiration, various reactive oxygen species (ROS) are formed. The balance of oxygenation and ROS is a critical aspect of homeostasis. We present the concept of using electrochemical methods to manipulate the levels of oxygen and ROS in biological systems in order to produce a desired outcome. Extensive oxygen depletion combined with generation of large concentrations of ROS can lead to cell death, which we propose is a novel and useful approach to precise electrosurgery of pathogenic tissues. Lower levels of oxygen reduction/ROS generation can alter the function of numerous biological pathways. Of particular interest is targeting ion channels using locally-generated hydrogen peroxide. This offers a completely new method to alter electrophysiology in vitro or in vivo. I will present several examples of faradaic electrochemistry on oxygen acting at the level of different targets. Controlled electrolysis can be used to reliably interrupt electrophysiological activity via rapid local changes in pH. Examples of nerve lesioning in animal models will be shown.

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Light-driven chemistry inside a phage head

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Photosynthetic reaction centres (RC) and light harvesting (LH) complexes are increasingly used as inspiration for new types of devices for solar energy utilization. We focus on engineering light-driven integrated pathways for the reduction of carbon dioxide into feedstocks for chemical industry. Efficient electron transfer requires proximity between RC and other components of the redox pathway. Virus-like particles (VLP) offer convenient platform for such confinement. We have adapted the P22 VLP self-assembly system, developed by Douglas and Prevelige labs for encapsulation of soluble enzymes [1], for incorporation of membrane proteins, namely RC-LH1 complex from *Rhodobacter sphaeroides*. We conjugated an engineered RC-LH1, which contains a hexa-histidine tag at the C-terminus of H-subunit [2], to an NTA-modified P22 scaffolding protein (SP) via transition metal coordination. Since integral membrane proteins cannot be assembled into VLPs in E. coli we have optimized P22 in vitro assembly [3] for use with detergents and subsequently incorporated the scaffolding-linked RC-LH1 into procapsid shells. Structural and enzymatic characterization of this initial VLP-based redox cascade will be presented.

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Lipid Membrane Behavior of Nitro-Fatty Acids and their Loading into POPC Liposomes to Activate Nrf2 Pathway

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Nitro-fatty acids (NO_2-FAs) are produced endogenously in the bloodstream, peripheral tissues and gastric fluid. They constitute a group of pleiotropically acting electrophilic signaling modulators, and some of them have been proposed as drug candidates. NO_2-FAs modulate inflammatory and fibrotic processes and generally participate in the redox processes of cells. The main representatives include nitro-oleic acid (NO_2-OA) and other derivatives of unsaturated fatty acids such as nitro-linoleic acid (NO_2-LA). In this study, we describe the behavior of 9/10-NO_2-OA, 10-NO_2-LA and the conjugated nitro-linoleic acid (9/12-NO_2-cLA) in a model POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) membrane using molecular dynamics (MD) and a battery of experimental approaches. NO_2-FAs were integrated into the structures of unilamellar POPC liposomes. We showed that when loaded in liposomes, NO_2-FAs undergo degradation (a decay reaction) to a very limited extent, in contrast to the free molecular form in an aqueous environment. This was confirmed by electron paramagnetic resonance (EPR) spectroscopic analysis of NO radical release and by electrochemical reduction of the nitro group by chronopotentiometry. Attenuated total reflection (ATR) spectroscopy, supported by MD calculations, enables the characterization of NO_2-OA insertion in membranes. The protonated form of NO_2-FAs penetrates into membranes more easily than the ionic form. We also found that NO_2-FAs integrated into POPC liposomes retained their ability to activate the Nrf2 pathway. This was documented by an increased expression of heme oxygenase-1 at the level of mRNA with a parallel decrease in protein levels of Keap1 in murine macrophage RAW 264.7 cells. For the purposes of comparative analyses, we used a DPPC:DPPE membrane model into which NO_2-FAs cannot penetrate. This mechanistic study supports the hypothesis that NO_2-FAs are distributed in cells and tissues in the lipid or aqueous phase, which basically affects whether they are mobile, stable, and thus biologically active.

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The Role of EDTA in Adsorption of Peptides on Model Biological Membranes

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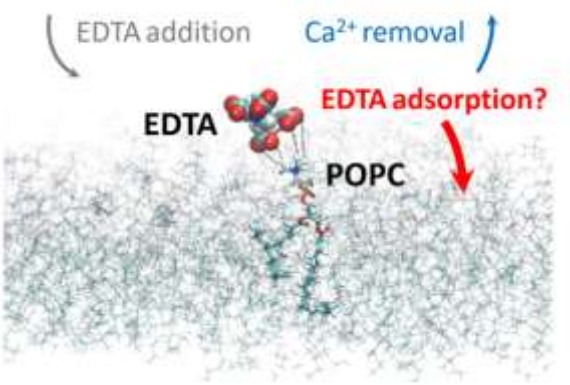
It has been established that cell penetration of positively charged cell-penetrating peptides (CPPs) consisting of arginine (Arg) only, such as Arg_9, is greatly facilitated compared to their equally charged lysine (Lys_9) peptides. Adsorption of CPPs to the outer cellular membrane leaflet typically composed of neutral lipids, is the first step of their translocation across cellular membranes, and it has been shown that Arg_9 efficiently adsorbs on zwitterionic neutral phosphatidylcholine POPC bilayers, in contrast to Lys_9.[1]

However, the adsorption of Arg_9 to POPC can be influenced by many factors, such as the ionic strength of the solution or the presence of various compounds. The addition of salts, and/or an increase in the concentration of charged peptides, diminishes the adsorption strength and in some cases even leads to peptide desorption from the membrane.[2] In this work we will show an additional effect on the peptide adsorption caused by negatively charged ethylenediaminetetraacetic acid (EDTA), often used in common biophysical experiments to remove metal cations from the system.

We will present three case study experiments – (i) EDTA effect on Arg_9 and Lys_9 adsorption on POPC large unilamellar vesicles (LUVs);[2] (ii) EDTA adsorption to POPC monolayers in a concentration-dependent manner[3] and (iii) EDTA control of the penetration of Arg_9 across the giant unilamellar vesicles (GUVs).[4] All experiments are supported by molecular dynamics simulations which will be discussed to explain the experimental findings.



Adsorption of EDTA at POPC monolayers

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Binding inhibition assay: theoretical and experimental optimization

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As the need for fast and sensitive techniques for the detection of a wide range of analytes grows, biosensors based on various transduction mechanisms have become an extensively researched field and have been applied to the detection of proteins, nucleic acids, saccharides, and even small molecules [1-2]. However, the detection of small molecules still represents a challenge for most biosensing methods as small molecules typically generate small signal and are unsuitable for signal enhancement e.g. by secondary antibody. Therefore, indirect detection methods, such as competitive or binding inhibition assay, are often employed. In the binding inhibition assay, the sample is spiked with a high-molecular-weight receptor (usually antibody against the analyte) targeting the analyte, and then, the remaining free receptor is detected. The assay is characterized by the calibration curve (with decreasing sigmoidal shape), its slope, half maximal inhibitory concentration (IC_{50}, the concentration of the analyte which binds 50% of the receptor), and the limit of detection (LOD).

Despite the extensive use of binding inhibition assays in biosensors, only a few studies concerning the effect of the assay parameters on its performance characteristic have been presented. The concentration of the spiked receptor had been identified as the parameter having the greatest impact on the assay performance. It was observed that decreasing the concentration of the spiked receptor shifts IC_{50} towards lower concentrations of analyte [3] and thus results in lower LOD. However, decreasing the concentration of the spiked receptor also results in lower sensitivity of the assay and lower signal-to-noise ratio, which have a negative impact on LOD. The optimal concentration of the spiked receptor, which yields the lowest LOD, has been so far determined empirically.

Our work aims to provide general guidance on how to estimate the optimal concentration of the spiked receptor for the particular binding inhibition assay. It represents the first study focused on the relationship between the concentration of the spiked receptor and IC_{50}. We performed theoretical calculations demonstrating that with decreasing concentration of the spiked receptor, IC_{50} asymptotically decreases to the value determined by the dissociation constant (K_D) of the interaction between the analyte and the spiked receptor. Then, we confirmed our results experimentally using the surface plasmon resonance (SPR) biosensor. We measured calibration curves for different concentrations of the spiked receptor and determined their IC_{50} and LODs. We conclude that in the binding inhibition assay, the concentration of the spiked receptor should be close to K_D of the interaction between the analyte and the spiked receptor in order to achieve the lowest LOD.

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Energy landscape steering mediates dynamic coupling in ATP-driven protein translocation by the bacterial Sec machinery

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The Sec translocon is a highly conserved membrane complex for transport of polypeptides across, or into, lipid bilayers. In bacteria, the core protein-channel SecYEG resides in the inner-membrane, through which secretion is powered by the cytosolic ATPase SecA. Here, we use single-molecule fluorescence to interrogate the dynamic state of SecYEG throughout the hydrolytic cycle of SecA. We show that the SecYEG channel fluctuates between open and closed states faster (~20-fold during transport) than ATP turnover; while the nucleotide status of SecA modulates the rates of opening and closure. Interestingly, a SecY variant (PrlA4), exhibiting faster protein transport, but unaffected ATPase rates, increases the dwell time in the open state, facilitating pre-protein diffusion through the pore; thereby improving the efficiency of translocation. Thus, contrary to prevailing structure-based models, SecYEG plays an integral part in the translocation mechanism through dynamic allosteric coupling in which SecA 'steers' the energy landscape of the protein-channel.

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Thermodynamics of chelate effect of Pd(II)-oxalate system

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Palladium(II) chemistry in aqueous solution is of interest as a model for analogous platinum(II) systems, due to higher reactivity, well-defined oxidation state and ease of preparation of the aqua ion. Thermodynamic and kinetic data for palladium(II) oxalato complexes are relevant *per se*, but also for example for the understanding of the *in vivo* reactivity of platinum(II) oxalato compounds used as cancerostatic drugs, *e. g.* oxaliplatin [1].

Oxalic acid (H_2ox) and other carboxylic acids are found in soil solutions in comparatively high concentrations (10^{-4}-10^{-3} mol.dm^{-3}) therefore they are typically present in surface waters in low concentration [1]. Since palladium as the isotope ^{107}Pd is abundant among the long-lived fission products in spent nuclear fuel [1], modelling of its chemical transformations and migration in natural waters requires reliable thermodynamic and kinetic sequestering data, including data for oxalate complex formation.

Complex formation between $[Pd(H_2O)_4]^{2+}$ and oxalate (ox = $C_2O_4^{2-}$) has been studied by molecular absorption spectroscopy in aqueous solution. Thermodynamic parameters ($\log_{10} K_{1,H}$ = 3.38(8), ΔH^0 = -33(3) kJ.mol^{-1}, ΔS^0 = -48(11) J.K^{-1}.mol^{-1}, T = 298.2 K, I = 1.00 mol.dm^{-3} $HClO_4$ [1]) have been determined for the reaction:

$$[Pd(H_2O)_4]^{2+} + H_2ox \rightleftharpoons [Pd(H_2O)_2(ox)] + 2\ H_3O^+$$

Stability constants for $[Pd(H_2O)_2(ox)]$ and $[Pd(ox)_2]^{2-}$ species ($\log_{10} \beta_1^0$ = 9.04(6), $\log_{10} \beta_2^0$ = 13.1(3), T = 298.2 K, I = 0 mol.dm^{-3}) [1] have been calculated by means of SIT.

Formation of $[Pd(H_2O)_2(ox)]$ from $[Pd(H_2O)_4]^{2+}$ is a two-step process, monitored by variable-temperature stopped-flow spectrophotometry. Rate-determining formation of a monodentate $[Pd(H_2O)_3(ox)]$ complex is followed by ring closure to the thermodynamically stable $[Pd(H_2O)_2(ox)]$ complex. Thermodynamic parameters calculated for both steps show that increased stability of the [Pd(ox)] species for the first step (formation of monodentate complex) is caused by increased higher entropic contribution, while the enthalpic contribution is important for the second step (chelate-ring closure). These results are compared with the ones obtained for Pd(II)-chloroacetate complex [2]. Structural and energetic data obtained by means of DFT (B3LYP-D3 functional with the def2-TZVPP basis set) calculations were carried out for the reaction between $[Pd(H_2O)_4]^{2+}$ and Hox^- with solvent effects introduced by the PCM approach and also with one additional water molecule. The calculated energy barriers for the formation of the bi-dentate complex are slightly higher for the ring closure step.

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Crystalline purines in unicellular eukaryotes: How Raman microscopy can contribute to understanding their origin, composition, and functions

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Despite the widespread occurrence of crystalline inclusions in unicellular eukaryotes and general awareness of their existence dating back to the end of the nineteenth century, scant attention has been paid to their chemical composition, functions, and evolutionary origins. The reason was the absence of detection methods that would enable reliable determination of the chemical composition of these inclusions *in situ*, together with the uncritical adoption of some assumptions of authorities based on indirect proofs. Using Raman microscopy, we revisited crystalline inclusions of the broad diversity of eukaryotes examining more than 200 species in all major supergroups [1]. We found that 80% of cellular crystalline inclusions consist of crystalline purines, such as anhydrous guanine (62 %), guanine monohydrate (2%), uric acid (12%), and xanthine (4%), which shifts the previous paradigm assuming the presence of calcite, and oxalates. Purine crystals emerge in microorganisms in all habitats, e.g., in freshwater algae, endosymbionts of reef-building corals, deadly parasites, anaerobes in termite guts, or slime mold amoebas. Hence, purine biocrystallization seems to be a general and ancestral eukaryotic process likely present in the last eukaryotic common ancestor. Purine crystalline inclusions were already shown to serve as high-capacity and rapid-turnover reserves of nitrogen in photosynthetic protists [2], however, even in unicellular eukaryotes they can have other functions, e.g., serving as optically active elements in light manipulation and sensing. In this contribution, we will present several aspects that could be found about this enigmatic phenomenon simply by using confocal Raman microscopy.

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Mechanisms of lipid membrane perturbations by antimicrobial polymers and a misleading side-effect

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The interaction of antimicrobial peptides or biomimetic polymers with lipid membranes can cause a variety of membrane perturbations. Systematic and mechanistic investigations are therefore crucial for the rational design of biomimetic compounds in the search for alternatives to classical antibiotics or therapeutic agents enhancing drug delivery.

Using various fluorescence methods and microcalorimetry, we thoroughly characterize membrane perturbations, most importantly membrane leakage induced by antimicrobial, biomimetic polymers. With a focus on varying lipid compositions, we distinguish different leakage mechanisms and find that changes in leakage mechanisms in different membranes constitute an important contribution to selectivity for different microbial species or cells (1).

Most importantly, we establish membrane fusion in model vesicles to be a common leakage mechanism that is less relevant in microbes (2,3,4). Even worse, vesicles composed of phosphatidylglycerol and phosphatidylethanolamine commonly used to model bacterial membranes are biased for this leaky fusion (4), potentially leading to misinterpretation in widespread model studies. Various implications of leaky fusion and vesicle aggregation are discussed alongside strategies to prevent them.

A positive aspect of our findings is that leaky fusion is probably useful for drug delivery applications.

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Zwitterionic Polymer Brushes as Biofunctional Antifouling Nano-coatings for Real-world Public-health Applications

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Advancing the frontiers in antifouling biofunctional materials and coatings toward real-world applications requires combined research across several disciplines, ranging from materials science, synthetic chemistry, and biophysics to cell biology. To address this challenge, we report an overview of our recent interdisciplinary collaborative research exploring the fascinating properties of ultrathin antifouling biofunctional polymer brushes with tailored zwitterionic structures. We report recent advances in the following areas: (i) new approaches in the design, fabrication, and characterization of antifouling functionalizable brushes, (ii) probing the brushes with advanced biophysical techniques, (iii) the research on microfluidic systems for the *on-chip* synthesis of polymer brushes, (iv) the development of biomaterials resistant to bacterial adhesion, and (v) the development of new zwitterionic brush-supported *cell-on-a-chip* systems capable of advanced studies on cellular responses to extracellular stresses. A variety of biophysical experimental techniques (QCM-D, electrochemical methods, FTIR, spectroscopic ellipsometry, SPR, AFM, and high-resolution spinning-disk confocal microscopy) has been employed within these studies. Our results show how important (and challenging) it is to precisely characterize the antifouling and biofunctional properties of polymer nano-coatings, especially when exposed to real-world biological fluids. We detail new approaches to probe brushes with emerging electrochemical impedance spectroscopy techniques as well as present a new antifouling terpolymer brush composition with a superior combination of antifouling and biofunctionalization capabilities over other coatings. This material has been successfully employed in several antifouling point-of-care biosensors, such as QCM biosensors for ultrasensitive detection of the SARS-CoV-2 virus in complex clinical samples and rapid detection of bacterial pathogens in crude food samples. Moreover, this newly developed functional coating has been directly employed in an extensive study examining monitoring of the surface contamination by SARS-CoV-2 in public transport vehicles in Prague Transportation System during the Covid-19 pandemic.

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Optical directionality of fluorescent proteins: fundamentals and applications

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Fluorescent proteins (FPs) serve as a basis of numerous genetically encoded sensors of biomolecular processes. Generally, FP-based sensors rely on various mechanisms of fluorescence quenching in order to convert a biomolecular event into an optical output. However, directionality of FP optical properties, until recently virtually unappreciated and underutilized, also provides appealing opportunities for development and applications of genetically encoded FP-based sensors. Here we describe the results of our investigations into directional optical properties of fluorescent proteins, along with applications of these properties in observations of molecular processes of cell signaling. Due to the wide range and importance of the observed molecular processes, we expect FP optical directionality to become a commonly known and used basis for development of genetically encoded fluorescent sensors for microscopy imaging.

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Membrane pore characterization using non-electrolytes

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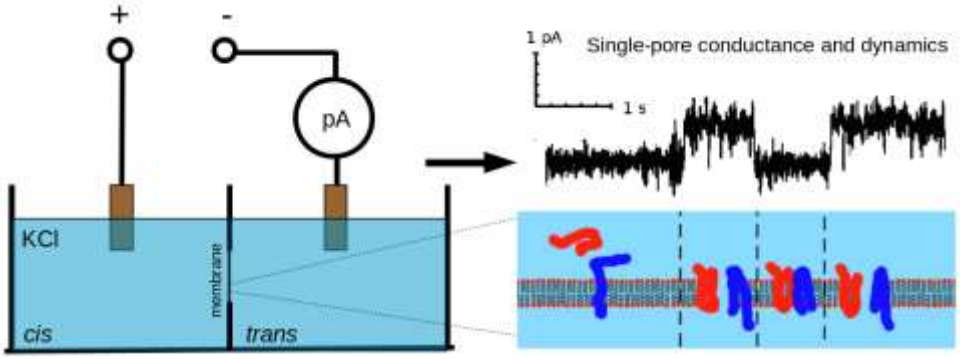
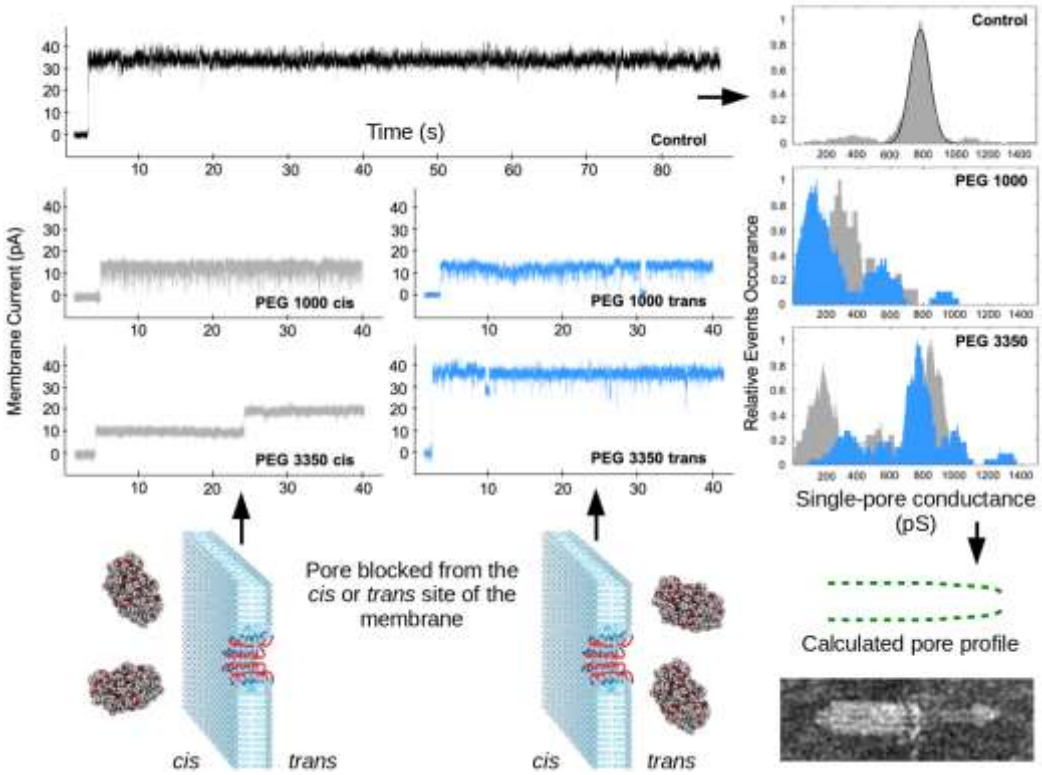
The measurement of electric current on planar lipid membranes enables the observation and characterization of individual molecules that create membrane pores. These are, for example, bacterial protein toxins, bacteriocins or selected antibiotics. Knowledge of the structure of the pore is necessary to understand its function. The permeability of individual pore for ions in aqueous solutions naturally reflects its geometry, i.e. the length and internal cross-section of the pore if the pore is simplified to a cylindrical conductor.



However, this simple method cannot be used to describe the structure a relatively narrow pores (~1nm) where the ions interact with pore lumen, or the pores with a more complicated internal profile. There is therefore the possibility of "blocking" the pore with non-conductive polymer molecules of non-electrolytes that have a defined molecular weight and shape (e.g., polyethylene glycol). Such a use of non-electrolytes will be demonstrated using examples of different types of symmetric and asymmetric membrane pores.



What can we learn from comparative characterization of materials on the macro- and nano-scale

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The physical and chemical properties of materials observed on the macro- or nano-scale may differ dramatically. For example, when measuring the Young's modulus of elasticity, we assume an “infinite” depth of material, which is not fulfilled especially when the Young’s modulus is measured at the nano-scale. Another example, the interaction of nanomaterials with biological environment and their toxicity is dependent on properties of individual nanoparticles, such as shape, surface passivation, zeta-potential (surface charge in colloidal state), chemical reactivity etc. and conflicting results about the same nanomaterial toxicity are published quite often. The chemical reactions of the same material or chemical compound also strongly depend on its form dramatically. For example, the chemical reactions observable on bulk materials only at high temperatures (>500°C) can be observed in nanomaterials at temperatures as low as 50-80°C. We decided to study those materials with our home-designed micro-spectroscopy setup combined with atomic force microscopy device equipped with several additional modules from different points of view. In this contribution we would like to present our knowledge about studying various systems at nanoscale and their comparison to bulk measurements. The new field of nano-reconnaissance can help us to understand for example the chemical reactivity of nanoparticles or reasons of secondary inflammatory reactions caused by deformation of proteins in the protein corona formed on the nanoparticle in a living organism.

References

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Poster Abstracts

(alphabetical order)

Continuous Monitoring Affinity Plasmonic Biosensor for Low Molecular Weight Analytes with the Use of Flexible Polymer Linkers **

Aktug G.[1], Asai N.[2], Cattozzo Mor D.[1], Forinova M. [1], Lisalova H.[1], Van-Truc, V.[3], Huang C-J[3], Dostalek J.[1]

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Plasmonic biosensors are pursued for direct label-free detection of chemical and biological species based on surface plasmon resonance (SPR)[1] as well as by the use of optical spectroscopy-based readout with plasmonically amplified optical signal[2,3]. Up to now, the translation of plasmonic biosensors to viable technologies in the medical field has been hampered due to the fouling of the sensor surface by the abundant matrix constituents when applied for the analysis of complex biological fluids. In addition, the majority of plasmonic biosensors with direct readout format are pursued for rapid detection at a single time and they lack the sensitivity for low molecular weight target molecules.This paper reports on the development of plasmonic sensor modality suitable for the continuous monitoring of low molecular weight analytes. It is based on ligands that reversibly interact with target low molecular weight analyte and plasmonically enhanced fluorescence (PEF) energy transfer[4] readout is proposed. The using of weak affinity ligands allows for reversible interaction with the target analyte constituting a direct continuous detection format. A specific aptamer-based ligand is anchored to a metallic sensor surface via a flexible polymeric linker (FPL) that is conjugated with a fluorophore label. The implementation of FPL based on single-stranded and double-stranded DNA chains is investigated for the readout mechanism that modulates the distance between the fluorophore label and metallic surface by the interaction with the target analyte, leading to reversible changes in the measured fluorescence signal based on switching between the plasmonic enhancement (distance >15 nm) and quenching (d<10 nm). The implementation of this concept for continuous monitoring of therapeutic drugs (blood anticoagulant Dabigatran Etexilate was selected[5]) is discussed and suitable biointerface with antifouling moieties and conformation switching of FPL is studied by combined SPR and quartz crystal microbalance with dissipation monitoring (QCM-D).

Acknowlegments
GA and JD acknowledge support from European Commission EIC Pathfinder project Versilib (#101046217). GA, DCM and JD are Czech Grant Agency through the project APLOMA (#22-30456J). NA was supported by the Austrian Science Fund (FWF) through the project DIPLAB (I 5119-B).

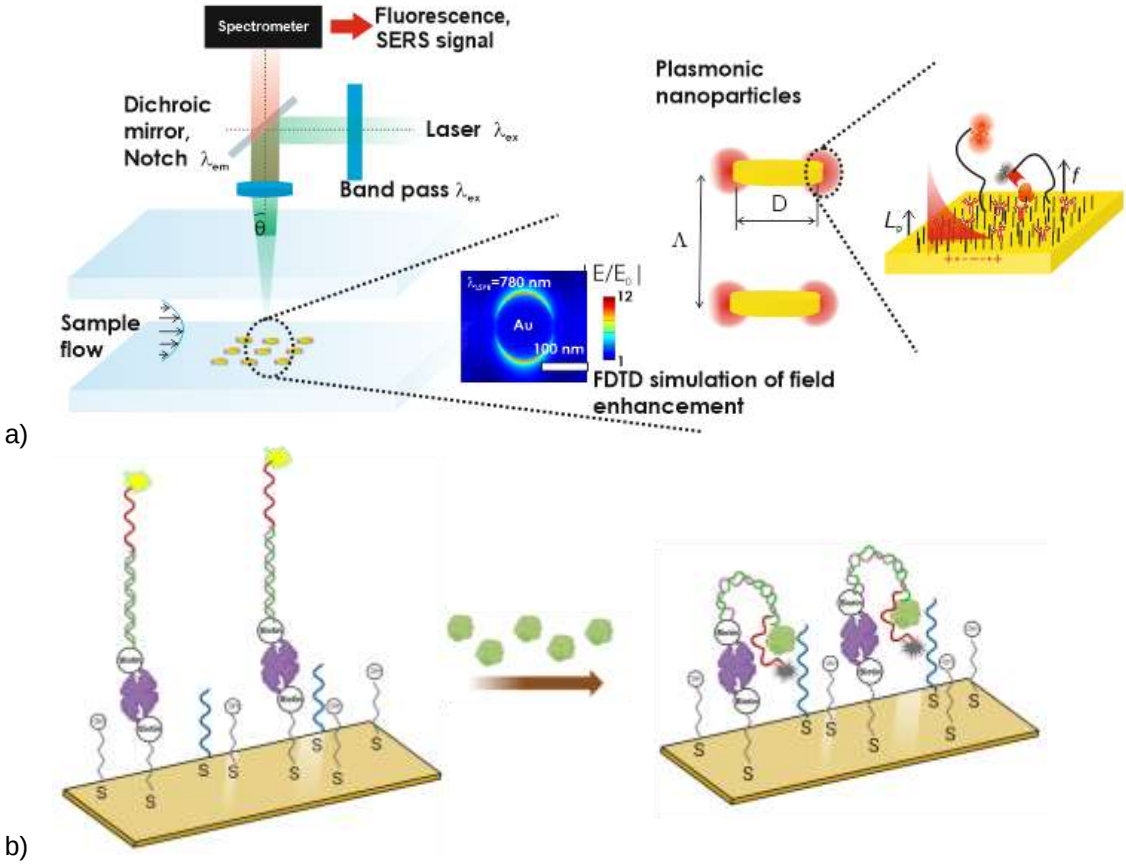

Figure (a) Schematic of the optical readout with plasmon-enhanced fluorescence (PEF) by the use of metallic nanostructures and biointerface with ligands immobilized by flexible polymer linkers and (b) the utilization double-stranded DNA brush architecture for flexible polymer linker – based reversible assay.

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** student flash talk eligible for the award of the Czech Biophysical Association

New psychoactive substance 25E-NBOH - electron transfer properties **

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This work deals with the study of in vitro oxidation of the new psychoactive substance 2-(((4-ethyl-2,5-dimethoxyphenethyl)amino)methyl)phenol also known as 25E-NBOH. Electrochemical and spectroelectrochemical methods were used during the research. The main goal of this work was to find first oxidation products and intermediates and to propose mechanism of the oxidation.

Substances of the NBOH group were synthetized for the first time in 2010 as a brain imaging agents for investigating 5-HT receptors by positron emission tomography. These substances are derived from phenethylamine and like other phenethylamine derivatives (e.g. MDMA, methamphetamine or DOB) are potent serotonin agonists and have stimulative and hallucinogenic effects. 25E-NBOH is due to its strong hallucinogenic and mild stimulative effects often abused and distributed as more affordable alternative to LSD. The exact mechanism of oxidation of 25E-NBOH is not known. Since the electron and proton transfers are inseparable parts of metabolism, electrochemical study of oxidation and identification of first intermediates and products may help in revelation of the exact reaction mechanism.

This work is based on cyclic voltammetry important for the research of the type of reaction scheme. Theoretical calculations of spatial distribution of the highest occupied molecular orbital predicted the electroactive site in molecule. In order to identify intermediates and products *in-situ* IR and UV-Vis spectroelectrochemistry were used. The oxidation mechanism is influenced by pH of solution. Also, oxidative electrolyses were performed and generated products were analyzed by HPLC-DAD.

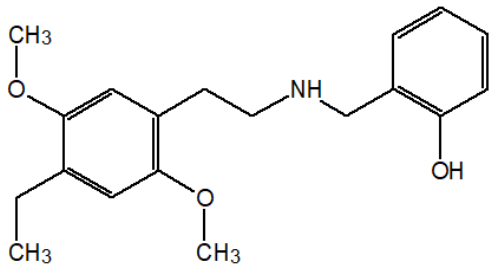

Fig.1.: Chemical structure of 25E-NBOH.

Acknowledgement
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** student flash talk eligible for the award of the Czech Biophysical Association

pH-sensitive polymers for drug delivery induce electrostatic lipid clustering, membrane permeability, aggregation and fusion of vesicles **

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Biomimetic polymers are hoped to serve as drug delivery tool through endosomal-escape. These polymers can induce electrostatic lipid clustering, membrane permeability, aggregation and fusion of vesicles and membranes.
The polymers P(NAiPP) and P(NAMP) used here are rather hydrophilic, positively charged depending on the pH, and have different transfection efficiencies (1).
First, a film balance was used to analyse the influence of the polymer on phosphoglycerol/ phosphocholine (PC/PG) or PC monolayer surface tension. A change in the tension can indicate an insertion or perturbation of the membrane. However, insertion was not observed. Complementary, the binding of P(NAiPP) to vesicles of different composition can be investigated using isothermal titration calorimetry (ITC).
Using fluorescence methods and microcalorimetry, we characterized membrane perturbations caused by the polymers.
Second, the ability of the polymers to induce lipid clusters was confirmed using Differential Scanning Calorimetry (DSC) in vesicles containing phosphoglycerol/ phosphoethanolamine (PG/PE) and (PG/PC). We tested the influence of polymer concentration, polymer charge, i.e. pH, and lipid composition on the clustering.
Most important for the drug delivery is the leakage and release of the drug from the endosome, also called endosomal-escape. Therefore, the effect of P(NAiPP)100 on the leakage and membrane fusion of PG/PE vesicles was investigated using calcein as fluorophore. As leakage occurs, the dye is released and the change in the fluorescence lifetime is measured. The polymers induce limited vesicle leakage and cause vesicle aggregation and/or membrane fusion. Preventing aggregation by PEG-lipids resulted in artefacts. As alternative, fluorescence microscopy on giant vesicles is planned.
We thus present insight to the potential mechanism of action of pH-sensitive biomimetic polymers designed for drug delivery.

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** student flash talk eligible for the award of the Czech Biophysical Association

Electrochemical properties of single-crystal boron doped diamond electrodes with vicinal crystal orientations and their application in electroanalysis **

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Boron doped diamond (BDD) is an attractive electrode material, which has been used in electrochemistry since 1992 [1]. A variety of beneficial electrochemical properties such as wide potential window either in aqueous or non-aqueous media, lower background current, high oxygen evolution overpotential, chemical stability in harsh environments including high temperatures and current densities, biocompatibility and a possibility of *in situ* hydroxyl radicals generation [2, 3] make BDD a material of choice for electroanalysis of organic compounds, wastewater treatment, electrosynthesis, electrocatalysis and even energy storage devices. Additionally, electroanalytical properties of BDD electrodes can be enhanced by various modifications of BDD surface. However, electrochemical response and another electrochemical properties of conventional polycrystalline BDD electrodes depend on many factors. Crucial are i) concentration of boron ii) surface termination (H- *vs*. O-), iii) sp^2-bonded carbon content and iv) crystal orientation [2, 4], which govern conductivity and affect heterogenous electron transfer kinetics.

Single-crystal BDD (SC-BDD) have not been studied well enough, nor employed as electrodes due to challenges in fabrication process. Usually, SC-BDDs with “traditional” or “conventional” crystal orientations {100}, {111} and {110}, naturally occurring in polycrystalline SC-BDD electrodes, are studied. Various crystal orientations present on surfaces of BDD films affect their electrochemical behavior [5]. Understanding the influence of crystal orientation on electrochemical behavior and other properties of BDD is an interesting option for targeted modification of BDD surface for electroanalysis of specific organic compounds. Ideally, the surface of SC-BDDs is flat and smooth, with none (or minimal) sp^2-bonded carbon content and no morphological defects.

In this work, SC-BDD electrodes with vicinal crystal orientations {113}, {115} and {118}, differing in concentration of boron (B/C = 250, 500, 1000 and 2000 ppm in the gas phase during deposition) were compared. The aim of this work was to investigate the influence of crystal orientation on electrochemical behavior and to assess suitability of these SC-BDD electrodes for electroanalysis of dopamine.

This research has been financially supported by The Czech Science Foundation [project 23-05688S] and Specific University Research [SVV 260690].

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** student flash talk eligible for the award of the Czech Biophysical Association

Multi-Responsive Photocrosslinked Hydrogels for Actuating and Sensing Applications **

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Hydrogels are materials composed from polymer networks that can uptake large amounts of water. Among other classes of hydrogels, responsive hydrogels can be on-demand toggled between a swollen and collapsed state, and they are increasingly used for actuation of miniature devices[1]. Furthermore, the possibility of appending biorecognition elements to the polymer matrix by post-modification, makes hydrogels a useful support in sensing applications[1]. Among the most common responsive hydrogels, N-isopropylacrylamide (NiPAAm) stands out, as it exhibits thermo-responsive behavior associated with its local critical solution temperature (LCST), that occurs in vicinity to human body temperature.

A versatile route for hydrogel preparation is provided by photocrosslinking of hydrophilic polymer chains, where chemical crosslinkers can be used to establish either reversible or irreversible bonds[1,2]. In this paper is discussed the implementation of a pNiPAAm – based hydrogel system with a dual actuating mechanism: temperature and light. This material was made from polymer chains of NiPAAm, copolymerized with additional monomers carrying benzophenone and coumarin groups. Benzophenone units were employed for the establishment of permanent crosslinks, via UV light irradiation, while cycloaddition of coumarin derivatives was utilized for reversible crosslinking, via visible light irradiation. This dual photocrosslinked hydrogel offers the possibility of spatially control over the hydrogel layer thickness by means of local temperature and illumination variation. These hydrogel systems are envisioned to serve in applications where microscopic actuation with precise intermediate control of thickness / size is needed.

In this contribution, we present the proof of concept and characterization of the swelling and actuating properties of thin hydrogel films, by the use of surface plasmon resonance (SPR) and optical waveguide spectroscopy (OWS) with spatially resolved measurements (surface plasmon resonance imaging - SPRI). The optical probing is performed upon variation of temperature and light irradiation, which in turn lead to changes in thickness and refractive index that can be optically observed and subsequently translated to swelling ratio characterization.

Acknowledgments
JD and DCM acknowledge support from the Czech Grant Agency through the project APLOMA (#22-30456J) and SoMICELL (# 23-05908K).

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** student flash talk eligible for the award of the Czech Biophysical Association

Interpretation of STED-FCS diffusion law plots for nanoscopically heterogeneous membranes**

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Stimulated emission depletion combined with fluorescence correlation spectroscopy (STED-FCS) is one of the few techniques that can nowadays detect nanoscopic membrane heterogeneities in biological membranes by the analysis of so-called diffusion law plots. These plots relate in-membrane probe diffusion coefficients to the size of the observation spot. Despite their frequent use in membrane biophysics, the interpretation of these dependencies is often ambiguous without exploiting their full potential. In this contribution, we show that a quantitative analysis of STED-FCS diffusion law plots provides unique information about the mobility of nanoscopic membrane heterogeneities as well as elucidates the diffusivity of individual lipids within these nanodomains. We demonstrate the applicability of this approach by performing STED-FCS experiments on nanoscopically heterogeneous membranes of giant lipid vesicles that contain ganglioside nanodomains of controllable size and membrane surface concentration. The final interpretation of experimental diffusion law plots corresponding to dynamics of model membrane is accomplished by their comparison with in-silico simulations of lipid probe diffusion in heterogeneous bilayers. By this combinatory approach we get better insight into the dynamics of nanosocpically heterogeneous membranes.

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** student flash talk eligible for the award of the Czech Biophysical Association

Transition metal ion complexes of triazacyclononane bearing fluorinated pendant arms as potential redox active ^{19}F MRI "smart" contrast agents **

Tereza Dobrovolná[1], Filip Koucký[1], Jana Havlíčková[1], Alan Liška[2], Jiří Ludvík[2], Ivana Císařová[1] and Jan Kotek[1]

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MRI (Magnetic resonance imaging) is an important diagnostic method in current medicine. This method usually uses measurement of water ^{1}H NMR signal. However, other nuclei can be also employed. One of such possibilities is use of ^{19}F nuclei, which brings advantages of similar frequency as ^{1}H and no natural background. To overcome a slow relaxation of ^{19}F nuclei, paramagnetic ion enhancing the relaxation rate is employed.

In this work, macrocyclic 1,4,7-triazacyclononane bearing three 2,2,2-trifluoroethylphosphinate pendant arms (H_3NOTP^{tfe}) was synthesized and its complexes with selected transition metal ions were prepared, characterized, and studied by relaxometric NMR measurements and electrochemistry.

The results show that the chosen concept is promising for ^{19}F MRI and the prepared complexes are applicable as ^{19}F MRI contrast agents. Especially the complex $[Co(NOTP^{tfe})]^-$ showed very promising properties – $Co^{II/III}$ redox potential laying in biologically relevant range, a high thermodynamic stability, kinetic inertness and significant difference in ^{19}F NMR relaxation (very fast relaxation of paramagnetic Co^{II} species, a slow relaxation of diamagnetic Co^{III} species). It predisposes it to serve as a "smart" contrast agent.

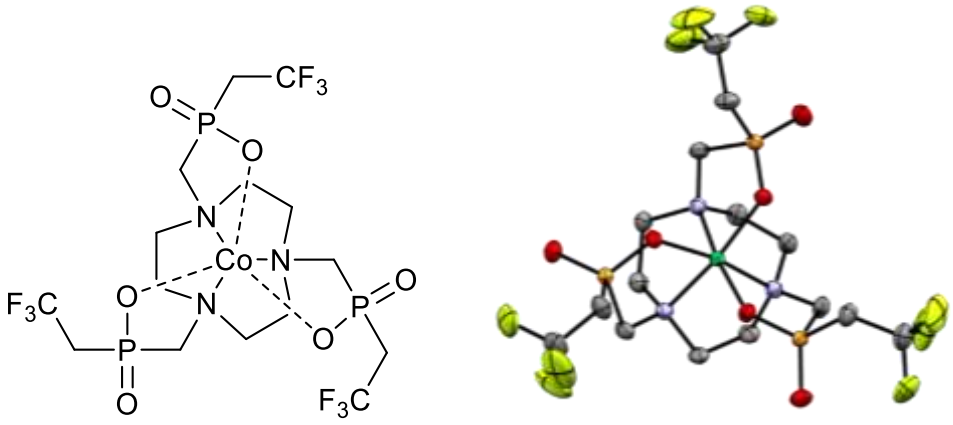

Figure: Schematic representation (left) and molecular structure determined by single crystal X-ray diffraction experiment (right) of the $[Co(NOTP^{tfe})]^-$ complex species.

** student flash talk eligible for the award of the Czech Biophysical Association

Ionic Strength and Solution Composition Dictate the Adsorption of

Cell-Penetrating Peptides onto Phosphatidylcholine Membranes **

Hüseyin Evci[1,2], Vandana Singh[1,3], Radek Šachl[1], Martin Hof[1], and Mario Vazdar[4]

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Interaction between arginine-rich positively charged peptides with neutral zwitterionic phosphocholine (PC) bilayers is a key step in the translocation of those potent cell-penetrating peptides into the cell interior. In the past, it is shown both theoretically and experimentally that polyarginines adsorb to the neutral PC-supported lipid bilayers in contrast to polylysines. Recent contributions showed some contradictions in characterizing this interaction[1,2]. Therefore, we systematically studied the interaction between R9 or K9 peptides and the POPC bilayer by means of fluorescence cross-correlation spectroscopy (FCCS) experiments and aided by molecular dynamics (MD) simulations. Using FCCS experiments with R9 and K9 fluorescently labeled with Oregon Green 488 and POPC liposomes stained with Atto633-DOPE, we first demonstrated that the binding of R9 to POPC is tighter by almost 2 orders of magnitude compared to that of K9. Finally, upon the addition of an excess of either Na+ or Ca2+ ions with R9, the total fluorescence correlation signal is lost, which implies the unbinding of R9 from the PC bilayer, in agreement with the predictions from MD simulations. Support from Grant 22-25953S from the Czech Science Foundation is greatly acknowledged.

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** student flash talk eligible for the award of the Czech Biophysical Association

Plasmon-Enhanced Multiphoton Polymer Crosslinking for Functionalization of Plasmonic Hotspots

Fossati S.[1], Morozov Y.[2], Cattozzo-Mor D.[1], Gruen J.J. [3], Diehl F.[3], Jonas U.[3], Dostalek J.[1]

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We present a new approach to prepare thermoresponsive biofunctional hydrogel microstructures using maskless multiphoton lithography. Unlike most commonly used polymerization-based methods[1,2], this technique relies on simultaneous photocrosslinking and attachment of pre-synthesized polymer chains onto solid substrates.[3] It offers a route for better control of the formed polymer network characteristics and allows facile incorporation of additional functions, e.g., postmodification by biomolecules at desired locations. By the synthesis of a series of poly(*N*-isopropyl acrylamide)-based co- and terpolymers, we explore the impact of photocrosslinker type (benzophenone- and anthraquinone-based compounds) and their conjugating to polymer chains on the efficacy of the photocrosslinking process. For precise control over the photocrosslinking conditions, we developed a custom-built lithographer equipped with a femtosecond laser operating at a wavelength of 785 nm. Through the combined characterization by surface plasmon resonance imaging, atomic force microscopy, and optical fluorescence microscopy, we investigate the swelling behavior of the prepared structures and demonstrate the possibility of their postmodification with biomolecular species. Interestingly, for a certain range of multiphoton photocrosslinking parameters, the prepared surface-attached microstructures exhibit a quasiperiodic topography that can be related to the so-called wrinkle-pattern formation. Leveraging the capabilities of advanced multiphoton photocrosslinking systems, this approach holds great promise for fabricating multifunctional 3D micro- and nanostructures. Such tailored responsive biofunctional materials with control over composition, swelling behavior, and spatially controlled postmodification. Among others, there will be discussed the utilization of responsive hydrogel binding matrix that is selectively attached to the distinct parts of lithographically made gold nanoparticles, where so called ‘plasmonic hotspot’ are located. At these locations, the electromagnetic field can be confined by the resonant excitation of localized surface plasmons, which is associated with increased field intensity and local density of optical states. In combination with such selectively attached responsive hydrogel binding matrix and its postmodification with biofunctional molecules, more precise probing of biomolecular species and their interaction by the use of plasmon-enhanced optical spectroscopy can be devised which is of interest in the fields of bionalaytical technologies and biomedical technologies.

Acknowledgments
YM was supported by the Austrian Science Fund (FWF) through the Lise Meitner Programme (M 2925). JD, SF, DCM acknowledge support from European Commission EIC Pathfinder project Versilib (#101046217) and Czech Grant Agency through the project APLOMA (#22-30456J) and SoMICELL (# 23-05908K).

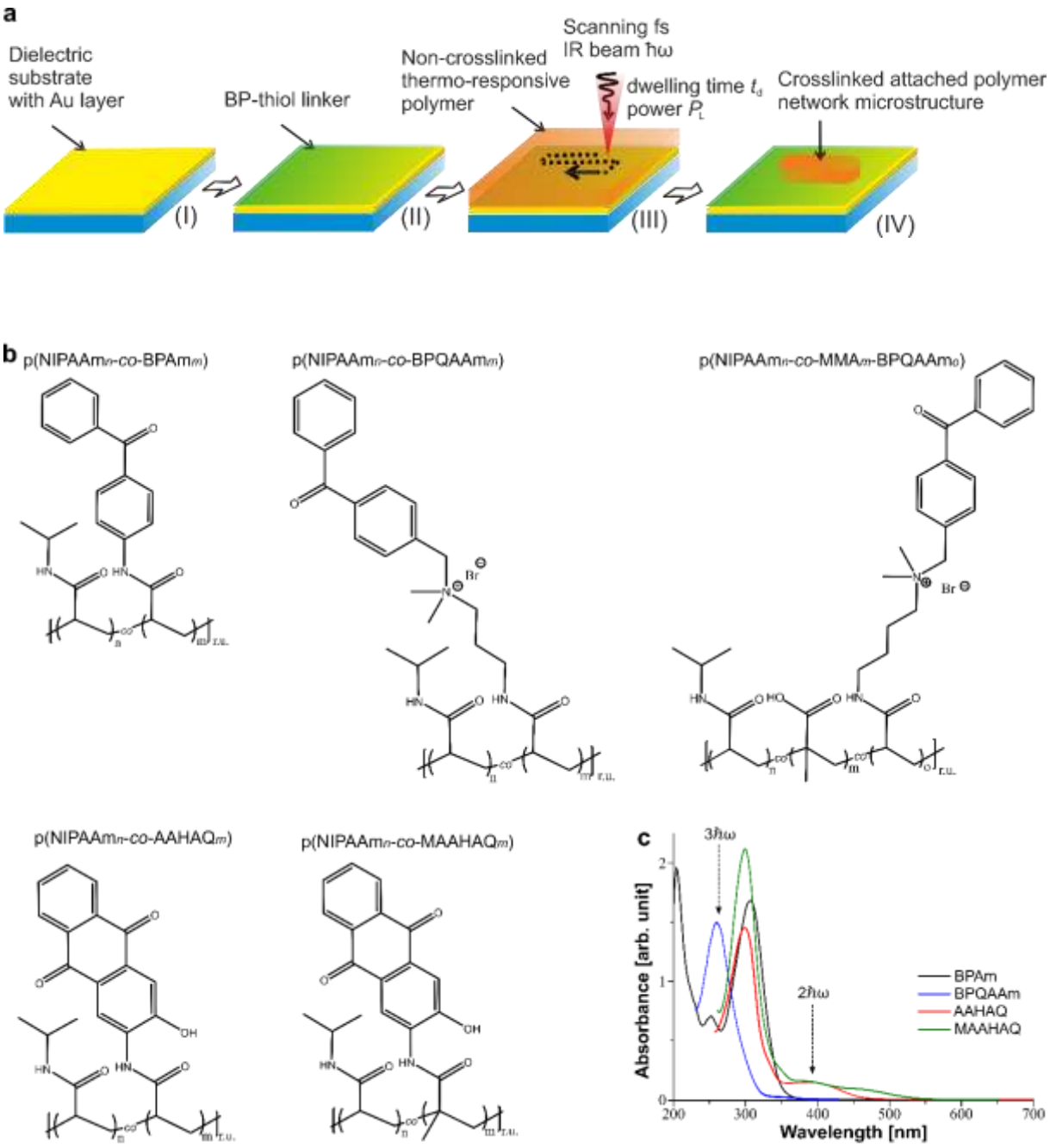

Figure (a) Schematic of the preparation of polymer network microstructures; (b) chemical structures of the polymers and (c) respective UV/VIS spectra of the photoactive monomers (BPAm, BPQAAm, AAHAQ, and MAAHAQ) at the concentration of 0.048 (in EtOH), 0.048 (in water), 0.040 (in DMSO), 0.027 g/l (in DMSO), respectively.

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Visualizing the Sub-diffraction Manipulation of Light by Plasmons using Single-Molecule Localization Microscopy **

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The project focuses on evaluating the magnitude of fluorescent shifts and understanding the enhancement mechanism in plasmonic nanostructures. Plasmonic coupling with fluorophores introduces a mislocalization in the emission position where the far-field microscopy detects an apparent fluorescence emission position that deviates significantly from the actual molecule position. The magnitude and nature of this deviation include information about the position and orientation of the plasmonically coupled fluorophore. By understanding the mechanism of the fluorescence shift, we can create tools for the spatial manipulation of light at the nanoscale.

To study the effect, we combine superresolution microscopy (single molecule localization microscopy), DNA nanotechnology, and plasmonic nanoparticles. To quantify the fluorescence shift, we use the DNA nanotechnology technique (DNA origami) that allows the positioning of fluorophores and plasmonic nanoparticles with nanometer precision. We design rod-like DNA origami structures with three attachment sites for the fluorophore, each 80nm apart, and one for the plasmonic nanoparticles. We use the DNA-PAINT technique for visualization of the structure with resolution below the diffraction limit. A transient binding of short imaging oligonucleotide containing a fluorophore of interest to a complementary oligonucleotide on the DNA origami binding sites takes place and allows single molecule localization. We record a sequence of images in the TIRF fluorescence microscope and gain the reconstructed super-resolved images by post-processing using the ImageJ Thunderstorm plugin and optimize the parameters.

We evaluate the magnitude of the fluorescence shift and enhancement in presence of nanoparticles with various sizes. We further aim to target the enhancement mechanism by controlling the spectral overlap between the plasmon resonance, fluorophore absorption, and emission and study the implications of the enhancement mechanism on position shifts. Understanding how the enhancement mechanism drives the shift will enable to control the shift by engineering the plasmonic structures for desired effects.

** student flash talk eligible for the award of the Czech Biophysical Association

Theoretical Aspects of Electronic Transport in Redox Proteins: Solution vs. EC-STM Junctions

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Redox proteins are well known for their ability to transfer electrons in important biological processes such as photosynthesis, respiration cycle, or denitrification reactions. For example, heme-containing cytochromes or copper proteins are often involved in these electron-transfer reactions. These are believed to proceed by the incoherent hopping mechanism described by Marcus theory, which predicts its long-range character and relatively strong dependence on temperature. However, when the conductance of single proteins on bio/metallic interfaces started to be probed by electrochemical scanning tunneling microscopy (EC-STM) and other methods, currents of large magnitudes and practically no temperature dependence were observed, suggesting coherent tunneling as the transport mechanism [1]. Yet, the concept of electronic charge coherently flowing through large and soft biomolecules is somewhat controversial.

We use theoretical modeling and computer simulation techniques based on the combination of classical molecular dynamics (MD) [2] and density functional theory (DFT) [3] to investigate electronic charge transport mechanisms in redox proteins surrounded by various environments. While the Marcus theory and combined quantum-mechanical/molecular-mechanical (QM/MM) approaches are well applicable to electron transfer in solvated proteins [4, 5], large-scale DFT calculations and state-of-the-art protocols [6, 7] are required to investigate the coherent tunneling in protein junctions. By exploring the electronic states and structures of redox proteins such as small-tetraheme cytochrome (STC), cytochrome b_{562}, and azurin, we identified the potential barrier at the bio/metallic interfaces as the key factor determining the transport mechanism in EC-STM junctions. At the same time, relatively slow distance decay of the transport results from large densities of electronic states present in the proteins [7]. The crossover between coherent tunneling and incoherent hopping is predicted to occur at several nanometer distances, substantially longer than in the case of small molecular junctions.

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Character of Cr-Cr bond in the SIYNAQ complex **

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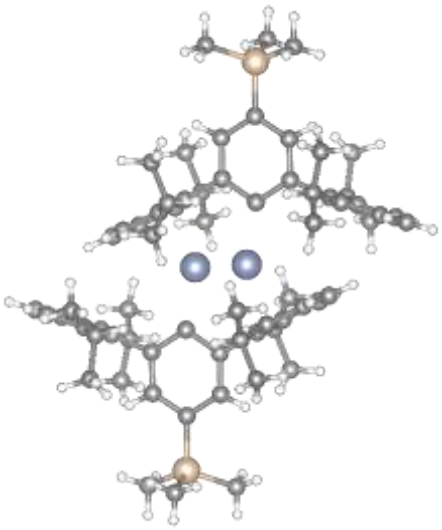
Cr-Cr dimer bond is formally a sextuple bond but contrary to the expectation it is a weak one. Larsson et al. compiled several theoretical and experimental works to conclude about the potential energy curve shape and the description of the Cr-Cr interaction. Their calculations are in a close agreement with the experiment, but they did not study the Cr-Cr bond character.[1]

Since Cr-Cr- bond is a weak one (and Cr is a strong reducing agent), the chromium dimer can be stabilized by different ligands. In the present study, we try to characterize the Cr-Cr bond/interaction in the SIYNAQ compound (Fig.1), from the Cambridge Crystallography database. SIYNAQ crystalizes in the form of dark red crystals which are spontaneously inflammable on air. Formally the chromiums have a quintuple bond between them, with Cr in the oxidation state 1+ (Cr^{1+}-Cr^{1+}).[2]

Computational chemistry tools are used to obtain information about the character of the Cr-Cr bond in the SIYNAQ complex. Standard DFT calculations with the use of two different functionals (BLYP and B3LYP) are performed. For further information, the Quantum theory of atoms in molecules is employed. From the nature of the chromium-chromium interaction, the Complete active space self-consistent field method and N-electron valence state perturbation theory were utilized. Localized and natural orbitals are also rendered to get pictographical viewpoint on the interaction. A comparison with CRAQAC13, Cr in the oxidation state 2+ (Cr^{2+}-Cr^{2+}), is also made.[3]



Fiq. 1. SIYNAQ complex[2]; chromium atoms are blue

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** student flash talk eligible for the award of the Czech Biophysical Association

Live-cell monitoring of proteins engaged in nucleolar stress response

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Nucleolar phosphoproteins play an important role in various cell processes, including sensing of nucleolar stress. In response to the stress, the phosphoproteins elevate their presence in nucleoplasm where they interact with other stress responsive proteins, such as p53 or Hdm2. Overexpression, fusions or mutations of nucleolar proteins appear in hematological disorders, such as acute myeloid leukemia (AML). In particular, C-terminal mutation of nucleolar phosphoprotein nucleophosmin (NPMmut) causes its delocalization to cytoplasm. Localization of nucleolar and regulatory proteins is an important factor in their ability to be engaged in various interaction networks. Delocalized proteins and their interactions become a target of cancer therapy. The efficient targeting calls for methods capable to monitor drug effects on the protein-protein interactions directly in live cells, such as time-resolved fluorescence or fluorescence correlation spectroscopy implemented on confocal fluorescence microscope [1, 2].

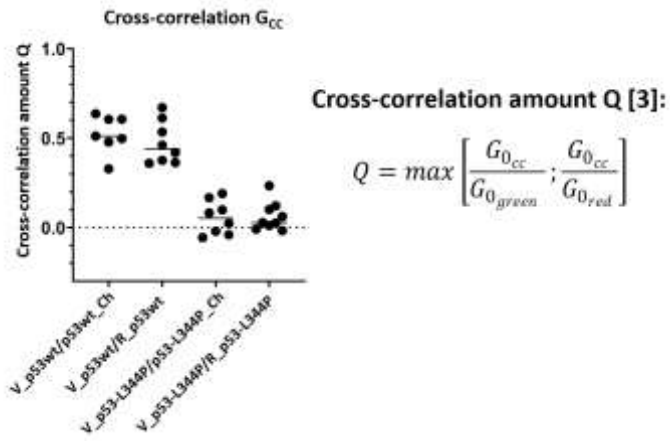

Figure 1. We introduced L344P point mutation expected to inhibit p53 oligomerization into our multicolored p53 constructs and we applied point FCCS (fluorescence cross-correlation spectroscopy) to monitor the oligomerization and protein-protein interactions in complexes engaged in the nucleolar stress response in live cells [4].

This work was supported by grant from the Czech Science Foundation No. 22-03875S and by MH CZ – DRO (IHBT, 00023736).

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New psychoactive substances, electrochemistry and spectroelectrochemistry **

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New psychoactive substances (NPS) are legal alternative to classical drugs (ecstasy, cocaine). These types of substances can be sold via Internet for example as a “bath salts” or “legal highs”. They are abused for their effects. NPS enhance mainly happiness, euphoria and libido. They are divided into groups according to their structure and pharmacological effects - synthetic stimulants, synthetic cannabinoids, synthetic hallucinogens, and synthetic depressants. European Monitoring Centre of Drug and Drug Addiction and the United Nations Office on Drug and Crime newly report many derivatives every year. Therefore, data about their toxicity are limited[1,2].

In our research we focus mainly on three of the new psychoactive compounds. Two stimulants and one fentanyl derivative. One of the compounds is cathinone derivative 4-methylpentedrone (4-MPD). Cathinone is a substance that can be get from the shrub *Catha edulis*. These substances are connected to an activity called “chemsex”, which is using chemicals during sex. In addition to side effects, it can increase their risk, for example HIV transmission may occur[3]. Another substance is also a stimulant. It is a fluorinated analogue of phenmetrazine (substance used in 1950s for obesity treatment), 3-fluorophenmetrazine (3-FPM) has similar effects including euphoria, increase of libido, stimulation. On the other hand, it can cause anxiety and excessive sweating[4]. Next studied substance is *meta*-fluoro methoxyacetyl fentanyl (FMAcF). This fluorinated fentanyl derivative belongs to the non-pharmaceutical fentanyls. It is used as a substitute to heroin, because it can produce longer effects, such as relaxation and sedation[5].

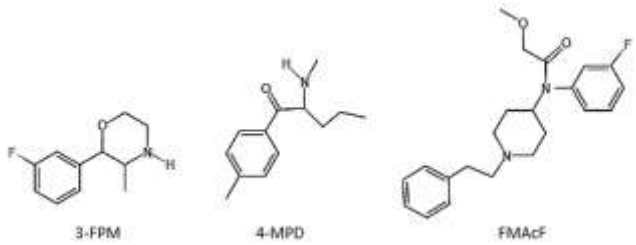

Figure 1: Chemical structures of 3-FPM, 4-MPD, FMAcF.

All of three drugs are examined by means of cyclic voltammetry, *in-situ* UV/Vis and IR spectroelectrochemistry. Using these methods we can detect also short-living reaction intermediates. It can be useful for identification of possible metabolic pathways in human organism. The research is supported by theoretical calculations of frontier orbitals energies and their spatial distribution.

Acknowledgement
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** student flash talk eligible for the award of the Czech Biophysical Association

MICROMANIPULATION OF GIANT LIPID VESICLES AS A NEW TOOL FOR THE RESEARCH OF BIOMEMBRANE STRUCTURES AND ITS MECHANICAL PROPERTIES**

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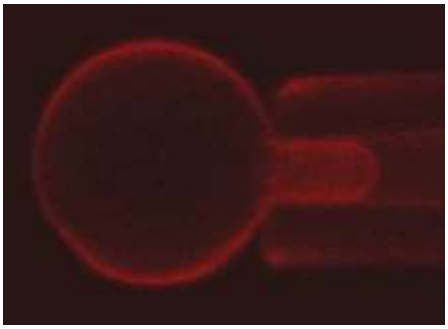
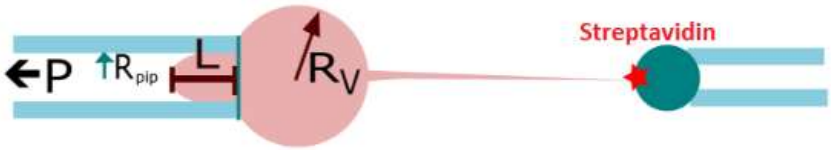
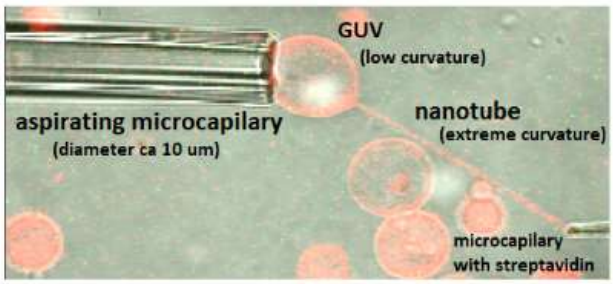
In cells, various membrane events are influenced and sometimes even controlled by changing the tension on the membrane or its curvature (for example movement of the cells, influence of the membrane proteins etc.). Micromanipulation technique brings new, so far not fully explored possibilities to this biomembrane research, as it enables direct modifications of membrane mechanical properties of giant unilamellar vesicles (GUVs), simulating cellular membranes. These experiments can be carried out by pulling out nanotubes from GUVs or by aspiration of GUVs into thin microcapillaries and thus affecting their membrane tension.







The combination of micromanipulation with other methods such as fluorescence microscopy will allow us to open the door to other exciting experiments we plan to perform in later stages of this project. Especially we are interested in the combination of the micromanipulation technology with our frequently used method MC-FRET, which allows us to detect and characterize membrane lipid clustering and formation of lipid nanodomains.

My poster will mainly focus on the progress we have achieved in the implementation of this new method at Heyrovsky Institute.

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** student flash talk eligible for the award of the Czech Biophysical Association

Spectroelectrochemistry in microfluidic cells with carbon working electrodes

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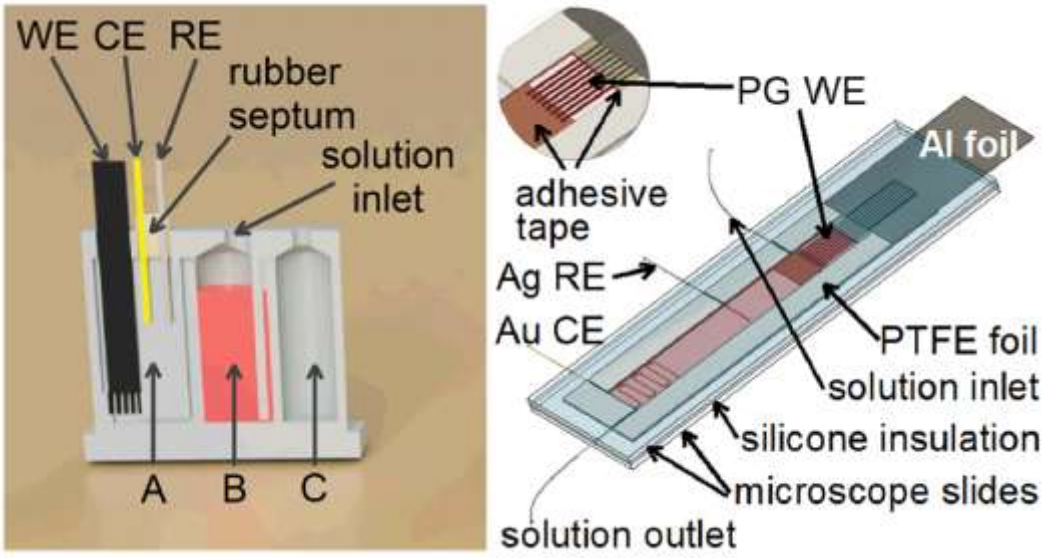
Spectroelectrochemistry is a valuable analytical tool for characterization, quantification and monitoring of intermediates and products in charge transfer reactions, finding applications in mechanistic studies or operando monitoring of electrocatalytic processes. Carbon working electrodes enable scrutinized charge transfer reaction to be performed without complications due to reactant or product adsorption on the electrode surface. However, due to complexity of manufacture, spectroelectrochemical cells with carbon working electrodes are rare. In this contribution, we present designs of two integrated microfluidic spectroelectrochemical cells with embedded carbon working electrodes.[1,2] The first cell design (Fig. 1 left) is manufactured by bi-material extrusion-based 3D printing, employing an optically transparent filament for the housing and windows and carbon black-based electrically conductive filament for forked-shape working electrode. The second cell design (Fig. 1 right) is manually assembled and involves an array of pencil graphite rods as the working electrode, housed in a pair of quartz slides used as optical windows. Both cells support an oxygen-free environment and enable long-term spectroscopic monitoring of electrogenerated reaction intermediates and products. Presented spectroelectrochemical experiments will be complemented by finite-element-method numerical simulations of charge transfer reactions coupled to diffusional mass transport.

Fig. 1 Left: Cell design with carbon black-based working electrode manufactured by extrusion 3D printing (A – measurement chamber, B – chamber for inspected solution, C – gas feed. Right: Cell design with pencil graphite (PG) working electrode manufactured by manual assembly.



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Unveiling the Behaviour of 4-Aminobenzenethiol Using a Combination of Interferometric Scattering Microscopy and Raman Spectroscopy **

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4-Aminobenzenethiol (4-ABT) has long captured the attention of researchers in the fields of surface-enhanced Raman scattering (SERS) and plasmon catalysis (PC) [1]. It exhibits a remarkable ability to undergo structural transformations upon exposure to excitation radiation, particularly when in proximity to plasmonic structures [2]. A notable example of such transformation is the dimerization of 4-ABT molecules leading to the formation of 4,4-dimercaptoazobenzene. While extensive studies have been conducted on this behavior, understanding the individual reaction steps and their collective impact on the spectral responses of 4-ABT remains a formidable challenge.

In our research, we employ a combination of interferometric scattering microscopy (iSCAT) and surface-enhanced Raman spectroscopy to investigate the photochemical pathways of 4-ABT adsorbed on aggregates of gold nanoparticles (AuNPs). iSCAT microscopy provides precise localization and tracking of the region from which Raman signals are collected, facilitating the acquisition of detailed structural insights about the molecules within areas of interest [3]. Furthermore, our instrumentation enables dynamic measurements with exceptional temporal resolution, on the order of 10 ms–100 ms, while maintaining single-molecule sensitivity. This outstanding temporal precision empowers us to unravel complex multi-step photocatalytic reactions and elucidate the molecular-level behavior of 4-ABT, along with the spatial rearrangement occurring at the aggregate surfaces.

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** student flash talk eligible for the award of the Czech Biophysical Association

Unique insights into the electrode/electrolyte interface of boron-doped diamond electrodes **

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Over the last two decades, boron-doped diamond electrodes (BDDEs) have begun to attract much attention in both basic and applied research. The originally non-conductive diamond can be used as an electrode material, as boron doping increases its conductivity. Particularly compared to traditional electrodes, BDDE is an outstanding durability electrode with unquestionable benefits, including uses in environmental monitoring, free chlorine biosensors, and wastewater treatment. These benefits are mostly explained by the boron element's doping, which contributes to the BDDE electrode's unique electrochemical characteristics. For example, a wide potential window in an aqueous solution, a very low background current, and different surface terminations can be used for different redox reactions [1-3].
Combining theory (idea to eliminate one of the current components) and experiment (LSV or CV) resulted in elimination voltammetry with linear scan (EVLS), a useful method of further processing of voltammetric data [4, 5]. Eliminating a selected (specified) current using the elimination functions *f(I)* allows a better understanding of how changes in a single current can affect the overall electrochemical reaction. For example, both the diffusion current (Id) and the kinetic current (I_k) can be removed, conserving only the capacitive current. Capacitive current is closely connected with the charging of the electrode/electrolyte interface and therefore changes in the electric double layer are reflected by means of the elimination function preserving only the capacitive current component.
We found that EVLS can effectively represent the change in the electrical double layer during the electrode reaction process. In the case of a reversible redox reaction with diffusion control and without kinetic complication, the simultaneous elimination of I_d and I_k should provide the zero conserved charging current component (I_c); any deviation from the zero line indicates a complication. Such a deviation in the form of a depression was observed both on the anodic (oxidation of $[Fe(CN)_6]^{4-}$) and on the cathodic side (reduction of $[Fe(CN)_6]^{3-}$), and the double-sided record reminded the form of a drop. It should be noted that this effect is not limited to BDDEs, but has been observed on other graphite electrodes with different sizes and shapes.
The EVLS approach to experimental data processing is revolutionary and distinctive. The following three EVLS functions with respect to the integer of 2 (I is the reference current, $I_{1/2}$ and I_2 are one-half and double of the reference current, respectively) were used.
$E4\ f(I) = -11.6570I_{1/2} + 17.4850I - 5.8284I_2$, which eliminates simultaneously charging and kinetic currents while retaining the diffusion current,
$E5\ f(I) = 6.8284I_{1/2} - 8.2426I + 2.4142I_2$, which eliminates simultaneously charging and diffusion currents while retaining the kinetic current, and
$E6\ f(I) = 4.8284I_{1/2} - 8.2426I + 3.4142I_2$, which eliminate simultaneously the kinetic and diffusion currents while retaining the charging current.
To confirm the superior performance of the BDDE electrodes, we first performed a short CV examination. It should be noted that these are screen-printed electrodes (i.e. SP-BDDEs) and SP-BDDEs surfaces were previously converted to the hydrogen termination by 5 mins polarization in 0.5 M H_2SO_4 at the potential -2 V. Using three scan rates, we performed EVLS diagnostics and found significant differences between the two methods of recording voltammetric recordings, when either a sample drop was applied to the SP-BDDE surface (drop mode - D) or the SP-BDDE was immersed in the sample solution (immersion mode - I). A big surprise for us was the differences in the values of charging and kinetic current components after EVLS procedures in both approaches.

After verifying the repeatability, we can state with certainty that:
(i) the rate of electron transfer in method D is higher than in method I;
(ii) oxygen affects the kinetic flow in the redox reaction and to some extent serves as a catalyst for the SP-BDDE used in this ferro/ferri redox reaction experiment.

(iii) the diffusion current is affected by extra forces in method D, which are now the subject of investigation.

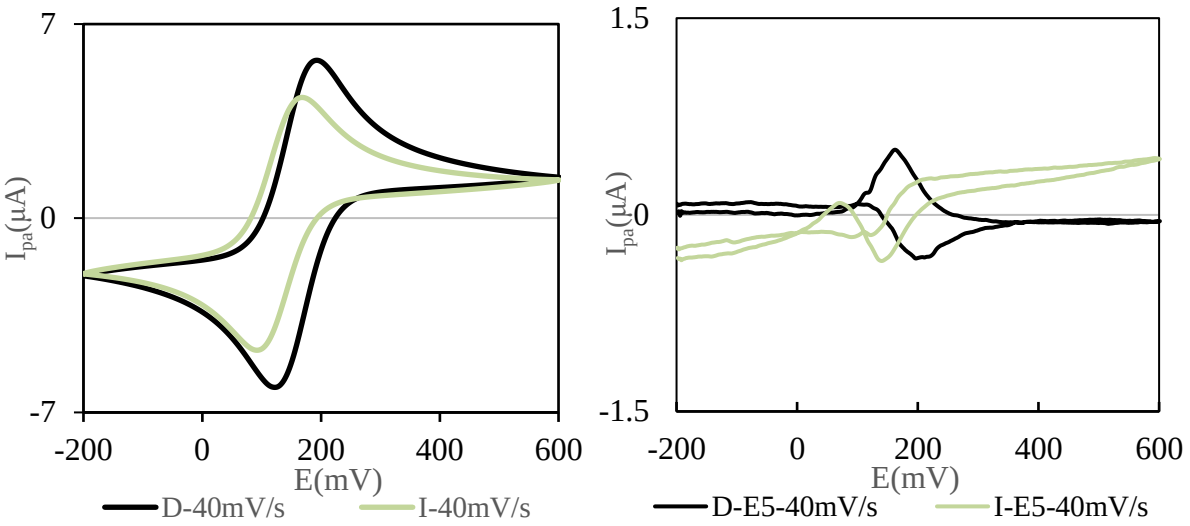

Fig.1 The two measure way's CV (method-D and method-I) and calculated EVLS function of 0.5 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl on SP-BDDE. The EVLS function E5 eliminates I_d and I_c and conserves I_k.

This contribution clearly demonstrates the importance of EVLS as an extension of voltammetric data processing methods that lead to deeper insight into electrode processes and their mechanisms.

Acknowledgment
The financial support provided by Masaryk University (project: Development of Methods and Instrumentation for the Analysis of Biologically Important Substances (MUNI/A/1539/2021 and MUNI/A/1421/2022).

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** student flash talk eligible for the award of the Czech Biophysical Association

Side on and end on interaction between iron/chromium decorated circumcoronenes and hydrogen molecule **

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Circumcoronene (CC) is a polyaromatic hydrocarbon. It is finite nanostructure, consisting of 54 carbons arranged in 19 hexagonal rings. This structure[1] was decorated with transition metals (TM) such as chromium (Cr) and iron (Fe). In both cases, the hydrogen molecule adsorption onto the TM decorated CC was probed in three ways, namely dissociation mode, side on and end on interaction modes. Both decorated CCs are able to stabilize up to three hydrogen molecules.[2] The energetically favored binding modes of a single hydrogen are the high spin, M_s = 5 (pentet) dissociated Fe-decorated CC and M_s = 7 (heptet) end on Cr-decorated CC high spin with one hydrogen molecule.[2] All systems are treated by density functional theory (DFT) and Bader quantum theory of atoms in molecules (QTAIM) analysis.

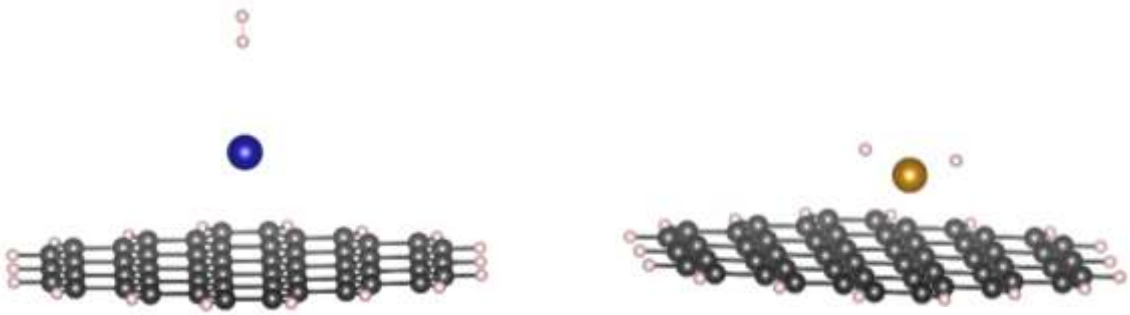

Figure 1. Optimized structures of end on Cr-decorated CC (left) and dissociated Fe-decorated CC (right) with one hydrogen molecule.

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** student flash talk eligible for the award of the Czech Biophysical Association

Characterization and optimization of SERS substrates using adenine as benchmark molecule and automation of data evaluation **

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Providing insight into biomolecular interactions at the nanoscale, Surface-Enhanced Raman Spectroscopy (SERS) is an important tool in biophysics and structural chemistry [1]. Factors such as morphology, shape, and type of nanostructured metal, laser wavelength, and analyte choice affect the performance of SERS substrates. Consequently, efforts invested in characterizing and improving these substrates to enhance their sensitivity, reproducibility, uniformity, and affordability, are paramount.

While the conventional use of fluorescent dyes for benchmarking of the SERS substrates introduces complexities due to resonance Raman scattering effects [2], we focus on adenine as an optimal reporting molecule for characterization of the SERS substrates. Adenine exhibits several advantageous properties making it an ideal choice, particularly for silver substrates. Importantly, adenine is non-resonant at visible wavelengths, but possesses strong Raman cross-section. Furthermore, in the 1 µM – 10 µM concentration range a unique surface complex between silver and adenine forms, providing additional Raman enhancement, and presenting opportunities for biophysical investigations [3]. At higher concentrations, we evidenced a layer of oriented adenine molecules on the surface of silver SERS substrates by atomically resolved 3D AFM.

The large-area SERS substrates characterization and optimization generally represent a demanding task, that requires evaluation of vast Raman datasets. Therefore, we present a novel approach enabling a precise automated SERS background removal and peak identification based on morphology operators [4].

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** student flash talk eligible for the award of the Czech Biophysical Association

Detection of prostate-specific antigen utilizing immunomagnetic assay with upconversion nanoparticles **

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Prostate cancer is the leading cause of death within the male population worldwide caused by oncologic diseases. The glycoprotein prostate-specific antigen (PSA) produced by the prostate is its most important biomarker. While blood serum concentrations up to 2.5 ng/mL are considered normal and can be generally detected by conventional immunoassays, more sensitive detection schemes are essential for early-stage disease diagnosis. Immunochemical methods, such as the enzyme-linked immunosorbent assay (ELISA), are considered the gold standard for PSA detection. However, there are several limitations that render ELISA insufficient regarding the sensitivity required for early-stage diagnosis.[1]

Photon-upconversion nanoparticles (UCNPs) are luminescent nanocrystals consisting of an inorganic matrix doped with lanthanide ions. They exhibit anti-Stokes luminescence (the ability to convert low-energy excitation to higher energy emission), thus avoiding optical background interference. When conjugated with biorecognition molecules (e.g., antibodies or streptavidin), UCNPs can be used as a sensitive label in various immunoassay formats, including microtiter plate-based upconversion-linked immunosorbent assay (ULISA).[2] Such labels can enhance the assay sensitivity significantly. However, as many biomarkers are extremely low-abundant in body fluids, even ULISA may still not be sensitive enough such that new, even more sensitive assay schemes are needed. Magnetic microparticles (MBs) are a promising alternative solid phase for microtiter plate immunoassays. The superparamagnetic properties of MBs allow for analyte preconcentration and thus improve the assay sensitivity.[3]

We have developed and optimized a sandwich immunoassay for the detection of PSA based on MBs and UCNP-based labels. We conjugated one type of monoclonal anti-PSA antibody to MBs and the other one to UCNPs via streptavidin-biotin binding, and tested their functionality. Optimal concentrations of MBs and serum were selected, followed by the fully optimized analysis of model real samples of spiked serum. The assay reached an LOD of 11.1 pg/mL, comparable to an ELISA using identical immunoreagents. An additional magnetic preconcentration step further improved the LOD to 0.25 pg/mL, which was 3-fold better compared to the ELISA, and 44-fold better than an MB-based ELISA using identical immunoreagents. These results demonstrate the potential of using MBs combined with UCNPs for the sensitive detection of cancer biomarkers.

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** student flash talk eligible for the award of the Czech Biophysical Association

Creation of Molecule-metal Surface Complexes and their Effect on SERS-spectra in the Systems of Amphetamine-based Drugs and Colloidal Nanoparticles **

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The misuse of addictive substances, particularly drugs, not only leads to health complications but also results in significant social issues, including increased criminal activity stemming from drug-induced aggressive behaviour. To address these challenges, the possession of addictive substances is a criminal offence. While there is a growing prevalence of new, non-regulated substances, traditional drugs remain popular. However, law enforcement faces difficulties in identifying these substances due to adulterants and minute sample quantities. To adapt to the evolving illicit drug market, continuous development of analytical methods is imperative.

One of the potentially suitable techniques for the analysis of these substances is Surface-enhanced Raman scattering (SERS) spectroscopy. This method, although highly useful in overcoming the limitations of standard Raman spectroscopy (mainly in the detection limit), is characteristic by a seemingly never-ending list of possible effects that could affect not only the intensity of the resulting signal but also the entire spectral profile of the measured species [1]. Consequently, bringing SERS into the field of forensic analytical chemistry appears to be a long-term goal. However, when the impact of as many occurring effects as possible is deciphered, SERS could not only serve as a typical analytical method but also provide additional information, such as the molecule's orientation to the surface, conformational changes, and the type of interaction with the surface, which is often challenging to obtain using standard analytical approaches.

This study explores the potential of SERS for the detection of amphetamine-based addictive stimulants, with a particular focus on possible surface-complex formation and its effect on the resulting surface-enhanced spectra's intensity and profile [2]. Among the compared alternatives (silver vs. gold surfaces), gold colloidal systems demonstrated superior performance, primarily because, in their case, the formation of surface complexes is more probable. Our findings contribute to the advancement of trace analysis techniques for combating substance misuse and associated societal challenges.

To extract the maximum amount of information from the obtained spectra, we used density functional theory computations. Our results show that in cases where molecule-metal complexes are formed, the SERS signal is much higher and more specific than in the opposite cases. We believe that the presented findings could prove useful when considering SERS spectroscopy as a tool for amphetamine-based drug analysis and from a physico-chemical perspective, where insight into the chemical enhancement mechanism is provided [3].

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** student flash talk eligible for the award of the Czech Biophysical Association

In vivo detection of AML-related proteins oligomerization by fluorescence anisotropy measurement

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Protein oligomerizations and protein-protein interactions are essential for the proper function of living organisms. Therefore, a number of biochemical and biophysical methods have been developed to detect them. In our laboratory, the identification of protein-protein interactions by FRET-FLIM (Förster resonance energy transfer – Fluorescence lifetime imaging) technique is a well-established procedure (for example [1]). Contrary to common FRET measurements, homoFRET measurement concerns energy transfer between identical fluorophores. HomoFRET requires labeling with a single fluorescent tag, and it is detectable by measuring the loss of anisotropy (i.e. loss of polarisation of emitted light). Our aim is to introduce homoFRET for measurement *in vivo* employing steady-state and time-resolved fluorescence anisotropy.

HomoFRET is known to affect the value of fluorescence anisotropy (r). After taking an initial anisotropy image (before photobleaching), we intended to reduce the homoFRET by fluorofore photobleaching to 30% of the initial intensity. Then we measured the final image (after photobleaching). By comparing anisotropy values in the images before and after photobleaching, the existence of homoFRET was either confirmed ($r_{before} < r_{after}$) or rejected ($r_{before} \sim r_{after}$).

As a model system to establish steady-state anisotropy measurements *in vivo,* we chose nucleophosmin (NPM), and its leukemia-related mutant NPMmut, which play a critical role in the development of acute myeloid leukemia (AML), and mVenus fluorescence protein as a suitable fluorescent label. Then, we applied the established method to study oligomerization of the p53 tumor suppressor, which is known to form monomers, dimers and tetramers *in vivo*. [2] The information obtained may help to elucidate mechanisms of the development or treatment of AML.

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Development of new substrates for super resolution studies of supported lipid bilayers (SLBs) by graphene induced energy transfer (GIET)

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Classical fluorescence microscopy is a widely used technique in biological research. Intrinsically it suffers from the limited spatial resolution on the order of hundreds of nanometers in lateral plane and several microns in the axial direction. A recent rapid development of novel techniques achieved nano-metric resolution in lateral dimension. In contrast, there still exists a high demand for robust techniques shrinking the axial resolution to the desired nanometer scale. One of the most promising approaches in this respect is Graphene Induced Energy Transfer (GIET) (1). In brief, fluorescence is quenched in a distance-dependent manner by the atomically-thin graphene sheet. As the range of the GIET occurs up to ~ 30 nm, lipid bilayers with the thickness of 5 nm can be resolved in detail. Here, we attempt to fabricate the novel graphene based supports suitable for GIET. Specifically, the graphene deposited on glass support is cushioned with Pyrene-PEG polymer. Pyrene promotes the interaction with the graphene, while PEG helps to minimize the effect of the graphene on the bilayer behavior. The experiments were focused on the formation of the supported lipid bilayers (SLBs) containing negatively charged lipids on these substrates. The SLB properties were monitored by time-resolved fluorescence microscopy and fluorescence correlation spectroscopy (FCS).

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Plasma activated water for agriculture application: first evidence about its effects to soil microbial communities and soil enzymatic activity **

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Due to plasma-liquid interactions, plasma activated water (PAW) contain various chemical species with high redox potential – so called short-lived species such as hydrogen or hydroxyl radicals, or long-lived species such as hydrogen peroxide, nitrites, nitrates, peroxynitrites, etc.(in general – reactive oxygen and nitrogen species – RONS) [1]. Those species can increase seed germination, the rooting speed and stimulate plant growth, which leads to higher crop yield [2]. However, due to the presence of H_2O_2, NO_2^- and NO_3^- at low pH values, PAW shows also a very strong antimicrobial effect [1, 3]. The aim of this work was to determine any possible negative effect of PAW application to soil microorganisms.

To prepare PAW, dielectric barrier discharge with the liquid electrode was used. This device was specially constructed for the agricultural applications. It was operating at high frequency voltage with the total power supply of (36±2) W. PAW (treatment time 0, 2, 5 and 10 min) was applied in a pot experiment to a model plant *Lactuca Sativa* for total of 90 days. After 90 days, OM decomposition rate was determined using a tea bag index introduced by Keuskamp [4], which estimates the decomposition constant (*k*) of an asymptote model of litter decomposition and a stabilized ratio of the hydrolysable fraction (stabilization factor *S*) using a single measurement of the mass loss ratios of green and rooibos tea. Soil dehydrogenase activity was determined using the 2,3,5-triphenyltetrazolium chloride (TTC) test. Plate count method was used to measure the population of P-solubilizing and N-fixing soil bacteria. Total culturable bacteria were enumerated by plating soil suspensions (100 µl) using Pikovskaya's and Ashby's mannitol agar respectively. The spread plates were incubated at 30°C for 7 days.

Summarized results are shown in table 1.

Tab. 1 *Concentrations of RONS in PAW samples and pH values, soil dehydrogenase activity and determined tea bag index parameters*

Sample	H_2O_2 ($mg \cdot l^{-1}$)	NO_3 ($mg \cdot l^{-1}$)	NO_2 ($mg \cdot l^{-1}$)	pH	DHA ($\mu g \cdot g^{-1} \cdot h^{-1}$)	Decomposition constant *k*	Stabilization factor *S*
0 min	0.00	0.00	0.00	6.50	2.23	0.03	0.54
2 min	0.78	3.47	1.15	4.47	2.08	0.03	0.47
5 min	0.95	19.48	2.09	3.68	2.40	0.03	0.50
10 min	0.88	49.68	4.17	3.33	3.15	0.03	0.15

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** student flash talk eligible for the award of the Czech Biophysical Association

Probing local protein/peptide microenvironment by GP-FRET**

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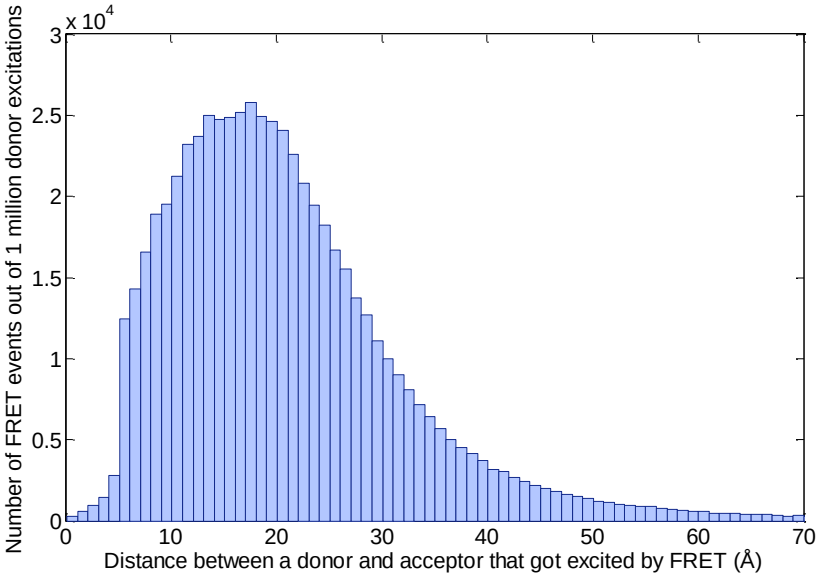
FRET–GP (Förster resonance energy transfer – General polarization) is a new method which, in contrast to the classical GP method [1], probes membrane environment selectively at a specific distance from an excited FRET donor. FRET is thus used as an efficient filter of the overall GP signal and probes selectively only the local donor environment and not the entire bulk membrane. The functionality of the method has been recently demonstrated by the experiments performed on proteoliposomes containing transmembrane peptide WALP[2]. In this system, tryptophan, which is part of the WALP peptide, serves as a donor and a membrane polarity probe Laurdan as an acceptor.

In this work, we explored the possibilities and limitations of the FRET-GP method by MC (Monte-Carlo) simulations[3,4], by investigating how the probed distance can be tuned by varying the experimental parameters, including: the acceptor concentration, the Förster radius (using different FRET pairs) and positions of the probes relative to the bilayer center. We show that the tested distance can be changed by using a different acceptor concentration. The extent to which this can be made, however, depends on the Förster radius. Overall, we provide a clear 'recipe' of how the GP-FRET experiment should be designed to probe specific local environment around single protein/peptide molecules.



Histogram of distances at which FRET occurrs, as calculated by Monte Carlo simulations. In this case, distances between tryptophan of WALP and Laurdan molecules that got excited by FRET over 1 million donor excitations. The size of the probed area around tryptophan can be described by the full width at half maximum (22 Å). Mean excitation distance for this distribution is 21 Å.

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** student flash talk eligible for the award of the Czech Biophysical Association

Simultaneous detection of tyrosine and tryptophan on polymer pencil graphite electrode

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L-tyrosine (Tyr) and L-tryptophan (Trp) are two aromatic essential amino acids playing a crucial role in biological systems as precursors of hormones and neurotransmitters, (thyroxin and dopamine) and other physiologically important biomolecules[1, 2] The both amino acids are necessary for humans to establish and maintain nutritional balance and their abnormal concentration levels are associated with certain diseases (e.g. dementia, Parkinson's disease, hypochondria, depression or alkaptonuria) [2-4]. Trp also very often coexists with Tyr in food processing, pharmaceutical formulations and biological fluids. Previously, the Tyr and Trp content in the blood serum were determined by using HPLC method [5], capillary electrophoresis [6], or some of these methods combined with either mass spectrometry [7].
Since the 1980s, it is known that some amino acids, including Cys, His, Met and also Tyr and Trp, are electrooxidized at carbon electrodes [8-11], and therefore many electrochemical techniques have been developed for the determination of free Tyr or Trp on modified electrode surface [12, 13].
These amino acids show similar electrochemical behaviour at non-modified carbon-based electrodes [14]. Due to this fact, many different strategies have been developed for the selective determination of Trp in the presence of Tyr, consisted in nanoparticles or nanocomposite carbon electrode surface modification [1, 15, 16]. Among the common carbon-based electrodes there are easily accessible pencil leads which are promising electrodes for sensitive and selective determination of Tyr and Trp [17, 18].
The Tyr and Trp undergo the irreversible oxidation process ($2e^-$, $2H^+$) at positive potentials far from zero and their oxidation mechanisms proposed more than 30 years ago are still used in literature (Fig. 1) [8-10].

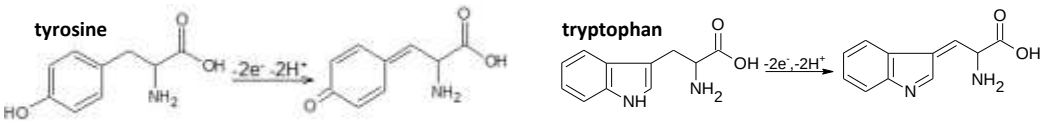

Fig. 1: Oxidation schema of L-Tyr and L-Trp [8]

Many authors consider these mechanisms somewhat controversial, and therefore they described various oxidation mechanisms of Tyr and Trp summarized by Dourado, et al.[19].
With respect to overlapped oxidation signals of Tyr and Trp, our research is focused on finding the promising approach for their successful resolution consisted not only in the choice of another electrochemical protocol (e.g. electrochemical method – cyclic voltammetry of differential pulse voltammetry, modification of electrode surface) but also in the change of pH, ionic strength and supporting electrolyte composition.
Our voltammetric experiments showed that tyrosine and tryptophan yield very close oxidation signals at ~ 0.7 V at pPeGE in aqueous buffered solutions. Similar electrooxidation behavior of Tyr and Trp is mainly manifested when both Tyr and Trp are present in solution and their overlapped oxidation signal is difficult to distinguish by using the classical voltammetric method. This statement could be supported by cyclic voltammetric results of Tyr and Trp mixture at different scan rates which showed that the better resolution of Tyr and Trp oxidation signals is possible only at low scan rates (Fig 2).

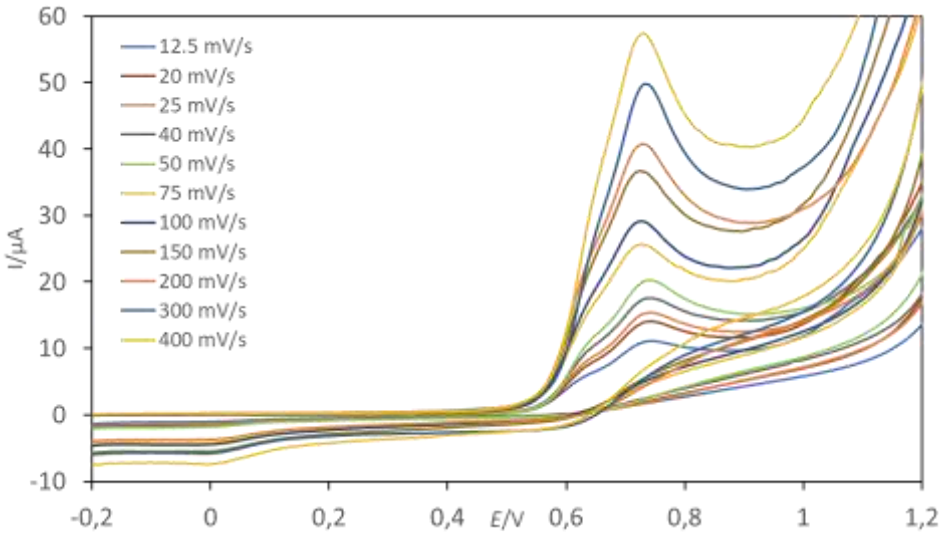

Fig. 2: Cyclic voltammogram of Tyr and Trp mixture at different scan rates on pPeGE (PBS, pH 7.4)

It follows from these results that cyclic voltammetry is not the most suitable method for the simultaneous determination of Tyr and Trp. For further experiments, the more sensitive differential pulse voltammetry (DPV) was used and can be considered the most optimal technique for the best resolution of Tyr and Trp oxidation signals with respect to pH, ionic strength and buffer composition. It was confirmed that compositions of the buffer and its ionic strength strongly affect the oxidation process of free Tyr and Trp and their resolution in the mixture. We found that the oxidation behavior of Tyr and Trp is influenced not only by chloride ions in the PBS buffer, which can be oxidized on the electrode surface or catalyze the oxidation of Tyr and Trp, but also by the acetate component of the buffer. Nevertheless, phosphate-acetate buffer can be considered the most suitable buffer for distinguishing Tyr and Trp oxidation signals. For deeper insight into the oxidation behavior of both amino acids, a detailed pH analysis was performed in phosphate-acetate buffer (pH 1.12 – 11). Tyr and Trp were shown to give oxidation signals over the entire pH range, but only at neutral and slightly alkaline pH (5.48–9.60) are these signals distinguishable, and the potential difference between these signals increases with increasing pH (Fig. 3). From the E_p vs. pH plot the apparent pK_a' values for Tyr (2.28, 9.17 and 9.77) and Trp (2.54 and 8.95) were determined.

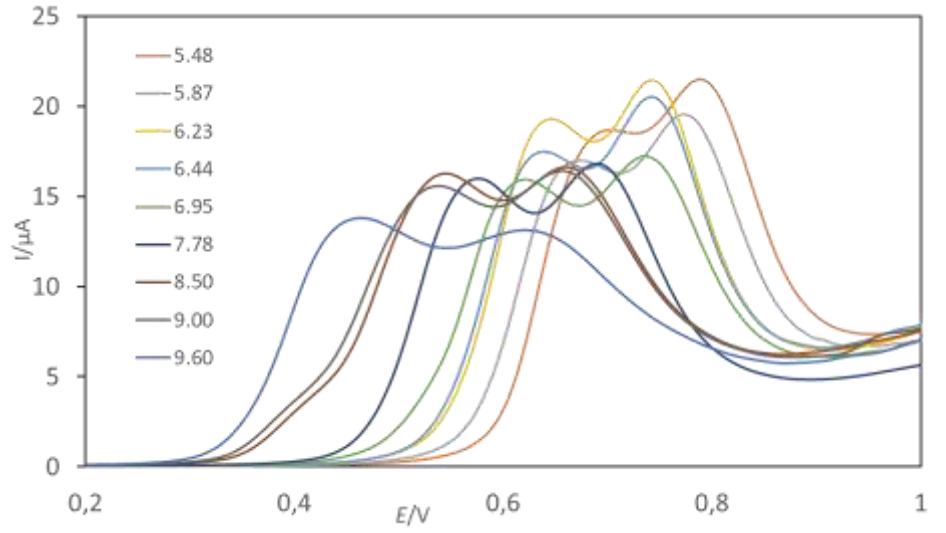

Fig.3: Differential pulse voltammograms of the mixture of Tyr and Trp (1:1) scanned on pPeGE in neutral and lightly basic pH (pH 5.48 – 9. 60; phosphate-acetate buffer).

The electrochemical analysis of Tyr and Trp mixture at different concentration ratios (1:1, 1:2, 1:3 and 1:6) showed that the oxidation peak resolution is worse with the ratio being 1:2 and lower. It should be noted that two distinct peaks may not mean that the one more positive peak corresponds only to Tyr and the second more negative peak corresponds only to Trp. Probably there both Tyr and Trp are present in the both signals and the current intensity is linked with the competitive reaction of Tyr and Trp on the electrode surface.

It can be concluded that DPV in combination with the polymer pencil graphite electrode and suitable experimental conditions enables the better resolution of Tyr and Trp. However, there are still many aspects including also different pH-dependent mechanism processes which should be clarified. Spectro-electrochemistry could be a promising tool for the study of redox electrode mechanisms and adsorptive stripping voltammetry, as well as impedance techniques, could answer the question of the adsorption of these amino acids on pPeGE surface. Moreover, many electrode modifications could be helping tools for the sensitive and selective detection of Tyr and Trp.

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High resolution near infrared imaging with DNA-PAINT **

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Abstract

One of the limiting factors in single molecule localization microscopy (SMLM) including DNA – Point Accumulation in Nanoscale Topography (DNA-PAINT), lies in the limited range of fluorophores which hinders the technique from reaching its full potential. DNA – PAINT is limited to the use of organic fluorophores that do not cover well the near-infrared and the infrared region, have small stoke shift leading to spectra crosstalk [1], thus, inhibiting its application.

We proffer a solution to this problem by using non-conventional inorganic fluorescent probes such as (NV-) fluorescent nanodiamonds and gold nanoclusters. Both probes can emit in the biologically inactive infra-red region, have large stoke shift, and high penetrating capability of infrared emission [2], thus enabling imaging of compact biological structures and deep into tissues, which is currently a problem with the DNA-PAINT; as well as, for other nanophotonic applications.

Here, the inorganic fluorescent probes will be synthesized, surface modified, and conjugated with single strand DNA. Fluorescent properties of inorganic probes such as quantum yield, fluorescent lifetime, and translational diffusion coefficient will be investigated and compared to conventional organic dyes. Inorganic fluorescent probes will be used for imaging of DNA nanostructures using DNA-PAINT technique, several intrinsic and extrinsic factors required for optimized DNA-PAINT imaging will be studied. The efficiency of the fluorescent probes and resolution of resulting image will be compared to that obtained using common organic dyes having near infrared emission.

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Tapinarof: Redox Chemistry, Stability and Photobiology Insight

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Tapinarof (3,5-dihydroxy-4-isopropylstilbene) belongs to the group of phenolic *trans*-stilbenes (Fig. 1). It is an isopropyl analogue of resveratrol, which is one of the most studied stilbenoids [1]. Tapinarof was approved by the FDA in 2022 (marked as VTAMA®, tapinarof 1% cream) for the treatment of psoriasis [2, 3]. In the scientific literature, this substance is alternatively referred to as benvitimod, or the following codes are used, DMVT-505, WBI-1001 or GSK2894512 [4].

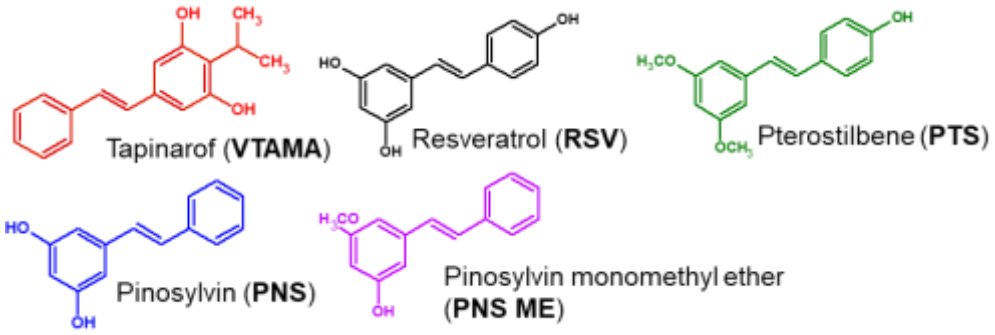

Fig. 1. Chemical structures of tested compounds.

The presented study aims to evaluate the redox transformations, (photo)stability and antioxidant effect of tapinarof. We used resveratrol (3,5,4′-trihydroxystilbene), as well as pinosylvin (3,5-dihydroxystilbene), pterostilbene (3',5'-dimethoxy-4-stilbenol) and pinosylvin monomethyl ether (Fig .1). Methylated derivatives were used due to the targeted blocking of hydroxyl groups, which effectively confirms their involvement in electron-donor mechanisms and antiradical/oxidation reactions. A similar approach was used to describe the mechanisms of oxidation and antioxidant action of quercetin [5], selected flavonolignans [6] and phytocannabinoids [7]; more details can be found in the summary [8].

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Investigating Functional and Dysfunctional Oligomeric States of Membrane-Associated Protein Oligomers Forming Membrane Pores on Giant Lipid Vesicles **

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Fibroblast Growth Factor 2 (FGF2) is a protein that has many extracellular roles, such as wound healing, cell differentiation or formation of metastasis. It is highly produced and released by malignant cells during tumorigenesis. FGF2 is secreted from cells via an unconventional secretory pathway. In the presence of phosphatidylinositol-4,5-bisphosphate PI(4,5)P2 lipids, FGF2 oligomerises in the membrane and forms membrane pores.To study the protein pore formation process at a single molecule level, we used synthetic free-standing lipid bilayers known as giant unilamellar vesicles (GUVs) and explored protein oligomerisation by appliying in-house developed method dual(+1)-FCS[1-3], which can investigate protein oligomerisation and correlate it to membrane-pore formation.

To distinguish between functional oligomers and non-functional protein aggregates, we developed a single-vesicle statistical approach based on determining the brightness of individually diffusing in-membrane protein oligomers and correlating the oligomeric state with membrane pore formation. Overall, our study shows that assessing oligomeric states alone does not provide a comprehensive knowledge about the structure-function relationship of membrane-inserted oligomers. It demosntrates that in order to get biologically relevant oligomeric states of functional protein oligomers, the oligomeric states have to be correlated with at least on read-out parameter that cross-checks protein functionality.

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Bioactive organic thin films with large variations of chemical structure and ζ-potential

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Surface reactivity and free energy play an essential role in the interactions of biomolecules and cells with the surfaces, especially if we think about nanomaterials that typically have huge surface area compared to their volume. I will discuss the plasma modification of surfaces by thin organic films containing amino or carboxyl groups, so-called amine or carboxyl plasma polymers. We used these bioactive films to construct immunosensors [1-5] or improve materials for tissue engineering [6-8]. I want to discuss our recent results [9,10] revealing that, unlike the endothelial cells, the nonendothelial cells (HaCaT, VSMC) behave differently on surfaces with different ζ-potential (-64.3 mV for carboxyl PP and -7.7 to 38.5 mV for amine PPs at pH 6.4). A simple experiment with trypsinization of attached endothelial (HSVEC) and nonendothelial (VSMC) cells demonstrated the difference in cell behavior. We also proved with a physical method, single-cell force spectroscopy, that the adhesion force of VSMA on amine PPs is higher than for VSMC. Since with the plasma polymerization, we can finely tune the film composition. It opens a large playground for cell-surface interaction studies. The open question is what film properties, such as the type and density of functional groups or ζ-potential, are critical parameters for such interactions.

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Monitoring the effects of a microsecond pulsed electric field on structure and function of tubulin

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Tubulins serve as the fundamental constituents of the largest class of filaments, known as microtubules (MTs), which play an indispensable role in a multitude of vital cellular processes. Notably, the tubulin protein exhibits an exceptionally high structural electric charge and dipole moment [1]. The functioning and interactions of tubulin proteins are greatly influenced by the electric charge present on the amino acid residues and atomic groups within them. Consequently, the application of an external electric field offers a promising approach to manipulating the functionality of cytoskeletal proteins such as tubulin [2]. The DLS (Dynamic Light Scattering) analysis revealed that the number of large particles increases in the PEF (pulse electric field)-treated tubulin samples. We observed a significant drop in the protein concentration as well in all the PEF-treated samples after the vast majority of aggregates were removed by ultracentrifugation. Polymerization assay proved that the 3 times PEF-treated tubulin lost the ability to form microtubules. The results were verified by phase contrast microscopy. The results of the 8-anilinonaphthalene-1-sulfonic acid (ANS) fluorescence spectroscopy showed an increasing trend in the fluorescence intensity at the treated tubulin samples, indicating that PEF might alter the structure of tubulin allowing the ANS to access more hydrophobic groups in the protein. However, the structural changes in the protein caused by PEF treatment decreased the ability of Ellman's reagent (DTNB) to interact with the tubulin's sulfhydryl groups. The PEF-treated samples exhibited decreased TRP/TYR autofluorescence. Our results contribute to the development of novel electromagnetic methods for modulating the function of biomolecular matter at the nanoscale. The PEF treatment causes structural changes in tubulin while preserving its basic ability to polymerize. We also found out the threshold of the irreversible function of tubulin and observed the aggregation effect of PEF treatment on tubulin.

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