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International workshop

Acoustic and electrochemical methods in the study of affinity interactions at surfaces

BOOK OF ABSTRACTS

20 JUNE 2016
BRATISLAVA, SLOVAKIA

International workshop

Acoustic and electrochemical methods in the study of affinity interactions at surfaces

Bratislava, Slovakia
20 June 2016

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The goal of the workshop is to provide a venue for advancing dynamic characterization of interfaces using acoustic and electrochemical techniques through a series of lectures and discussions. The workshop brings together experts in fundamental and applied aspects of in situ- acoustic and electrochemical diagnostics from Europe, Canada and USA. Workshop is organized in framework of the project FORMILK funded by European Commission under the programme H2020-MCSA-RISE-2015.

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PLENARY LECTURES, KEY NOTE AND ORAL PRESENTATIONS

Surface chemistry for an anti-fouling ultra-high frequency acoustic wave biosensor

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Adsorption or fouling of substrates by the components of biological fluids, especially blood, has constituted an extremely difficult problem with respect to the employment of various biosensors, including acoustic wave devices, in medical science. Over many years a variety of strategies have been employed to attempt reduction of fouling with some emphasis on control of surface free energy and imposition of a plethora if surface coatings. In our work we are addressing the physical chemistry of *covalent* surface modification through the synthesis of new silane-based, linker molecules. These moieties are bifunctional, medium-chain length trichlorosilanes containing a PEG backbone. One of these has shown remarkable properties in terms of the reduction of adsorption as detected by biosensor in undiluted serum. The structure of water intercalated in the adlayer appears to be crucial and has been studied by a number of surface analysis techniques including neutron reflectometry.

This research has led to the development of a chemi-passive surface that is now being used in our lab to modify the interface of an ultra-high frequency acoustic wave device that is capable of operation at frequencies up to 1 GHz (EMPAS). This sensor uses acoustic waves which are instigated in a piezoelectric substrate via the secondary electric field associated with a fat spiral coil in close proximity. In particular, we are working on an off-line theranostic configuration for the detection of endotoxin in whole human serum to be employed in tandem with removal of the molecule by special cartridge. Further research is being conducted on the detection of early stage biomarkers for ovarian cancer, lysophosphatidic acid and heat shock protein 10. The latter involves the use of aptamer technology on the EMPAS surface. Finally, we are also studying the detection of signaling species associated with the breast cancer metasthesis process.

Acoustic devices as a powerful tool for biophysics and molecular diagnostics

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The use of acoustic devices has expanded considerably during the last years in the fields of biophysics and analytical and clinical chemistry. Their planar geometry, simple fabrication, high sensitivity to both mass and viscoelastic properties of the attached matter, and integration capability in a Lab-on-Chip (LOC) platform, are some of their advantages. Currently, their use goes beyond that of a mere mass sensor as a result of the development of new models and acoustic measuring platforms.

In our group we are using Quartz Crystal Microbalance (QCM) and Surface Acoustic wave (SAW) devices to probe the attachment of protein and DNA molecules at the device surface through the use of a suitable anchor. Based on the measured acoustic ratio of wave energy dissipation over frequency we can derive quantitative information on the shape and size of the attached molecule. In the case of DNA, we showed that it is possible to discriminate between straight double stranded DNA molecules of different lengths, or same size DNAs of various shapes (straight, curved, triangle) [1,2]. This novel way of characterizing DNA conformation was subsequently applied to the development of an assay for molecular diagnostics; specifically we demonstrated that by producing in the same PCR reaction amplicons of a different length, each one corresponding to a different target, we were able to perform multiplexed amplification and acoustic detection of two or three targets simultaneously [3]. We have further applied the above label-free assay in combination with micro-nano devices and technologies to produce an integrated platform combining several sample pretreatment modules with a SAW detection biochip. This LOC platform was applied to bacteria capture and lysis and DNA purification using two plasma etched nanotextured surfaces [4], followed by DNA amplification in a micro-PCR device. The direct acoustic detection of *Salmonella* pathogenic DNA in the PCR mixture without any further purification was demonstrated for as few as 5 microbial cells. The above concepts are currently used in our lab for application in human samples and development of a point-of-care device.

References

[1] A. Tsortos, G. Papadakis, K. Mitsakakis, K.A. Melzak, E. Gizeli, Biophysical J. 94 (2008) 2706-2715
[2] A. Tsortos, G. Papadakis, E. Gizeli, Biosens. Bioelectr. 24 (2008) 836-841
[3] G. Papadakis, A. Tsortos, A. Kordas, I. Tiniakou, E. Morou, J. Vontas, D. Kardassis, E. Gizeli, Scientific Reports 3:2033 (2013) DOI: 10.1038
[4] K. Tsougeni , G. Papadakis , M. Gianneli , A. Grammoustianou , V. Constantoudis, B. Dupuy, P. N. Petrou, S. E. Kakabakos, A. Tserepi, E. Gizeli, E. Gogolides, Lab on a Chip 16 (2016) 120-131

Immunosensing of pathogens: comparison of piezoelectric, electrochemical and optical transducers

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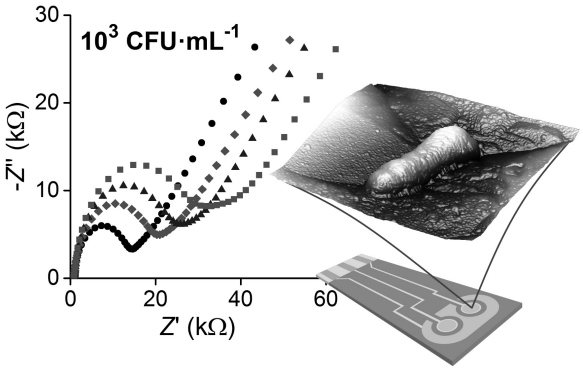

Rapid detection of low levels of bacteria remains challenging for point-of-care clinical diagnosis, food testing and environmental screening. Here, research focused on *Salmonella enterica* var. Typhimurium, which one of the leading agents of gastrointestinal diseases; humans become infected particularly by consumption of the contaminated food such as meat, eggs and unpasteurized dairy products. is

A label-free immunosensing employed screen-printed sensors combined with electrochemical impedance spectroscopy [1], piezoelectric quartz crystal microbalance (QCM) and surface plasmon resonance (SPR / Biacore, BioNavis). Specific antibody was covalently immobilized either directly to gold (cysteamine monolayer and glutaraldehyde) or through carboxymethylated dextran layers (EDC/NHS activation). These approaches provided limits of detection at 1000 CFU/mL within 10-20 min, with negligible interference from other bacteria. To improve sensitivity, signal enhancement was carried out using labeling of the captured microbes with secondary antibody-peroxidase conjugate. Afterwards, substrate mixture consisting of H_2O_2 and 4-chloro-1-naphthol formed insoluble benzo-4-chlorocyclohexadienone which precipitated on the sensing surface and provided excellent signal amplification [2]. In this way, 100 CFU/ml of S. Typhimurium was detectable. Several variants of immunosensors were compared, including evaluation on milk samples.

Different ways for sample treatment (combinations of heat and sonication) were tested and their impact on the assay performance was evaluated. Atomic force microscopy was used to study the effect of the treatment on the cell shape and to confirm the specific binding of microbes to the sensing surface, cells were visible even on the rough screen-printed surfaces. AFM images and height profiles also helped to quantify effect of the precipitating product generated by the enzyme label on the improved performance of immunosensors.

References

[1] Z. Farka, T. Jurik, M. Pastucha, D. Kovar, K. Lacina,P. Skladal. Rapid Immunosensing of Salmonella Typhimurium Using Electrochemical Impedance Spectroscopy: the Effect of Sample Treatment. Electroanalysis 28 (2016) 1 – 8.

[2] T. Jurik, P. Skladal. Detection of hydrogen peroxide and glucose by enzyme product precipitation on sensor surface. Chem. Papers 69 (2015) 167-175.

Nanomotor-based biosensing: Moving the receptor around the sample

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This presentation will describe new motion-based bioassays based on autonomously moving receptor-functionalized nanomotors. The new motor-based sensing approach relies on new capabilities of modern nano/microscale motors. Particular attention will be given to catalytic nanowire and microtube motors propelled by the electrocatalytic decomposition of a chemical fuel, The increased cargo-towing force of new man-made nanomotors, along with their precise motion control within microchannel networks, versatility and facile functionalization, can be combined for developing advanced microchip systems based on active transport.

The motion-based biosensing strategy relies on the continuous movement receptor-modified microengines through complex samples in connection to diverse 'on-the-fly' biomolecular interactions of nucleic acids, proteins, bacteria or cancer cells. A variety of receptors, attached to self-propelled nanoscale motors, can thus move around the sample and, along with the generated microbubbles, lead to greatly enhanced fluid transport and accelerated recognition process. Selective capture and transport of target DNA and cancer cells from raw complex body fluids will be demonstrated. Key factors governing such motion-based sensing will be covered. The resulting assays add new and rich dimensions of analytical information and offer remarkable sensitivity, coupled with simplicity, speed and low costs. We will discuss the challenges of implementing molecular recognition into the nanomotor movement and for generating well-defined distance signals. New microengines with a 'built-in' recognition capability, based on boronic-acid or molecularly-imprinted outer layers, will also be discussed. The latter obviated the need for the receptor immobilization. The greatly improved capabilities of chemically-powered artificial nanomotors could pave the way to exciting and important bioanalytical applications and to sophisticated nanoscale and microchip devices performing complex tasks.

References:

1. J. Wang, Nanomachines: Fundamental and Applications, Wiley, 2013.
2. J. Wang, Can Man-Made Nanomachines Compete with Nature Biomotors?, *ACS Nano*, 3 (2009) 4.
3. K. Manesh and J. Wang, Motion Control at the Nanoscale, *Small* 6 (2010) 338.
4.. J. Wu, D. Kagan, S. Balasubramanian, K. Manesh, S. Campuzano and J. Wang, Motion-based DNA Detection using Catalytic Nanomotors, *Nature Communications,* 1 (2010) 1.
5. S. Campuzano, D. Kagan, J. Orozco, J. Wang, Motion-based Sensing and Biosensing using Electrochemically-Propelled Nanomotors, *Analyst*, 136 (2011) 4621.
6. S. Balasubramanian, D. Kagan, C.M. Hu, S. Campuzano, M. J. Lobo-Castañon, N. Lim, Dae Y. Kang, M. Zimmerman, L. Zhang, J. Wang, Micromachine Enables Capture and Isolation of Cancer Cells in Complex Media, *Angew Chemie Ind Ed*. (VIP Paper) 50 (2011) 4161.
7. J. Wang, Self-propelled affinity biosensors: Moving the receptor around the sample, Biosensors Bioelectronics, 76 (2016) 234-242.

Affinity sensing: recent advances

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We will report about recent developments and trends in affinity sensing to achieve ultra sensitive analytes detection for real application to real matrices, from drug development to clinical diagnostic to cultural heritage.

Both QCM and SPR analysis will be presented and discussed for protein and DNA based sensing with an eye to bioreceptor development. In particular coupling affinity sensing to nanotehcnology will be discussed from the point of view of analytical chemists [1]. Improvement in detection levels by building molecular architecture using nanostructurs differing in material, size and shapes will be presented. Behind this novel strategies to develop biochip based on nanostructured material for advances in SPR transduction i.e. SPR imaging (SPRi) [2] and localized SPR (LSPR)-based sensing will be finally discussed in their applications to some analytical problem [3]

References

[1] S. Mariani, S. Scarano, M.L. Ermini, M. Bonini, M. Minunni, Chem. Comm., 51 (2015) 6587-6590.
[2] G. Spoto, M. Minunni, J. Phys. Chem. Lett., 3 (2012) 2682–2691.
[3] M.L. Ermini, S. Scarano, S. Mariani, M. Minunni, Biosens. Bioelectron., 61 (2014) 28-37.

Addressing the challenge of in situ structural-functional characterization of thin polymer films

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The possibility to tune the functional response of thin polymer films through synthesis or design of hierarchical assembly opened a universe of materials-by-design with functional and adjustable structural motives to meet the requirements of applications ranging from photovoltaics, flexible electronics to miniaturized wearable sensors. An understanding of the relationship between structure and functionality of polymer films is a critical part of the theory-guided research, which could enable efficient search for novel materials, hierarchical structure of the device which could withstand complex environment conditions.
In this presentation we will review recent developments in the characterization of thin films using scattering techniques (neutron and X-ray), spectroscopic techniques (Raman and ellipsometry), and resonant techniques (techniques to enable structural characterization of thin films). We will discuss the challenges and benefits of in-situ imaging systems which could enable simultaneous characterization of structure and functionality. We will also look at how computational science could facilitate the development of structural and functional materials of the future. This research was conducted at the Center for Nanophase Materials Sciences, which is a DOE Office of Science User Facility.

References

[1] E.S. Muckley, J. Lynch, R. Kumar, B. Sumpter, I.N. Ivanov, PEDOT:PSS/QCM-Based Multimodal Humidity and Pressure Sensor, Sensors Actuators B Chem. (2016). doi:10.1016/j.snb.2016.05.054.
[2] E.S. Muckley, A.J. Nelson, C.B. Jacobs, I.N. Ivanov Effect of UV irradiation on adsorption /desorption of oxygen and water on carbon nanotubes - SPIE OPTO, 2016.
[3] C. B. Jacobs, A. V. Ievlev, L. F. Collins, E. S. Muckley, P.C. Joshi, I. N. Ivanov, Spatially resolved resistance of NiO nanostructures under humid environment. Proc. SPIE 9749, Oxide-based Materials and Devices VII, 97491Q (February 27, 2016); doi:10.1117/12.2214459.

Single molecule sensing: From AFM tip chemistry to receptor – ligand energy landscapes

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Biorecognition, i.e. the molecular interaction of biological molecules, plays a pivotal role in life. Cell adhesion, genome replication and transcription, signaling, and immune response are prominent examples of biorecognition based processes. Single molecule approaches give the opportunity of elucidating important aspects of these processes that are inaccessible by common ensemble measurement techniques. Molecular Recognition Force Spectroscopy (MRFS) is an atomic force microscopy (AFM) based technique to probe inter- and intramolecular interaction forces on the single molecule level. MRFS requires the upgrade of conventional silicon(nitride) or gold coated AFM tips to bio-functionalized single molecule sensors by tethering a biomolecule to the apex of the tip (ligand). The corresponding interaction partner (receptor) has to be immobilized on the surface. In force distance cycles receptor and ligand are repeatedly brought into contact to monitor formation and rupture of complexes and measure the unbinding force upon rupture. Varying the force loading rate allows exploration of the energy landscape of the interaction, and provides information on the dissociation rate koff and the width of the energy barrier xβ. In the rapidly evolving field of nanomedicine biosensing based AFM techniques are of significant interest. They permit in depth investigation of novel nanoparticle based drug delivery vehicles, as well as their adhesion and uptake by cells. For instance using MRFS with AFM tip-bound bio-functionalized carbon nanotubes, selective binding to cancer cells could be quantified, demonstrating the potential of a single molecule approach for the investigation of drug delivery approaches and targeting capacity. In contrast to force spectroscopy, recognition imaging – which is based on the same biosensing cantilever technology – also enables to laterally locate relevant receptor binding sites. Recognition imaging has shown to be a powerful technique for investigation of normal and pathological erythrocytes ranging from functional exploration to molecular diagnostics.

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Bio-medical AFM imaging using self-sensing cantilever

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Advances in micro-, nano-, and biotechnology put increasing demands on nano-scale microscopy and characterization. Atomic force microscopy (AFM) is one of the highest resolution microscopy methods used in this area. While traditional AFMs using optical deflection detection of the cantilever sensor yield very high resolution images they show disadvantages in terms of stability, they are difficult to parallelize and automate as well as the integration with other analysis techniques is limited due to the required optical components.

Self – sensing cantilever can help overcoming the mentioned limitations and give a big benefit in usability. New developments in self – sensing cantilever technology brought this strain sensing deflection detection cantilevers to a level where resolution and sensitivity are equal to conventional optical readout cantilevers. Nevertheless are these cantilevers up to now mainly limited to special AFMs or homebuilt prototype equipment on hard samples in dry state.

In this work we present the implementation of self – sensing cantilevers to a conventional bio – AFM without any changes to the AFM hardware itself. Therefore a self – sensing cantilever holder with incorporated amplification and voltage supply for the cantilever integrated Wheatstone bridge. A second adjustable amplifier and a signal splitter box complete the add-on hardware and the optical deflection signal is subsequently replaced by the electrical strain sensing signal from the self – sensing cantilever.

Hardware and cantilevers have been tested and characterized on standard calibration gratings. After characterization these unique system has been applied on different cell types at ambient conditions and in physiological buffer solutions using intermittent contact mode with acousto- mechanical or thermal excitation. The activation state of human platelets has been characterized in terms of its time dependence. The bio medical imaging results have been compared to conventional optical readout by imaging the same sample positions with both readout methods using cantilevers with similar physical parameters.

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Acoustics and AFM studies of protein cleavage by plasmin

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Plasmin is serine protease cleaving peptide bonds in molecules of fibrin and milk protein. Degradation of milk proteins highly influences cheese ripening through development of desirable changes in flavor and consistency. In contrast, proteolysis is undesirable when it results in gelation and bitterness due casein breakdown as observed in ultra high temperature (UHT) treated milk [1]. In this work we report an acoustic method of detection based on a specific peptide substrate (PS) for in situ monitoring of enzymatic activity of plasmin [2]. Additionally, the activity of plasmin on the PS is visualized and examined in real time conditions by AFM. These imaging experiments served for optimizing the preparation of sensing layer as well as helped to elucidate the mechanism into which the peptide undergoes by the action of plasmin at different intervals of time. The biorecognition element consists of a PS specific to plasmin immobilized on a piezoelectric quartz crystal electrode. After enzymatic reaction with plasmin, it cleaves a short fragment of the peptide causing increase in the resonance frequency of the piezocrystal. Plasmin was detected in the range of concentrations 1–20nM, a target interval in which its presence presumably affects the quality of milk. The PS exhibited negligible response against to similar protease trypsin. This has been confirmed also by electrochemical detection method. Limit of detection of this acoustic transducer was found to be 0.65nM. Formation of the sensing surface and kinetic effect of plasmin on the peptide substrate was studied by atomic force microscopy (AFM). The PS response was also validated in pretreated milk samples spiked by known concentrations of plasmin achieving an average recovery of 63±0.6%.

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References
[1] N. Datta, H. C. Deeth, Food Sci.Technol. 36 (2002) 173–182.
[2] K. Oohtsuka, I. Maekawa, M. Waki, S. Takenaka, Anal. Biochem. 385 (2009) 293–299.

Electrochemical enzyme activity assays in raw milk samples – application in a regional quality control survey

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Electrochemical enzyme activity assays are highly sensitive alternatives of optical measurements. Electroactive tag modified specific peptide substrates of hydrolytic enzymes can be used either in solution phase or as an electrode surface confined film to detect enzyme cleavage activity. Lower detection limits can be achieved by electrochemical assays than by conventional optical detection, thus extremely low enzyme activity values can be measured.

Proteolytic enzyme, plasmin is a very important component of the blood thrombolytic system, it disconnects peptide bonds in case of unnecessary clot formation. It is filtered in the milk of mammals in very small concentration, but can result in unwanted change of protein composition of the milk. Electrochemical enzyme assays were used to detect plasmin activity in milk.

A regional survey has been carried out to measure the indigenous plasmin enzyme activity of milk samples produced by several farms in the cross-border region. Physico-chemical and microbiological tests have been also carried out parallel to the enzymatic tests. The magnitude of enzyme activity can be correlated to the vulnerability of milk proteins to proteolytic breakdown resulting in unwanted changes in texture and taste of raw milk and different milk products.

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References

[1] G. Castillo, K. Pribransky, G. Mező, L. Kocsis, A. Csámpai, K. Németh, Z. Keresztes, T. Hianik: Electrochemical and photometric detection of plasmin by specific peptide substrate; Electroanalysis 27 (2015) 789 – 798.

Applications of high resolution ultrasonic spectroscopy for analysis of complex formulations

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The presentation describes major principles and instrument configurations of high resolution ultrasonic spectrometers (HR-US), as well as their applications for molecular and microstructural characterisation of liquids and semi-solid materials. The technique provides simultaneous measurements of two ultrasonic parameters, velocity and attenuation with an exceptionally high resolution in the frequency range 1 to 20 MHz. It requires small sample volumes, typically 1-2 ml (down to 0.03 ml). Measurements can be performed in a broad range of temperatures (-30 to 120 ^{o}C), at excessive pressures, in concentrated and dilute samples and in aggressive solvents, which allows analysis of molecular and microstructural transformations in conditions corresponding to industrial processes.

The application examples include real-time monitoring of chemical reactions, stability and activity of enzymes, protein denaturation and fusion of protein particles, gelation phenomena, molecular binding, analysis of microstructural organization, microemulsion phase diagrams and encapsulation of active ingredients in microemulsion droplets, monitoring of sedimentation and creaming.

Phononic crystals - A device for inside sensing?!

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Sensor Principle

Phononic crystals (PnC) have been introduced in the early 1990s and consist of two or more materials arranged in a spatially periodic manner. Modulation in density and sound velocities is used to create band gaps at wavelengths commensurate to the lattice constant. Introduction of a defect into the crystal lattice is a technique to create resonant modes. If located within the band gap and if well elevated above ground such a resonant mode can be used as unique sensor signal. We could show that sensitivity to speed of sound of liquids confined in the defect is sufficient to determine the concentration of a component in mixtures.

Inside sensing?

The majority of recent publications on resonant chemical and biochemical sensors, e.g. the QCM - quartz crystal microbalance address their application to specific problems like the detection of biomolecules. Characteristic for all these sensors is that the properties of the outermost layer of the sensors are affected by the environment that causes changes in the resonance conditions of the sensor. Consequently the sensor measures interfacial properties. By contrast, the complete analyte-filled cavity resonates in case of a phononic crystal sensor. Consequently the sensor measures volumetric properties. It does not necessarily mean that the well-established methods for enhancement of sensitivity and selectivity cannot be applied here; however, this aspect is outside scope of my presentation. The same applies to any steps of analyte pre-treatment like mixing or separation, including those performed within microfluidic systems. However, standard binary mixtures of water and alcohols with well-known acoustic properties used for proof-of-concept especially feature strong intermolecular interactions resulting in a significant excess contribution to Gibb's Free Energy and hence an extreme in sound velocity *vs*. molar ratio, hence our sensor opens a window to molecular properties.

Challenges

Starting from our first 1D and 2D realizations of phononic crystal sensors we introduce current investigations on four design concepts, a Sandwiched Phononic Crystal (SPC), a disposable capillary phononic crystal (DCPC), a cylindrical phononic crystal (Tubular Bell), and a cubic 3D phononic crystal (C3D PnC). The SPC applies a resonant cavity located between two stacked layers. Key issue is avoiding scattering of the guided waves into the bulk of the sensor chip. The DCPC follows the idea of a disposable analyte-filled glass capillary based on standard components used e.g. in blood analysis instead of a complete disposable sensor chip. Here insensitivity to unavoidable variations in the coupling layer between phononic crystal and capillary is of major importance. The Tubular Bell is a cylindrical microfluidic pipe containing the analyte. The design of the outer rim of the cylinder realizes dynamic cavities inside the pipe when acoustically excited at certain frequencies. The inner surface is not modified. The cubic sensor is our newest approach combining 3D phononic crystal design and microfluidics. 3D printing technology allows for both rapid prototyping and customized devices, however, has not yet been widely applied to fabricate phononic crystals.

Electrochemistry of biomacromolecules and affinity interaction at electrode surfaces

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First paper on electrochemistry of proteins was published in 1930 showing the ability of proteins to catalyze hydrogen evolution at Hg electrodes. About 30 years later it was shown that DNA produced reduction signals reflecting DNA structure [1,2]. At present electrochemistry of nucleic acids (NAs) is a booming field, based predominantly on affinity interactions of NA complementary strands at electrode surfaces, and dealing largely with DNA hybridization and damage [2]. Electrochemistry of proteins was oriented mainly on a small group of conjugated proteins yielding reversible reactions of their non-protein components (e.g. metals in metalloproteins). Recently we have shown that using constant current chronopotentiometric stripping (CPS) practically any protein produces electrocatalytic peak H at Hg and solid amalgam electrodes (SAEs). Using peak H at low current densities, proteins can be determined down to nM and subnanomolar concentrations. Proteins do not denature when adsorbed to Hg electrodes or SAEs at p.z.c., but can be denatured at negative potentials. Protein structure-sensitive CPS analysis was developed using high negative current densities (where the rate of potential changes is extremely fast) [3]. At thiol-modified electrodes, changes in properties of mutant proteins were detected. Using CPS, detection of sequence-specific DNA-protein binding was shown. Recently it has been shown that also voltammetric methods, such as fast CV and normal pulse voltammetry can discriminate native from denatured proteins but their protein structure sensitivity is much lower than that of CPS. For decades polysaccharides (PSs) were considered as electroinactive biopolymers. Recently it was shown that some PSs produce peak H_{PS}, similar to peak H of proteins. Moreover, facile modification of PSs and oligosaccharides with osmium(VI) complexes can transform the electroinactive carbohydrates in electroactive Os(VI) adducts, detectable down to pM concentrations. Glycan detection in glycoproteins without deglycosylation was shown at carbon electrodes. Our results show new possibilities in glycoprotein analysis [4]. At present many types of biomacromolecules can be analyzed electrochemically, including NAs, proteins, polysaccharides, lipoproteins, lipopolysaccharides etc., offering challenges for development of new methods of their sensing highly desirable in diagnostics and therapy of various diseases, including cancer.

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References
[1] E. Palecek, Nature 188 (1960) 656
[2] E. Palecek, M. Bartosik M., Chem. Rev. 112 (2012) 3427
[3] E. Palecek et al., Chem. Rev. 115 (2015) 2045.
[4] E. Palecek, Electrochim Acta 187 (2016) 375.

Chronopotentiometric analysis of protein interactions

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Development of label-free and reagent-free electrochemical methods for analysis of practically all proteins represents a great challenge for electrochemistry to enter wide fields of proteomics and glycomics. Last decades we have been developing label-free and reagent-free electrochemical methods for protein [1] and protein-nucleic acid interaction analysis [2]. Almost 15 years we studied peptides and proteins using constant current chronopotentiometric stripping (CPS) analysis at mercury-containing electrodes. Well-developed peak H, due to the catalytic hydrogen evolution, displays sensitivity to local and global changes in protein structure [1] at bare and thiol-modified mercury electrodes [3]. The method was applied in the analysis of tumor suppressor p53 protein [4], α-synuclein [5] and peptides involved in neurodegenerative diseases [6], membrane proteins [7], for studies of sequence-specific p53-DNA binding [2] and also for the detection of lectin-glycoprotein interactions. Incubation of lectin concanavalin A with glycoprotein ovalbumin resulted in an increase of the CPS peak H of the complex as compared to the CPS peaks of individual ovalbumin and concanavalin A proteins. Qualitatively similar results were also obtained with other glycoprotein-lectin couples (concanavalin A-RNase B and lectin from *Sambucus nigra*-fetuin). These results together with previous ones obtained with oncoproteins [1], such as p53, AGR-2 [8] showed interesting properties of surface-attached proteins and offered simple and inexpensive tools for protein research important in present glycomics and biomedicine.

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References

[1] E. Palecek, J. Tkac, M. Bartosik, T. Bertok, V. Ostatna, J. Palecek, Chem. Rev.115 (2015) 2045.
[2] E. Palecek, H. Cernocka, V. Ostatna, L. Navratilova, M. Brazdova, Anal. Chim. Acta, 828 (2014) 1.
[3] V. Ostatna, H. Cernocka, E. Palecek, J. Am. Chem. Soc. 132 (2010) 9408.
[4] E. Palecek, V. Ostatna, H. Cernocka, A. C. Joerger, A. R. Fersht, J. Am. Chem. Soc. 133 (2011) 7190.
[5] E. Palecek, V. Ostatna, M. Masarik, C. W. Bertoncini, T. M. Jovin, Analyst 133 (2008) 76.
[6] K. Kurzatkowska, V. Ostatna, I. W. Hamley, T. Doneux, E. Palecek, Electrochim. Acta, 106 (2013) 43.
[7] M. Zatloukalova, E. Orolinova, M. Kubala, J. Hrbac, J. Vacek, Electroanalysis 24 (2012) 1758.
[8] V. Ostatná, V. Vargová, R. Hrstka, M. Ďurech, B. Vojtěšek, E. Paleček, Electrochim. Acta 150 (2014) 218.

POSTERS

P1
Study of plasmin activity on specific peptide substrate by photometric and electrochemical methods

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Plasmin is a serine protease that plays the major role in the enzymatic activity in milk. Proteolysis by plasmin is associated to breakdown of caseins resulting in bitterness of milk which affects its quality and could have important industrial implications. For analyzing protease activity, we used a specific peptide substrate that was partially cleaved after interaction with plasmin [1]: Lys-Thr-Phe-Lys-Gly-Gly-Gly-Gly-Gly-Gly-Cys. In the photometric setup, plasmin was generated from plasminogen of bovine plasma after its activation by urokinase type activator by measuring the initial hydrolysis rates of the peptide substrate by UV−VIS spectroscopy. For electrochemical experiments, the peptide was additionally modified by a ferrocene Fc redox group at the lysine-end and by a thiol group at the cystamine-end for enabling the chemisorption of peptide (Fc-P) at gold electrode, following the cleavage by plasmin with cyclic voltammetry (CV). The photometric assay achieved a limit of detection LOD=3.68 ± 0.04 nM, whereas the electrochemical transducer exhibited 6 times improved sensitivity LOD=0.56 ± 0.03 nM also demonstrating lower cross-reactivity with thrombin and β-casein. The electrochemical assay has been also validated in treated milk samples and the matrix effect of milk components was analyzed [2].

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References
[1] Ohtsuka, K., Maekawa, I., Waki, M., Takenaka, T., Analytical Biochemistry 385, (2009) 293.

P2
Real-time electrochemical follow-up of enzyme activity in milk-based gels

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The concept to follow in real-time the progression of enzymatic reaction front in heterogenous phase media, such as milk-based gels, is presented.

Surface immobilised ferrocene-tagged enzyme specific peptide substrate of plasmin can be prepared according to [1]. Electrochemical measurements give information on the quantity of surface confined redox tags. Enzymatic cleavage results in a decrease of measured cyclic voltammetric peak current, thus the calculated surface concentration change can be used to derive enzymatic rate contants. Multichannel potentiostate connected to microelectrode arrays was developped to follow in time the position change of lytic front.

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References
[1] G. Castillo, K. Pribransky, G. Mező, L. Kocsis, A. Csámpai, K. Németh, Z. Keresztes, T. Hianik: Electrochemical and photometric detection of plasmin by specific peptide substrate; Electroanalysis 27 (2015) 789 – 798

P3
Proteases activity detection on immobilized layer of casein by biosensor based on transverse shear mode transducer

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Quality of milk and cheese is highly dependable on their flavour and consistency influenced by milk protein degradation. Desirable ripening, unwanted bitterness and gelation as aftermaths of UHT (ultra high temperature) breakdown of casein are main aims of food industry and its research to control these chemical structural changes [1]. Proteolytic activity of protease plasmin, starting with abundant transfer from blood circulation into the bovine milk, is known for being connected with casein breakdown [2]. Our work primary objective was to study β-casein degradation mechanism by plasmin and trypsin proteases. Transverse shear mode method (TSM) enabled monitoring of changes in frequency and in motional resistance for casein layer cleaved by the proteases and their consequent analysis. Short casein fragments were cleaved by protease causing increase in resonance frequency of TSM transducer. Plasmin concentrations were in range 1-20 nM as it corresponded to concentration which causes milk quality changes. Similar, but more substantial effects were observed during trypsin application on casein layer. Limit of detection was evaluated as 0,65 nM for our TSM transducer. On the contrary to the positive signals for plasmin and trypsin cleavage of casein layer, thrombin caused only small frequency decrease and dynamic resistance increase, as it doesn´t cleave casein and it is evidently performing nonspecific adsorption of thrombin on casein layer. Atomic force microscopy was utilised to study changes in topography of casein layer.

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References
[1] K. Hyunjun, S. Park, K. Kim, J. Electroanal. Chem. 742 (2015) 70 - 73.
[2] M. Ouanezar, F. Guyomarch, A. Bouchoux, Langmuir 28 (2012) 4915 – 4919.

P4
Structural characterization possibilities of milk-derived colloidal particles: from casein micelles towards extracellular vesicles

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Structurally, milk is a complex matrix of proteins (casein and whey), fat globules, minerals and water. Casein micelles are naturally created particles composed of a complex of associated casein and calcium phosphate. Although casein micelles are essential for nutrition (neonate) and for dairy industry, there is still conflict regarding their structure. Small-angle X-ray scattering (SAXS) was recently used to study the internal structure of the casein micelles and to verify the models advocated in the literature [1]. Milk functions not only as a nutrition source, but delivers also immune modulatory factors to neonates. Extracellular vesicles (EV) have been identified in human breast milk and in bovine milk. EVs are lipid bilayer enclosed structures, released by cells and are recognized as potent vehicles for intercellular communication. The isolation of native populations of milk-derived EVs is, however, challenging encumbering further analysis [2].

The Biological Nanochemistry Research Group has a unique potential in the characterization of nanosized systems and provides wide-ranging structural information from the atomic level up to several micrometers. For example, the morphology of the complex milk matrix can be visualized directly by freeze fracture combined with transmission electron microscopy (**FF-TEM**). Size and size distribution of separated milk-derived colloid particles can be determined by dynamic light scattering (**DLS**). An in-house designed, general-purpose laboratory small-angle X-ray scattering (**SAXS**) apparatus (http://credo.ttk.mta.hu) was optimized for the study of nanoparticles (NPs). Application examples include sterically stabilized liposomes, functionalized silica NPs, or red blood cell (RBC) derived nanoerythrosomes. The low-resolution structures of several proteins in aqueous solutions have also been successfully described. **FTIR** spectroscopy can give direct explanations for configurations and molecular interactions. Casein secondary and tertiary structures were revealed preparing casein stabilized Au@NPs elucidating the structure-function relationship. A reproducible isolation and purification protocol for Jurkat cell and RBC derived **EV**s has been elaborated by our group. By providing the first exploratory FTIR investigations of EVs, we proposed a fast and cheap method for EV preparation screening by estimation of protein-to-lipid ratio.

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References

[1] C.G. de Kruif, Th. Huppertz, V.S. Urban, A.V. Petukhov, Adv. Coll. Interface Sci. 36 (2012) 171-172
[2] M.I. Zonneveld et al., J. Extracellular Vesicle 3 (2014) 24215.

P5
The study of the interaction of cytochrome C with DNA aptamers by electrochemical methods

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Cytochrome C (cyt C) is a small peripheral membrane heme protein that takes part in essential biological functions as electron carrier and metal−ion mediator. Under physiological conditions it is positively charged owing to lysine and arginine amino acid residues. It is responsible for the activation of apoptopic pathway and can be used as an indicator of apoptotic process in the cells [1]. The monitoring of the cyt C release was suggested to use for the screening of anti-cancer drugs [2]. Therefore development of rapid and easy to use method of cyt C detection is urgently needed. The biosensor technology based on DNA aptamers allowing to fulfill these requirements [3].

We report the study of interaction of cyt C with DNA aptamer composed of 76 bases (apt 76) [2]. For this purpose we used electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) in HEPES buffer containing 75 mM NaCl, 5 mM $MgCl_2$ and 1 mM $CaCl_2$ on gold support. The electrochemical activity of cyt C and its interaction with DNA aptamers was studied by cyclic voltammetry (CV) in a multi−layer configuration. The detection of cyt C has been performed at the surface of gold electrode modified by polyamidoamine dendrimers (PAMAM) of fourth generation (G4) with covalently attached apt 76 [4]. EIS was applied for controlling layer formation and cyt C detection using 5 mM $K[Fe(CN)_6]^{-3/-4}$ as redox probe and 100 mM KCl. Charge transfer resistance, R_{ct}, was the parameter used to evaluate the binding response of cyt C to the aptamers. The limit of detection for cyt C was LOD = 15.18 ± 0.62 nM. The specificity of detection has been analyzed using other proteins such as lysozyme and thrombin. In this case approx 30-40 % lower changes of R_{ct} have been observed.

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References
[1] Y.L.P. Ow et al., Nat. Rev. Mol. Cell Biol. 9 (2008) 532−542
[2] F.G. Loo, et al., Sens. Act. B 198 (2014) 416-423
[3] V.B. Stepanova et al., Sens.Act. B. 225(2016) 57-65
[4] G. Castillo et al., Food Control 52 (2015) 9−18

P6
Synthesis, characterization and antibacterial activity of gold nanoparticles prepared by citrus sinensis extracts

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Nowadays, metallic nanoparticles are widely used in biomedicine and biotechnology. New techniques for its synthesis using biomass from fruits, vegetables and plants have been recently developed in order to make the preparation more feasible and environmentally−friendly [1]. In this work, we report the synthesis of gold nanoparticles (AuNps) by reduction of chloroauric acid using peels and juice extracts from orange (*citrus sinensis*) fruit. The obtained AuNps were characterized by UV−VIS Spectroscopy, Dynamic Light Scattering (DLS), Laser Doppler Velocimetry (LDV) and Transmission Electron Microscopy (TEM). Parameters such as temperature, pH and ionic strength were analyzed to determine its effect on the properties of AuNps. Furthermore, the prospective application of AuNps as antibacterial agents against Gram−positive *Bacillus cereus* and Gram−negative *Escherichia coli* bacteria was examined. Obtained results revealed that AuNps synthesized by using peels extract were smaller ($d = 75.23 \pm 1.53$ nm) compared to AuNps prepared by orange juice ($d = 180.6 \pm 5.29$ nm). The zeta potential for AuNps−peels was $\zeta = -41.13 \pm 1.95$ mV whereas for AuNps−juice this value was $\zeta = -34.7 \pm 1.04$ mV. The fruit−assisted AuNps exhibited quasi−spherical and hexagonal morphologies heterogeneously distributed as it was demonstrated by TEM imaging. By UV−VIS spectroscopy the effect of temperature after AuNps synthesis was shown to have minimal impact on their optical properties, but it provided better size distributions. Similarly, AuNps were quite stable after addition of NaCl in concentrations up to 0.5 M. The effect of pH demonstrated that acidic media ($pH \leq 3$) induced to aggregation of AuNps. Experiments on bacteria revealed that AuNps−juice had a pronounced antibacterial effect against Gram−negative cultures.

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P7
Synthesis and characterization of folic acid conjugated chitosan nanoparticles – a possible platform for targeted drug delivery

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Chitosan is naturally derived polymer that is nontoxic, biocompatible and biodegradable. These properties make it highly attractive functional biomaterial for various applications. Chitosan-based materials have gained interest in tissue engineering [1], quartz crystal microbalance (QCM) biosensing [2], and drug delivery [3]. When functionalized with special cell targeting ligands, it can be also used as a platform for cancer detection [4].

In order to use chitosan nanoparticles in targeted drug delivery into the cancer cells, we have functionalized chitosan with folic acid ligand. Folic acid can specifically recognize folate receptors, which are over-expressed on the surface of some tumor cells [5].

In this work, we have focused on the preparation and characterization of folic acid-chitosan conjugates. Chitosan nanoparticles were prepared by ionic gelation method and folic acid ligand was covalently bound to its surface via carbodiimide crosslinking chemistry [6]. Prepared conjugates were characterized by means of size and zeta potential measurements. Polydispersity index (PDI) of samples was also monitored. Blank chitosan nanoparticles had size 193.3 ± 3.18 nm, PDI was 0.258 ± 0.014 and zeta potential +17.57mV. Conjugation of folic acid to chitosan decreased the size of nanoparticles to 83.35±1.30 nm and zeta potential to +8.82±0.52mV; PDI was 0.279±0.034. Prepared nanoparticles will be further tested for targeted delivery of chemoterapeutics into the folate expressing tumor cells According to the obtained results, optimization in the formulation protocol can be performed.

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References
[1] F. Croiser, Ch. Jérôme, Eur. Polymer J., 49 (2013) 780-792
[2] E. De Giglio et al., Anal. Bioanal. Chem., 400 (2011) 1997 -2002
[3] M.P. Patel et.al., J. Pharm. Sci., 13 (2010) 536 – 557
[4] S. Zhang et. al., Analyst, 139 (2014) 6259 -6265
[5] P.M.S.D. Cal et. al., Chem. Commun., 50 (2014) 5261-5263
[6] J. Ji et al., Polym. Bull., 68 (2012) 1707–1720

P8
The effect of polyethylene glycol-modified lipids on the interaction of HIV-1 derived peptide-dendrimer complexes with lipid monolayers

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The aim of the work was to investigate the interaction of dendriplexes formed from polyanionic HIV peptide Nef and cationic carbosilane dendrimer (CBD) with model lipid membranes - large unilamellar vesicles (LUVs) and monolayers containing various molar ratio of zwitterionic 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[Methoxy (polyethylene glycol) – 2000] (DSPE-PEG_{2000}). The thermodynamics and mechanical properties of the lipid membranes were examined by ultrasonic spectroscopy and densitometry and for dendriplexes-membrane interaction studies we used monolayer surface pressure measurements and Brewster angle microscopy (BAM). The presence of PEG at polar part of the membrane resulted in an increase of the temperature phase transition of lipid bilayer of LUVs, in decrease of specific volume and adiabatic compressibility. The interaction of dendriplexes with monolayers was accompanied by formation of the clusters as revealed from BAM experiments and by increase of the surface pressure. Increase of surface pressure was maximal for monolayers composed of DMPC, however, with increased concentration of DSPE-PEG_{2000} the changes of surface pressure were less expressed. The data obtained on lipid monolayers can be useful for optimization and potential use of dendriplexes as carriers of HIV-1 peptides. At the same time the experiments performed by ultrasonic spectroscopy and densitometry allowed to obtain important information on the thermodynamics and mechanical properties of lipid membranes that is important for interpretation data of dendriplex-membrane interactions.

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P9
Design and fabrication of polymeric microsurfaces with 2photon photopolymerisation method.

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Two-photon polymerization provides an advantageous technique for fabrication of well defined home-designed miniaturized microstructures and surfaces. We demonstrate the use and application of the two-photon polymerization of organically-modified ceramic composite materials initiated by femtosecond laser pulses. Polymer microsurfaces were 3D printed from OrmoComp (Micro Resist Technology GmbH) using 2-photon photopolymerisation (laser microFAB workstation by Newport with Spirit ultrafast amplified laser, 520 nm). Different conditions of 2P photopolymerisation were tested, including distinct energy levels and the speed of scanning for the laser and different microscope lenses, to achieve the best polymerization results for micro-structures. Scanning electron microscopy (SEM, LEO 1550, Carl Zeiss, Germany)and confocal microscopy (LSM 510 META, Zeiss, excitation at 488nm) was employed to obtain images of micro-surfaces. Presented approach is the first step towards evaluation of the potential applicability of biocompatible constructs in the biomedical field at micro-scale level in controlled conditions, necessary for their use for example in the presence of living cells [1]. Design and evaluation of properties of materials and structures with mesoscopic arrangement and their interaction with biological objects is a prerequisite for development of a new generation of nano / micro / bio-sensors.

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References
[1] T. Teplicky, J. Horilova, J. Bruncko, C. Gladine, I. Lajdova, A. Mateasik, D. Chorvat Jr., A. Marcek Chorvatova, Flavin fluorescence lifetime imaging of living peripheral blood mononuclear cells on micro and nano-structured surfaces. Multiphoton Microscopy in the Biomedical Sciences XV Book Series, eds. Periasamy A, Konig K, So PTC. Proceedings of SPIE, the International Society for Optical Engineering Vol. 9329: 93290D-01 to 93290D-10, 2015.

P10
SERS surfaces for bio applications

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Here we present comparison of different substrates and its performance for usage in surface enhanced Raman spectroscopy (SERS) [1]. Substrates are screened for its possibility to be used in wet procedures, which are needed for molecules of biological significance. Substrates were chosen for their simplicity of preparation and claimed efficiency. All of them were prepared by simple means of thermal evaporation. Surface plasmons were measured to pick up line of laser closest to exciton. We have evaluated enhancement factors for all substrates. Strongest enhancement measured was 4.15×10^5. This was achieved on well known concept of metal film over nanosphere (MFON), where silver was used as metal (AgFON).

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References

[1] T. Dieing, O. Hollricher, J. Toporski, Confocal Raman Microscopy. Springer Series in Optical Sciences, ISBN 978-3-642-12521-8. (2011) p. 289

P11
Application of confocal Raman spectroscopy for intracellular localization of graphene oxide nanoplatform toward development of targeted drug delivery to cancer cell

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The live imaging of cells using confocal Raman spectroscopy (CRM) is nowadays very popular. The immersion CRM allows real time observation of cells in their natural environment without any fixation and fluorescent dyes. Diagnostics of oncological diseases stays at the forefront of the current medical research. A lot of oncological markers are available for early diagnostics. The CA IX (Carbonic Anhydrase IX) is a cell surface, hypoxia-inducible enzyme that is expressed in aggressive tumors, hence, it can be used as a tumor biomarker [1]. In this work we studied the interaction of pristine GO nanoflakes with cell line c33 (cervix cancer cells). Used cells were transfected and overexpressing human CA IX protein. The cells were studied in PBS (Phosphate-buffered saline) buffer with water immersion objective (63×, NA = 1). As a proof of principle, we present the localization capabilities of current CRM for nanoscale GO flakes with the lateral dimensions smaller than 300 nm, which is below the diffraction limit of the objective used. The small lateral size of GO flakes guarantees a successful penetration through the cell membrane. With CRM it was possible to identify GO inside the cell without using any additional dyes. This opens new opportunities of the CA IX real-time tracking by means of GO-antibody conjugated nanoplatforms in living cells as well as analysis of targeted drugs delivery into cancer cells.

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References
[1] E. Švastová et al., FEBS Letters, 557 (2004) 439-445.

P12
Highly sensitive detection of Jurkat cells using an acoustic aptasensor

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The diagnostic of cancer at early stage of disease is on high demand. The biosensor technology using specific receptor that recognize cancer markers at the surface of cancer cells can help in solving this problem. Among receptors the DNA/RNA aptamers are of high advantages over antibodies, due to their higher stability and lower price having similar and even better affinity to cancer markers. In this work we used DNA aptamer (Sgc8c) specific to the protein tyrosine kinase-like 7 (PTK7). This protein is overexpressed in various cancer cell lines. PTK7 was identified as a potential biomarker for T-cell acute lymphoblastic leukemia (ALL). ALL is a type of leukemia that starts from white blood cells in the bone marrow. It develops from cells called lymphocytes or from lymphoblasts. We tested the human T lymphocyte cells - Jurkat and myeloma cells U266.DNA aptamer interactions with the cells were studied using thickness shear mode acoustic method (TSM) in flow system. TSM is sensitive to mass and surface viscosity variation per unit area by measuring the change in frequency and motional resistance of a quartz crystal transducer. We used two concepts of the gold support coating: 1. neutravidine with biotinylated aptamer or 2. thiol-modified aptamer, chemisorbed on a gold. In later case naked gold surface was blocked by dodecanethiol. Addition of Jurkat cells ($0{,}5 \times 10^6$ / ml) caused the decay in frequency by approx. 95 Hz for biotinylated system and about 84 Hz for thiol system. The aptamer specificity were verified using non-specific aptamer or control cells U266. Non-specific interactions with neutravidine and DDT were excluded.

Using atomic force microscopy (AFM) we studied the topography, size, shape and the distribution of the cells on the glass slide after the cultivation and adhering.

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References

[1] D. Shangguan, Z. Cao, L. Meng, P. Mallikaratchy, K. Sefah, H. Wang, Y. Li, W. Tan, J. Proteome Res., 7 (2008) 2133-2139.

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