Session 21: Biophysics application

Oral presentations

O.21.1

Novel insights into the role of BK_{Ca} channel in cellular DNA damage response induced by particulate matter

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Recent studies have shown that exposure to particulate matter (PM) can lead to cancer, cardiovascular and neurological disorders. The potential mechanisms of the PM on these diseases' progression are connected with oxidative stress, mitochondrial dysfunction, and inflammation at the cellular level, whereas at the genomic level with genotoxicity. However, the current knowledge about molecular mechanisms of DNA damage response (DDR) to PM exposure is limited. Although the mechanisms of DDR have been extensively studied, most work has focused on signaling mediated by cytosolic or nuclear proteins, rather than ion channels. The precise molecular mechanisms underlying their involvement in DDR remain unclear. We focused on the role of the large-conductance Ca^{2+}-regulated potassium channel (BK_{Ca}) and its potential role in DDR. Our preliminary studies have demonstrated that exposure to standardized PM induced apoptosis *via* caspase-3 cleavage of PARP in human bronchial epithelial cells depleted for the BK_{Ca} channel, G2/M cell cycle arrest and an increase of DNA double-strand breaks occurrence suggesting the role of this ion channel in DDR.
The results may contribute to the development of novel therapeutic strategies targeting BK_{Ca} channels to enhance cellular resilience to DNA damage and reduce the risk of many diseases associated with exposure to environmental pollutants.

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O.21.2

Silver nanoparticles as doxorubicin carriers – direct interactions and modulation of biological effects

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Cancer is one of the most prevalent challenges of modern medicine. The increasing number of afflicted and death rate justifies great interest and funding directed towards discovery of new drugs and potential modifications of actual therapies. Utilization of the metallic nanoparticles as a carriers and modulators of the classic anticancer drugs, such as doxorubicin (DOX), is one of the postulated solutions. Noble metal nanoparticles seems to be promising candidates for such vessels, as they are relatively stable, possess large surface to volume ratio and potentially exhibit anticancer activity. In our work we utilized silver nanoparticles (AgNPs) as a vessel for DOX delivery and modulation. We employed vast number of physicochemical and biological techniques to analyze effects of configuration of DOX with AgNPs. Namely, we used molecular modelling to assess theoretical interactions between these compounds. Subsequently, we analyzed their interactions using spectroscopic methods, dynamic light scattering and atomic force microscopy. Finally, biological effects of DOX-AgNPs were examined using MTT and 3D Matrigel assays.
Our findings show that DOX interacts with AgNPs, which in consequence enhances cytotoxic effects of DOX, as visualized in 3D Matrigel assays. Promising results of our experiments build a solid ground for the further research on metallic nanoparticles acting not only as carriers but also modulators in anticancer therapy.

O.21.3

Tractor beams and single molecules: How to visualize and manipulate single biomolecules in real-time

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Imagine you could directly see the location and dynamics of individual proteins binding to a piece of single DNA. What if you could assemble your biological complex step by step and see it in action in real-time? What if you could manipulate the structure of your biomolecule and quickly change buffer conditions to test your experimental hypotheses? By using "molecular tractor beams", our fluorescence-correlated optical tweezers, the C-trap, makes that a reality, in real time and on the single molecule level.
Here, we present our efforts further enabling discoveries in the field of biology and biophysics using the C-Trap, the world's first dynamic single-molecule microscope that combines the power of high-resolution optical tweezers, confocal microscopy or STED nanoscopy, with advanced microfluidics in a truly integrated system. We present several examples in which using dynamic single molecule analysis enhanced the understanding of protein folding, genome structure and organization, cytoskeletal mechanics, and molecular condensates dynamics. The ability to control, visualize and manipulate single molecules in real time, gives researchers the power to directly prove molecular mechanisms, in ways not previously possible, allowing you to answer mechanistic questions faster. These experiments show that technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument with the ability to open new avenues in many research areas.

O.21.4

Spectroscopic characteristics of perivascular adipose tissue lipid profile and its alterations due to cardiometabolic diseases.

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The perivascular adipose tissue (PVAT) surrounding the aorta is linked to cardiovascular and lifestyle diseases i.e. atherosclerosis and obesity. As PVAT is crucial in the pathology of lipid-related disorders, new tools are needed to assess the lipid profile of PVAT and understand its connection to vascular function. Raman spectroscopy is ideal for studying lipid-rich biomedical samples. By analyzing Raman spectra, we can establish spectroscopic markers i.e. the lipid unsaturation degree (I_{1660}/I_{1444}), which can serve as an indicator of inflammation. In this study, we examined changes in lipid unsaturation in thoracic and abdominal PVAT to understand the PVAT status in relation to atherosclerosis ($ApoE^{-/-}/LDLr^{-/-}$ mice model) and obesity induced by a high-fat diet. *In vitro* mechanistic studies on isolated adipocytes were also conducted.
Results showed that lipid unsaturation degree varies in PVAT based on location, pathology, and age of animals. Changes in lipid composition during disease development are more pronounced in white adipose tissue depots. Raman spectroscopy can distinguish thoracic and abdominal PVAT, with thoracic PVAT having a consistent lipid profile along the aorta compared to the mixed composition of abdominal PVAT. We demonstrated that the lipid unsaturation degree reveals the chemical heterogeneity of PVAT, providing insights into its biochemistry.

O.21.5

Nanoscale Differential Scanning Fluorimetry (nanoDSF) as a valuable tool for characterising proteins derived from extremophilic sources

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Nanoscale Differential Scanning Fluorimetry (nanoDSF) is a powerful biophysical technique that allows precise measurement of the thermal and chemical stability of proteins by monitoring changes in their intrinsic fluorescence. It also enables the assessment of ligand-induced conformational stabilisation.

In this study, we showcase the application of nanoDSF in the study of proteins derived from thermophilic microorganisms, phages or metagenomic samples from terrestrial hot springs in Iceland and Arctic deep-sea hydrothermal vents. Many enzymes from extremophilic sources display features desired by industry, therefore we focused our interest on DNA polymerases, single-strand DNA binding proteins, laccases or lytic enzymes, all of which have potential biotechnological applications either as tools for molecular diagnostics, bioremediation or as alternative antimicrobial agents.

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O.21.6

Multisite phosphorylation of human HP1a provides another layer of heterochromatin regulation by metal ions as demonstrated by solution NMR spectroscopy

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Chromatin organization controls DNA's accessibility to regulatory factors and influences gene regulation. Heterochromatin, or condensed chromatin, containing mostly silenced genes, self-assembles through weak, multivalent interactions with its associated proteins that possess intrinsically disordered regions (IDRs) and undergo liquid-liquid phase separation (LLPS).

HP1a is a very conserved protein involved in chromatin regulation. In human this 44 kDa protein consists of globular domains flanked by IDRs. While the well folded fragments bind numerous protein and DNA targets, the IDRs control HP1a function by undergoing post-translational modification. Notably, phosphorylation of HP1a serine-rich cluster by casein kinase II induces LLPS, an effect that was previously overlooked and seems to have biologically relevant consequences.

Using solution NMR spectroscopy we follow this reaction in a real time at the atomic resolution and show that modification of Ser14 is a prerequisite step for installation of three other phosphate moieties. Dramatic consequences of this multi-site phosphorylation include changes in inter- and intramolecular long-range interactions that we probe with paramagnetic relaxation enhancements (PRE). Moreover, upon phosphorylation HP1a becomes sensitive to divalent metal ions, which, within the physiological concentration range, promote or disrupt droplets formation. Our results shed light on possible regulation of chromatin structure by biometals.