8^{th} Regional Biophysics Conference

Book of Abstracts

Zreče, Slovenia
16^{th} to 20^{th} May 2018

Organized by:

DRUŠTVO BIOFIZIKOV SLOVENIJE
SLOVENIAN BIOPHYSICAL SOCIETY

Laboratory of Biophysics
Condensed Matter Physics Department
Jožef Stefan Institute

#ReBiCon2018

Title: 8^{th} Regional Biophysics Conference
Subtitle: Book of Abstracts

Editors: Hana Majaron, Petra Čotar, Monika Koren, Neža Golmajer Zima
Photo: Janez Štrancar

Published by Slovenian Biophysical Society
This book is available at http://www.rbc2018.si/documents/boa_final.pdf
June 1^{st} 2018

CIP - Kataložni zapis o publikaciji
Narodna in univerzitetna knjižnica, Ljubljana

577.3

REGIONAL Biophysics Conference (8 ; 2018 ; Zreče)
Book of abstracts [Elektronski vir] / 8th Regional Biophysics Conference, Zreče, Slovenia, 16th to 20th May, 2018 ; organized by Slovenian Biophysical Society [and] Laboratory of Biophysics, Condensed Matter Physics Department, Jožef Stefan Institute ; [editors Hana Majaron ... [et al.] ; photo Janez Štrancar]. - El. zbornik. - Ljubljana : Slovenian Biophysical Society, 2018

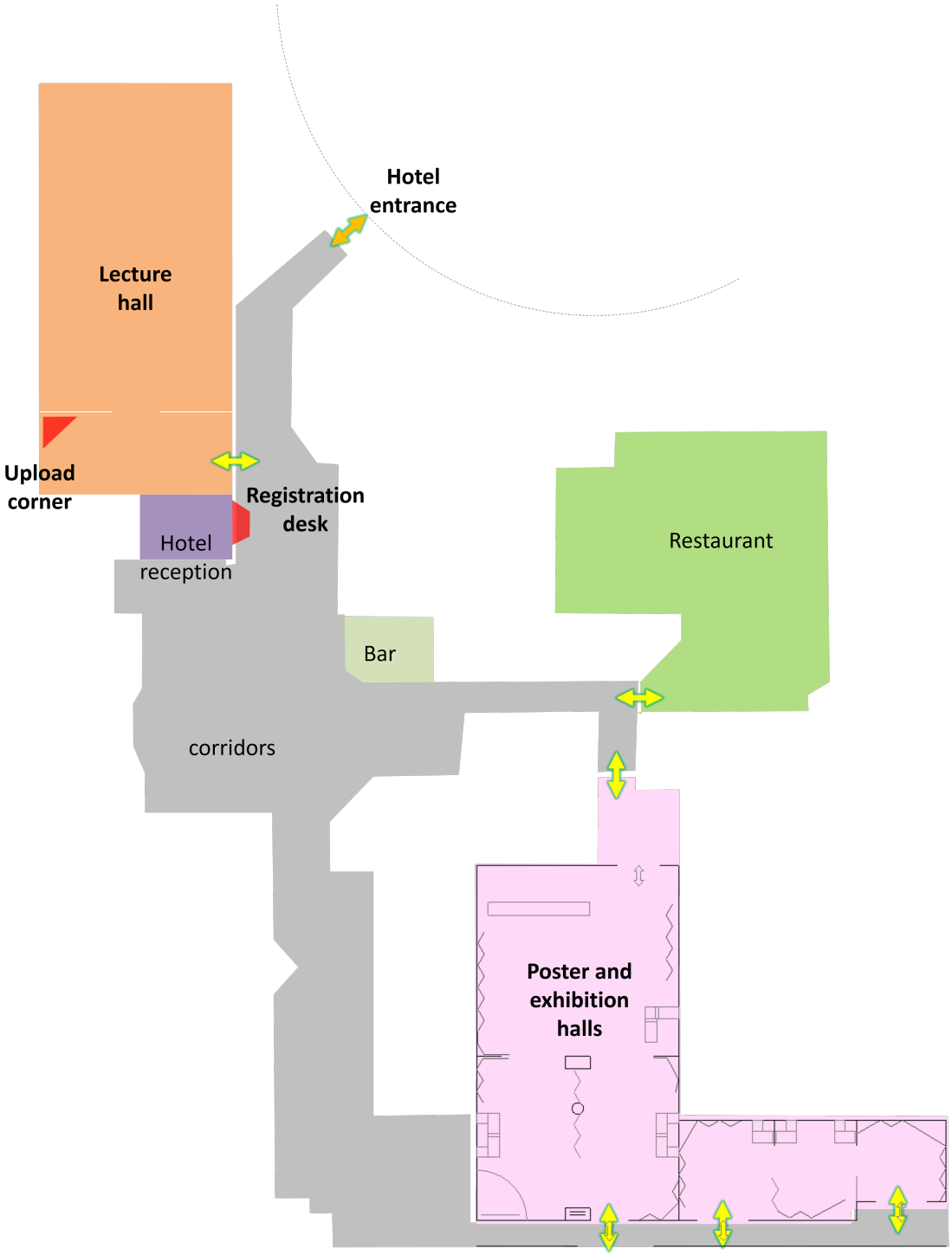
Način dostopa (URL): http://www.rbc2018.si/documents/boa_final.pdf

ISBN 978-961-90942-4-2
1. Majaron, Hana

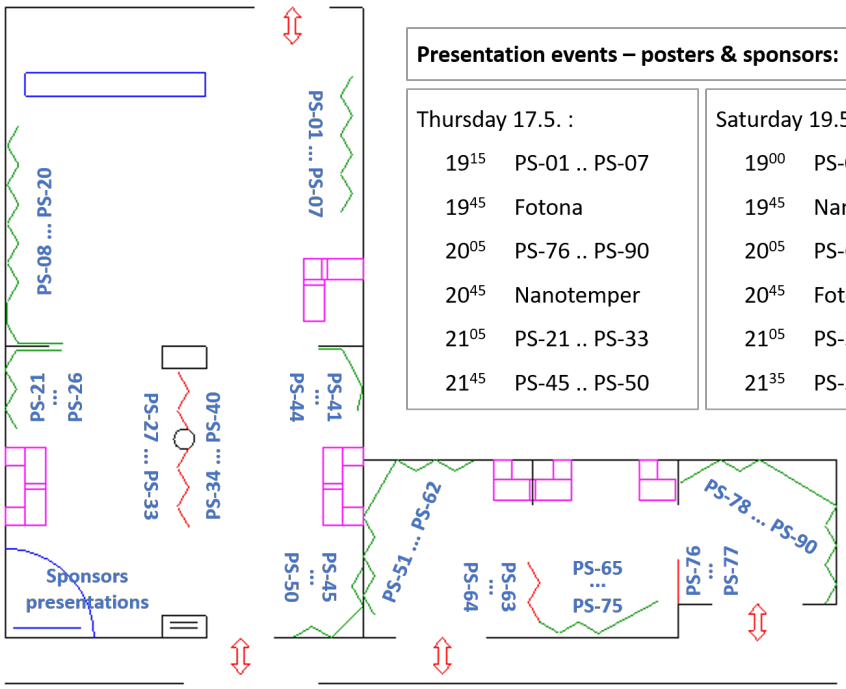
4509297

Contents

1 Maps

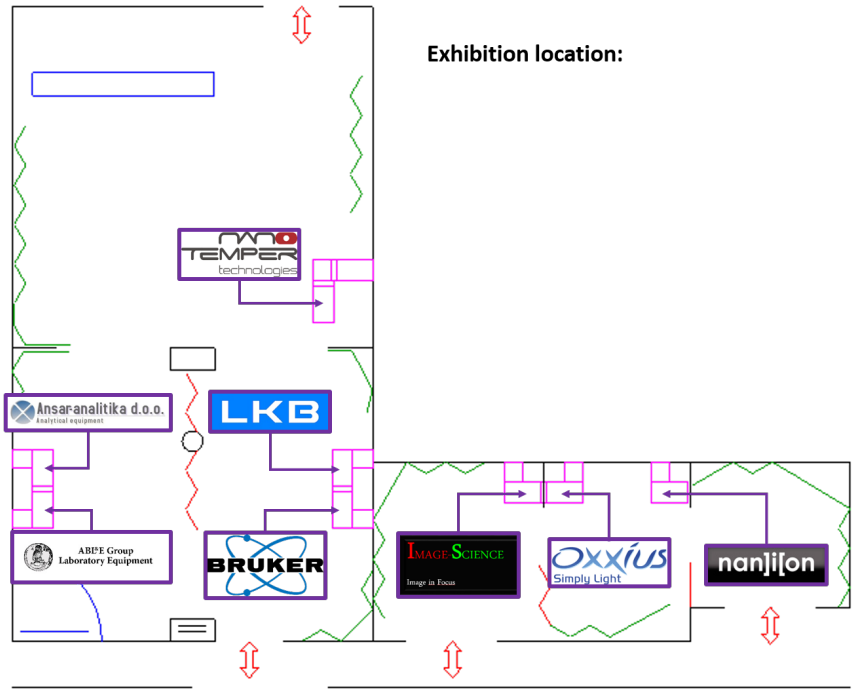

Hotel
entrance
Lecture
hall
Upload
corner
Registration
desk
Hotel
reception
Restaurant
Bar
corridors
Poster and
exhibition
halls

Poster and exhibition halls:



Presentation events – posters & sponsors:

Thursday 17.5. :		Saturday 19.5. :	
19^{15}	PS-01 .. PS-07	19^{00}	PS-08 .. PS-20
19^{45}	Fotona	19^{45}	Nanion
20^{05}	PS-76 .. PS-90	20^{05}	PS-65 .. PS-75
20^{45}	Nanotemper	20^{45}	Fotona
21^{05}	PS-21 .. PS-33	21^{05}	PS-34 .. PS-44
21^{45}	PS-45 .. PS-50	21^{35}	PS-51 .. PS-64



Exhibition location:

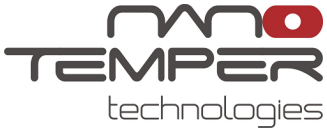
2 Programme

Wednesday, 16. 5. 2018

11:15 *Registration (11:15 - 12:45)*

14:00 *Registration (14:00 - 19:00)*

15:45 *Opening*



System biology, bioinformatics, omics

16:00 Plenary lecture: Mario Cindrić (HR) - Identification of microorganisms by mass spectrometry

16:45 Invited lecture: Marko Djordjević (RS) - Biophysical modeling of bacterial immune system regulation

17:15 Oral: Gergely J. Szöllősi (HU) - Gene transfers, like fossils, can date the tree of life

Ultrafast phenomena

17:30 Plenary lecture: Janos Hajdu (HU) - X-ray Diffraction from Single Particles and Biomolecules

18:15 Contributing lecture: Petar H. Lambrev (HU) - Ultrafast energy transfer dynamics in photosynthetic light-harvesting complexes probed by two-dimensional electronic spectroscopy

18:45 Oral: Sofia M. Kapetanaki (UK) - Understanding the interplay of heme and carbon monoxide in ion channel regulation

19:15 *Get-together dinner*

Thursday, 17. 5. 2018

8:00 *Breakfast & Registration*

Advanced imaging and spectroscopies

8:45 Plenary lecture: Michal Cagalinec (SK) - Cell biophysics of fluorescent probes for super-resolution optical microscopy

9:30 Invited lecture: Iztok Urbančič (SI) - Advanced STED microscopy of the membrane organisation in activating T-cells

10:00 Contributing lecture: Mario Brameshuber (AT) - Monovalent T-cell antigen receptor complexes drive T-cell antigen recognition

10:30 Oral: Josef Lazar (CZ) - 2P or not 2P: single-photon vs. two-photon polarization fluorescence microscopy

10:45 *Break*

Advanced imaging and spectroscopies

11:15 Contributing lecture: Jurij Sablić (SI) - Open Boundary Molecular Dynamics of DNA

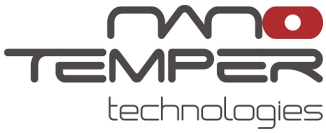
Biomedical applications and cancer

11:45 Invited lecture: Imre Derényi (HU) - Cancer Risk and the Somatic Cell-lineage Tree

12:15 Invited lecture: Gregor Bano (SK)- Phosphorescence kinetics of singlet oxygen produced by pulsed laser excitation - selected special cases

12:45 Contributing lecture: Loredana Casalis (IT) - Cell biomechanics as a marker of disease development: the case of calcific aortic valve disease

13:15 *Lunch*



Biomedical applications and cancer

14:45 Contributing lecture: Pavle R. Andjus (RS) - Subcellular astrocytic markers of neuroinflammation in amyotrophic lateral sclerosis as revealed by synchrotron-based spectromicroscopy

15:15 Oral: Irene Jiménez Munguía (RU) - Bilayer lipid membrane as a model of photodynamic therapy processes in cancer cells

15:30 Oral: Michal Nemergut (SK) - Affinity chromatography based on DARPin and maltose binding protein complex formations

15:45 Oral: Attila Gergely Végh (HU) - Membrane tethers reshape intercellular de-adhesion dynamics

16:00 Oral: Janja Mirtič (SI) - Alginate based microcapsules for local probiotic delivery: Evaluation of bacterial entrapment, release, and growth

16:15 Oral: Aleksandra Pavićević (RS) - EPR spin labeling of erythrocytes as a tool for diagnosis and the follow-up of the treatment of Gaucher disease

16:30 *Break*

Computational biophysics

16:45 Invited lecture: Juraj Szavits-Nossan (HR) - mRNA sequence determinants of protein production rate

17:15 Invited lecture: Marko Gosak (SI) - Network science as a gateway to understanding of emerging dynamics and function of multicellular systems

17:45 Contributing lecture: Magdalena Majekova (SK) - Ligand-based drug design considering specific features of aldose reductase

18:15 Oral: Andrej Dobovišek (SI) - The maximum entropy production principle in enzyme kinetics: novel theoretical insights

Poster session *with buffet dinner (18:45-22:30)*

Oral invitations and sponsor presentations at:

19:15 posters from PS-01 to PS-07,

19:45 Fotona,

20:05 posters from PS-76 to PS-90,

20:45 Nanotemper,

21:05 posters from PS-21 to PS-33,

21:45 posters from PS-45 to PS-50.

Friday, 18. 5. 2018

8:00 *Breakfast & Registration*

Single molecule and interactions

8:45 Plenary lecture: Gerhard J. Schütz (AT) - Varying label density to probe membrane protein nanoclusters in STORM/PALM

9:30 Contributing lecture: Miklós Kellermayer (HU) - Mechanically-driven ejection of viral DNA

10:00 Oral: Saša Vrhovec Hartman (SI) - Diffusiophoresis of blood cells and vesicles in transient chemical gradients

10:15 Oral: Jakub Nowak (PL) – Cutting edge biophysical tools to determine biomolecular interactions and characterize protein stability in solution

10:30 Oral: Andreas M. Arnold (AT) - Overcoming Blinking Artifacts in Nanocluster Detection with Two Color STORM

10:45 *Break*

Materials & Nanobiophysics

11:15 Invited lecture: Erik Reimhult (AT) - Tailoring biomolecular interactions of core-shell nanoparticles and their application to magnetoresponsive drug delivery vehicles

11:45 Invited lecture: Zuzana Garaiova (SK) - Nanoparticles as drug delivery systems. The interaction with biomembrane models

12:15 Contributing lecture: Jaroslaw Jacak (AT) - Bio-applications for 3D Nano-lithography in Microfluidics

12:45 Contributing lecture: Orestis G. Andriotis (AT) - Poking and pulling collagen: Nanomechanical assessment of native collagen fibrils

13:15 Oral: Hana Majaron (SI) - A relevant in vitro model of the alveolus enables reliable studies of nanomaterial-cell interactions

13:30 *Lunch*

15:00 *Excursions*

Remote session **at Ptuj Castle**, »Quo vadis, biophysics«

16:20 Janez Štrancar, opening

16:25 Gerhard Schütz (AT) - Future perspectives of superresolution microscopy

16:30 Mauro Dalla Serra (IT) - COST action ARBRE-MOBIEU

16:35 Andrej Korenić (RS) - AUTOIGG: MSC RISE Action

16:40 Pavle R. Andjus (RS) - Can Belgrade School of Physiology help SpaceX mission to Mars and beyond?

16:45 Laszlo Zimanyi (HU) - ELI-ALPS presentation

16:50 Géza I. Groma (HU) - Ultrafast kinetics of the intramolecular energy migration in NADH

16:55 Tilen Koklič (SI) - In vitro models and H2020 SmartNanoTox

17:00 Jaroslaw Jacak (AT) - Lithography for microfluidics: organ on a chip systems

17:05 András Kincses (HU) - Effects of shear stress on a lab-on-a-chip endothelial model

17:10 General discussion

20:00 *Conference dinner*

Saturday, 19. 5. 2018

8:00 *Breakfast & Registration*

Cell biophysics

8:45 Plenary lecture: Massimo Vassalli (IT) - Mechanosensing in living cells: the emerging role of piezo channels in human patho-physiology

9:30 Invited lecture: Silvia Caponi (IT) - Mechanical and chemical analysis of single living cells investigated by non-contact micro-spectroscopic techniques

10:00 Invited lecture: Nadica Ivošević-de Nardis (HR) - Single cell at the charged interface

10:30 Oral: Edina Szabó-Meleg (HU) - Visualization of transport properties of membrane nanotubes with live cell laser-scanning confocal and super-resolution (SIM) microscopes

10:45 Oral: Miroslav Živić (RS) - ATP dependency of osmotically activated outwardly rectified current in the membrane of cytoplasmic droplets obtained from sporangiophore of model filamentous fungus Phycomyces blakesleeanus

11:00 *Break*

Cell biophysics

11:30 Invited lecture: Gabriella Viero (IT) - Towards understanding of translation biology by coupling positional sequencing and nano-imaging

12:00 Contributing lecture: Matjaž Humar (SI) - Bio-integrated micro-laser particles for sensing, imaging and cell barcoding

12:30 Oral: Peter Galajda (HU) - Fast evolution of antibiotic resistance in microfluidic devices

12:45 Oral: Špela Zemljič Jokhadar (SI) - The role of Arp2/3 complex in cyto-mechanics measured by AFM and optical tweezers

13:00 *Lunch*

Neurobiophysics

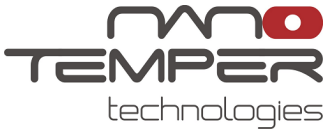
14:30 Plenary lecture: Vladana Vukojević (RS) - Functional Fluorescence Microscopy Imaging (fFMI) of fast dynamic processes in live cells by massively parallel Fluorescence Correlation Spectroscopy

15:15 Invited lecture: Johann G. Danzl (AT) - Improving high-resolution optical imaging for (neuro-)biology

15:45 Contributing lecture: Jernej Jorgačevski (SI) - Exocytotic properties of astrocytic vesicles

16:15 Contributing lecture: Jasna Šaponjić (RS) - Altered sleep oscillations as early biomarkers of Parkinson's disease cholinopathy

16:45 *Break*



Computational biophysics

17:00 Contributing lecture: Péter Horváth (HU)

17:30 Oral: Daniel Grajzel (HU) - Tissue size regulation amplifies the effect of asymmetrical cell divisions on cancer incidence

17:45 Oral: Nebojša Milošević (RS) - Artificial neural networks and multi-dimensional approach in the classification: 2D images of neurons from the human dentate nucleus

18:00 Oral: Alexandra Zahradníková (SK) - Energetics of the open - closed transition in the RYR N-terminal region: importance for the CPVT phenotype

Poster session *with buffet dinner (18:30-22:30)*

Oral invitations and sponsor presentations at:

19:00 posters from PS-08 to PS-20,

19:45 Nanion,

20:05 posters from PS-65 to PS-75,

20:45 Fotona,

21:05 posters from PS-34 to PS-44,

21:35 posters from PS-51 to PS-64.

Sunday, 20. 5. 2018

8:00 *Breakfast*

Proteins & supramolecular structures

8:45 Plenary lecture: Gregor Anderluh (SI) - Mechanism of membrane interactions of Nep1-like proteins

9:30 Invited lecture: Aleksandar J. Krmpot (RS) - Mapping of hemoglobin residuals in erythrocyte ghosts using two photon excited fluorescence microscopy

10:00 Invited lecture: Beáta Bugyi (HU) – Coordination of actine-microtubule dynamics

10:30 Oral: Parveen Akhtar (HU) - Self-aggregation of light-harvesting complex II in reconstituted membranes mimics non-photochemical quenching in plants

10:45 Oral: Gergö Fülöp (AT) - Probing lipid interactions of plasma membrane proteins: a micropatterning approach

11:00 *Break*

Proteins & supramolecular structures

11:30 Contributing lecture: Manuela Basso (IT) - RNA-mediated intercellular miscommunication: role of extracellular vesicle cargos in Amyotrophic Lateral Sclerosis

12:00 Contributing lecture: Isabella Derler (AT) - The multiple roles of Orai N-terminus in CRAC channel function

12:30 Oral: Pavol Hrubovčák (SK) – Location of the general anesthtics in model membranes

12:45 Oral: Adéla Melcrová (CZ) - Influence of a transmembrane domain on calcium-membrane interaction

13:00 Oral: Tea Lenarčič (SI) - Structural basis for NLP toxicity

13:15 *Closing*

13:30 *Lunch*

3 Abstracts

Session S01: System biology, bioinformatics, omics

Session S02: Ultrafast phenomena

Session S03: Advanced imaging and spectroscopies

Session S04: Biomedical applications and cancer

Session S05: Computational biophysics

Session S06: Single molecule and interactions

Session S07: Materials and Nanobiophysics

Session S08: Cell biophysics

Session S09: Neurobiophysics

Session S10: Proteins and supramolecular structures

Session PS: Poster session

S01-PL-01, Session: System biology, bioinformatics, omics

Identification of microorganisms by mass spectrometry

Mario Cindric (1)

(1) Rudjer Boskovic Institute, Division of Molecular Medicine, Bijenicka 54, 10000, Zagreb, Croatia

Currently used techniques for species identification are mostly relied on protein database matching or alternatively on immunological procedures. Although widely used, such an approach does not always provide satisfactory matching, sequence coverage or specific antigen-antibody reaction to unambiguously identify DNA, RNA, lipid, and sugar or peptide/protein of selected species. Mass spectrometry as a tool for species determination was introduced about ten years ago. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identifies microbes using either intact cells or cell extracts. The most abounded proteins from the cell detected in the instrument are matched against commercially available databases that cover relatively limited number of microbes. On the other hand, high resolution mass spectrometers in contrast to MALDI-TOF MS could provide more accurate and precise results after de novo sequencing of analyzed proteins.

Keywords: Mass spectromety;biotypization; microorganisms

Back to programme

S01-IL-02, session: System biology, bioinformatics, omics

Biophysical modeling of bacterial immune system regulation

Marko Djordjevic (1)

(1) Faculty of Biology, University of Belgrade

Bacterial immune systems (CRISPR-Cas and restriction-modification systems) defend bacterial cells against invasion by viruses or plasmids. CRISPR-Cas has also found major biotechnology applications, but to optimally exploit them, it is necessary to understand native function of this system in bacteria. CRISPR-Cas is typically silent under normal conditions, and one of the main questions in understanding CRISPR-Cas functioning is how this normally silent system is induced, which is very hard to directly experimentally observe. Consequently, CRISPR-Cas induction mechanism is currently unknown, and the main goal of this work is to use computational modeling to understand the role of the key features in CRISPR-Cas activation, and to propose a realistic experimental model for CRISPR-Cas induction. To address these questions, we use thermodynamical modeling of the system transcription regulation, and dynamical modeling of the relevant molecular species (RNA and proteins). Furthermore, much more rudimental bacterial immune systems (restriction-modification) exhibit similar features in their transcription regulation as CRISPR-Cas, and likely face similar dynamical constraints in their functioning. Consequently, we also model regulation of bacterial restriction-modification systems with different architectures, and propose that the same design principles may be behind regulation of both bacterial immune systems. Finding the same design principles behind mechanistically otherwise different systems (in this case CRISPR-Cas and restriction-modification systems) is also a major goal of systems biology.

Financing: Ministry of Education and Science of Serbia (OI173052), Swiss National Science Foundation SCOPES project (IZ73Z0_152297)

Keywords: biophysical modeling, computational systems biology, CRISPR-Cas, restriction modification systems, gene expression regulation

Back to programme

S01-OR-03, session: System biology, bioinformatics, omics

Gene transfers, like fossils, can date the tree of life

Gergely J Szöllősi (1)

(1) Eötvös University, Budapest

The geological record provides the only source of absolute time information to date the tree of life. But most life is microbial, and most microbes do not fossilize, leading to major uncertainties about the ages of microbial groups and the timing of some of the earliest and most important events in life's evolutionary history. I discuss our recent results, which show that patterns of lateral gene transfer deduced from analysis of modern genomes encode a novel and highly informative source of information about the temporal coexistence of lineages throughout the history of life. We use new phylogenetic methods to reconstruct the history of thousands of gene families and show that dates implied by gene transfers are strongly correlated with estimates from relaxed molecular clocks in Bacteria, Archaea and Eukaryotes. A comparison with mammalian fossils shows that gene transfer in microbes is potentially as informative for dating the tree of life as the geological record in macroorganisms.

Back to programme

S02-PL-01, Session: Ultrafast phenomena

X-ray Diffraction from Single Particles and Biomolecules

Janos Hajdu (1,2)

(1) Uppsala University, Uppsala, Sweden
(2) The European Extreme Light Infrastructure, Prague, Czech Republic

Theory predicts that with an ultra-short and extremely bright coherent X-ray pulse, a single diffraction pattern may be recorded from a large macromolecule, a virus, or a cell before the sample explodes and turns into a plasma. The talk will summaries the experimental results in this area.

Back to programme

S02-CL-02, session: Ultrafast phenomena

Ultrafast energy transfer dynamics in photosynthetic light-harvesting complexes probed by two-dimensional electronic spectroscopy

Parveen Akhtar (1), Cheng Zhang (2), Nhut Thanh Do (2), Howe-Siang Tan (2)
Presented by: Petar H. Lambrev (1)

(1) Hungarian Academy of Sciences, Biological Research Centre
(2) Nanyang Technological University, School of Physical and Mathematical Sciences

Efficient photosynthetic light energy conversion depends on the ability of the photosynthetic apparatus to transfer the captured solar energy to the photochemical reaction centres without losses. Resolving the ultrafast kinetics of photosynthetic energy and electron transfer is a challenging experimental task. Two-dimensional electronic spectroscopy (2DES) has proven to be a powerful technique for mapping energy transfer pathways with high spectral and temporal resolution (1, 2). By correlating the frequencies of the molecular states interacting with the excitation (pump) and detection (probe) pulses, 2DES reveals coupled donor-acceptor pairs and the time dependence of energy transfer between them. We have measured the excited-state dynamics in plant light-harvesting complex II (LHCII) by 2DES at cryogenic and physiological temperatures. Using broadband excitation pulses that overlap with the lowest-lying electronic excited state of all chlorophyll a molecules in LHCII, we were able to simultaneously resolve dowhhill and uphill exciton equilibration pathways. By this means the spectral equilibration dynamics could be clearly identified and separated from, e.g., radiative or nonradiative excitation decay. The results punctuate the necessity, and provide the experimental basis, to develop a unified model of excitation energy transfer that can explain the high resolution spectroscopy results obtained at low temperatures as well as the kinetics in physiological conditions.

(1) Fuller FD, Ogilvie JP. Experimental implementations of two-dimensional Fourier transform electronic spectroscopy. Annu Rev Phys Chem. 2015;66:667-90.
(2) Oliver TA. Recent advances in multidimensional ultrafast spectroscopy. Royal Society Open Science. 2018;5(1):171425.

Keywords: multidimensional spectroscopy; exciton relaxation; ultrafast spectroscopy; photosynthesis; chlorophyll-protein complexes

Financing: The work was supported by grants from the Singapore Ministry of Education Academic Research Fund (Tier 2 MOE2015-T2-039), the Hungarian Ministry for National Economy (GINOP-2.3.2-15-2016-00001), and the National Research, Development and Innovation Office (NKFIH NN 124904).

Back to programme

S02-OR-03, Session: Ultrafast phenomena

Understanding the interplay of heme and carbon monoxide in ion channel regulation

Sofia M. Kapetanaki (1), Mark J. Burton (2), Morgan Thomas (1), Jaswir Basran (2), Manuel J. Llansola-Portoles (3), Nicola Portolan (2), Chiasa Uragami (3), Bruno Robert (3), Tatyana Chernova (4), Peter C. E. Moody (2), Ralf Schmidl (2), Jérôme Santolini (3), Pierre Dorlet (3), John S. Mitcheson (2), Noel W. Davies (2), Marten H. Vos (5), Nina M. Storey (2), Emma L. Raven (1)

(1) Department of Chemistry, University of Leicester, Leicester, LE1 7RH, UK
(2) Department of Molecular and Cell Biology, University of Leicester, Leicester, LE1 9HN, UK
(3) I2BC, CEA, CNRS, Université Paris-Saclay, 91198, Gif-sur- Yvette cedex, France
(4) †MRC Toxicology Unit, Leicester LE1 9HN, UK
(5) LOB, Ecole Polytechnique, CNRS, INSERM, 91128 Palaiseau Cedex, France

Heme is essential for living organisms, functioning as a crucial element in the redox-sensitive reaction centre in hemeproteins. An emerging role of heme is its ability to regulate ion channel function (1). At the same time, the well-known intracellular signaling molecule carbon monoxide (CO) is recognized as playing a key biological role in regulation of ion channel response.Using electrophysiological and biochemical methods and a combination of spectroscopic techniques (electron paramagnetic resonance, vibrational spectroscopy and ultrafast absorption spectroscopy) we have identified and quantified the interaction of heme with cytoplasmic domains of the channels and its interplay with CO (2, 3, 4).We use this information to present molecular-level insight into the dynamic processes that control the interactions of CO with heme-regulated channel proteins, and we present a structural framework for understanding the complex interplay between heme and CO in ion channel regulation.

(1) Tang, X.D.et al. Haem can bind to and inhibit mammalian calcium-dependent Slo 1 BK Channels. Nature. 2003; 425:531-535.doi:10.1038/nature02003
(2) Burton, M.J.et al. A heme-binding domain controls regulation of ATP-dependent potassium channels.Proc. Natl. Acad. Sci. U.S.A. 2016;113: 3785-3790.doi: 10.1073/pnas.1600-211113
(3) Kapetanaki, S.M., et al. A mechanism for CO regulation of ion channels Nat. Commun. 2018; 9, 907.doi: 10.1038/s41467-018- 03291-z
(4) Kapetanaki S.M.et al. (unpublished results)

Back to programme

S03-PL-01, session: Advanced imaging and spectroscopies

Cell biophysics of fluorescent probes for super-resolution optical microscopy

Michal Cagalinec (1)

(1) Institute of Molecular Physiology and Genetics Centre of Biosciences Slovak Academy of Sciences Dubravska cesta 9 Pavilion of Medical Sciences 2B 3.66 P.O. BOX 63 840 05 Bratislava

Several light microscopy methods how to break the classical Abbe's optical resolution limit exist nowadays. The most developed and at the same time providing the best theoretical resolution of 20 nm is the PhotoActivation Localization Microscopy (PALM)/STochastic Optical Reconstruction Microscopy (STORM). For PALM, unique optical property of photoswitchable/photoactivatable fluorescent proteins, namely irreversible photoconversion is principal factor to achieve desired resolution. These proteins were originally found in reef corals and their optical and physico-chemical properties, as excitation-emission spectra, quantum yield and monomeric forms were improved by genetic technologies and molecular modelling. In case of STORM photoswitching is facilitated by chemically synthetized chromophores. Due to high resolution demands, PALM/STORM work with fixated samples only. To be possible to acquire living cells, although by reduction in resolution, several new approaches were introduced including STimulated Emission Depletion (STED) microscopy. In case of STED microscopy resolution drops down to 60 nm by rapid bleaching of a donut around the central excited point by a STED laser. Additional benefit of STED microscopy represents use of standard fluorescent proteins. Independently, advanced fluorophores attached to antibodies are commercially available in these days as well. In this lecture we will present principles of the methods mentioned above and the properties of chromophores available for PALM/STORM. We will present several practical applications of PALM/STORM including mitochondrially targeted photoconvertible protein KikumeGR1 and outer mitochondrial membrane targeted protein Fis-EOS. Moreover, we will introduce the STED microscopy available in our laboratory and will present practical aspects to optimize the image acquisition.

Financing: SASPRO 0063/01/02, VEGA 2/0169/16, APVV 15-0302, ITMS 26230120006

Keywords: Super-resolution microscopy, PALM, STORM, STED, photoconvertible proteins

Back to programme

S03-IL-02, session: Advanced imaging and spectroscopies

Advanced STED microscopy of the membrane organisation in activating T-cells

Iztok Urbancic (1,2), Erdinc Sezgin (1), Falk Schneider (1), Francesco Reina (1), Ana Mafalda Santos (1), Dominic Waithe (3), Simon Davis (3), Christian Eggeling (1,4)

(1) MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK
(2) Condensed Matter Physics Department, "Jožef Stefan" Institute, Ljubljana, Slovenia
(3) MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK UK
(4) Institute of Applied Optics, Friedrich-Schiller-University amp; Leibniz Institute of Photonic Technology (IPHT), Jena, Germa

Nanoscale organization of the membranes of living cells plays crucial roles in numerous vital processes, including during the activation of T-cells and their formation of the immunological synapse. However, the detailed nature and function of reorganization of lipids during this key initiating event of immune response remain largely unclear. To gain further insight into the mechanisms, we employed two techniques that probe complementary properties of the membranes at the molecular level: STED-FCS to reveal detailed picture of the diffusion of the lipids, and spectral imaging with environment-sensitive membrane probes, i.e. microspectroscopy, to map differences in local molecular order within the lipid bilayer. We correlated both properties with the locations of the key membrane proteins responsible for triggering the activation of T-cells, such as T-cell receptor. In search for the most sensitive description of the membrane order, we systematically compared the established methods of analysis and representation: generalized polarization, lineshape fitting, and spectral phasors.

Financing: European Commission, Welcome Trust, Wolfson Foundation, MRC, EPSRC, BBSRC, University of Oxford (John Fell Fund)

Keywords: Fluorescence microscopy; Fluorescence correlation spectroscopy; STED; T-cells; immunology

Back to programme

S03-CL-03, session: Advanced imaging and spectroscopies

Monovalent T-cell antigen receptor complexes drive T-cell antigen recognition

Mario Brameshuber (1)

(1) TU Wien, Institute of Applied Physics - Biophysics

T-cells recognize MHC-embedded antigenic peptide fragments (pMHCs) on antigen presenting cells (APCs) through their clonotypic T-cell antigen receptors (TCRs), which typically feature only moderate affinities towards their pMHC ligands. Higher order TCR-structures, either constitutively present or dynamically formed upon pMHC binding, have thus long been considered instrumental for intracellular T-cell signaling and for maintaining high antigen sensitivity. Here we have determined the stoichiometry of the TCR and their non-covalently associated CD3 signaling chains in living antigen-experienced T-cells with the use of (i) single molecule Fluorescence Recovery After Photobleaching, (ii) Förster Resonance Energy Transfer (FRET) and (iii) photon arrival time - based fluorescence correlation spectroscopy (FCS) measurements. Using these approaches, we find exclusively monomeric TCR entities prior to and during pMHC binding. Hence, molecular events other than TCR clustering drive the recognition pMHCs at both high and low densities (1).

(1) Brameshuber et al. Monomeric TCRs Drive T-Cell Antigen Recognition. Nature Immunology. Accepted.

Financing: FWF, projects I953-B20

Keywords: immunology; single molecule fluorescence; FRET; photon antibunching; FCS; T-cells; TCR/CD3

Back to programme

S03-CL-05, Session: Advanced imaging and spectroscopies

Open Boundary Molecular Dynamics of DNA

Jurij Sablić (1)

(1) Laboratory for Molecular Modeling, National Institute of Chemistry, Ljubljana, Slovenia

The composition and electrolyte concentration of the aqueous bathing environment have important consequences for many biological processes and can profoundly affect the behavior of biomolecules. Nevertheless, because of computational limitations, many molecular simulations of biophysical systems can be performed only at specific ionic conditions: either at nominally zero salt concentration, i.e., including only counterions enforcing the system's electroneutrality, or at excessive salt concentrations. In this talk, we present an efficient molecular dynamics simulation approach for an atomistic DNA molecule at realistic physiological ionic conditions. The simulations are performed by employing the open boundary molecular dynamics method that allows for simulation of open systems that can exchange mass and linear momentum with the environment. In the explicit domain, the water molecules and ions are both overtly present in the system, whereas in the implicit water domain, only the ions are explicitly present and the water is described as a continuous dielectric medium. Water molecules are inserted and deleted into/from the system in the intermediate buffer domain that acts as a water reservoir to the explicit domain, with both water molecules and ions free to enter or leave the explicit domain. Our approach is general and allows for efficient molecular simulations of biomolecules solvated in bathing salt solutions at any ionic strength condition.

[1] Julija Zavadlav, Jurij Sablić, Rudolf Podgornik, Matej Praprotnik, Biophys. J., DOI: https://doi.org/10.1016/j.bpj.2018.02.042

Back to programme

S03-OR-04, session: Advanced imaging and spectroscopies

2P or not 2P: single-photon vs. two-photon polarization fluorescence microscopy

Olga Rybakova (1), Alexey Bondar (2), Josef Lazar (3)

(1) Inst. of Organic Chemistry and Biochemistry CAS
(2) Microbiology Institute CAS
(3) Inst. of Microbiology CAS Vídeňská 1083 142 20 Praha 4

Polarization fluorescence microscopy, also called fluorescence resolved linear dichroism microscopy, has in the past several years been applied to a number of biological and models systems. It has been used for observations of molecular events in living cells, as well as for gaining insights into molecular organization. The two main implementations, utilizing single-photon and two-photon excitation, possess distinct advantages and disadvantages. Here we present the results of our direct comparisons of the two techniques in applications ranging from observations of molecular processes in living cells to gaining insights into membrane protein structure.

Financing: INTERREG, C4Sys, GACR

Keywords: fluorescence microscopy; cell signaling

Back to programme

S04-IL-01, session: Biomedical applications and cancer

Cancer Risk and the Somatic Cell-lineage Tree

Imre Derenyi (1), Gergely J Szollosi (1), Marton Demeter (1), Daniel Grajzel (1), Mate Kiss (1)

(1) Eotvos University

All the cells of an organism are the product of cell divisions organized into a single binary tree. This somatic cell-lineage tree is not uniform in the sense that its lineages have different lengths. As cell divisions are accompanied by replication errors, longer cell lineages are more prone to the accumulation of mutations and, thereby, to somatic evolution, which can potentially lead to the development of cancer. By mapping the accumulation of driver mutations along a somatic cell-lineage tree into a graph theoretical problem, we have been able to derive an analytical formula for the probability of carcinogenesis in an arbitrary cell-lineage tree with a given rate of driver mutations per cell division. The result is consistent with epidemiological data and highlights the significance of the longest cell lineages. We also show how tissues can minimize the length of their longest lineages through differentiation hierarchies.

Keywords: cancer; evolution; mutations

Back to programme

S04-IL-02, Session: Biomedical applications and cancer

Phosphorescence kinetics of singlet oxygen produced by pulsed laser excitation - selected special cases

Gregor Bano (1)

(1) Department of Biophysics, Fakulty of Science, P. J. Safarik University in Kosice Jesenna 5 04001 Kosice Slovakia

Singlet oxygen is a highly reactive molecule, which is studied intensively because of its essential role in photodynamic therapy (PDT) [1]. During PDT, the oxidative stress induced by singlet oxygen in living cells leads to cell death. Singlet oxygen is mostly produced by energy transfer between light-activated photosensitizer molecules and ground state oxygen molecules, and can be detected through its phosphorescence at 1270 nm. The time-course of the phosphorescence signal is usually measured after short-pulse laser excitation of the photosensitizer.
Two special cases of singlet oxygen phosphorescence kinetics will be discussed. First, the conventional formula describing the singlet oxygen phosphorescence signal needs to be modified, when working with long excitation pulses [2]. The major advantage of this quasi-continuous excitation is the suppression of photo-bleaching. Second, modified phosphorescence kinetics applies in the situation when singlet oxygen is produced inside of nano-particles (e.g. drug nano-carriers). In this case, singlet oxygen may diffuse out of the particles, which affects the time-course of the phosphorescence signal.

(1) Ogilby, P. R., Singlet Oxygen: There is Indeed Something New Under the Sun. Chemical Society Reviews, 2010;39(8): 3181-3209.
(2) Varchola J, Huntosova V, Jancura D, Wagnieres G, Miskovsky P, Bano G. Temperature and oxygen-concentration dependence of singlet oxygen production by RuPhen as induced by quasi-continuous excitation, Photochem. Photobiol. Sci., 2014;13: 1781-1787.

Keywords: singlet oxygen; photodynamic therapy

Financing: This work was supported by the APVV-15-0485 grant of the Slovak Ministry of Education and the project (ITMS code: 26220220107) of the Operation Program Research and Development funded by the European Regional Development Fund.

Back to programme

S04-CL-03, Session: Biomedical applications and cancer

Cell biomechanics as a marker of disease development: the case of calcific aortic valve disease

Loredana Casalis (1), Luisa Ulloa Severino (1,2) , Ilaria Rago (1,2) , Fabio Perissinotto (1,2), Denis Scaini (1,3), Rosaria Santoro (4) , Maurizio Pesce (4)

(1) Elettra Sincrotrone Trieste, Trieste, Italy
(2) Università di Trieste, Trieste, Italy
(3) Scuola Superiore di Studi Avanzati (SISSA), Trieste, Italy
(4) Centro Cardiologico Monzino, Milano, Italy

Cells sense forces from the extracellular matrix (ECM) and transduce them into biochemical signals. The molecules produced cause in turn remodeling of the ECM. Molecular altered expression will affect this force sensing mechanism changing cellular properties as migration, differentiation, etc. Therefore, cells mechanical properties can be used as a marker for the early diagnosis of pathologies as cancer or cardiovascular diseases. In this framework, Atomic Force Microscopy (AFM) represents an excellent tool to evaluate the mechanical properties of different cellular systems.
In this talk, we will analyze the mechanical properties of aortic valve interstitial cells (VICs), the predominant constituent of aortic valves, governing ECM structure and composition, in the onset of calcific aortic valve disease (CAVD).
In particular, we obtained adhesion polymeric substrates with different stiffness onto which human AoV VICs were plated, and subsequently investigated for the cytoskeleton dynamics and the activity of the mechanosensing-activated transcription factor YAP. We found that cells were subject to a reversible stiffness-dependent nuclear translocation of the transcription factor in concert with an increase in cytoskeleton tensioning and loading of the myofibroblast-specific protein αSMA onto the F-actin cytoskeleton.
Then, we studied the interaction between porcine VICs and optically transparent, vertically aligned carbon nanotube (CNT) substrates, mimicking the chemical/morphological role of natural ECM. Here we found that the number of myofibroblasts (correlated to disease-associated phenotype) was similar to the case of healthy valves, and that fibroblasts on CNT matrix resulted in higher stiffness and higher number of focal adhesions, with respect to reference glass. AFM imaging of the inner membrane of VICs broken up by osmotic shock allowed to observe that CNTs are piercing and pinching the plasma membrane, in this way facilitating the creation of clusters of FAs that contribute to increase cellular rigidity.

Back to programme

S04-CL-04, session: Biomedical applications and cancer

Subcellular astrocytic markers of neuroinflammation in amyotrophic lateral sclerosis as revealed by synchrotron-based spectromicroscopy

Pavle R Andjus (1), Stefan Stamenkovic (1), Tanja Ducic (2)

(1) University of Belgrade Faculty of Biology
(2) CELLS-ALBA synchrotron light source

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. Although this is a neuronal disease, non-neuronal cells play a crucial role. We will present our investigations of cell morphology and subcellular organization of glial cells – astrocytes isolated from hSOD1 G93A transgenic ALS rats as compared to nontransgenic littermates. Cortical astrocytes were cultured and whole intact cells were islotaed vitrified i.e. cryopreserved, close to their native state, without any staining or chemical embedding or mechanical modification. Elemental content including trace elements and their cellular localization in situ was investigated using X-ray fluorescence (XRF) on the synchrotron beamline. "Water window" low energy range (520 eV) high resolution "soft" transmission X-ray tomography (TXM) was also used to obtain 3D tomography of intact astrocytes. Comprehensive bio-macromolecular subcellular compartmentalization was also obtained by means of Fourier transform infrared spectro-microscopy (FTIR). All these techniques are chemically sensitive and provide complementary information on single cell biophysical chemistry. Subcellular changes with clear cytosol ultra-structural reorganization with multivesicular organelle clustering probably resulting from protein aggregation was observed by TXM on ALS astrocytes. XRF imaging revealed higher concentrations of Cu, S and P in the hSOD1 G93A mutant astrocytes. In addition, FTIR spectro-microscopy, sensitive to the macromolecular changes, showed a shift in Amide I band towards the lower frequency, suggesting the increase of anti-parallel -sheet structure of proteins in ALS astrocytes. Furthermore, FTIR choline peak disappeared in ALS cells. These data thus point to conformational changes of proteins (presumably SOD1) leading to aggregation and subcellular organelle reorganization in ALS astrocytes.

Financing: MESTD RS grant no III41005, Biostruct-X

Keywords: glia, neuroinflammation, ALS, synchrotron light source

Back to programme

S04-OR-05, session: Biomedical applications and cancer

Bilayer lipid membrane as a model of photodynamic therapy processes in cancer cells

Irene Jiménez Munguía (1), Kirill Birin (2), Yulia Gorbunova (3), Valeri Sokolov (2)

(1) National University of Science and Technology-MISiS Leninskiy prospekt 4 (, 4). Russian Federation ( ) Moscow ()
(2) A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, 31/4 Leninskiy pr. 119071 Moscow, Russia;
(3) A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, 31/4 Leninskiy pr. 119071 Moscow, Russia; N.S. Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, 31 Leninskiy pr. 1

The photodynamic therapy of cancer is based on using of photosensitizers (PS) which bind to cells and kill them by singlet oxygen (SO) generated under illumination. These processes were studied in vitro on a model system – bilayer lipid membrane (BLM). We have studied adsorption and photodynamic efficiency of various derivates of porphyrins. The binding of PS to BLM as well as damage of incorporated into it special molecules — SO targets — were monitored by measuring of boundary potential (BP) by a method developed by us earlier. The BP was negative in the case of anions of sulfonated tetraphenylporphyrines (TPPS4) and positive one with cations of b-imidazolyl substituted tetraphenylporphyrine (b-ImTPP) and corresponding Zn(II) complex (b-ImTPPZn). The photodynamic activity of these PS was evaluated as rate R of damage of the SO targets (T) — molecules of di-4-ANEPPS adsorbed on the surface of BLM, under illumination. The rate of oxidation of T molecules adsorbed on (cis) side of the membrane containing PS molecules was compared with that when T were adsorbed on opposite (trans) side. The value of R at cis- side was higher than that at trans- side in contrast to our earlier study when SO was generated by sulfonated aluminum(III) phthalocyanines (1,2). The difference between porphyrins and phthalocyanines was explained by different positions of these PS on the boundary of the membrane.

1. Sokolov V. S., Gavrilchik A. N., Kulagina A. O., Meshkov I. N., Pohl P., Gorbunova Y. G. Voltage-sensitive styryl dyes as singlet oxygen targets on the surface of bilayer lipid membrane. J Photochem Photobiol B. 2016;161: 162-169.
2. Sokolov V. S., Shcherbakov A. A., Tashkin V. Y., Gavril'chik A. N., Chizmadzhev Y. A., Pohl P. Oxidation and lateral diffusion of styryl dyes on the surface of a bilayer lipid membrane. Elektrokhimiya. 2017; 53: 1171-1181.

Financing: Russian Scientific Foundation (14-13-01373).

Keywords: photodynamics therapy, porphyrins

Back to programme

S04-OR-06, session: Biomedical applications and cancer

Affinity chromatography based on DARPin and maltose binding protein complex formations

Michal Nemergut (1), Erik Sedlák (2)

(1) Department of Biophysics, Pavol Jozef Šafárik University in Košice, Slovakia
(2) Center for Interdisciplinary Biosciences, Pavol Jozef Šafárik University in Košice, Slovakia

DARPins (designed ankyrin repeat proteins) as a novel class of binding proteins with non-immunoglobulin scaffolds are in the center of interest of protein engineering research. DARPins can be rapidly engineered to detect diverse target proteins with high specificity and affinity. From this point of view, they offer an attractive alternative to antibodies. In this work, we study DARPin, which was selected with high specificity and affinity against maltose binding protein (MBP), with an intention to explore its application potential. MBP is a globular protein that is commonly used as a tag for MBP-conjugates purification with purpose to increase their solubility and expression yield. The main goal of this study is to develop the affinity chromatography for MBP-conjugates purification based on interaction between the DARPin and MBP. In an effort to find optimal working conditions, we study the influence of solvent pH on the stability of these proteins as well as on affinity of interaction between them. The results we have obtained suggest that our model of affinity chromatography has strong potential in purification of proteins containing MBP tag with comparable or higher expression yield of purified protein than commercial affinity chromatography.

(1) Binz H.K. High-affinity binders selected from designed ankyrin repeat protein libraries. Nat. Biotechnol. 2004, 22: 575-582.
(2) Wetzel S. Folding and unfolding mechanism of highly stable full-consensus ankyrin repeat proteins. J. Mol. Biol. 2008. 376: 241-257.

Financing: APVV-15-0069; VEGA-1/0423/16

Keywords: affinity chromatography; DARPin; maltose binding protein

Back to programme

S04-OR-07, session: Biomedical applications and cancer

Membrane tethers reshape intercellular de-adhesion dynamics

Attila Gergely Végh (1), Reka Anita Domokos (2), Béla Varga (1,3), Csilla Fazakas (1), Imola Wilhelm (1,4), Istvan Krizbai (1,4), Zsolt Szegletes (1), György Váró (1)

(1) Institute of Biophysics, Biological Research Centre, Temesvári Krt. 62, H-6726 Szeged
(2) Babes-Bolyai University, Faculty of Physics, 1 Mihail Cogalniceanu street, RO-400084 Cluj-Napoca
(3) Laboratoire Charles Coulomb, UMR 5221 CNRS – Université Montpellier, Place Eugen Batallion CC074 Bat.21, 34095 Montpellier, France
(4) Institute of Life Sciences, Vasile Goldis Western University, 86 Liviu Rebreanu street, RO-310045, Arad, Romania

Proper homeostasis of the central nervous system relies on the structural and functional integrity of the neurovascular unit (NVU). The most important physiological function of the NVU is formation and maintenance of the blood-brain barrier (BBB). Anatomically the cerebral microvascular endothelium together with pericytes, astrocytes, neurons and the extracellular matrix builds up the NVU. From a mechanical point of view, the endothelium is the most exposed to mechanical stress they represent the first defense and signaling line of the BBB. Unfortunately, most of the neoplasms found in the CNS are of metastatic origin. The first and crucial step of brain metastasis formation is the establishment of firm adhesion between the blood travelling tumor cells and the tightly connected layer of the endothelium. Amongst all tumors, the melanoma exhibits elevated frequency to metastasise to the brain. Intercellular dynamics might present crucial nanomechanical aspects, therefore direct investigation with high accuracy provides important information. Using single-cell force spectroscopy, de-adhesion dynamics of melanoma cells with different level of malignancy (WM35, A2058 and A375) from a confluent layer of brain micro-capillary endothelial cells (hCMEC/D3) was investigated. Based on simple mechanical assumptions, hereby we present our latest data on comparing the intercellular deüadhesion dznamics. Apparent mechanical properties showed altered characteristics pointing towards cell type dependent aspects. Our results show that nanomechanical properties can be associated to higher metastatic potential and invasive characteristics may rely on stronger adhesive properties mediated by altered tether formation dynamics.

Financing: National Science Fund of Hungary OTKA K116158, PD115697, PD121130, the GINOP-2.3.2-15-2016-00001 / 00020 / 00034 and GINOP-2.3.3-15-2016-00030 programs. IM Wilhelm BO/00334/16/8 and AG Végh and BO/00598/14/8 were supported by the János Bolyai Fellowship.

Keywords: single cell force spectroscopy; membrane tether; metastatic potential

Back to programme

S04-OR-08, session: Biomedical applications and cancer

Alginate based microcapsules for local probiotic delivery: Evaluation of bacterial entrapment, release, and growth

Janja Mirtič (1), Tomaž Rijavec (2), Špela Zupančič (1), Aleš Lapanje (2), Julijana Kristl (1)

(1) Faculty of Pharmacy, University of Ljubljana, Slovenia
(2) Institute Josef Stefan, Ljubljana, Slovenia

The delivery of probiotics to different sites of action within the human body can help to prevent or even treat several diseases. The action of priobiotics is either based on the shaping of local microbiota or interacting with host cells resulting in the reversal of disease progression. The primary aim of this study was to develop microcapsules for delivery of probiotic bacteria that promote their prolonged survival and efficient revival and successful colonisation of the target surface. Encapsulation was based on alginate crosslinking with calcium ions and was performed by prilling the polymer dispersion supplemented with the probiotic using membrane vibration technology, followed by chitosan coating by polyelectrolyte complexation. The microcapsules were further dried by lyophilisation. Up to 2 10 10 CFU/g dry microcapsules of probiotic spore was encapsulated, achieving 100% of microcapsules with incorporated revivable probiotics. The chitosan coating improved their bioadhesion potential, but did not have negative impact on viability and growth kinetics of the bacteria. Alginate matrix was sufficient for bacteria entrapment, allowed the inward diffusion of nutrients and oxygen, and was simply disentangled to enable fast cell release. Surprisingly, already nanometer thin chitosan layer, represented a barrier, which enabled also sustained release of the probiotic bacteria. The collected results propose that the developed probiotic-loaded microcapsules are suitable for local delivery into periodontal pockets (1).

1. Mirtič J, Rijavec T, Zupančič Š, Zvonar Pobirk A, Lapanje A, Kristl J. Development of probiotic-loaded microcapsules for local delivery: physical properties, cell release and growth. Journal of Pharmaceutical Sciences. 2018;Under Review.

Financing: Slovenian Research Agency through the P1-0189 research programme and the J1-6746 and J4-7640 projects.

Keywords: microcapsules, probiotics, alginate matrix, cell growth

Back to programme

S04-OR-09, session: Biomedical applications and cancer

EPR spin labeling of erythrocytes as a tool for diagnosis and the follow-up of the treatment of Gaucher disease

Aleksandra Pavićević (1), Milan Lakočević (2), Ana Popović-Bijelić (1), Marko Daković (3), Miloš Mojović (3)

(1) EPR laboratory, Faculty of Physical Chemistry, University of Belgrade
(2) Clinic of Endocrinology, Diabetes and Metabolic Diseases, Clinical Centre of Serbia
(3) EPR laboratory, Faculty of Physical Chemistry,University of Belgrade

Gaucher disease is the most common lysosomal storage disorder caused by more than 300 identified mutations in the GBA1 gene that encodes -glucocerebrosidase. This enzyme is a lysosomal membrane protein which catalyzes the degradation of glucocerebroside to glucose and ceramide (1). GBA1 gene mutations can result in an altered/impaired function of -glucocerebrosidase, causing glucocerebroside to accumulate inside the cells. Consequently, due to the altered lipid composition, the membrane fluidity may be affected. In this study, membrane fluidity of erythrocytes from type 1 Gaucher disease (GD) patients (receiving two different doses of enzyme replacement therapy (ERT), and therapy-naive) was evaluated using the electron paramagnetic resonance (EPR) spin labeling method. Spin labeling of the cell membranes was performed using a 5-doxyl stearic acid, due to its preferential incorporation into the both inner and outer leaflet of the less ordered parts of the membrane. It was observed that there is a statistically significant difference in the order parameter between erythrocyte membranes of healthy controls and therapy-naive GD patients. Furthermore, based on the order parameter values, it was found that introduction of the ERT leads to restoration of the physiological membrane fluidity. Accordingly, spin labeling of erythrocyte membranes could serve as a simple test for the diagnosis of GD, and the efficiency of ERT.

(1) Horowitz M, Elstein D, Zimran A, Goker-Alpan O. New Directions in Gaucher Disease. Hum. Mutat. 2006;37: 1121–1136. doi: 10.1002/humu.23056

Financing: Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number III41005]

Keywords: Electron paramagnetic resonance; Gaucher disease; Membrane fluidity; Peripheral blood mononuclear cells; Spin labeling

Back to programme

S05-IL-01, session: Computational biophysics

mRNA sequence determinants of protein production rate

Juraj Szavits-Nossan (1), Luca Ciandrini (2), Maria Carmen Romano (3)

(1) School of Physics and Astronomy, University of Edinburg
(2) DIMNP, Universite de Montpellier, CNRS
(3) Institute for Complex Systems and Mathematical Biology, Department of Physics, University of Aberdeen

Protein synthesis is of paramount importance to every living cell. The major challenge in molecular biology is to understand how information that is encoded in an mRNA transcript affects the rate of mRNA translation and therefore the overall protein production (1). Computational models of mRNA translation that aim to address this challenge have a long history dating as back to 1960s (2,3). So far these models had very limited power in predicting determinants of translation efficiency, owning to the fact that ribosome dynamics operates out of equilibrium and is therefore beyond the realm of equilibrium statistical physics. I present here a novel mathematical method for solving a standard biophysical model of translation. The solution shows an excellent agreement when compared to numerical genome-wide simulations of S. cerevisiae transcript sequences and predicts that the first 10 codons, together with the value of the initiation rate, are the main determinants of protein production rate. I also discuss potential experiments for testing these predictions.

(1) Gingold H, Pilpel Y. Determinants of translation efficiency and accuracy. Molecular Systems Biology. 2011;7: 481. Available from: doi:10.1038/msb.2011.14.
(2) MacDonald CT, Gibbs JH, Pipkin AC. Kinetics of biopolymerization on nucleic acid templates. Biopolymers. 1968;6: 1-25. Available from: doi:10.1002/bip.1968.360060102.
(3) Zur H, Tuller T. Predictive biophysical modeling and understanding of the dynamics of mRNA translation and its evolution. Nucleic Acids Research. 2016;44(19): 9031-9049. Availabe from doi:10.1093/nar/gkw764.

Financing: The Leverhulme Trust

Keywords: protein synthesis, mRNA translation, ribosome dynamics

Back to programme

S05-IL-02, session: Computational biophysics

Network science as a gateway to understanding of emerging dynamics and function of multicellular systems

Marko Gosak (1)

(1) University of Maribor, Slovenia

The past two decades have witnessed the coming of age of network science as the central paradigm behind some of the most fascinating discoveries of the 21st century. In the complex networks framework, system elements are network nodes, and connections between nodes represent some form of interaction between system elements [1]. The flexibility to define network nodes and edges to represent different aspects of biological systems has been employed to model numerous diverse systems at multiple scales [2]. In the lecture I shall review the recent advances in the study of complex biological systems that were inspired and enabled by methods of network science. Specific focus will be given to the extraction of functional connectivity patterns in multicellular systems, with emphasis on insulin secreting beta cell populations in islets of Langerhans. Describing the intercellular activity patterns by means of network language has not only revealed that beta cell networks share many architectural similarities with several other real-life networks, such as small-worldness, heterogeneity, and modularity, but also leads to important new insights into the relationship between cellular metabolic activity and the orchestration of collective behavior [2]. Finally, I will concentrate on the emerging field of multilayer networks and highlight the potential offered by this framework in exploring the complex organization of tissues in both health and disease.

[1] Barabási A-L. Network Science. Cambridge: Cambridge University Press; 2016.
[2] Gosak M, et al., Network science of biological systems at different scales: A review, Physics of Life Reviews. 2017; 10.1016/j.plrev.2017.11.003.

Financing: Slovenian Research Agency

Keywords: network science; biological networks; intercellular communication; calcium imaging

Back to programme

S05-CL-03, session: Computational biophysics

Ligand-based drug design considering specific features of aldose reductase

Magdalena Majekova, Milan Štefek, Marta Šoltésová Prnová, Lucia Kováčiková, Jana Grobarčík Balleková

(1) Department of Biochemical Pharmacology, Centre of Experimental Medicine of SAS, Institute of Experimental Pharmacology amp; Toxicology, Slovak Academy of Sciences Dubravska cesta 9, 84104 Bratislava, Slovakia

Aldose reductase is as a rate-limiting enzyme in the polyol pathway, one of the main mechanisms of glucose toxicity. Inhibitors of aldose reductase showed to be helpful in prevention of diabetic complications and other disorders connected related to a glucose toxicity. Aldose reductase (EC: 1.1.1.21), the three-dimensional structure of AR has been explored in many research works, belongs to the aldo-keto reductase superfamily. Aldose reductase catalyzes many substrates, as lipid aldehydes, methyl glyoxal, aliphatic and aromatic aldehydes, daunorubicin, doxorubicin, etc. With the exception of PGH2 transformation to PGD2, which proceeds in the absence of cofactors NADPH or NADP + , all other known catalytic processes of AR are NADPH-dependent. We present a survey of ligand-based drug design, which resulted in identification of efficient inhibitors (1), with highlighting the specific features of aldose reductase.

(1) Majekova M, Ballekova J, Prnova M, et al.. Structure optimization of tetrahydropyridoindole-based aldose reductase inhibitors improved their efficacy and selectivity. Bioorg. Med. Chem. 2017;25: 6353-6360. Available from: doi: 10.1016/j.bmc.2017.10.005.

Financing: Slovak Research and Development Agency under the contract No. APVV-15-0455 and the Scientific Grant Agency VEGA grant 2/0127/18

Keywords: aldose reductase, ligand-based drug design, electrostatics

Back to programme

S05-OR-04, session: Computational biophysics
PS-67, session: Poster Session

The maximum entropy production principle in enzyme kinetics: novel theoretical insights

Andrej Dobovišek (1,2), Rene Markovič (1,3,4), Marko Šterk (1), Marko Marhl (1,2,3), Aleš Fajmut (1,5)

(1) University of Maribor Faculty of Natural Sciences and Mathematics Department of Physics Koroška cesta 160 2000 Maribor
(2) University of Maribor, Faculty of Medicine, Taborska 8, 2000 Maribor, Slovenia
(3) University of Maribor, Faculty of Education, Koroška cesta 160, 2000 Maribor, Slovenia
(4) University of Maribor, Faculty of Energy Technology, Hočevarjev trg 1, 8270 Krško, Slovenia
(5) University of Maribor, Faculty of Health Sciences, Žitna ulica 15, 2000 Maribor, Slovenia

Enzymatic reactions in cells and in certain types of biotechnological reactors operate as open thermodynamic systems with incoming and outgoing fluxes of matter. As a rule, such systems are often shifted far from thermodynamic equilibrium. While behaviour of systems operating in (or close to) equilibrium state is well understood, the behaviour of open non-equilibrium systems is much less clear. The maximum entropy production principle is a type of thermodynamic optimization, which states that open non-equilibrium systems spontaneously approach towards stationary state with maximal entropy production. It is also claimed that such a state is statistically most probable state of the system and thus characterised by the maximum in the Shannon information entropy. We investigated whether the most likely thermodynamic states of enzymatic reactions are indeed characterised by the maximal entropy production. For five different enzymes: Glucose Isomerase, three different types of b-Lactamases and Triose Phosphate Isomerase, we derived the entropy production and the Shannon information entropy as a function of enzyme rate constants. Under the conditions of mass and Gibbs free energy conservations the co-existence of well-defined global maxima in entropy production and Shannon information entropy is demonstrated, with respect to arbitrary chosen kinetic parameter. By the use of local stability analysis we demonstrate that co-existence of maxima in entropy production and information entropy might be a consequence of flexible enzymatic structure, which is required for fast transitions between different enzyme macromolecular states.

Financing: ARRS

Keywords: enzyme kinetics; maximum entropy production; maximum Shannon information entropy; stability analysis;

Back to programme

S05-OR-06, session: Computational biophysics

Tissue size regulation amplifies the effect of asymmetrical cell divisions on cancer incidence

Daniel Grajzel (1), Imre Derényi (1), Gergely János Szöllősi (1)

(1) ELTE-MTA "Lendulet" Biophysics Research Group, Department of Biological Physics, Eötvös University, Budapest, Hungary

The cells in our tissues go through many divisions during our lifetime. Every time a cell divides, there is a possibility that it collects a mutation. If these mutations accumulate they can cause cancer. How can multicellular organisms maintain their tissues' homeostatic function and keep the risk of cancer sufficiently low? According to our former results in hierarchically organised tissues divisional load (the number of divisions along cell lineages) can be greatly minimised in order to avoid the accumulation of somatic mutations.

Here we extend our model to take into consideration the fact that the cells interact with each other. In this model the rate of the different types of divisions depends on the number of the cells on the hierarchical levels. Normally, this regulation prevents the tissue from growing bigger than its homeostatic size. Using simulations we examine the conditions necessary for the emergence of cancer by introducing mutations that increase the rate of different types of cell division events.

When cells only undergo symmetric differentiation and symmetric division, and are not able to differentiate asymmetrically, it is harder for mutations to be fixed in the cell population. The reason behind this is that the critical number of mutations for the fixation is higher. Our results indicate that tissue size regulation considerably amplifies this effect and results in a surprisingly large reduction in the occurrence of cancer if the cells in the tissue do not undergo asymmetrical divisions.

Back to programme

S05-OR-07, session: Computational biophysics

Artificial neural networks and multidimensional approach in the classification: 2D images of neurons from the human dentate nucleus

Nebojša T Milošević (1), Veličko Vraneš (2), Damjan Stojić (3)

(1) Department of Biophysics, Medical faculty, University of Belgrade Laboratory for image analysis, Institute of Biophysics, Medical faculty, University of Belgrade, Serbia
(2) Laboratory for image analysis, Institute of Biophysics, Medical faculty, University of Belgrade, Serbia Department of Biophysics, University INTEC, Santo Domigo, Republica Dominicana
(3) Laboratory for image analysis, Institute of Biophysics, Medical faculty, University of Belgrade, Serbia

Introduction: Neurons in the human dentate nucleus are classified into four types according to their morphology (1) and into two types according to their topology (2). Thus this study have two major aims: i) verify or improve previous classification and ii) investigate whether border neurons express the same features or they belong to a different morphological types (3). Material and Methods: Fifteen parameters quantifying four aspects of neuron morphology (surface area and shape of whole neuron, dendritic length and branching complexity) were measured (1). Classification scheme was investigated using neural networks and multidimensional approach (3). Results: The use of neural network didn't confirm the previous classification on central and border cells, but it showed four neuronal types, based on soma area and dendritic length. Further analysis showed significant differences between two types of border neurons, mainly in parameters which quantify dendritic branching complexity and dendritic length. All methodological approaches demonstrated slight clustering of data: cluster analysis showed two data clusters and separate unifactor analysis indicated inter-cluster differences. Discriminant and correlation-comparison analysis further proved and explained the result on a more cohesive manner. Conclusion: Human dentate nucleus neurons can be classified into four neuron types, according to their quantitative properties. Border neurons can be divided into two different topological types. The obtained neuronal differences were discussed further in relation to the structure and function of the cerebellar network.

(1) Grbatinić I, Marić DL, Milošević NT. Neurons from the adult human dentate nucleus: neural networks in the neuron classification. J Theor Biol. 2015; 370: 11-20.
(2) Marić D. Qualitative and quantitative analysis of adult human dentate nucleus neurons morphology (Ph.D. thesis). Medical faculty, University of Novi Sad, Serbia, Balkans, 2010.
(3) Grbatinić I, Milošević NT. Classification of adult human dentate nucleus border neurons: artificial neural networks and multidimensional approach. J Theor Biol. 2016; 404: 273-284.

Financing: Instituto Technologico de Santo Domingo (INTEC), Santo Domingo, Republica Dominicana

Keywords: Dentate nucleus; Border neurons; Classification; Neural networks; Cluster analysis;

Back to programme

S05-OR-08, session: Computational biophysics

Energetics of the open - closed transition in the N-terminal region: importance for the CPVT phenotype

Jozef Sevcik (1), Andrea Faltinova (2), Alexandra Zahradníková (1,2)

(1) Institute of Molecular Biology, Slovak Academy of Sciences
(2) Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences

The central helix of the N-terminal region (NTR) of human cardiac ryanodine receptor (RyR) was shown to be crucial for NTR stability (1). Mutations in this helix were shown to cause the hereditary arrhythmia CPVT (http://triad.fsm.it/cardmoc/), characteristic by increased open probability of the RyR. Using structural modelling, we compared energetics of the NTR in the open and closed conformation for wild-type NTR, for each of the seven known CPVT mutants, and for a putative complex of NTR with a domain peptide that was found to increase RyR open probability. In both conformations, peptide binding induced a decrease of NTR energy due to formation of new H-bonds, while all mutations induced an increase of NTR energy due to loss of H-bonds. However, the energetic difference between the closed and open conformation was lower in the complex as well as in six of the seven mutations, because H-bonds were preferentially formed in the open conformation of the complex, while they were preferentially lost in the closed conformation of the mutated NTR. We hypothesize that impairment of ryanodine receptor function in CPVT arrhythmias may be caused at least in part by the decrease of the energetic difference between the closed and the open conformation of the NTR.

Supported by APVV-15-0302.

(1) Borko L, Bauerová-Hlinková V, Hostinová E, Gašperík J, Beck K, Lai FA, Zahradníková A, Sevčík J. Structural insights into the human RyR2 N-terminal region involved in cardiac arrhythmias. Acta Crystallogr D Biol Crystallogr. 2014;70(11): 2897-912. Available from: doi: 10.1107/S1399004714020343.

Financing: Agentúra na podporu výskumu a vývoja

Keywords: structural modelling; ryanodine receptor; allosteric activation; CPVT mutations

Back to programme

S06-PL-01, session: Single molecule and interactions

Varying label density to probe membrane protein nanoclusters in STORM/PALM

Gerhard J. Schütz (1)

(1) Wiedner Hauptstrasse 8, 1040 Wien, Austria

Superresolution microscopy has facilitated the investigation of cellular structures at length scales far below the optical diffraction limit. When applied to the plasma membrane, the presence of a variety of protein nanoclusters was revealed, which lead to speculations whether nanoclustering was a general feature of plasma membrane proteins. Recently, however, doubts were raised whether imaging artifacts inherent to PALM/STORM might have influenced or even caused the observation of some of those protein clusters. To approach these concerns, we developed a method to robustly discriminate clustered from random distributions of molecules detected with single molecule localization microscopy-based techniques like PALM and STORM (1).The approach is based on deliberate variations of the labeling density of the samples and quantitative cluster analysis. Our method circumvents the problem of clustering artifacts generated by the blinking statistics of the fluorophores used. It can be readily applied to PALM and STORM experiments where either overexpressed proteins are present over a broad range of expression levels or antibody concentrations are titrated to achieve different degrees of labeling. In my talk I will present the application of this technique particularly for the understanding of the spatial distribution of the T cell receptor complex in the plasma membrane of T cells.

1. Baumgart F, Arnold AM, Leskovar K, Staszek K, Fölser M, Weghuber J, et al. Varying label density allows artifact-free analysis of membrane-protein nanoclusters. Nat Meth. 2016;13(8):661-4.

Financing: FWF, WWTF

Keywords: Superresolution microscopy; plasma membrane; Immunology

Back to programme

S06-CL-02, session: Single molecule and interactions

Mechanically-driven ejection of viral DNA

Miklos Kellermayer (1), Zsuzsanna Vörös (1), Gabriella Csík (1), Levente Herényi (1)

(1) Department of Biophysics and Radiation Biology, Semmelweis University

Viruses are parasitic infectious agents with a nanoscale shell, known as the capsid, that encapsulates the genomic material. Most bacteriophage viruses invade bacteria by transferring their genome inside the host cell while leaving the capsid outside. Thus, the foremost event of bacteriophage infection is the ejection of genomic material into the host bacterium after the virus has recognized and bound to surface receptor sites. How ejection is triggered is yet unknown. We show, by manipulating individual mature T7 phage particles, that tapping the capsid wall with an oscillating atomic-force-microscope cantilever triggers rapid DNA ejection via the tail complex. Triggering rate increases exponentially as a function of force, hence follows transition-state theory, across an activation barrier of 23 kcal/mol at 1.2 nm along the reaction coordinate. The conformation of the ejected DNA molecule revealed that it had been exposed to a propulsive force. This force, arising from intra-capsid pressure, assists in initiating the ejection process and the transfer of DNA across spatial dimensions beyond that of the virion. Chemical immobilization of the tail fibers also resulted in enhanced DNA ejection, suggesting that the triggering process might involve a conformational switch that can be mechanically activated either by external forces or via the tail-fiber complex. Considering the emerging interest in artificial micro- and nanocapsules capable of triggered material release, understanding how viral DNA ejection is triggered carries important application potential. The unique features of the single-particle mechanics method employed here may be useful in uncovering the fine details of viral DNA ejection.

Financing: FP7, Hungarian Office for Research, Development and Inovation

Keywords: virus, nanomechanics, AFM, DNA

Back to programme

S06-OR-03, session: Single molecule and interactions

Diffusiophoresis of blood cells and vesicles in transient chemical gradients

Saša Vrhovec Hartman (1), Bojan Božič (1), Jure Derganc (1)

(1) Institute of Biophysics, Faculty of Medicine, University of Ljubljana

Microcavities provide a well-controlled flow-free microenvironment and thus play an important role in many microfluidic systems, for example in the form of cell-culturing microchambers. Here we show that transient concentration gradients that emerge during diffusive exchange of solutes in microcavities can induce noticeable passive migration of blood cells and synthetic phospholipid vesicles, an effect that has been broadly termed as diffusiophoresis. We observed migration during the exchange of different electrolyte and non-electrolyte solutions that all had the same osmolarity. Depending on the type of solutions, cells and vesicles migrated towards the entrance or towards the end of the cavity, with migration distances on the order of ten micrometres. Thus, the observed phenomena can significantly affect cell behaviour in many applications of microfluidic devices in biomedicine and biotechnology.

Financing: The study was supported by Slovenian Research Agency, Grant P1-0055

Keywords: Diffusiophoresis; Transient solute gradient; diffusion; erytrocytes; leukocytes; giant lipid vesicles; microfluidics; microcavities;

Back to programme

S06-OR-04, session: Single molecule and interactions

CUTTING EDGE BIOPHYSICAL TOOLS TO DETERMINE BIOMOLECULAR INTERACTIONS AND CHARACTERIZE PROTEIN STABILITY IN SOLUTION

Jakub Nowak (1)

(1) NanoTemper Technologies

Introduction to Micro Scale Thermophoresis Analysis of bio-molecular interactions, such as protein-protein, protein-nucleic acid or protein-small molecule, not only helps to develop therapeutics or diagnostics techniques, but it also provides important insights into cellular processes. Here we present MicroScale Thermophoresis (MST) for the investigation of affinities of biomolecular interactions. MST is a biophysical technique that measures the strength of the interaction between two molecules by detecting variations in fluorescence signal as a result of an IR-laser induced temperature change. The range of the variation in the fluorescence signal correlates with the binding of a ligand to the fluorescent target Introduction to nanoDSF The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid protein unfolding analysis, providing an unmatched scanning speed and data point density. This yields ultra-high resolution melting profiles of proteins which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required, protein solutions can be analyzed independent of buffer compositions. In addition, information on protein aggregation can be recorded in parallel, providing insight into colloidal stability of the sample. The presentation will cover biophysical concepts of the techniques showing benefits of MST and nanoDSF technologies platforms, and will be followed by specific examples of applications towards various experimental systems.

Keywords: biomolecular interactions, complex buffer systems, protein stability, aggregation

Back to programme

S06-OR-05, session: Single molecule and interactions

Overcoming Blinking Artifacts in Nanocluster Detection with Two Color STORM

Andreas Maximilian Arnold (1), Florian Baumgart (1), Gerhard J. Schütz (1)

(1) Institute of Applied Physics, TU Wien

Observations using single molecule localization microscopy have led to the belief that the majority of tested membrane proteins are organized in clusters at sizes below the diffraction limit. These nanoclusters are thought to play an important role in cellular signaling. However, concerns about the existence of nanoclusters have been fueled by the notion that virtually all fluorescent probes show complex blinking behavior including long-lived dark states. This results in artificial localization clusters due to the repeated observation of single molecules. Existing post-processing approaches commonly struggle to reliably distinguish real molecular clustering from such blinking artifacts. Recently, we proposed an experimental approach relying on the deliberate titration of fluorescent labels in a number of samples. Here, we present a supplementary approach using information from two-color STORM experiments. Targeting the same protein species with differently labelled antibodies allows for the calculation of distance distributions between localizations from both color channels. Molecular clusters exhibit a characteristic bias towards short distances. The use of two-color information overcomes the detection of apparent clustering due to fluorophore blinking, which is problematic in other methods, such as Ripley's K analysis. Furthermore, rotation of one color channel provides intrinsic controls, thus allowing for statistical significance tests without the necessity of additional calibration. We evaluate the limits of the method with Monte Carlo simulations and experiments on clustered and randomly distributed membrane proteins.

Financing: Austrian Science Fund (FWF); Austrian Acadamy of Sciences (ÖAW)

Keywords: Superresolution Microscopy; Molecular Clustering; Fluorophore Blinking

Back to programme

S07-IL-01, session: Materials and Nanobiophysics

Tailoring biomolecular interactions of core-shell nanoparticles and their application to magnetoresponsive drug delivery vehicles

Erik Reimhult (1)

(1) University of Natural Resources and Life Sciences, Vienna

Core-shell nanoparticles can be used in biomedical applications, e.g., as biomedical imaging contrast agents, for hyperthermia and in drug delivery, as well as for biotechnological applications such as separation and purification. Unique functions can be achieved by using nanoscale inorganic cores, such as superparamagnetic iron oxide nanocrystals for controlling interactions with magnetic fields. Using densely grafted polymer shells with controlled morphology and topology interactions with proteins can be tuned in biological fluids and we show this ultimately affects cell interaction and uptake.
However, to enable advanced materials functions in a biological environment, e.g. specific binding, aggregation and drug release, integration of grafted stimuli-responsive polymer shells or into structured amphiphile and polymer assemblies must be carefully designed. We will describe multiple developments from our lab regarding the experimental design and characterization of such hierarchically structured nanomaterials. Our focus is on using localized magnetic heating of nanoparticles in lipid and block copolymer vesicles to control membrane permeability through local phase transitions and thereby release of encapsulated molecules.

Financing: ERC, FWF, BMWFW

Keywords: core-shell nanoparticle; lipid membrane; block copolymer membrane; drug release; thermoresponsive polymer; cell uptake

Back to programme

S07-IL-02, session: Materials and Nanobiophysics

Nanoparticles as drug delivery systems. The interaction with biomembrane models

Zuzana Garaiova (1), Sopio Melikishvili (1), Sylwia Michlewska (2), Maksim Ionov (2), Elzbieta Pedziwiatr-Werbicka (2), Iveta Waczulikova (1), Maria Angeles Muñoz-Fernández (3), Rafael Gomez-Ramirez (4), Francisco Javier de la Mata (4), Maria Bryszewska (2), Tibor Hianik (1)

(1) Comenius University in Bratislava, Faculty of Mathematics, Physics and Informatics
(2) University of Lodz, Faculty of Biology and Environmental Protection
(3) Hospital Gregorio Marañon, Dr. Esquerdo, Madrid
(4) University Alcala, Alcala de Henares, Spain

Nanoparticles (NPs) may help to protect drugs including new classes of therapeutic biomolecules (e.g. nucleic acids, proteins) from enzymatic degradation as well as from undesirable interactions with biological components, and efficiently transport them into the site of their therapeutic action. Different kind of nanoparticles has been synthesized for these purposes (1). We will focus on the polymer nanoparticles such as dendrimers and on their potential to be used as carriers for delivery of HIV synthetic peptides into dendritic cells serving as antigen-presenting cells, and thus, as a possible HIV vaccination platform. Since the cell membrane represents one of the most significant obstacles on the route of delivering cargo into the intracellular space, the analysis of biomembrane interactions is of utmost importance. This can be accomplished by using various biomimetic membrane models such as Langmuir monolayers and lipid vesicles. We have demonstrated that interaction between model membranes and peptides is stronger when being complexed with NPs than for uncomplexed (free) peptides. The interaction depended on the lipid composition; polyethyleneglycol modified lipids made this interaction weaker (2).

(1) Peña-González C E, et al. Gold nanoparticles stabilized by cationic carbosilane dendrons: synthesis and biological properties. Dalton Transactions. 2017; 46: 8736-8745. Available from: doi: 10.1039/C6DT03791G.
(2) Melikishvili S, et al. The effect of polyethylene glycol-modified lipids on the interaction of HIV-1 derived peptide-dendrimer complexes with lipid membranes.Biochim Biophys Acta. 2016; 1858(12):3005-3016. Available from: doi:10.1016/j.bbamem.2016.09.005.

Financing: This work was supported by Slovak Research and Development Agency, APVV (projects No. APVV-14-0267 and SK-PL-2015-0021), Polish Ministry of Science and Higher Education and VEGA 1/0152/15.

Keywords: Nanoparticles; Synthetic peptides; HIV/AIDS; Vaccine; Biomembrane models

Back to programme

S07-CL-03, session: Materials and Nanobiophysics

Bio-applications for 3D Nanolithography in Microfluidics

Jaroslaw Jacak (1), Bianca Buchegger (2), Sandra Mayr (3), Richard Wollhofen (2), Birgit Plochberger (3), Markus Axmann (4), Christian Gabriel (5), Thomas A. Klar (6)

(1) University of Applied Sciences, Department of Medical Engineering, Linz
(2) Institute of Applied Physics, Johannes Kepler University Linz, Altenberger Straße 69, 4040 Linz, Austria
(3) Upper Austria University of Applied Sciences, Campus Linz, Garnisonstraße 21, 4020 Linz, Austria
(4) Medical University of Vienna, Center for Pathobiochemistry and Genetics, Institute of Medical Chemistry and Pathobiochemistry, 1090 Vienna, Austria
(5) Red Cross Transfusion Service Linz, 4020 Linz, Austria
(6) Institute of Applied Physics, Johannes Kepler University Linz, Altenberger Straße 69, 4040 Linz, Austria

Multiphoton polymerization (MPP), optionally combined with stimulated emission depletion (STED) lithography, allows two and three dimensional polymer fabrication with structure sizes and resolution below the diffraction limit. Structuring of polymers with photons gives new opportunities to a large field of applications e.g. in the field of biotechnology and tissue engineering. Radical photoinitiator molecules in a photoresist are excited with a near infrared laser via multiphoton absorption; this allows writing of features as small as 100 nm with a resolution of 200 nm. To achieve spatial polymerization restriction similar to STED-microscopy, the excited photoinitiators are depleted in the outer rim of the excitation volume via stimulated emission by a second laser beam. An appropriate beam shaping shrinks the volume of excited photoinitiators. Thereby, polymerization initiation is furtherly confined.
In a further application, the 3D lithography scaffolds have been structured inside microfluidic channels for studying platelet aggregation. Therefore, protein-adsorptive nanoanchors were polymerized on top of 3D structures and functionalized with von Willebrand Factor . Platelets were pumped through the microfluidic channel and their time dependent activation has been observed. For visualization with fluorescent microscopy, the platelets were fixed and immunostained. The characterization of the activation inside of a 3D thrombclot has been realized using localisation microscopy (in particular direct stochastic optical reconstruction microscopy) using various thrombocyte activation markers (e.g CD62p). For characterization, 3D nanoscopic-images of the mimicked 'thrombus' at different activation stages have been compared.

Financing: Interreg, FWF

Keywords: 3D Nanolithography, Fluorescence microscopy, Biomaterials

Back to programme

S07-CL-04, session: Materials and Nanobiophysics

Poking and pulling collagen: Nanomechanical assessment of native collagen fibrils

Orestis G Andriotis (1), Mathis Nalbach (1), Jakob Scheruebl (1), Sylvia Desissaire (2), Mark G. Jones (3), Donna E Davies (3), Philipp J Thurner (1)

(1) Institute of Lightweight Design and Structural BiomechanicsVienna University of Technology Getreidemarkt 9, 1060 Vienna Austria
(2) Medical University Vienna, Center for Medical Physics and Biomedical Engineering, General Hospital Vienna, 4L
(3) The Brooke Laboratory, Clinical and Experimental Sciences, University of Southampton, Faculty of Medicine

Recent advances in mechanical signaling between cells and the extracellular matrix (1), brought interest in understanding the mechanical properties of tissues at the sub-cellular level i.e. of native collagen fibrils. Nanomechanical assessment has been achieved via atomic force microscopy (AFM) on native collagen fibrils from human and murine samples (2). The versatility of AFM allows both indentation and tensile experiments to be carried out in air-dry and fully hydrated environments. When fully hydrated, collagen fibrils become three orders of magnitude softer under indentation loading, compared to air-dried state. This suggests that hydration plays an important role in collagen fibril mechanics. Partial addition of an osmotic pressure-inducing agent, namely polyethylene glycol, results in partial dehydration followed by an up to 6-fold increase in tensile stiffness. Interestingly, this partial dehydration-based tuning mechanism is reversed and could be used by cells to tune their microenvironment e.g. by secreting proteoglycans. The identification of such structure-function relationships of individual collagen fibrils in combination with biochemical and biological analyses could be used to manufacture tissue-engineered scaffolds with desired mechanical properties and help enhance our understanding of the development and progression of pathologies at the nanoscale.

1. DuFort CC, Paszek MJ, Weaver VM. Balancing forces: architectural control of mechanotransduction. Nature reviews Molecular cell biology. 2011;12(5):308-19.
2. Andriotis OG, Manuyakorn W, Zekonyte J, Katsamenis OL, Fabri S, Howarth PH, et al. Nanomechanical assessment of human and murine collagen fibrils via atomic force microscopy cantilever-based nanoindentation. Journal of the mechanical behavior of biomedical materials. 2014;39:9-26.

Financing: EPSRC, MRC

Keywords: Atomic force microscopy; Collagen fibrils; Mechanical properties; Hydration; Structure-function relationships

Back to programme

S07-OR-05, Session: Materials & Nanobiophysics

A relevant in vitro model of the alveolus enables reliable studies of nanomaterial-cell interactions

Hana Majaron (1)(2), Boštjan Kokot (2), Tilen Koklič (2), Janez Štrancar (2)

(1) Jozef Stefan International Postgarudate School, Ljubljana, Slovenia
(2) Laboratory for Biophysics, Condensed matter department, Jožef Stefan Institute, Slovenia

Although we are constantly being exposed to airborne nanomaterial, we are still unable to predict its potential adverse outcomes - an issue arising from our lack of understanding of the basic interactions of nanomaterial with biomolecules and our cells. In order to study these biophysical interactions, we designed a simple in vitro model of the lung alveolus, exposed to nanomaterial, which would more closely resemble the actual physiological conditions than the widely-used submerged in vitro models. As such, it would result in more reliable determination of relevant molecular interactions for description of nanomaterial toxicity.
We have tested several methods of application of exogenous lung surfactant onto alveolar type II cells, and have found nebulization of lung surfactant to be the superior to pipetting with regard to the homogeneity of the surfactant layer. The subsequent nebulization of highly-concentrated nanoparticles enables a uniform exposure of cells to nanomaterial eliminating the need to disperse nanomaterial using physiologically irrelevant proteins, mainly albumin. Using these two improvements results in a simple in vitro model of lung alveoli, exposed to airborne nanomaterial, which enables studying biophysical interactions on a relevant system in real-time using super-resolution STED microscopy. I will also present the drawbacks of our current in vitro model and some suggested future upgrades.

Keywords: Fluorescence microscopy; molecular biology; super-resolution microscopy; STED microscopy; in vitro model; lung in vitro model; lung surfactant

Back to programme

S08-PL-01, Session: Cell biophysics

Mechanosensing in living cells: the emerging role of piezo channels in human patho-physiology

Massimo Vassalli (1)

(1) Institute of Biophysics of the National Research Council of Italy

Cellular adaptation is not only regulated by chemical messengers. They process physical and mechanical signals by modulating gene expression that lead to the onset of an altered phenotype. Mechanobiology is the emerging branch of biophysics studying how cells sense mechanical cues and local stresses, and translate them into molecular messages for the nucleus.
Several mechanisms have been proposed to contribute to cellular mechanotransduction. Among them, a key role is played by the MechanoSensitive ion Channels (MSCs), molecular structures harboring the plasma membrane and directly converting forces/stresses experienced by the cell into a chemical signal (ion exchange). MSCs were identified in bacteria about 35 years ago (Guharay et al. 1984) but only recently, the long-sought mammalian MSCs were identified by the group of Patapoutian (Coste et al. 2010) who named them Piezo1 and Piezo2. This discovery opened new avenues in mechanobiology, providing a key to interpret physiological and pathological processes that have been elusive before.
After a general introduction, the organization of piezo1 in the plasma membrane will be addressed, showing the role of cholesterol content in the plasma membrane and trying to provide an interpretation of the observed phenomenology.

(1) Guharay F, Sachs F; Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. J. Physiol., 352 (1984), pp. 685-701.
(2) Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A; Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels; Science. 2010 Oct 1;330(6000):55-60

Back to programme

S08-IL-02, session: Cell biophysics

Mechanical and chemical analysis of single living cells investigated by non-contact micro-spectroscopic techniques

Sara Mattana (1), Maurizio Mattarelli (1), Lorena Urbanelli (2), Krizia Ssgini (2), Carla Emiliani (2), Mauro Dalla Serra (3), Daniele Fioretto (1), Silvia Caponi (4)

(1) Dipartimento di Fisica e Geologia, Università di Perugia, Via A. Pascoli, I-06100 Perugia, Italy
(2) Laboratory of Biochemistry and Molecular Biology, Department of Chemistry, Biology and Biotechnology, University of Perugia, via del Giochetto, I-06123 Perugia, Italy.
(3) Istituto di Biofisica CNR (IBF-CNR), Unità di Trento, and FBK, Via Sommarive 18, 38123 Trento, Italy
(4) CNR-Consiglio Nazionale delle Ricerche, Italy

Mechanical and chemical properties of cells and tissues are intimately connected: their imbalances can be symptoms or effects of pathologies. We developed an innovative method that can simultaneously characterize the elastic and chemical properties of biological materials with sub-cellular spatial resolution [1-2]. The technique use Raman and Brillouin spectroscopies, established techniques for nondestructive contactless and label free readout of materials properties. In a proof-of-principle experiment, the ability of the set-up to characterize subcellular compartments distinguish cell status was successfully tested.

From the Brillouin signal, the elastic properties of single living cells immersed in buffer solution are analyzed [2]. The existence of mechanical heterogeneity inside the cell has been point out: a 20% increase is observed in the elastic modulus passing from the plasmatic membrane to the nucleus as distinguished by comparison with the Raman spectroscopic marker. Brillouin line shape analysis is even more relevant for the comparison of cells under physiological and pathological conditions. Following oncogene expression, cells show an overall reduction in the elastic modulus (15%) and apparent viscosity (50%).

[1] Scarponi F, Mattana S, Corezzi S, Caponi S, Comez L et al. High-performance versatile setup for simultaneous Brillouin-Raman micro-spectroscopy. Phys Rev X (2017) 7: 031015.
[2] S. Mattana , M. Mattarelli , L. Urbanelli , K. Sagini , C. Emiliani , M. Dalla Serra , D. Fioretto, S. Caponi Non-contact mechanical and chemical analysis of single living cells by micro-spectroscopic techniques, Nature- Light: Science Applications (2018) 7, e17139; doi: 10.1038/lsa.2017.139

Financing: PAT (Autonomous Province of Trento) (GP/PAT/2012) 'Grandi Progetti 2012' Project 'MaDEleNA'. European Commission under the EU Horizon 2020 Programme Grant Agreement No: 644852, PROTEUS. Consiglio Nazionale delle Ricerche-Istituto Officina dei Materiali.

Keywords: Raman and Brillouin spectroscopy, elastic moduli, imaging

Back to programme

S08-IL-03, session: Cell biophysics

Single cell at the charged interface

Nadica Ivosevic Denardis (1)

(1) Rudjer Boskovic Institute, Zagreb

The single cell-electrode interaction is relevant to fundamental biological processes involving cell adhesion, cell fusion, cell activity, and membrane response to environmental stress. Our focus is on single-celled marine microalgae as the globally important primary producers, drivers of essential biogeochemical cycles and present the basis of the aquatic food web. Selected algal cell specie possesses only cell membrane, pronounced motility and tolerates a wide range of salinity. It will be shown changes of algal cell nanomechanical properties and adhesion dynamics during their growth using complementary surface methods and mathematical modelling. AFM measurements reveal that nanomechanical properties change during cell growth, and this is reflected in the interfacial adhesion dynamics. AFM results show that: (i) cells are larger in exponential than in the stationary phase, which is in an agreement with the amperometrically determined larger amount of interfacial displaced charge and (ii) cells in the exponential phase are stiffer and more hydrophobic than those in the stationary phase, which suggests molecular modification of cell envelope during aging. Results obtained with the reaction kinetics model of the three-step process (1) indicate that adhesion and spreading at the charged interface is slower when cells are in exponential phase, which may be attributed to larger volume and stiffer and more hydrophobic cellular material. In the future, cell mechanical properties could be considered as a marker for environmental stress in order to better understand algal behaviour at the aquatic interfaces.

(1) Ivošević DeNardis N, Pečar Ilić J, Ružić I, Pletikapić G. Electrochimica Acta. 2015, 176 (743–754). Available from:doi:10.1016/j.electacta.2015.07.068. Financing: This work was supported by the Croatian Ministry of Science, Education and Sports through the projects (i) 098-0982934-2744, (ii) Croatia–France bilateral programme Cogito (0411B014), and (iii) Grants provided by the French Centre National de la Recherche

Keywords: AFM, amperometry, cell adhesion, kinetic, mathematical model, nanomechanics

Back to programme

S08-IL-06, session: Cell biophysics

Towards understanding of translation biology by coupling positional sequencing and nano-imaging?

Gabriella Viero (1)

(1) Institute of Biophysics - CNR Italy

The process of protein synthesis, or translation, is driven by the ribosome that has been widely studied in various organisms, providing intriguing evolutionary and mechanistic insights about translation. In cells, groups of ribosomes form the so-called polyribosome when are simultaneously translating a single mRNA. As such, polyribosomes are among the biggest cytoplasmic machines and integrated platforms of molecules (ribosomes, mRNA, RNA binding proteins (RBPs) and nc-RNAs), whose organization is not yet studied in detail.
Our work stems from the following questions: what if the current ribosome-centered paradigm of translation hinged on a restrictive, flattened view of the polyribosome? what if the architecture and control of the super-organization of polyribosomes was multi-dimensional and at least as rich as that of transcription? We are trying to answer these questions and dissect the organization of polyribosomes with a multi-level approach by integrating: i) imaging data, by Atomic Force Microscopy and ii) positional information about ribosome footprints along the mRNA, by cutting-edge sequencing methods with a single nucleotide resolution. Our work illustrates a novel interdisciplinary approach to connect sequencing and imaging data, trying to reconciling biophysics and systems biology. This integrated approach have been employed for better understanding the role of ribosome biology in motor neuron diseases such as SMA and ALS.

Keywords: protein synthesis; ribosome; atomic force microscopy; omics

Back to programme

S08-OR-04, session: Cell biophysics

Visualization of transport properties of membrane nanotubes with live cell laser-scanning confocal and superresolution (SIM) microscopes

Henriett Halász (1), Alireza Ghadaksaz (1), Tamás Madarász (1), Krisztina Huber (2), Miklós Nyitrai (3), János Matkó (2), Edina Szabó-Meleg (3)

(1) University of Pécs, Hungary
(2) Eötvös Loránd University, Hungary
(3) University of Pécs, Medical School, Department of Biophysics and University of Pécs, Szentágothai Research Centre

Cell protrusions appearing on the surface of the cells are exceptionally heterogeneous structures facilitating communication and matter transport. A group of these protrusions - membrane nanotubes (NTs) - provide special, physical connection between distant cells transferring not only cell organelles (lysosome, mitochondria), lipids, proteins, vesicles, or DNA/RNA molecules, but also pathogens as viruses and bacteria. Many cell types in culture including immune cells, fibroblasts, epithelial cells, neurons have NT forming capacity. Although among immune cells, the NTs connecting B cells still remained poorly known. Recently we have shown that B cell NTs contain both actin filaments and microtubules[1].
Therefore, using confocal and structured illumination microscopic imaging we focused on the characterization of intercellular transport of cytoplasmic microvesicles, revealing possible motor proteins mediating these transport procedures, and the visualization of intercellular exchange of CD86 costimulatory molecules (important immunoregulators) via the NTs of murine B cells and macrophages.
Extended intercellular transfer of microvesicles and immunregulatory molecules via the nanotubes were clearly demonstrated by our in vitro live cell imaging data which may spread antigens, costimulators and consequently improve the efficiency of antigen-dependent T cell activation that playing role in the formation of immunological memory.

[1] Osteikoetxea-Molnar A. et al. The growth determinants and transport properties of tunneling nanotube networks between B lymphocytes. Cellular and Molecular Life Sciences 2016;73(23): 4531-4545. Available from: doi: 10.1007/s00018-016-2233-y.

Keywords: superresolution microscopy; membrane nanotube; intercellular communication

Back to programme

S08-OR-05, session: Cell biophysics

ATP dependency of osmotically activated outwardly rectified current in the membrane of cytoplasmic droplets obtained from sporangiophore of model filamentous fungus Phycomyces blakesleeanus

Miroslav Živic (1), Strahinja Križak (2), Marina Stanić (3), Milan Žižić (3), Nataša Todorović (4)

(1) University of Belgrade - Faculty of Biology, Serbia
(2) Medigroup, Belgrade, Serbia
(3) Institute for Multidisciplinary Research, University of Belgrade, Serbia
(4) Institute for Biological Research, University of Belgrade, Serbia

ORIC is outwardly rectified, rapidly inactivating anionic current that is activated in hypo-osmotic conditions in the membrane of cytoplasmic droplets obtained from sporangiophore of filamentous fungus Phycomyces blakesleeanus . It shares biophysical properties (rectification and inactivation dynamics, ion selectivity order) and activating stimuli (hypo-osmotic conditions or presence of intracellular GTP under isoosmotic conditions) with volume regulated anionic current (VRAC) present in animals. Since VRAC is ATP regulated channel, we tested if ATP affects ORIC as well. For these purposes patch-clamp experiments in whole-cell configuration with 2mM MgATP in the patch pipette were performed. Inclusion of ATP increased maximal ORIC while strikingly reducing its run-down. Time to full activation of ORIC was longer in the presence of MgATP. Additionally, decrease of Mg +2 to 32M, with 2mM ATP present, did not affect the activation speed, but ORIC activated to a larger value and plateau of maximal activity was longer compared to 2mM Mg +2 with ATP. This suggests that the intracellular Mg +2 exert an inhibiting effect on ORIC, also similar to the effect of Mg +2 on VRAC. Furthermore, in order to establish if respiration blockers affect ORIC, we tested the efficiency of two respiration blockers, antimycin A and azide, on ORIC in concentrations that inhibit cytochrome respiratory pathway completely. Both metabolic blockers inhibited ORIC, thus confirming the ATP is necessary for ORIC activity.

Financing: Serbian Ministry of Education, Science and Technological Development, Grant no. 173040

Keywords: filamentous fungi;osmotically activated ionic current; ATP

Back to programme

S08-CL-07, session: Cell biophysics

Bio-integrated micro-laser particles for sensing, imaging and cell barcoding

Matjaž Humar (1)

(1) Jožef Stefan Institute Faculty of Mathematics and Physics, University of Ljubljana

Micro-sized lasers completely embedded within single live cells (1) and biological tissues (2) have been demonstrated. The lasers were made out of solid beads including biocompatible and biodegradable materials. The lasers inside cells can act as very sensitive sensors, enabling us to better understand cellular processes. Further, lasers were used for cell tagging. Each laser within a cell emits light with a slightly different fingerprint that can be easily detected and used as a barcode to tag the cell (3), providing the ability the study cell migration including cancer metastasis. We have also demonstrated that small lasers embedded in the sample can be used for novel nonlinear microscopy, including super resolution imaging (4). The narrow spectra and nonlinear power dependence of stimulated emission from the laser particles yield optical sectioning, sub-diffraction resolution, and low out-of-focus background. Small lasers embedded into cells and tissues may enable new diagnostic, treatment and imaging tools in medicine and biology (5).

(1) M. Humar and S. H. Yun. Intracellular microlasers, Nature Photonics 9, 572 (2015).
(2) M. Humar, A. Dobravec, X. Zhao, S. H. Yun. Biomaterial microlasers implantable in the cornea, skin, and blood. Optica 4, 1080-1085 (2017).
(3) M. Humar, A. Upadhya, S.H. Yun. Spectral reading of optical resonance-encoded cells in microfluidics. Lab on a Chip 17, 2777-2784 (2017).
(4) S. Cho, M. Humar, N. Martino, S. H. Yun, Physical Review Letters 117, 193902 (2016).
(5) M. Humar, S.J.J. Kwok, S.Y. Cho, M. Choi, A.K. Yetisen, S.H. Yun. Toward biomaterial-based implantable photonic devices. Nanophotonics 5, 60–80 (2016).

Keywords: lasers, cells, sensing

Back to programme

S08-OR-08, session: Cell biophysics

Fast evolution of antibiotic resistance in microfluidic devices

Peter Galajda (1), Krisztina Nagy (1), Ágnes Ábrahám (1)

(1) Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences

In natural microbial habitats environmental factors often exhibit heterogeneous spatial distribution. This may have a profound effect on various biological processes from the single cell level to populations or ecosystems. Still, laboratory experiments mostly use homogeneous environmental conditions (mixed batch cultures). Research involving spatial heterogeneity of factors exerting stress or selective pressure is especially lacking.We have studied the effect of heterogeneous distribution of antibiotics on E. coli bacteria. For this we have developed a microfuidic device to create linear concentration gradients of antibiotics without fluid flow. We have followed the spatial distribution and growth of cells within the device. We have seen that certain antibiotic agents induce a chemotactic response in E. coli. On a longer timescale we have seen increased growth and collective migration of cells to areas with seemingly lethal antibiotic concentrations. Analysis of cells extracted from the device has shown that the presence of antibiotic gradients induces the appearance of resistant cells extremely fast, within 12-24 hours.Our results demonstrate that in order to study certain microbial processes in the lab often the chemical heterogeneity and complexity of natural environments need to be reproduced.

Financing: Hungarian National Research, Development and Innovation Office

Keywords: antibiotic resistance, microfluidics, evolution

Back to programme

S08-OR-09, session: Cell biophysics

The role of Arp2/3 complex in cytomechanics measured by AFM and optical tweezers

Spela Zemljic Jokhadar (1), Jure Derganc (1), Jose Luis Toca-Herrera (2), Jagoba Iturri (2)

(1) Institute of Biophysics, Faculty of medicine, Ljubljana, Slovenia
(2) Institute of Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Science Vienna, Austria

The sub-membrane actin cortex in animal cells undergoes a continuous turnover and enables a quick mechanical response to stimuli. The majority of the cortical F-actin (which represents roughly 50 % of total cellular F-actin) is generated only by two nucleators: formin Diaph 1 and Arp 2/3 complexes. To analyze the potential influence of Arp2/3 on the cellular mechanics we measured cell stiffness of endothelial cells before and after the treatment with CK-869, which is a known suppressor of Arp2/3 activity. Complementarily, cells were also treated with F-actin disrupting drugs Cytochalasin-D (an inhibitor of actin polymerization) and Jasplakinolide (an actin polymerization promoter) for reference purposes. The cellular stiffness was measured in parallel by means of two different tools that operate in different force regimes: atomic force microscopy (AFM), which induces large deformations and probes bulk cellular stiffness, and optical tweezers (OT), which induce small deformations and probes stiffness at the level of the cellular membrane. In agreement with previous studies, the results with both methods showed a reduction in cell stiffness for Cytochalasin-D treated cells, and no significant difference for Jasplakinolide. Importantly, the CK-869 treatment caused a significant reduction in cell stiffness when measured by OT but, at the same time, almost no impact when measured by AFM, which implies that the effects due to Arp2/3 inhibition influence only the cortical, sub-membrane part of the cell. Hence, the combined employment of AFM and OT techniques appears as an excellent tool for a more complete, level wise, characterization of cell stiffness.

Financing: Slovenian Research Agency Grant P1-0055

Keywords: AFM, Optical tweezers

Back to programme

S09-PL-01, session: Neurobiophysics

Functional Fluorescence Microscopy Imaging (fFMI) of fast dynamic processes in live cells by massively parallel Fluorescence Correlation Spectroscopy

Vladana Vukojevic (1)

(1) Center for Molecular Medicine (CMM) Department of Clinical Neuroscience Karolinska Institutet 17176 Stockholm Sweden

Fluorescence Correlation Spectroscopy (FCS) is a quantitative analytical method with the ultimate, single-molecule sensitivity that enables us to measure in live cells the local concentration of fluorescent molecules, characterize their transporting properties (diffusion and trafficking) and the kinetics of their interactions (1). To enable quantitative characterization of the underlying dynamical reaction-diffusion networks through which biomolecules are integrated over space and time and perform specialized biological functions, such as gene transcription and signal transduction, we have developed a new quantitative confocal fluorescence microscopy imaging technique. This new technique relies on the use of massively parallel FCS (mpFCS) to simultaneously excite fluorescent molecules in multiple spots (32x32=1024) in the focal plane, thus enabling quantitative confocal fluorescence microscopy imaging at a rate of 21 s/frame (2,3). (1) Vukojevic V, Papadopoulos DK, Terenius L, Gehring WJ, Rigler R. Quantitative study of synthetic Hox transcription factor-DNA interactions in live cells. PNAS 2010; 107(9):4093-4098. Available from: doi:10.1073/pnas.0914612107. (2) Krmpot AJ, Nikolic SN, Vitali M, Papadopoulos DK, Thyberg P, Tisa S, Nilsson L, Gehring WJ, Terenius L, Rigler R, Vukojevic V. Quantitative confocal fluorescence microscopy of dynamic processes by multifocal fluorescence correlation spectroscopy. Proc. SPIE 9536, Advanced Microscopy Techniques IV; and Neurophotonics II, ed. E Beaurepaire, PTC So, F Pavone, EM Hillman. 2015 95360O. Available from: doi:10.1117/12.2183935. (3) Papadopoulos DK, Krmpot AJ, Nikolic SN, Krautz R, Terenius L, Tomancak P, Rigler R, Gehring WJ, Vukojevic V. Probing the kinetic landscape of Hox transcription factor-DNA binding in live cells by massively parallel Fluorescence correlation Spectroscopy. Mech. Dev. 2015; 138Pt 2:218-225. Available from: doi:10.1016/j.mod.2015.09.004.

Keywords: Quantitative fluorescence microscopy; Fluorescence Correlation Spectroscopy; Dynamical reaction-diffusion landscapes; Live cell; Transcription factor

Back to programme

S09-IL-02, session: Neurobiophysics

Improving high-resolution optical imaging for (neuro-)biology

Johann Georg Danzl (1)

(1) Institute of Science and Technology Austria

Far-field optical super-resolution microscopy or nanoscopy techniques "super-resolve" features residing closer than the diffraction-limit by transiently preparing fluorophores in distinguishable (typically on- and off-) states and reading them out sequentially. In coordinate-targeted super-resolution modalities, such as stimulated emission depletion (STED) microscopy, this state difference is created by patterns of light, driving for instance all molecules to the off-state except for those residing at intensity minima.

I will discuss our recent efforts to improve coordinate-targeted nanoscopy. As a specific example, I will highlight how the use of multiple off-state transitions for nanoscopy can improve repeated imaging capability and on/off state contrast, enhancing image resolution and signal-to-background ratio in an approach that we dubbed "protected STED" (1). This allowed e.g. decoding the elaborate 3D structure of dendritic spines in living brain tissue. I will also give an outlook on the activities of my recently founded group at IST Austria.

(1) Danzl, Sidenstein et al.. Coordinate-targeted fluorescence nanoscopy with multiple off-states. Nature Photonics 2016; 10: 122.

Keywords: super-resolution microscopy; STED; nanoscopy

Back to programme

S09-CL-03, session: Neurobiophysics

Exocytotic properties of astrocytic vesicles

Jernej Jorgacevski (1)

(1) Faculty of Medicine, University of Ljubljana, and Celica BIOMEDICAL d.o.o., Ljubljana, Slovenia

An important mode of intercellular communication involves the release of molecules from cells by exocytosis. Exocytotic transmitter release is most likely regulated by the SNARE complex, which contains a vesicular protein, synaptobrevin2 (Sb2). By using super-resolution stimulated emission depletion (STED) microscopy and structured illumination microscopy (SIM), we showed that in astrocytes smaller vesicles contain amino acid and peptidergic transmitters and larger vesicles contain ATP (1). We determined that an average astrocytic vesicle contains 15–25 endogenous Sb2 molecules (2). Then we monitored the interaction of a single vesicle with the plasma membrane by a high-resolution membrane capacitance approach. Astrocyte stimulation increases the frequency of predominantly transient fusion exocytotic events in smaller vesicles, whereas larger vesicles proceed more likely to full fusion exocytosis. Treatment of astrocytes with botulinum neurotoxins D and E, and dnSNARE peptide (coding the cytoplasmic domain of Sb2) stabilized the fusion-pore diameter to narrow, release-unproductive diameters in both vesicle types, regardless of vesicle diameter (1). Astrocytes also exhibit strong vesicle related phenotype in certain disorders, like X-linked non-syndromic intellectual disability (XLID), that have traditionally been linked to neurons.

(1) Guček A, Jorgačevski J, Singh P, Geisler C, Lisjak M, Vardjan N, Kreft M, Egner A, Zorec R. Dominant negative SNARE peptides stabilize the fusion pore in a narrow, release-unproductive state. Cellular and molecular life sciences. 2016;73: 3719-3731.
(2) Singh P, Jorgačevski J, Kreft M, Grubišić V, Stout RF, Potokar M, Parpura V, Zorec R. Single-vesicle architecture of synaptobrevin2 in astrocytes. Nature communications. 2014;5: 1-12.

Financing: Research Agency of Slovenia (Grant Numbers P3 0310, J3 4051, J3 4146, J3 7605 and J3 3632) and CipKeBip

Keywords: Astrocytes; exocytosis, vesicles

Back to programme

S09-CL-04, session: Neurobiophysics

Altered sleep oscillations as early biomarkers of Parkinson's disease cholinopathy

Jasna Saponjic (1)

(1) University of Belgrade, Institute for Biological Research - Sinisa Stankovic, Department of Neurobiology

Rhythmic oscillations in the electroencephalographic (EEG) local field potentials underlie the basis of behavioral states and neurological diseases through coherent binding of the cooperating neuronal ensembles. Biophysical methodological approaches enabled us to evidence an augmented cortical activation during non-rapid-eye-movement (NREM) and rapid-eye- movement (REM) sleep, expressed as a long-lasting augmentation of EEG beta vs. delta amplitude attenuation during NREM, with no change of sleep architecture during Parkinson's disease (PD) cholinopathy in rat. Moreover, two REM states emerged in the motor cortex ("theta coherent REM" and "sigma coherent REM"), with distinct cortical drives, expressed as the impaired theta and sigma oscillations propagation . T he hallmarks of earlier aging onset during PD cholinopathy is the REM sleep "enriched" with sigma activity in the motor cortex alongside the broadly augmented propagation of EEG oscillations to the dorsal nuchal muscles. This unique REM sleep was underlined by the altered sleep spindles pattern and dynamic, reflecting the reorganization at thalamocortical level. Furthermore, there was a sustainable increase of EEG delta vs. beta amplitude decrease in the hippocampus during NREM sleep alongside the altered high voltage sleep spindle (HVS) dynamic during REM sleep, followed by hypokinesia.

In order how they occurred, the h ippocampal NREM sleep disorder, altered HVS dynamics during REM sleep in the hippocampus and motor cortex, and delayed „hypokinesia" may serve as biomarkers of PD onset and progression. Understanding the role of brain oscillations as possible biomarkers of brain function is still a challenge, and despite extensive research, their role is still not well established.

Financing: This work was supported by Serbian Ministry of Education, Science and Technological Development Grant OI 173022.

Keywords: sleep; EEG; brain oscillations; sleep spindle; biomarkers; Parkinson' disease

Back to programme

S10-PL-01, session: Proteins and supramolecular structures

Mechanism of membrane interactions of Nep1-like proteins

Gregor Anderluh (1), Tea Lenarčič (2), Vesna Hodnik (3), Marjetka Podobnik (2)

(1) National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology
(2) National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology
(3) National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology and Department of Biology, Biotechnical Faculty, University of Ljubljana

Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are large protein family of virulence factors that are secreted by various plant pathogens and can be found in bacteria, fungi and oomycetes. Upon membrane interactions they are able to trigger plant tissue necrosis and immunity-associated responses in various dicotyledonous plants. The molecular details of their cytolytic mechanism are not known in a great detail. Recently we have identified glycosyl inositol phospho ceramides (GIPC), plant-related sphingolipids, as a target molecule for NLPs binding to plant membranes. Surface plasmon resonance showed that NLP proteins can bind sugars glucosamine and mannosamine, which are often present as the terminal sugars in GIPCs, by millimolar affinity. Structural analysis of NLP protein from oomycete Pythium aphanidermatum in complex with the sugar have indicated structural changes in the protein induced by sugar binding and allow to elucidate the importance of the metal ion coordination for binding. Furthermore, the structure of the non-toxic representative of NLPs, HaNLP3, from oomycete Hyaloperonospora arabidopsidis indicated differences between the proteins that are important for the cytotoxicity. Collectively, we have provided important molecular insights into the mechanisms of cytolytic activity of toxic NLP proteins towards plant plasma membranes.

Financing: Slovenian Research Agency

Keywords: protein-membrane interactions; cytolysins; Nep1-like proteins

Back to programme

S10-IL-02, session: Proteins and supramolecular structures

Mapping of hemoglobin residuals in erythrocyte ghosts using two photon excited fluorescence microscopy

Ivana Drvenica (1), Katarina Bukara (2), Svetlana Jovanić (3), Ana Stančić (1), Vesna Ilić (1), Mihailo D Rabasović (3), Dejan V Pantelić (3), Branislav M Jelenković (3), Branko Bugarski (2), Aleksandar Jovan Krmpot (3)

(1) Institute for Medical Research, University of Belgrade, Dr Subotića br 4, 11 129 Belgrade, Serbia
(2) Department of Chemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11 000 Belgrade, Serbia
(3) Photonics Center, Institute of Physics Belgrade, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia

We have utilized the two photon excitation fluorescence (TPEF) microscopy to map the spatial distribution of residual hemoglobin. The home-made experimental set up for NLSM utilizes the train of the femtosecond pulses from Ti:Sapphire laser at 730nm, repetition rate 76MHz, and pulse duration 160fs. Porcine slaughterhouse blood and human outdated blood were used as a starting biological material. The erythrocyte ghosts were prepared by gradual hypotonic hemolysis [3]. First we used TPEF microscopy to image the intact erythrocytes at single cell level and to study their morphologies, discocyte of human and echynocite of porcine erythrocytes. We have shown that the distribution of hemoglobin in intact erythrocytes follows the cells' shape with pronounced abundance in the proximity of cell membrane [4]. The TPEF images have also revealed that despite an extensive washing out procedure after gradual hypotonic hemolysis, residual hemoglobin localized on intracellular side of the ghost membranes [4]. The TPEF estimated hemoglobin distribution in intact erythrocytes and residual hemoglobin distribution in erythrocyte ghosts could be of importance in biotechnological processes but also in diagnosis of different pathological conditions.

[1] W. Zheng et al, Biomed Opt Express 2, 71-79 (2010)
[2] V. Leuzzi, et al, J. Inherit. Metab. Dis., 1-12 (2016)
[3] I. Kostić, wt al, Colloids Surf B 122, 250-259 (2014)
[4] K. Bukara et al, J. Biomed. Opt. 22, 26003 (2017)

Keywords: multiphoton fluorescence microscopy, ultrafast lasers, hemoglobin, label-free imaging, erythrocytes, erythrocyte ghosts

Back to programme

S10-IL-03, session: Proteins and supramolecular structures

Coordination of actin-microtubule dynamics

Beáta Bugyi (1)

(1) University of Pécs, Department of Biophysics Pécs, Szigeti str. 12. H-7624 Hungary

The crosstalk between the actin and microtubule cytoskeleton is essential for diverse cellular functions, including cell motility, division, adhesion and intracellular trafficking. The dynamic and structural features of these cytoskeletal elements are tightly co-regulated, however, the underlying molecular players and mechanisms are still elusive. Dishevelled-associated activator of morphogenesis (DAAM), the member of the Diaphanous-related formin (Drf) protein family consists of the canonical Formin homology domains (FH1-FH2) flanked by N-, and C-terminal regulatory elements (DID, DAD, CT). We set out to analyze the activities of DAAM in cytoskeletal polymer dynamics by in vitro protein biophysical and biochemical approaches. Our results revealed that DAAM is a profilin-gated processive actin polymerase, which antagonizes with Capping proteins to maintain actin polymer growth. DAAM can organize individual actin polymers, as well as microtubules into higher-order bundled structures. Moreover, DAAM can mediate the formation of inhomogeneous polymer structures by co-aligning actin filaments and microtubules. Our work extends to the investigation of the importance of the different regions of DAAM in these activities. In conclusion, our work establishes DAAM as an important regulator of actin dynamics, and also reveals a novel role of DAAM as a possible regulator of the functional coordination between the actin and microtubule cytoskeleton.

Back to programme

S10-CL-06, session: Proteins and supramolecular structures

RNA-mediated intercellular miscommunication: role of extracellular vesicle cargos in Amyotrophic Lateral Sclerosis

Manuela Basso (1)

(1) Laboratory of Transcriptional Neurobiology Centre for Integrative Biology (CIBIO) University of Trento via Sommarive 9 38123 Trento (TN)

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder that primarily targets upper and lower motor neurons. The progression of the disease is mediated by altered intercellular communication in the spinal cord between neurons and glial cells. Intercellular communication, mainly happening through extracellular vesicles (EVs) is responsible for the horizontal transfer of proteins and RNAs to recipient cells. Recently, we showed that EVs released from ALS mutant astrocytes selectively induced toxicity in wild type motor neuron, thus reinforcing the notion that astrocytes exert toxicity in ALS disease propagation and suggesting EVs as mediators of toxicity. Although, multiple factors can play a role in motor neuron degeneration, recent evidences point toward a fundamental role for RNA and RNA-binding protein dys-homeostasis as crucial players in ALS pathogenesis. A thorough characterization of spinal astrocytes-EVs with multi-disciplinary approaches will be presented and implications in ALS spreading will be discussed.

Financing: EU-Marie-Sklodowska Curie Actions (individual fellowship)

Keywords: microvesicles, exosomes, neurodegeneration, cargo, nanotechnologies

Back to programme

S10-CL-07, session: Proteins and supramolecular structures

The multiple roles of Orai N-terminus in CRAC channel function

Isabella Derler (1)

(1) University of Linz, Institut of Biophysics Gruberstraße 40 4040 Linz

Calcium (Ca 2+) is an essential second messenger required for diverse signaling processes in immune cells. Ca 2+ release-activated Ca 2+ (CRAC) channels represent one main Ca 2+ entry pathway into the cell. They are fully reconstituted via two proteins, the stromal interaction molecule 1 (STIM1), a Ca 2+ sensor in the endoplasmic reticulum and the Ca 2+ ion channel Orai in the plasma membrane. After Ca 2+ store depletion, STIM1 proteins undergo a conformational rearrangement which leads to STIM1 oligomerization and their translocation into ER-PM junctions. Here, STIM1 proteins couple to Orai Ca2+ ion channels which results in Ca 2+ influx into the cell. STIM1 binding to and communication with Orai1 is predominantly achieved via a direct interaction of their C-terminal strands. Although binding of STIM1 to Orai1 C-terminus is an absolute premise, it is not sufficient for channel gating and still requires additional cytosolic domains of Orai1. Our studies uncovered a segment of the cytosolic Orai N-terminus, that is fully conserved among the Orai family and forms an elongated helix of the pore region, as indispensable for CRAC channel function. Several hot spots within this region control Orai channel activation, while other parts therein fine tune together with STIM1 the open Orai channel conformation. A communication of the N-terminus with a cytosolic loop region of Orai channels maintains their activation in an isoform-specific manner. Moreover, cholesterol has been identified to modulate Orai channel activation via its N-terminus. In summary, we present a multi-facetted role of the Orai N-terminus in CRAC channel activation.

Financing: FWF (P27641, P30567 to Isabella Derler, P28123 to Marc Fahrner, P28498 to Martin Muik, P28701 to Rainer Schindl, P27263, W 1250 to Christoph Romanin); program Inter-COST (project LTC17069 to Rudi Ettrich)

Keywords: ion channels, calcium, CRAC channels, STIM1, Orai1, Patch-Clamp, FRET

Back to programme

S10-OR-04, session: Proteins and supramolecular structures

Self-aggregation of light-harvesting complex II in reconstituted membranes mimics non-photochemical quenching in plants

Parveen Akhtar (1), Marijonas Tutkus (2), Fanni Görföl (1), Gediminas Trinkunas (2), Danielis Rutkauskas (2), Petar H. Lambrev (1)

(1) Hungarian Academy of Sciences, Biological Research Centre
(2) Centre for Physical Sciences and Technology, Institute of Physics

Photosynthetic organisms encounter large variations in the light intensity, often in a short period of time. Under high light conditions, the excess absorbed energy becomes potentially harmful and a mechanism of heat dissipation called non-photochemical fluorescence quenching (NPQ) is activated [1]. In plants, NPQ primarily occurs in the light-harvesting complex II (LHCII) and involves changes in the molecular and supramolecular structure [2]. Quenching can be triggered in isolated LHCII by aggregation, but it is not clear whether the same mechanism applies in vivo where protein contacts are restricted by the bilayer membrane. In this work we used reconstituted LHCII-lipid membranes in combination with time-resolved fluorescence spectroscopy and single molecule microscopy to monitor the changes in fluorescence yield as a function of vesicle size, curvature and protein density. Reconstituted membranes displayed a high degree of heterogeneity with respect to size, protein density and fluorescence yield. Protein-protein and lipid-protein interaction forces establish an equilibrium of multiple structural states/phases, whereby the median and average values of these important physical parameters differ by a large margin. This complicates the interpretation of results from traditional macroscopic, or ensemble-averaged, measurements. A strong correlation between the fluorescence yield and the lipid:protein ratio was found. The steady-state and time-resolved fluorescence spectra exhibited features reminiscent of NPQ in vivo, suggesting a common mechanism of quenching.

[1] Demmig-Adams B, Garab G, Adams III W. Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria: Springer; 2014.
[2] Ruban AV. Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. Plant Physiol. 2016;170(4):1903-16.

Financing: Hungarian Ministry for National Economy (GINOP-2.3.2-15-2016-00001); National Research, Development and Innovation Office (NKFIH NN 124904)

Keywords: LHCII; time-correlated single photon counting; membrane proteins; proteoliposomes; thylakoid membranes

Back to programme

S10-OR-05, session: Proteins and supramolecular structures

Probing lipid interactions of plasma membrane proteins: a micropatterning approach

Gergö Fülöp

It is well established that lipids and proteins are not just independent components of the plasma membrane of eukaryotic cells but that their arrangement, dynamics and function are interdependent.Besides direct lipid-protein interaction, transmembrane proteins are thought to bind a shell of annular lipids, which are more or less tightly associated with the proteins. Furthermore, highly ordered nanoscopic membrane domains have been proposed to act to compartmentalize proteins and their interactions, but have thus far not been directly observed. In this study, we use protein micropatterning combined with single-molecule tracking to directly measure lipid-protein interactions in the plasma membrane of living cells: different fluorescently labelled transmembrane proteins of interest (POIs) were captured and enriched within well-defined areas in the plasma membrane, leaving regions depleted of POI, which function as reference areas. From the distribution and diffusion behaviour of lipids and proteins with respect to the POI patterns, we were able to conclude on the local membrane environment of the POI. We found that a palmitoylated protein based on the transmembrane domain of hemagglutinin (HA-mGFP) influences its membrane environment well beyond the size of the transmembrane helix. The same effect was observed for a palmitoylation-deficient mutant allowing us to rule out formation of a more ordered membrane domain around HA-mGFP as the cause for this apparently increased protein size.

Keywords: membrane proteins; lipids; single molecule microscopy

Back to programme

S10-OR-08, session: Proteins and supramolecular structures

Location of the general anesthetics in model membranes

Pavol Hrubovcak (1), Tomas Kondela (2), Norbert Kucerka (1)

(1) Frank laboratory of neutron physics, Joint institute for nuclear research, Dubna, Russia
(2) Faculty of pharmacy, Comenius University in Bratislava, Bratislava, Slovakia

The anesthetic effect of primary aliphatic alcohols and alkanes is known generally, however, the question of its origin has not been sufficiently answered yet. Since there is a very close relation between changes in a structure of biomembrane and its specific functions, there is a hypothesis, which suggests the anesthetic effect stemming from the interaction of aliphatic molecules with cellular membrane constituents. A case study of bilayer systems doped with n-decane put forward the model, which assumes that the location and orientation of n-decane molecules within bilayer depends on their concentration. According to the model, molecules of n-decane most likely prefer one of the two possible positions. Either they concentrate among the lipid molecules with parallel orientation to their amphiphilic chains or they are located in the center of the bilayer, perpendicular to the lipid chains. The drawback of the hypothesis is in the ambiguous interpretation of experimental data. In order to shed more light on this question, we designed and performed a small-angle neutron diffraction experiment. We prepared model phospholipid (DOPC) membranes containing deuterium-labelled and unlabeled molecules of n-decane. Employing contrast variation method and mutual comparison of neutron diffraction data obtained for labeled and unlabeled samples, we were able to determine the location of n-decane molecules within the membrane.

Financing: VEGA Grant 1/0916/16; JINR Project 04-4-1121-2015/2017; ILL through Central European Neutron Initiative

Keywords: lipid bilayer; neutron diffraction; decane; membrane structure

Back to programme

S10-OR-09, session: Proteins and supramolecular structures

Influence of a transmembrane domain on calcium-membrane interaction

Adéla Melcrová (1), Piotr Jurkiewicz (1)

(1) J. Heyrovský Institute of Physical Chemistry of the CAS, v. v. i.

Calcium plays a significant role in various cellular processes including cell signaling or membrane fusion. Calcium ions have strong interactions with the negatively charged inner leaflet of plasma membrane (PM) where are the target molecules of many signaling pathways. Changes in biophysical properties of phospholipid membranes upon calcium binding were recently investigated (1). We took another step in further understanding the calcium binding to biologically relevant model of PM by incorporating a transmembrane peptide. We designed the peptide as a single transmembrane helix with either zero total charge or with a mild amount of positive charge. The positively charged peptide follows the so called positive-inside rule stating that the excess positive charge of helical TM proteins resides at the cytoplasmic side of the PM. The positive charge is however expected to repel the calcium ions. We use time-resolved emission spectroscopy of lipid vesicles accompanied by zeta potential measurements. The fluorescence technique gives us information on hydration and mobility of molecules in the vicinity of a fluorescent probe whereas the zeta potential shows trends in surface charge on the studied membranes. We found out that both transmembrane peptides indeed rigidify phospholipid bilayers. The positive charge only strenghtens this effect. Surprisingly, the positive charge of the peptide has no influence on calcium binding to membranes which is the same regardless the peptide content.

1. Melcrova et al. The complex nature of calcium cation interactions with phospholipid bilayers. Scientific Reports. 2016;6(1):38035. Available from: doi: 10.1038/srep38035.

Financing: Czech Science Foundation, Martina Roeselová Memorial Fellowship, Charles University project SVV

Keywords: Fluorescence spectroscopy, Time-resolved emission spectroscopy, Calcium-membrane interaction, Transmembrane peptide

Back to programme

S10-OR-10, session: Proteins and supramolecular structures

Structural basis for NLP toxicity

Tea Lenarcic (1)

(1) National Institute of Chemistry, Slovenia

Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are secreted by several phytopathogenic bacteria, fungi and oomycetes. They trigger leaf necrosis and immunity-associated responses in various dicotyledonous plants. Our recent studies showed that glycosylinositol phosphorylceramides (GIPC), a major class of plant sphingolipids in plant cell plasma membranes, are target molecules for NLP proteins. Structural studies using X-ray crystallography complemented with other biochemical and biophysical experiments revealed that a NLP protein from the phytopathogenic oomycete Pythium aphanidermatum (NLPPya) forms complexes with terminal hexose moieties of these sphingolipids. NLPPya binding to GIPC head groups induces several conformational changes, such as widening of the L2-L3-lined crevice and a 2.9Å movement of magnesium ion towards the center of the protein relative to its position in ligand-free NLPPya, which is followed by membrane attachment and host cell lysis. Structure based point mutations of residues in NLPPya protein predicted to be crucial for membrane anchoring resulted in plant toxin resistance. These results propose a model of early steps of NLPPya membrane interaction as well as suggest an explanation for selectivity of NLP proteins for dicotyledonous plants due to steric limitations of GIPC glycoside modules binding into a ligand binding cleft of NLPPya.

Keywords: phytopathogenic microorganisms; NLP proteins; X-ray crystallography

Back to programme

PS-01, session: Poster session

Open boundary molecular dynamics of sheared star-polymer melts

Jurij Sablić (1), Rafael Delgado Buscalioni (2), and Matej Praprotnik (1)

(1) Laboratory for Molecular Modeling, Theory Department, National Institute of Chemistry, SI-1001 Ljubljana, Slovenia
(2) Departamento Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid, Campus de Cantoblanco, E-28049 Madrid, Spain

We present Open Boundary Molecular Dynamics (OBMD) simulation of a star-polymer melt [1,2]. The simulation box is open in one direction, through which the system exchanges mass and momentum with the surroundings. The simulations are carried out under constant normal load, which is, together with shear flow, imposed to the system as an external boundary condition at the open ends. We study rheological, structural, and dynamical properties of the sheared melt under constant normal load and compare them with the ones obtained from simulations under constant volume. In the latter, the shear flow is introduced by modification of the equations of motion and boundary conditions. The results indicate that in equilibrium the melt under constant load exhibits grand-canonical behavior. Furthermore, the melt under shear flow expands, which results in the decrease of its density with the increase of the strength of shear flow, as opposed to the constant volume case, where it remains constant. The open boundaries also affect the pressure and rheology of the melt, while the results of both setups coincide in the majority of the structural and dynamical properties.

[1] R. Delgado-Buscalioni, J. Sablić in M. Praprotnik, Eur. Phys. J. Special Topics 224 , 2331 (2015).
[2] J. Sablić, M. Praprotnik in R. Delgado-Buscalioni, Soft Matter 12 , 2416 (2016)

Back to programme

PS-02, session: Poster session

Archaeosomes as model system to study and use listeriolysin O

Saša Rezelj (1), Gregor Anderluh (1)

(1) Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia

Archaeosomes are vesicles prepared of archaeal lipids from extremophile Archaea. The advantage of archaeal lipids is their chemical and physical stability. Cholesterol incorporation into archaeosomes further increases their stability and makes them suitable model system to study and use cholesterol dependent cytolysins (CDCs). In our studies, we used CDC listeriolysin O (LLO). Although, LLO is one of the most studied CDC, its exact binding and pore formation mechanism is yet to be elucidated. Until recently, we believed LLO can recognize only cholesterol on the membranes and thus binding and pore formation to be cholesterol dependent. Surprisingly, by using archaeosomes without cholesterol we observed binding and pore formation of LLO on archeal lipids. We hypothesize LLO can, next to cholesterol, recognize also sugars as membrane binding sites, since archaeal lipids have carbohydrate inositol in their lipid headgroup. We were able to prepare Giant Unilamellar Vesicles (GUV) with up to 20mol% cholesterol by electroformation. Since GUVs are close model system of cells due to their size, we were able to observe the binding of LLO to archaeal GUVs with confocal microscopy. Furthermore, we used synthetic biology approach to produce LLO with cell-free expression inside GUVs. Archaeal lipids enabled us to form GUVs bigger than 100 mm, which were stable for several days. Next to the wild type LLO, we are also using pH dependent mutant LLO Y406A, which is active only in acidic pH. With all those systems combined, we are developing specifically controllable vesicles for applications in synthetic biology and biotechnology.

Financing: Slovenian Research Agency

Keywords: listeriolysin O, Giant Unilamellar Vesicles, archaeal lipids, archaeosomes, syntehtic biology

Back to programme

PS-03, session: Poster session

A robust approach from nano indentation experiments

Alice Bartolozzi (1), Francesca Baldini (2), Michele Basso (1), Morgan Barbieri (3), Gemma Palazzolo (4), Laura Vergani (2), Massimo Vassalli (3)

(1) University of Florence, Italy
(2) University of Genova, Italy
(3) Biophysical Institute (CNR, Genova), Italy
(4) Italian Institute of Technology (IIT), Italy

In the last years, the exploitation of nanotechnology to the study of biological systems opened new avenues towards innovative clinical approaches based on single cell mechanical characterization.The present work is focused particularly on nano-indentation measurements for the characterization of single cell mechanical properties.The proposed approach aimed at providing a more robust parameter in terms of cell stiffness and was based on the Force Integration to Equal Limits (FIEL) method (1), a relative method that prevents several critical points of the standard fitting procedure (2). In order to increase and supplement the information gained from nanoindentation experiments, the FIEL method was extended to obtain the "Elastography" of the sample, computing stiffness trend depending on the indentation depth. This method allowed to identify different stiffness layers putatively associated with inner components and compartments of the cell. This approach was applied to FAO cells, a differentiated rat hepatoma cell line treated to mimic nonalcoholic fatty liver disease.

(1)E. Hassan, W. F. Heinz, J. H. Hoh. Relative Microelastic Mapping of Living Cells by Atomic Force Microscopy, Biophysical Journal 1998 74(3): 1564–1578, doi: 10.1016/S0006-3495(98)77868-3(2)Y. Ding, G. Xu, G. Wang. On the determination of elastic moduli of cells by AFM based indentation, Scientific Reports volume 7 2017, doi: 10.1038/srep45575.

Back to programme

PS-05, session: Poster session

The blood brain barrier integrity and the brain tissue redox status in the transgenic 5xFAD mouse model of Alzheimer's disease

Ana Belča Vesković (1), Aleksandra Pavićević (1), Đura Nakarada (1), Bogomir Prokić (2), Saša Petričević (3), Milka Perović (4), Selma Kanazir (4), Ana Popović-Bijelić (1), Miloš Mojović ( )

(1) 1. EPR laboratory, University of Belgrade – Faculty of physical chemistry, Studentski trg 12-16, Belgrade, Serbia
(2) University of Belgrade – Faculty of veterinary medicine, Bulevar oslobođenja 18, Belgrade, Serbia
(3) Galenika a.d., Sector for development, registration and quality, Batajnički drum bb, Belgrade, Serbia
(4) University of Belgrade - Institute for Biological Research "Siniša Stanković", Bulevar despota Stefana 142, Belgrade, Serbia

This study investigates the blood brain barrier (BBB) integrity and the brain tissue redox status in the transgenic 5xFAD mouse model of Alzheimer's disease using in vivo L-band, and ex vivo X-band EPR spectroscopy. 3-Carbamoyl-proxyl reduction experiments were performed on 9-month-old female 5xFAD mice (Tg), and age- and gender-matched non-transgenic (non-Tg) controls. Ex vivo results showed that the brains of Tg mice contain 2 more 3CP per mg of tissue than the non-Tg. This implies that more spin probe was able to enter the brain of 5xFAD mice, indicating a compromised BBB. This is also predicted by the rate constants obtained from the pharmacokinetic model that was used to fit the in vivo measured reduction decay of 3CP EPR signal. Namely, the results show that the BBB crossing rate constant is significantly higher in 5xFAD, compared to non-Tg. Furthermore, the percent of spin probe reduction in the brain tissues, assessed from ex vivo measurements, showed that it was comparable for both, Tg and non-Tg mice. This may lead to the conclusion that the brain redox state of both groups is the same. However, if we acknowledge that more 3CP was detected in the brains of Tg mice, meaning that quantitatively more spin probe was reduced, this in turn could imply that the redox capacity of 5xFAD brains was increased. Future experiments will be performed on male mice, several age groups, and also using another spin probe, less membrane permeable than 3CP.

Financing: The Ministry of Education, Science and Technological Development of the Republic of Serbia, projects 41005 and 173056

Keywords: Alzheimer's disease; 5xFAD mouse; EPR; 3-Carbamoyl-proxyl; blood brain barrier; redox status

Back to programme

PS-06, session: Poster session

Bioinformatics study of the structures of different curcumin formulations with enhanced bioavailability

Janja Belinc (1), Timotej Brumec (1), Eva Jovan (1), Peter Marinko (2), Vesna Postružnik (1), Vita Petek Regoršek (3), Marko Šterk (2), Tina Zorec (3), Nataša Latinović Antić (2), Tadej Emeršič (4), Andrej Dobovišek (2), Maša Hojnik Niderl (5), Aleš Fajmut (2)

(1) University of Maribor, Faculty of Health Sciences, Žitna ulica 15, 2000 Maribor, Slovenia
(2) University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška cesta 160, 2000 Maribor, Slovenia
(3) University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia
(4) University of Ljubljana, Faculty of Medicine, Institute of Biophysics, Vrazov trg 2, 1000 Ljubljana, Slovenia
(5) Vitiva proizvodnja in storitve d.d., Nova vas pri Markovcih 98, 2281 Markovci, Slovenia

Curcumin, a phenol isolated from turmeric, exhibits many beneficial health-promoting characteristics, however, its poor pharmacokinetic properties, in particular low systemic bioavailability, remains an obstacle in achieving them. Curcumin exhibits low aqueous solubility, thus poor gastrointestinal absorption and a high rate of metabolism. As a consequence, it occurs in the blood primarily in the physiologically and pharmacologically inactive sulphate and glucuronide. Over the last decade, research efforts have focused on improving its bioavailability. This has resulted in several commercialised curcumin formulations with different carriers, such as micelles, liposomes, as well as solid (nano)particles and (nano)structures, formed from either natural or artificial compounds, such as galactomannans, lecithin, phospholipids, oligosaccharides, polysorbates and polyethylene glycols. Their common goal is to mask the hydrophobic nature of curcumin, to protect it from rapid metabolism and deliver it at a highest possible proportion unmodified to the tissues. Recently, various nanoparticulate preparations such as nanoemulsions, nanomicelles, dendrimers, conjugates, polymers, nanogels, and solid dispersions were also proposed, though, a number of these formulations have limitations because some ingredients are not recommended for food use. Here we will present a systematic review of several different structures and the compounds of curcumin formulations with increased bioavailability, and in another presentation we will focus on their pharmacokinetic properties.

The work presented here is a part of the ongoing project PKP 2018 involving students of Bioinformatics, Physics and Chemistry and a partner company Vitiva d.o.o. The investment is co-financed by the Republic of Slovenia and the European Union under the Cohesion Fund EKP 2014-2020.

Keywords: Curcumin; Formulation; Bioavailability

Back to programme

PS-07, session: Poster session

Study of the influence of negatively charged mixed micelles on properties of cytochrome c

Martin Berta (1), Nataša Tomášková (1), Erik Sedlák (1)

(1) P. J. Šafárik University, Košice

Cytochrome c (cyt c) is known not only for its electron transfer activity in electron transport chain in mitochondria, but also for its function as initiator of apoptosis. It is generally acknowledged that cyt c function in apoptosis is accompanied by its increased peroxidase-like activity, which is triggered by its interaction with negatively charged inner mitochondrial membrane. In the present research, we attempt to determine amount of negative charge, which is necessary to induce the peroxidase activity as well as conformational changes in cyt c induced by interaction with the negatively charged surface. The interaction of cyt c with negatively charges membrane surface is of electrostatic and hydrophobic natures, and triggers methionine decoordination and formation of non-native low spin, and high spin forms of cyt c. In order to understand the structural modifications of cyt c induced by interaction with membrane surface, we used mixed micelles as a model system. This approach allowed us to assess the role of electrostatic interaction in conformational changes of cyt c. The effect of mixed micelles (SDS:DDM) on conformation, p K a , T m values, and peroxidase-like activity of cyt c was analyzed by absorption spectrometry, fluorimetry and spectropolarimetry. Our results indicate that the peroxidase-like activity of cyt c significantly increases in the presence of the mixed micelles with negative charge of about 10-15. Interestingly, under these conditions, the conformation of cyt c was not significantly affected, which suggests that the increased peroxidase-like activity of cyt c is associated with an increased dynamic of its heme region.

Financing: Slovak grand agency VEGA no. 1/0426/16, APVV-15-0069

Back to programme

PS-08, session: Poster session

Determining the Oligomeric States of the Metabotropic Glutamate Receptor mGlu2 by using Single Molecule Techniques

Clara Bodner (1), Jonathan Javitch (2), Wesley Asher (2), Gerhard Schütz (1), Mario Brameshuber (1)

(1) Institute of Applied Physics, TU Wien, Vienna, Austria
(2) Department of Pharmacology, Columbia University, New York

We present single-molecule studies of the Glutamate receptor - a constitutive dimer serving as a model system of a protein with known subunit stoichiometry. In future experiments, results based on mGlu2 dimerization will be used to characterize the unknown oligomeric state of the Dopamine receptor D2s. The Dopamine receptor is a member of the rhodopsin-like G protein-coupled receptors (GPCRs) that mediates the physiological function of the neurotransmitter dopamine [1].
Recently, single particle tracking and FRET experiments on mGlu2 and D2s have been performed at low receptor surface densities. Results suggested, that dopamine receptors form dimeric or higher-order oligomeric complexes with distinctive signaling profiles and functions, while the pure dimeric nature of mGlu2 was confirmed. In order to be able to characterize these receptors at physiologically high surface densities, we utilized in-house developed single molecule methods. A two-color TOCCSL (thinning out clusters while conserving stoichiometry of labeling) [2][3] approach was utilized in order to identify the subunit stoichiometry of the glutamate receptor based on co-localization analysis of the two different fluorophores. In addition, brightness analysis of individual fluorescent spots and single-molecule FRET (Förster Resonance Energy Transfer) between differently labeled molecules was used to support the two-color TOCCSL based results.

[1] Smith NJ, Milligan G. Allostery at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. Pharmacol Rev. 2010;62:701–25. [2] M. Moertelmaier, M. Brameshuber, M. Linimeier, G. J. Schütz, H. Stockinger, Applied Physics Letters 2005, 87.26, 263903. [3] V. Ruprecht, M. Brameshuber, G. J. Schütz, Soft Matter 2010, 6, 568-81.

Keywords: TOCCSL; smFRET; Fluorescence microscopy

Back to programme

PS-09, session: Poster session

Theoretical model describing the osmotic phenomena due to the transmembrane pores induced by a pore-forming agent

Bojan Božič (1), Grega Hrovat (1), Luka Kristanc (1), Gregor Gomišček (1,2)

(1) Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
(2) Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia

Nystatin is a polyene antifungal agent, which binds to the cell membrane. A group of nystatin molecules forms a pore. Studies of antifungal agents on phospholipid vesicles can explain the role of transmembrane pores. In the experiments the phospholipid vesicles contain a sucrose solution. When they are transferred to nystatin and glucose solution, three characteristic types of tension-pore occur: transient pore, slow rupture and explosion [1]. The type of pores depends on the nystatin concentration. To explain the vesicle behavior the number of nystatin pores in the model has to vary [2]. Taking into account the time dependence of the growing number of nystatin pores, the theoretical results are close to the behavior of the observed tension-pores in the phospholipid vesicles. The model presented predicts the same three types of tension-pores as they are predicted by the model with a constant number of pores. However, considering the variation of the number of nystatin pores a later occurrence of tension-pores can be explained, which is consistent with experiments. The results show that the concentration of glucose solution in the vesicle increases whereas sucrose in the vesicle solution monotonically decreases in all cases. We found that the changes in mechanical properties of the membrane influence the number of nystatin pores required for the same transition between different types of tension-pores in a linear manner.

[1] Kristanc L, Svetina S, Gomišček G, Effects of the Pore-Forming Agent Nystatin on Giant Phospholipid Vesicles, Biochim Biophys Acta. 2012;1818:636-644. Available from: doi: 10.1016/j.bbamem.2011.11.036.
[2] Kristanc L, Božič B, Gomišček G. The role of sterols in the lipid vesicle response induced by the pore-forming agent nystatin, Biochim Biophys Acta. 2014;1838:2635-2645. Available from: doi: 10.1016/j.bbamem.2014.05.019.

Financing: Slovenian Research Agency (https://www.arrs.gov. si, P1-0055)

Keywords: lipid vesicles; tension-pore; nystatin pore

Back to programme

PS-10, session: Poster session

Interferon augments the expression of MHC class II molecules and modulates single vesicle interaction with the plasma membrane in cultured rat astrocytes

Mićo Božić (1,2), Eva Lasič (1,2), Matjaž Stenovec (1,2), Robert Zorec (1,2)

(1) Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia
(2) Celica Biomedical, Tehnološki park 24, 1000 Ljubljana, Slovenia

Astrocytes are the most abundant and heterogeneous glial cells that control homeostasis, provide for defense of the central nervous system, and demonstrate remarkable adaptive plasticity. Upon exposure to the cytokine IFNγ, astrocytes acquire (re)active phenotype characterized by the increased expression of major histocompatibility complex class II molecules (MHC II) and enhanced mobility of endolysosomes. The nature of astrocytic vesicles delivering MHC II molecules to the plasmalemma and the vesicle interactions with it are less well understood. Here, we activated cultured rat astrocytes with IFNγ (600 U/ml, 48h) to immunofluorescently examine subcellular localization of MHC II by confocal and by structured illumination microscopy. In addition, we examined single vesicle interactions with the plasmalemma by cell-attached membrane capacitance measurements. Cell activation with IFNγ augmented expression of MHC II molecules; the MHC II immunofluorescence (per cell) increased from 4% (controls) to 13% (IFNγ-activated; $P<0.001$). The number of MHC II-positive vesicles in the peri-plasmalemmal space increased by 14fold, from 0.007 ± 0.001 vesicles/μm in controls to 0.098 ± 0.021 vesicles/μm in IFNγ-activated cells ($P<0.001$). The diameter of MHC II-positive vesicles was larger in IFNγ-activated cells than in controls; 266.8 ± 3.1 μm vs. 224.5 ± 5.7 μm ($P<0.01$). In activated astrocytes, reversible fusion of larger vesicles with broader fusion pores was observed, while the frequency of complete exo- and endocytotic events decreased when compared to controls. IFNγ treatment favors increased vesicular MHC II expression and possible interaction with the plasmalemma, and affects dynamics of single vesicle exo-/endocytosis in activated astrocytes.

Financing: Javna agencija za raziskovalno dejavnost RS (ARRS) / Slovenian Research Agency

Keywords: astrocytes; interferon γ; major histocompatibility complex class II; endolysosomes; single vesicle interactions; exocytosis; endocytosis; membrane capacitance; patch clamp; confocal microscopy; structured illumination microscopy;

Back to programme

PS-11, session: Poster session

Properties of Bio-compatible Polymers for 3D Nanostructuring

Boris Buchroithner (1)

(1) PhD Student Johannes Kepler University Linz

Johannes Kepler University, Institute of Applied Physics, Altenbergerstr. 69, 4040 Linz, Austria Abstract: Two-Photon Polymerization (2PP) allows fabrication of arbitrary three-dimensional structures in the sub-micrometer range with controllable spatial and material properties. For 2PP a liquid resin consisting of cross-linkable monomers and a small amount of a photo initiator is used as matrix for writing. Femtosecond laser pulses and a high numerical aperture objective are used to create a focal volume with a high energy density. Therefor a simultaneous absorption of two photons by the photo initiator is probable. The excited photo initiator decays than into a chemical active radical, which starts the radical polymerization. Thus the cross-linking of the monomers yields the solidification of the polymer. Recent advances in three-dimensional nanolithography for tissue engineering requires for non-toxic materials mimicking tissue properties (eg. the extracellular matrix or cartilage). Typically for 3D lithography acrylate monomers are used because of their high reactivity. However, they are toxic. Up to now mostly methacrylates are used. However they are less reactive and therefore require a lower manufacturing velocity. In this work we present a new type of biocompatible polymer, which combines the reactivity of acrylates, the biocompatibility of methacrylates and the stability of thiols. The resins are structured using 2PP with a 515nm light source, a writing speeds up to several mm/s and sub-micrometer feature sizes. In order to characterize the mechanical properties of the manufactured scaffolds, atomic force microscopy (AFM) was used. The polymers Young's modulus have been characterized and compared to different available resins.

Financing: Interreg ATC14 CAC-SuMeR

Keywords: Two-Photon Polymerization; biocompatible polymer; Young's modulus

Back to programme

PS-12, session: Poster session

Conformational biases of DC8E8 antibody tetratope on the molecule of intrinsically disordered protein tau

Ondrej Cehlar (1), Rostislav Skrabana (2), Michal Novak (3)

(1) Axon Neuroscience RD Services SE, Bratislava, Slovakia Institute of Neuroimmunology of SAS, Bratislava, Slovakia
(2) Axon Neuroscience RD Services SE, Bratislava Institute of Neuroimmunology of SAS, Bratislava
(3) Institute of Neuroimmunology of SAS, Bratislava

Accumulation of intrinsically disordered protein tau in the form of insoluble aggregates is a common feature of neurodegenerative tauopathies. Monoclonal antibody DC8E8 is able to inhibit tau-tau interaction and therefore it holds promise for the immunotherapy of Alzheimer's disease. The active vaccine based on the DC8E8 epitope peptide has successfully passed the phase 1 clinical trial. Minimal epitope of DC8E8 represents amino acid motif HXPGGG that is present in each of the four microtubule binding repeats (MTBRs) of tau. However, the affinity of DC8E8 for its MTBR epitopes differs and descends as follows: MTBR2 > MTBR1 > MTBR3 > MTBR4. These differences in the antibody affinity for highly homologous epitopes can be attributed to different conformational biases of epitope peptides for the bound conformation. The crystal structure was solved so far only for the complex of MTBR2 peptide with DC8E8 Fab. We have performed molecular dynamics simulations of 18 amino acids long peptides from all four MTBRs and compared the percentage of sampled bound-like conformation with the antibody affinity. Unravelling the unique mode of recognition of DC8E8 antibody and conformational biases of tau protein repeat regions can aid to reveal the hindered structural features of tau protein biology. Acknowledgement: This work was supported by VEGA grant 2/0177/15. Calculations were performed in the Computing Centre of the Slovak Academy of Sciences using the supercomputing infrastructure acquired in project ITMS 26230120002 and 26210120002 (Slovak infrastructure for high-performance computing) supported by the Research Development Operational Programme funded by the ERDF

Keywords: Molecular dynamics simulation; tau protein; antibody

Back to programme

PS-13, session: Poster session

Development of High-Precision Laser-assisted Cell Printing System

Chia-Yu Chang (1), Bing-Sian Li (1), Shih-Chi Hung (1), Tsung-Jen Wang (1), Long-Sheng Lu (1), Tzu-Sen Yang (1)

(1) Institute of Nanomedicine and Medical Engineering, College of Biomedical Engineering, Taipei Medical University

Tissue engineering is a technology that seeds cells on the preformed scaffold along with growth factors and culture to form functional substitutes. However, seeding cells on the preformed scaffold cannot control over cell position and tissue architecture, which has the inability to mimic the complex microstructures of tissues. 3D bioprinting shows a promise to bridge the divergence between artificially engineered tissues and native tissues. Among 3D bioprinting methods, laser-based bioprinting has the highest spatial resolution. For this reason, we developed a 830nm laser induced forward transfer (LIFT) bioprinting system with high resolution. The laser was focused on an energy-absorbing layer and induces a vapor bubble through a 20X objective. The bubble formation process produced a jet that transfers a droplet of bioinks onto a receiving substrate. A customized 2D pattern can be printed by controlling both the shutter and XY stage motion simultaneously. Gold film was used as the energy-absorbing layer for near-IR laser at the present time. The results showed that alginate droplets were successfully generated and the diameter of these droplets was about 200 to 250 m. We continued to modify printing conditions by adjusting laser energy, laser exposure time and direct-writing height for printing performance improvement. We anticipate the proposed LIFT technology can provide potential for treating corneal endothelial decompensation.

Lee VK and Dai G. Printing of Three-Dimensional Tissue Analogs for Regenerative Medicine. Ann Biomed Eng. 2017;45(1):115-131.

Keywords: tissue engineering; 3D bioprinting; laser induced forward transfer (LIFT)

Back to programme

PS-14, session: Poster session

Numerical study of electrochemotherapy treatment of spinal metastases using transpedicular approach

Helena Cindrič (1), Bor Kos (1), Giuseppe Tedesco (2), Matteo Cadossi (2), Alessandro Gasbarrini (2), Damijan Miklavčič (1)

(1) Laboratory of Biocybernetics, Faculty of Electrical Engineering, University of Ljubljana, Slovenia
(2) Department of Oncologic and Degenerative Spine Surgery, Rizzoli Orthopedic Institute, Bologna, Italy

Spinal metastases are a common complication in cancer patients that greatly decrease their quality of life. It has been demonstrated in previous studies that bone metastases can be efficiently treated by electrochemotherapy [1]. We numerically investigated a novel approach to treat spinal metastases, i.e. transpedicular approach, in which needle electrodes for electrochemotherapy are inserted into the vertebral body through the pedicles. Our analysis was based on electric field distribution within the tumor and surrounding tissues. Three clinical cases, each expressing a different stage of vertebral body, arch and spinal canal involvement, were used. Complete coverage of tumor tissue with electric field above reversible electroporation threshold (400 V/cm) is a prerequisite for successful electrochemotherapy. Two cases exhibited above 99 % tumor coverage; coverage of the third tumor was 98,9 %. Potential damage to spinal cord and nerves was also evaluated. In one case, in which the tumor had grown into the spinal canal, 12 mm 3 of spinal cord tissue was covered with electric field above the assumed threshold for irreversible electroporation (ablation) of neural tissue, indicating potential for neural damage. Our study shows that transpedicular approach enables successful treatment. However, if the tumor is in immediate vicinity of critical neural structures potential neural damage needs to be carefully considered.

[1] Gasbarrini A, Campos WK, Campanacci L, Boriani S. Electrochemotherapy to Metastatic Spinal Melanoma: A Novel Treatment of Spinal Metastasis? Spine. 2015 Dec;40(24):E1340-1346.

Financing: Slovenian Research Agency (ARRS), within the scope of the European Associated Laboratory on the Electroporation in Biology and Medicine (LEA-EBAM)

Keywords: Electrochemotherapy; Electroporation; Numerical modelling; Spinal metastases;

Back to programme

PS-15, session: Poster session

Optimal hierarchical tissue organization in the presence of selection

Demeter Márton Csaba (1)

(1) ELTE-MTA "Lendulet" Biophysics Research Group, Department of Biological Physics, Eötvös University, Pázmány Péter stny. 1A, H-1117 Budapest, Hungary

Cancer development is a somatic evolutionary process: cells must divide and as a result mutations that can ultimately lead to neoplastic progression may accumulate. Our previous results demonstrated that hierarchically organized tissues can greatly reduce divisional load , the number of divisions along cell lineage trees [Derenyi and Szollosi 2017 Nat.Comm.]. These results, however, did not consider the selective effects of mutations.
Here we explore the role of selection by introducing mutations that change the rate of different types of cell divisions and differentiation events implementing the tissue hierarchy. We derive a generic threshold value that determines the critical number of mutations necessary for cancer initiation in hierarchically organized tissues. Using extensive stochastic simulations we show that we are able to analytically estimate the probability of neoplastic progression in our model using the theory of birth death processes and the statistical characteristics of the cell-linage tree.
Our results demonstrate that hierarchically organized tissues optimized to reduce the accumulation of mutations with selective effects, i.e. to reduce to probability of neoplastic progression, are not identical to those that minimize divisional load. In particular we find that in tissues with a physiologically realistic number of levels the division rate of stem cells is higher than the extremely low rates required to minimize divisional load. This suggest that the optimum induced by selection gives rise to an elevated functional diversity of tumor cells due to an increased propensity for neoplastic progression in less differentiated levels of the hierarchy.

Keywords: Cancer; hierarchical organization; statistical physics; computational biophysics; stem cells

Back to programme

PS-16, session: Poster session

Modulated Photophysics of Curcumin Entrapped in Polymeric Micellar Assembly: The Role of the Hydrophilic/Hydrophobic Ratio of 2-oxazoline Based Gradient Copolymer

Shubhashis Datta (1), Annamaria Jutkova (2), Petra Sramkova (3), Dusan Chorvat (), Pavol Miskovsky (4), Daniel Jancura (4), Juraj Kronek (4)

(1) Postdoctoral fellow
(2) PhD student
(3) Post doc student
(4) Professor

Polymeric micelles have been extensively used for the targeted delivery of anticancer drugs to tumor sites by passive as well as active mechanism. Present study provides important information on the nano-formulation of curcumin, a bioactive compound using poly(2-oxazoline) which are considered as the new generation polymer therapeutics. Here we have investigated the role of the hydrophobic block of 2-oxazoline based gradient copolymers on the modulation of the photophysical properties of curcumin trapped inside the micellar assembly. The solubility and chemical stability of curcumin is largely enhanced due to encapsulation inside the micelle and it depends very much on the hydrophobicity of core of the polymeric micelle. The fluorescence quantum yield is also enhanced along with the blue shifting of the emission maxima which is a consequence of the reduced interaction of curcumin with water. The rate of the major excited state deactivation pathway of curcumin through intramolecular proton transfer process is retarded due to the intermolecular hydrogen bonding with micellar core.

Financing: The authors are thankful to the Slovak Grant Agency VEGA for the financial support in the project No. 2/0124/18 and the Slovak Research and Development Agency for financial support in the project No. APVV-15-0485.

Keywords: Polymeric micelle; 2-ethyl-oxazoline; EPST;

Back to programme

PS-17, session: Poster session

GPCR Transactivation Observed by Two-photon Polarization Microscopy

Ilaria D'Augello (1), Veronika Bělíková (2), Alexey Bondar (3), Peter McCormick (4), Josef Lazar (5)

(1) Institute of Organic Chemistry and Biochemistry AS CR
(2) Inst. of Microbiology CAS
(3) Laboratory of Cell Biology, Center for Nanobiology and Structural Biology, Institute of Microbiology, ASCR, Nove Hrady
(4) School of Pharmacy, University of East Anglia, Norwich, UK
(5) Inst. of Microbiology CAS

Many G-protein coupled receptors exist as homo- or heterodimers. While GPCR dimerization has been known for many years, many of its functional aspects remain to be elucidated. We have now employed the technique of two-photon polarization microscopy (2PPM), known to allow sensitive observations of activation of the Galphai signaling pathway, to investigate functional dimerization of the cannabinoid receptor CB1R with other GPCRs. Our results show that the presence of the CB1R receptor, which normally couples to the G protein Galphai, allows activation of the Galphai pathway by other GPCRs. Our findings provide a possible explanation for known side effects of pharmaceutical drugs targeting various GPCRs.

Keywords: G-protein coupled receptors; two-photon polarization microscopy

Back to programme

PS-18, session: Poster session

Comprehensive biophysical assays: From single channel electrophysiology to overall cell behavior

Elena Dragicevic (1)

(1) Nanion Technologies GmbH

Integral membrane proteins, predominantly ion channels and transporters, have been the focus of basic biophysical research, as well as drug discovery and safety projects for decades. Electrophysiological experiments on fully functional artificial lipid bilayers enable the investigation of basically any membrane-affecting agent. In combination with automated patch clamp and impedance/electrical field potential (EFP)-like recordings of relevant targets expressed in heterologous systems, as well as of human iPSC-derived cardiomyocytes and neurons, we demonstrate broad biophysical application assays, connecting single channel electrophysiology with overall single cell and cell population behavior. Here, we present the temperature dependent activation or deactivation of different Transient Receptor Potential (TRP) channels by means of planar patch clamping on our medium and high throughput screening (HTS) platforms Patchliner and SyncroPatch 384PE, as well as with highest resolution on a single channel level on our recently introduced Orbit mini setup. Additionally, the effect of drugs on action potentials as recorded in iPSC-cardiomyocytes is important for assessing the interaction of the cardiac ion channel ensemble. We present our advances in development of iPSC-cardiomyocytes "ready-to-use" assays for automated patch clamp. We also show, short and long-term impedance/EFP-like recordings of diverse cell-types, such as drug safety experiments on iPS cardiomyocytes and cancer tox-assays. In summary, medium and high throughput screening (HTS) assays such as automated electrophysiological patch clamp and impedance-based assays allow for the determination of drug effects on a whole cell level whereas artificial bilayers provide a robust environment for the assessment of single ion channel molecules.

Keywords: ion channels, automated patch clamp, bilayers, cardiomyocytes, cancer

Back to programme

PS-19, session: Poster session

The ins and outs of plastid membrane trafficking under normal development and stress conditions

Jean-Marc Brillouet (1), Henrik Aronsson (2), Katalin Solymosi (3)

(1) UMR SPO INRA, SupAgro, UM I, Montpellier
(2) Department of Plant and Environmental Sciences, Göteborg University, Göteborg
(3) Department of Plant Anatomy, ELTE Eötvös Loránd University, Budapest

Plastid inner membranes harbour several proteins and play vital roles in plastid metabolism. Their components (e.g. lipids) are synthesized in the envelope membranes and need to be transported through the aqueous stroma towards the inner membranes. Similarly, some of the plastid-derived metabolites need to be transported from the inner membranes towards the envelope or even beyond. However, comprehensive data about membrane trafficking of plastids are scarce. Here we present systematic ultrastructural evidence about the occurrence and role of vesicles in membrane trafficking in various plastid types present in different cell types and organs of several plant species. Vesicles occur at ambient conditions but their number increases under various stress conditions (e.g. cold pretreatment, salt stress, oxidative stress). These data along with molecular data showing the presence of protein-mediated vesicle transport components similar to cytosolic coat protein complex II system unambigously outline the importance of vesicle transport in plastids, especially during thylakoid biogenesis. On the other hand, we show that small, thylakoid-derived vesicles containing condensed tannins or their precursors, and termed tannosomes are 'excreted' out by the plastids in tannosome shuttles to the cytoplasm and are directed towards the vacuole. Under salt stress conditions, peculiar envelope membrane invaginations are produced and part of the plastid stroma seems to be prone to autophagy-like processes. Similarly, we present data about peculiar plastid autophagy processes with multilamellar membrane structures observed under nutrient starvation related to low light conditions and probably being involved in nitrogen recycling. Clearly, plastid membrane trafficking processes have important physiological roles.

Financing: This work has been financed by the Ministry of Human Capacities under the project ÚNKP-17-4-III-ELTE-128, and by the National Research, Development and Innovation Office (grant OTKA FK124748).

Keywords: thylakoid biogenesis; plastid autophagy; tannin formation; plastid vesicle formation

Back to programme

PS-20, session: Poster session

Hydrogen attachment and release in Fe(H)2(H2)(PEtPh2)3. Theoretical predictions

Vjeran Gomzi (1)

(1) Faculty of electrical engineering and computing, University of Zagreb

A special class of hydrogen-binding metal complexes consists of complexes able to bind hydrogen molecules at one or more of their ligand positions. Some time ago, it has been found that one of the metal complexes with dihydrogen-binding capabilities is Fe(H)2(H2)(PEtPh2)3 [1]. More recently it has been predicted that this complex actually possesses two dihydrogen binding sites of almost the same energy [2]. It has been shown that its specific properties may be related to the asymmetry of the non-hydrogen ligands. Following this reasoning, the processes of binding and release of hydrogen molecule to the metal center in the complex have further been investigated. Here, the attachment and detachment of hydrogen molecule to the 5-coordinate and 6-coordinate complex respectively have been investigated theoretically by performing relaxed potential-energy scans. Ligand asymmetry seems to reflect on the different barrier energies for the approach to and detachment from the two possible dihydrogen ligand positions. This asymmetry further leads to the possible behavior best represented by hysteresis-like diagrams for the H2 approach and detachment at the two minima.

[1] Van der Sluys LS, Eckert J, Eisenstein O, Hall JH, Huffman JC, Jackson SA, Koetzle TF, Kubas GJ, Vergamini PJ, Caulton KG. An attractive cis-effect of hydride on neighbor ligands: experimental and theoretical studies on the structure and intramolecular rearrangements of Fe(H)2(.eta.2-H2)(PEtPh2)3. JACS. 1990;112:4813-4841.
[2] Došlić N, Gomzi V, Mališ M, Matanović I, Eckert J. Fluxionality of hydrogen ligands in Fe(H)2(H2)(PEtPh2)3. Inorg Chem. 2011; 50(21):10740-10747

Keywords: iron complex, hydrogen, DFT, potential-energy scan

Back to programme

PS-21, session: Poster session

Fluorophores in the urine of patients with neuropsychiatric disorders

Lucia Ilavská (1), Marcela Morvová Jr. (2), Jana Trebatická (3), Zdeňka Ďuračková (4), Libuša Šikurová (5)

(1) Student of Master's degree at Faculty of Mathematics, Physics and Informatics of the Comenius University in Bratislava, Mlynská Dolina, 842 48 Bratislava
(2) Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská Dolina, 842 48 Bratislava
(3) Department of Child Psychiatry, Faculty of Medicine of the Comenius University and National Institute of Child Diseases, Limbova 1, 833 40 Bratislava
(4) Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Sasinkova 2, 813 72 Bratislava
(5) Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics of the Comenius University, Mlynská Dolina, 842 48 Bratislava

The prevalence of psychiatric disorders in population permanently increases and their occurrence shifts to earlier years. A hypothesis has been formulated that many mental diseases, rise from a disturbance of the brain serotoninergic system. The only known serotonin precursor is tryptophan. Tryptophan is metabolized mainly through the methoxyindole and kynurenine pathways. Also guanosine triphosphate metabolites (neopterin, biopterin, pterin) are associated with tryptophan metabolism. The goal of this work was to determine the levels of neopterin, biopterin, pterin and tryptophan in the urine of children with depressive disorder and compare them to the levels in urine of healthy volunteers. These metabolites were determined by high performance liquid chromatography (HPLC) with fluorescence detection 350/450 nm (excitation/emission wavelength) and 280/370 nm for tryptophan. We found decreased tryptophan in the urine of depressed patients compared to healthy volunteers. Biopterin also showed similar tendencies. Biopterin metabolism enters the conversion of tryptophan to serotonin. Decreased levels of tryptophan may be due to a faster degradation in kynurenic way.

Financing: This study was supported by APVV–15–0063 and VEGA 1/0703/13.

Keywords: neuropsychiatric disorders; urine; high performance liquid chromatography

Back to programme

PS-22, session: Poster session

Radical(s) in cytochrom c oxidase

Daniel Jancura (1), Marian Fabian (2)

(1) Department of Biophysics, Faculty of Science University of P.J. Safarik Jesenna 5 041 54, Kosice, Slovakia
(2) Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P.J. Safarik, Jesenna 5 040 01 Kosice, Slovakia

Catalytic mechanism of cytochrome c oxidase (CcO) involves formation of ferryl intermediates P and F . The production of these intermediates is accompanied by a formation of protein-based radical(s). The reaction of oxidized CcO with hydrogen peroxide also leads to the formation of P and F and corresponding radical species. However, the application of electron paramagnetic spectroscopy (EPR) to detect such radical has resulted only in the observation of low amounts relative to the concentration of the P intermediate. A possible reason for this fact is a coupling of the unpaired electron of radical with the paramagnetic metal center(s) within the catalytic site of CcO. We have developed a new approach, a moderate destabilization of the enzyme structure by protein denaturant, guanidinium chloride (Gnd.Cl), to detect stoichiometric amount of the radical in CcO. In this situation, a coupling between protein-based radical and a metal center(s) is broken. As our results show, the yield of the EPR observed radical in P is significantly increased in the presence of Gnd.Cl relative to that in the absence of denaturant. In a sample with 2 M Gnd.Cl, the yield of the detected radical reached 50% of the P population. The origin of EPR detected radical(s) and their possible roles in the catalytic cycle of CcO is discussed.

Financing: Slovak Research and Development AGency, APVV-0485-15

Keywords: cytochrome c oxidase; radical

Back to programme

PS-23, session: Poster session

Detection of polymorphism in pharmaceutical products using 14N NQR spectroscopy

Vojko Jazbinšek (1), Janez Pirnat (1), Zvonko Trontelj (1), Zoran Lavrič (2), Stane Srčič (2)

(1) Institute of Mathematics, Physics and Mechanics
(2) University of Ljubljana, Faculty of Pharmacy

The golden standard in determination of polymorphism in an active pharmaceutical ingredient (API) is the X-ray diffraction method. However, it usually requires a special sample preparation and is less suitable for checking the possible appearance of polymorphism in drugs during the production process and shelf life. In our studies of some APIs in pharmaceutical products we have noticed that nitrogen nuclear quadrupolar resonance (14 N NQR) reveals nondestructively, quickly and reliably the appearance of polymorphism [1,2].
In this study, we examined polymorphism in antibacterial drug sulfanilamide in order to demonstrate good and reliable selective property of 14 N NQR spectroscopy and its applicability in determination of polymorphism. There are three known polymorphs of sulfanilamide, which gives two sets of three 14 N NQR transition frequencies, corresponding to two different nitrogen sites in the crystal structure for each of three polymorphs. One of the three 14 N NQR frequencies is recognized to be enough to determine the polymorph. This quick and reliable proof of polymorphism appearance could become a method of choice in determination and/or confirmation of polymorphism, especially in solid drugs containing nitrogen.

[1] Lužnik J, Pirnat J, Jazbinšek V, Lavrič Z, Žagar V, Srčič S, Seliger J, Trontelj Z. 14 N Nuclear Quadrupole Resonance Study of Polymorphism in Famotidine. J Pharm Sci. 2014; 103(9): 2704–2709. Available from: doi:10.1002/jps.23956.
[2] Lavrič Z, Pirnat J, Lužnik J, Seliger J, Žagar V, Trontelj Z, Srčič S. Application of 14 N NQR to the study of piroxicam polymorphism. J Pharm Sci. 2010; 99(12): 4857–4865. Available from: doi:10.1002/jps.22186.

Financing: Slovenian Research Agency

Keywords: NQR spectroscopy; polymorphism; sulfanilamide

Back to programme

PS-24, session: Poster session

The radioprotective effects of the aminothiol GL2011 administered to Wistar rats after irradiation

Miloš Jovanović (1), Aleksandra Pavićević (2), Bojana Šećerov (3), Pavle Andjus (1), Ana Popović-Bijelić (2), Miloš Mojović (2)

(1) University of Belgrade – Faculty of Biology, Studentski trg 12, Belgrade, Serbia
(2) EPR laboratory, University of Belgrade – Faculty of Physical Chemistry, Studentski trg 12-16, Belgrade, Serbia
(3) Vinča Institute of Nuclear Sciences, Mike Petrovića Alasa 12-14, Belgrade, Serbia

Previous studies have shown an effective radioprotective action of a new non-toxic aminothiol GL2011 (1,2). According to the data showing modulation of proteins involved in protection against oxidative stress (1) we hypothesised that this affect may be also partly due to free radical scavanging that could be beneficial even with post-irradiation administration. This was investigated by L-band EPR spectroscopy monitoring the in vivo reduction kinetics of the spin probe 3-carbamoyl-proxyl (3CP) recorded from the liver of 2-month old male albino Wistar rats irradiated with 6.7 Gy. The half-elimination times of 3CP (t 1/2) were determined from the time course of EPR spectra measured after 1h, 4h, 8h, 3 days and 5 days after irradiation of rats without radioprotection. 3CP reduction after 1 and 4h was faster than in non-irradiated rats (11.4 0.4 min, and 10.7 0.4 min, vs. 14.9 0.4, respectively). Suggesting that at these time points following irradiation, there is a significant endogenous oxygen/nitrogen reactive species production, most likely causing 3CP reduction. Furthermore, to evaluate the effect of GL2011 administered after irradiation, a group of rats received a dose of GL2011 3h after irradiation, and in vivo 3CP reduction was measured subsequently after 1 h. The results indicated that GL2011 had a protective effect of free radical scavanging even when administered after irradiation, as t 1/2 was found to be 15.1 0.4 min, comparable to that of non-irradiated rats. The protective effect of post-irradiation administered GL2011 was also confirmed by the rat survival study (1).

(1) Ganesan MK et al. Amino Acids. 2014; 46:1681-1696.
(2) Okić-Djordjević I et al. Croatian Medical Journal. 2014; 55:45-49.

Financing: Ministry of Education, Science and Technological Development of the Republic of Serbia, project III41005.

Keywords: EPR spectroscopy; radioprotection; aminothiol

Back to programme

PS-25, session: Poster session

Micelles of Poly(2-oxazoline) Based Gradient Copolymer Enhancing Chemical Stability and Bioavailability of Curcumin

Annamaria Jutkova (1), Shubhashis Datta (1), Petra Sramkova (2), Lenka Lenkavska (1), Veronika Huntosova (1), Dusan Chorvat (3), Pavol Miskovsky (1), Daniel Jancura (1), Juraj Kronek (2)

(1) Pavol Jozef Safarik University in Kosice
(2) Polymer Institute, Slovak Academy of Sciences, Bratislava
(3) International Laser Centre, Bratislava

Tunable properties and biocompatibility of poly(2-oxazoline) (POx) based nanoparticles make them ideal for the development of new generation drug formulations in cancer therapy. Here we report on the synthesis of a POx based gradient copolymer and its self-assembly to prepare polymeric micelles for the targeted delivery of an anticancer drug, curcumin (CM). Initial solvent used for dissolving either POx copolymer or POx/CM system plays an important role on the size, morphology and drug loading capacity of these nanoparticles. The chemical stability and solubility of CM is highly enhanced due to encapsulation inside micelles. CM within these spherical nanoparticles has become stable for more than 3 weeks. The targeting efficiency of curcumin loaded nanoparticles was evaluated using two different types of cell lines (U87 MG and HeLa cells). POx based nanoparticles are efficient to encapsulate CM and deliver it into the cancer cells.

Financing: Slovak Research and Development Agency (APVV-15-0485)

Keywords: poly(2-oxazoline); nanoparticles; curcumin; drug delivery

Back to programme

PS-26, session: Poster session

The topology of LPS-lipid interaction

Balint Kiss (1)

(1) Semmelweis University Budapest, Department of Biophysics and Radiation Biology

Lipopolysaccharides (LPS) are membrane components present in the cell wall of Gram negative bacteria. These molecules play a key role in bacterial toxicity and immunogenicity, they are also crucial in the host recognition process of T7 bacteriophage.

To better understand supramolecular assemblies composed of LPS and their effect on bacterial membrane structure, we have created LPS-containing lipid bilayers by preparing liposomes. The observations were made with atomic force microscopy (AFM) and dynamic light scattering techniques (DLS). Liposomes were put on mica surface, on which they create patch like structures. Using AFM, we have identified the LPS molecules in lipid bilayers, in addition to observing their distribution and their effect on the bilayer structure. The LPS molecules are mostly distributed in the solid-state membrane regions, creating rough surfaces. We have studied the effect of temperature change and buffer composition on the sample, with special attention to divalent cations, which are needed for proper surface adhesion. Upon raising the temperature above the lipid's phase transition temperature the rough LPS regions became denser. Storing the sample at room temperature causes the dissociation of LPS from the lipid membrane. We have also studied the vesicles created by LPS on mica on different temperatures. Understanding the effect of LPS on lipid bilayers not only helps us to study the structure of bacterial cell walls, but it also enables us to create a model system which can be used to study the molecular properties of T7 bacteriophage infection process.

Financing: NKFI-OTKA-124966

Keywords: lipopolysaccharide; atomic force microscopy; T7 bacteriophage

Back to programme

PS-27, session: Poster session

Minimizing the number of cell divisions in spatially fixed tissues

Máté Kiss (1)

(1) Eötvös Lóránd University, Department of Biological Physics

Protecting genetic information from mutations is an essential task of all living cells. These mutations arise largely from replication errors during cell division and if they accumulate, they can cause the development of cancerous tumours. For this reason the self-renewing and growing tissues which have to produce a large number of functional cells during the lifetime of an organism are subjected to increased risk. To mitigate this risk there must be a biological mechanism fine tuned by natural selection that is capable of keeping the number of divisions low along any individual cell lineage.

Here we describe a mathematical model of tissue dynamics in which the rate of cell division is governed only by the spatial position of any given cell. We consider a spatially explicit tissue whose geometry does not change and as a result its cells must compete for available space.

We derive analytical results on the optimal pattern of division rates that lead to sufficiently low number of cell divisions to explain the resistance of constantly growing tissues, such as meristems, to somatic evolution. We also developed a genetic algorithm to explore optimal tissue differentiation structures at the organism level. Using this approach we investigate how spatially constrained tissue structures can produce the low amount of genomic differences observed between cells located on far away branches of large plants.

Financing: MTA-ELTE "Lendület" Evolutionary Genomics Research Group

Keywords: Tissue dynamics; Optimal tissue hierarchy; Meristems; Somatic evolution;

Back to programme

PS-28, session: Poster session

Fast and affordable predictions of hydrophobic association in cyclodextrin

Gerhard Koenig (1), Darrin M York (1)

(1) Laboratory for Biomolecular Simulation Research, Center for Integrative Proteomics Research, Rutgers University

Cyclodextrins are cyclic oligosaccharides that can be employed to improve the solubility and bioavailability of drugs. Mechanistically, this is achieved by forming hydrophobic inclusion complexes, which also makes cyclodextrins an interesting model system for the biophysics of hydrophobic association. By using GPU assisted computing, such binding simulations can now be performed within hours instead of weeks. To further improve the efficiency of such calculations, we provide a simple framework for absolute binding calculations without dedicated free energy code. In addition, we combine the resulting cyclodextrin binding data with the corresponding solvation free energies and partition coefficients in solvents of varying hydrophobicity, which allows us to evaluate the different contributions of polar and apolar interactions to the binding free energy. This improves our understanding of hydrophobic association and provides further guidance for the future development of molecular force fields.

Financing: NIH, NSF

Keywords: Free energy simulations; Hydrophobic association, GPU computing

Back to programme

PS-29, session: Poster session

Europium doped anatase TiO2 nanoparticles for NP localization in vitro in living cells and ex vivo tissues

Maja Garvas (1), Tilen Koklic (1), Polona Umek (1), Hana Majaron (1), Rok Podlipec (1), Janez Štrancar (1)

(1) Laboratory of Biophysics, Condensed Matter Physics Department, Jozef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia

Numerous adverse health effects have been linked to a variety of nanoparticles including asbestosis, lung cancer, and cardiovascular disease. Especially concerning is TiO 2 , since it is most prevalent engineered nanomaterial, which is used extensively in many commercial products.

In order to understand the mechanism of interaction of nanoparticles with an organism on a cellular level and subsequent aberrations of molecular pathways, leading to an adverse health outcome, one has to track a nanoparticle in a living organism. For this purpose, we synthesized Europium doped TiO 2 spherical shaped nanoparticles and nanotubes. The highest intensity of europium photoluminescence at 610 nm is usually achieved at excitation wavelengths in the ultraviolet range. However, excitation with wavelengths below 500 nm results in high autofluorescence signal, which complicates the interpretation of results from cells in vitro.

Here we show that spherical and tubular anatase TiO 2 nanoparticles doped with europium can be excited with 640 nm and can be clearly distinguished from the autofluorescence signal in vitro in living cells and ex vivo tissues. Both types of nanoparticles enter the LA4 lung epithelial cells, however the nanotubes were also found co-localized with plasma membranes of the cells. The nanoparticles can also be distinguished from autofluorescence in ex vivo mouse lung tissue after instillation. We localized the nanoparticles inside mouse lung macrophages using two photon excitation at 800 nm and spectral lifetime imaging.

Financing: Slovenian Research Agency (research core funding No. (P1-0060)); Horizon 2020 SmarNanotox grant agreement No. 686098

Keywords: TiO2 nanoparticles; Fluorescence microscopy; Fluorescence lifetime

Back to programme

PS-30, session: Poster session

Integrated optical investigation of the photocycle of dried Photoactive Yellow Protein films in environments of controlled humidity

Szilvia Krekic (1), Dávid Nagy (1), László Fábián (1), László Zimányi (1), András Dér (1)

(1) Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

In the near future, demand of faster data transfer and processing will further increase, however microelectronics might not be able to keep up regarding the problems of miniaturization and heat production. When considering options to overcome the disadvantages of integrated electronics, integrated optics (IO) is one of the most widely researched fields. In all-optical circuits data transfer and processing is performed by solely optical means. The main objective of integrated optical research is to find materials with suitable nonlinear optical characteristics that can play an active role in IO circuits. Our current research focuses on the nonlinear optical properties of Photoactive Yellow Protein (PYP).

Dried protein films were put into glass cuvettes with controlled relative humidity. Light-induced spectral changes in the visible range were monitored by an Optical Multichannel Analyzer (OMA) and the data obtained were evaluated by singular value decomposition and global multiexponential fit to determine the number of intermediates. Based on our earlier results this procedure was followed by so called target testing to obtain the intermediate spectra. The calculated spectra were used as input for spectrotemporal fit using a photocycle scheme with reversible transitions. This yielded the rate coefficients of the transitions, the kinetics of the intermediates and the final spectra.

Photocycle kinetics combined with measurements of light-induced refractive index changes may contribute valuable results to our further plans, namely, to develop integrated optical devices where PYP can be used as an active optical component.

Keywords: integrated optics; photoactive yellow protein; biophotonics

Back to programme

PS-32, session: Poster session

Curcumin anti-inflammatory properties revealed by a theoretical model

Nataša Latinović Antić (1), Tadej Emeršič (2), Andrej Dobovišek (3), Aleš Fajmut (4)

(1) University of Maribor, Faculty of Natural Sciences and Mathematics
(2) University of Ljubljana, Faculty of Medicine, Institute of Biophysics University of Maribor, Faculty of Natural Sciences and Mathematics
(3) University of Maribor, Faculty of Natural Sciences and Mathematics University of Maribor, Faculty of Medicine
(4) University of Maribor, Faculty of Natural Sciences and Mathematics University of Maribor, Faculty of Health Sciences

Curcumin is a natural phenol and is sold as a dietary supplement. Recently, its role in preventing inflammation has been intensively studied and identified by experimental methods and clinical studies. Its major problem remains poor absorption and fast metabolism after oral dosing. Hence, different formulations with increased bioavailability have been developed. In this systems-pharmacology based model, which couples pharmacokinetics and enzyme kinetics on the cellular level with force and diameter changes in the airways, we simulate the effects of oral dosing of different commercially available curcumin formulations and their action on the metabolites of arachidonic acid, prostaglandins and leukotrienes, the products of the cyclooxygenase (COX) and the lipoxygenase (LOX) pathways, respectively. The model is significantly upgraded from our previous version that was addressing hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs). In contrast to NSAIDs curcumin does not inhibit only enzymes COX 1 and 2 but also 5-LOX and prostaglandin E 2 synthase (PGES). Moreover, experiments reveal also its inhibitory effect on the expression of COX-2, 5-LOX and phospholipase A 2 (PLA 2) via interleukin 1 (IL-1) and nuclear factor B (NF-B) -dependent pathway. The model shows that on the levels of eicosanoids the effect of high curcumin doses is similar to that of the NSAIDs and supports the idea of using curcumin either as a partial substituent of NSAIDs or as an add-on therapy e.g. in treatment of rheumatoid arthritis. It is also tested by the model if high doses of curcumin induce bronchoconstriction, characteristics of NSAID hypersensitivity.

Financing: ARRS P1-0055

Keywords: Systems pharmacology model; Curcumin; NSAID; Arachidonic acid; Eicosanoids; Inflammation

Back to programme

PS-33, session: Poster session

Integrate Laser-Guided Direct Writing and Optical Tweezers for Fast Serial Analysis of Single Living Cells

Bing-Sian Li (1), Chia-Yu Chang (1), Shenq-Yang Shy (1), Shih-Chi Hung (1), Tzu-Sen Yang (1)

(1) Department of Mechanical Engineering, National Central University

Cell array technology represents the most straightforward method for investigating drug-cell interactions. High-throughput living-cell microarrays in general are based on microtiter plates, high-density spotting, microfabrication, and microuidics technologies. In the study, we integrate laser-guided direct writing(LGDW) and optical tweezers for fast serial analysis of single living cells. Here, we apply 830 nm laser to construct a LGDW bioprinting system. The LDGW system is integrated into an inverted microscope, together with microfluidic flow chamber. In addition, LGDW bioprinting system is further incorporated an optical tweezers system to manipulate single living cell in three dimension and then pattern 2D living cell arrays in well-defined manner. Therefore, this integrated cell array platform allows us to probing real-time cell morphology and cell-cell interactions in the absence and presence of doxorubicin (DOX), one of the most famous anticancer drug. It has been confirmed that DOX generated reactive oxygen species (ROS) and induced oxidative damage. To probe the ROS formed by DOX, NIH3T3 and MG63 cells printed by this integrated cell array platform, a 3 x 5 2D array, were treated with 2,7-dichloro-fluorescein diacetate (DCFH-DA). We anticipate this integrate LGDW and optical tweezers can be applied for fast drug screening at the single living cell level.

Qian Yang and Jinrong Peng. Porous Au@Pt Nanoparticles: Therapeutic Platform for TumorChemo-Photothermal Co-Therapy and Alleviating Doxorubicin-Induced Oxidative Damage. ACS Appl. Mater. Interfaces 2018, 10, 150164

Keywords: Cell array; Laser-Guided Direct Writing; Optical tweezers;

Back to programme

PS-34, session: Poster session

Aquaporin-4 e isoform regulate rapid cell volume changes in astrocytes

Marjeta Lisjak (1), Maja Potokar (1,2), Boštjan Rituper (1), Jernej Jorgačevski (1,2), Robert Zorec (1,2)

(1) Laboratory of Neuroendocrinology - Molecular Cell Physiology, Institute of Pathophysiology, University of Ljubljana, Faculty of Medicine
(2) Celica BIOMEDICAL d.o.o., Ljubljana

Aquaporin 4 (AQP4) is the predominant water channel in the brain. It is mainly expressed in astrocytes at blood-brain and blood-liquor interfaces. This unique position of AQP4 indicates, that it has critical role in controlling water balance in the brain. In the mammalian brain several AQP4 isoforms have been identified. Two of them (AQP4a (M1) and AQP4c (M23)) have been confirmed to cluster into plasma membrane supramolecular structures, termed orthogonal arrays of particles (OAPs), which may be important for water permeability through the plasma membrane. However, the role of the newly described water conductive mammalian isoform AQP4e is unknown.

In our research we investigated the dynamics of AQP4e aggregation into OAPs and its role in the regulation of astrocyte water homeostasis. With super-resolution structured illumination microscopy we found out, that AQP4e isoform is co-localized in OAPs in rat astrocytes. We observed that in hypoosmotic conditions, which elicit cell edema, OAP formation is enhanced by overexpressed AQP4e. This suggests that AQP4e may be involved in regulatory volume changes in astrocytes. We tested this hypothesis by using confocal microscopy. Our results revealed that AQP4e affects the mechanism of regulatory volume decrease in hypoosmotic conditions in astrocytes (1).

(1) Lisjak M, Potokar M, Rituper B, Jorgačevski J, Zorec R. AQP4e-based orthogonal arrays regulate rapid cell volume changes in astrocytes. The Journal of Neuroscience. 2017;37(44): 10748–10756. Available from: doi:10.1523/JNEUROSCI.0776-17.2017.

Financing: ARRS - Javna agencija za raziskovalno dejavnost Republike Slovenije

Keywords: aquaporin 4; orthogonal arrays of particles; astrocytes; structured illumination microscopy

Back to programme

PS-35, session: Poster session

DSC and isoperibolic calorimetry in the diagnostics of septic arthritis

Árpád Dandé (1), László Gábor Nöt (1), Norbert Wiegand (1), Béla Kocsis (2), Dénes Lőrinczy (3)

(1) Department of Traumatology and Hand Surgery School of Medicine, University of Pécs
(2) Department of Microbiology School of Medicine, University of Pécs
(3) Department of Biophysics, School of Medicine, University of Pécs

The synovial fluid analysis is important for diagnosing septic arthritis. To reach a quick diagnosis, preferably faster than the microbiological cultures, the DSC (differential scanning calorimetry) could be a useful tool for it. Different type of bacterial strains were inoculated in 200 L bouillon to reach the $10^3 - 10^5$ CFU/mL concentrations. Then we added 500 L of aseptic human synovial fluid and the mixture was incubated for 24 hours at 37 C. The inoculated samples were denaturated in pair with reference samples containing only bouillon. The thermal characteristics (maximal denaturation temperature (Tm) and calorimetric enthalpy change (d H)) were monitored by SETARAM Micro DSC-II calorimeter between 37 - 90 C with 0.3 K min -1 heating rate. The denaturation scans clearly demonstrated specific, representative thermograms in case of different grades of arthritis, as well as with each individual bacterial strain.

We monitored the proliferation characteristics too of inoculated synovial fluid samples at 37 C by isoperibol calorimeter: bacteria in 200 L bouillon mixed with 500 L of aseptic human synovial fluid. The detected scans clearly demonstrated specific, representative thermograms in case of each bacterium. Therefore, thermoanalyzes of human synovial fluid samples by isotherm calorimetry too could be a useful tool in the differential diagnosis of human septic arthritis.

Financing: OTKA CO-272

Keywords: Synovial fluid; Septic arthritis; DSC; Isoperibol calorimetry

Back to programme

PS-36, session: Poster session

Functional dynamics of two photolyase mutants revealed by ultrafast spectroscopy

Jonatan Pasitka (1), Andras Lukacs (2)

(1) Department of Biophysics, University of Pecs, Hungary

Photolyase and cryptochrome are members of the same photoactive flavoprotein family having highly homologue structures with very different functions. A major difference between photolyases and cryptochromes is the redox state of the flavin cofactor as for photorepair the flavin should be in its fully reduced state while in cryptochromes the flavin is in oxidized state. We performed site directed mutagenesis in E. coli photolyase and exchanged the crucial asparagine to an aspartic acid (N378D) and investigated the changes in the photocycle imposed by the alteration of the redox state of the flavin. Ultrafast spectroscopy measurements has shown that the N378D photolyase mutant behaves like a cryptochrome, showing a photocycle very similar known in plant cryptochromes. We also constructed a second mutant photolyase in which the asparagine was altered to a cysteine (N378C) owing to the fact that in the animal cryptochromes mainly cysteine is located in the given position. Regarding the behaviour of the cysteine containing mutant we found that the flavin acquired and remained in a more stable oxidized state compared to the N378D mutant. We might conclude that the alteration of the asparagine to either an aspartate or a cysteine resulted in the change of the redox state of the flavin to the oxidized form that is characteristic of cryptochromes. This oxidized state endured longer in the N378C mutant than in the N378D.

Financing: UNKP-2017-1

Keywords: photolyase, ultrafast spectroscopy, flavoproteins

Back to programme

PS-37, session: Poster session

Giant lipid vesicles shape transformations in a microfluidic diffusion chamber

Mojca Mally (1), Bojan Božič (1), Saša Vrhovec Hartman (1), Urška Klančnik (1), Maruša Mur (1), Saša Svetina (1), Jure Derganc (1)

(1) Institute of Biophysics, Faculty of Medicine, University of Ljubljana

Lipid bilayers represent a basis of cell membranes and are a framework for all cells and cellular organelles. We present a study of controlled transformation of an artificial membrane shape by incorporating a chosen substance into only one layer of a membrane bilayer. Bacterial lipopolysaccharide (LPS) is used as a model molecule as it inserts itself into the upper layer of the membrane and due to its size it cannot flip to the lower layer. We demonstrate the method and experiments that enable the analysis of the response of cell size flaccid lipid vesicles to the LPS insertion. The experiments are carried out in the environment of a microfluidic diffusion chamber, where the changes in substance concentration are highly controlled, reversible, and take place slowly, through the diffusion of LPS molecules from the main microfluidic channel to the vicinity of the vesicle inside the chamber. The flaccid vesicles immediately respond even to extremely small changes in LPS concentrations (order of 10 ng /ml) by changing their shape. Most often a spherical vesicle forms a protrusion in the form of a string of small spheres. With the continuous observation of individual vesicles, the time dependence of the formation of spheres and the size of the spheres can be monitored. Through a theoretical analysis of morphological changes the partition coefficient of the active substance (LPS) binding into the lipid vesicle membrane can be quantitatively evaluated due to a high sensitivity of the method.

Financing: Slovenian Research Agency

Keywords: Flaccid membranes; Giant lipid vesicles; Microfluidics; Shape transformations;

Back to programme

PS-38, session: Poster session

Chelidonine interferes with IL-6R/STAT3 signaling in uveal melanoma cells

Istvan Csomos (1), Csenge Filep (1), Marton Nadasi (1), Eniko Nizsaloczki (2), Laszlo Matyus (2), Andrea Bodnar (2)

(1) Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen
(2) Division of Biomathematics, Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen

STAT3 is a transcription factor with a critical role in the regulation of cell cycle, cell proliferation/survival and cell migration. As a consequence, it is a promising target in antitumor therapies. Canonical function of STAT3 demands phosphorylation of a Tyr705 residue followed by dimerization and nuclear translocation. STAT3 can also be phosphorylated on Ser727, with a putative role in fine tuning STAT3 activation. One of the major activators of STAT3 is interleukin-6, a cytokine present in an elevated concentration in various tumors, including uveal melanomas. Herein we aimed to study whether chelidonine, a potential antitumor agent, interferes with the IL-6/STAT3 pathway in human uveal melanoma cells. According to previous data, this alkaloid provokes cell death in numerous cancer cells, inhibits microtubule assembly and affects cell cycle progression. Using flow cytometry and confocal microscopy experiments we have demonstrated that chelidonine increases the basal level of pS-STAT3 in a significant fraction of cells. This effect was accompanied with abrogation of IL-6-induced STAT3 activation, which developed in a time-dependent fashion. Reduced efficiency of IL-6 induced STAT3 activation was also observed in cells with unaffected level of pS-STAT3. Chelidonine did not affect the level of IL-6R or total STAT3, but reduced significantly expression of gp130, the signaling subunit of functional IL-6R. According to our data chelidonine may interfere with STAT3 activation via two distinct mechanisms: one involves processes associated with serine phosphorylation, whereas the other one may be related to decreased expression of gp130.

Financing: EFOP-3.6.3-VEKOP-16-2017-00009

Keywords: IL-6, chelidonine, STAT3, uveal melanoma, flow cytometry, confocal microscopy, signaling

Back to programme

PS-39, session: Poster session

Development of a protocol for unbiased sequencing of monoclonal antibodies combining proteomics and bioinformatics

Klaudia Mihalovičová (1), Rostislav Skrabana (1,2), Andrea Legenova (2), Ondrej Cehlar (1,2), Petra Majerova (1,2), Eva Kontsekova (1,2), Michal Novak (1)

(1) Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
(2) AXON Neuroscience Ramp;D Services SE, Bratislava, Slovakia

Fragments of monoclonal antibodies (Fab or scFv) can serve as crystallization chaperones of otherwise hardly crystallizing targets (1). Fab fragment was successfully employed in structural study of the core segment of Alzheimer's disease-associated aggregated tau protein (2). Recombinant antibody fragments are even more suitable for crystallization. Expression of such recombinant proteins requires the knowledge of the sequence of antibody variable region, which can be achieved by PCR based methods combined with molecular cloning. Amplification of antibody variable region using degenerated forward primers often lead to problems associated with primer design and a polyploidy nature of hybridoma cells that may lead to detection of aberrant and/or nonproductive immunoglobulin-like transcripts.

Aim of this work was to develop a protocol for unbiased design of unique forward primer, annealing to the leader peptide preceding variable region. We adopted MALDI in-source decay (ISD) top down sequencing of purified antibody chains coupled with bioinformatics. Identified peptides from variable region were compared with known mouse immunoglobulin sequences (protein BLAST, IMGT database), the most probable germline was selected and a unique forward primer designed. Proposed methodology has been validated on more than 15 monoclonal antibodies specific to human tau protein.

(1) L. Griffin, A. Lawson. Antibody fragments as tools in crystallography. Clinical and Experimental Immunology. 2011;(165): 285-29. Available from: doi:10.1111/j.1365-2249.2011.04427.
(2) R. Skrabana, R. Dvorsky, J. Sevcik, M. Novak. Monoclonal antibody MN423 as a stable mold facilitates structure determination of disordered tau protein. Journal of Structural Biology. 2010;(171): 74-8. Available from: doi: 10.1016/j.jsb.2010.02.016 .

Financing: COST BM1405

Keywords: MALDI in-source decay, antibody sequencing, crystallization chaperones

Back to programme

PS-40, session: Poster session

Spectroscopic analysis of intrinsically disordered protein tau, involved in the pathogenesis of Alzheimer's disease

Jana Moravcsíková (1), Rostislav Skrabana (2), Ondrej Cehlar (2)

(1) Faculty of Mathematics, Physics and Informatics of the Comenius University in Bratislava
(2) Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava; Slovakia

Several intrinsically disordered proteins (IDPs) play principal role in the neurodegenerative processes of various types. Among them, tau protein is involved in Alzheimer's disease and related tauopathies. A precise and accurate method for determination of tau protein concentration is the first prerequisite to study structural processes underlying its pathological transitions by biophysical means (1).

Method for determination of protein concentrations has to be both technically simple, and quick. The measurement of ultraviolet absorbance at 280 nm is the most frequently used method, since the molar absorptivity at 280 nm can be predicted directly from the content of tryptophan, tyrosine and disulphides. Absorbance at 205 nm relying on the electronic transition in peptide bonds has been used as an alternative, as it is more sensitive and can be used in proteins with a low content of tryptophan and tyrosine, e.g. , in IDPs (2). However, the peculiar character of disordered proteins requires independent determination of their peptide bond molar absorptivity. Therefore, we dedicated this work to find out factors affecting molar absorptivity in IDPs at 205 nm, using tau protein isoforms as the IDP representative.

(1) Skrabana R, Sevcik J, Novak M, Intrinsically Disordered Proteins in the Neurodegenerative Processes: Formation of Tau Protein Paired Helical Filaments and Their Analysis. Cellular and Molecular Neurobiology. 2006; vol.26, Nos.7:1085-1097.
(2) Anthis N J, Clore G M, Sequence-specific determination of protein and peptide concentrations by absorbance at 205 nm. Protein Science. 2013; vol.22:851-858.

Back to programme

PS-41, session: Poster session

Native skin fluorophores in young healthy adults

Marcela Morvová Jr. (1), Peter Jeczko (1), Libuša Šikurová (1)

(1) Faculty of Mathematics, Physics and Informatics of the Comenius University in Bratislava

Nowadays in vivo fluorescence spectroscopy finds application in characterization of specific fluorophores in biological sample and can also be applied in monitoring of human skin. It is known large number of fluorophores, which occur naturally in any biological tissue and are jointly responsible for the fluorescence properties of biological tissues. However human skin performs weak autofluorescence based on native fluorophores with different concentration and spatial distribution within the skin. The primary native fluorophores in human skin are: the aromatic amino acids tyrosine and tryptophan, pepsin digestible collagen cross-links (PDCCL), collagenase digestible collagen cross-links (CDCCL), elastin and porphyrins. The goal of this study is to monitor differences in skin fluorescence which are related to gender and to anatomical regions in young healthy individuals (aged 20-30). For this purpose, the Luminescence Spectrometer (Perkin Elmer LS 45) and quartz optical fiber (Remote Fiber Optic accessory, Perkin Elmer), were used. Three fluorescent bands were recognizable: 280/340 nm; 325/390 nm and 400/600 nm (excitation/emission wavelength). The main significant gender and regional differences were found in the amino acid wavelengths.

Financing: This study was supported by grants VEGA 1/0136/18 and APVV-15-0119, APVV-15-0063 and ITMS: 26240120027.

Keywords: fluorophores; human skin; autofluorescence

Back to programme

PS-42, session: Poster session

Electrochemical behavior of neurotransmitters in the presence of hydroquinone and avarol

Đura Nakarada (1), Boris Pejin (2), Zorica Mojović (3), Miloš Mojović (1)

(1) Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
(2) Department of Life Sciences, Institute for Multidiscipinary Research IMSI, University of Belgrade, Kneza Višeslava 1, 11030, Belgrade, Serbia
(3) University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Department of Catalysis and Chemical Engineering, Njegoševa 12, 11000 Belgrade, Serbia

Avarol is sesquiterpene hydroquinone extracted from Mediterranean sponge Dysidea avara that has shown antibacterial, cytotoxic and wide spectrum of other biological activities (1). Literature data shows that electrochemical oxidation of avarol proceeds through 2e - – 2 proton process, similarly to hydroquinone (2). The interaction of avarol with several neurotansmitters was investigated by cyclic voltammetry and compared with the behavior of hydroquinone, its active moiety. Experiments with avarol were performed in 50 % (V/V) ethanol – phosphate buffer (pH=7) mixture due to the low solubility of avarol in water. Hydroquinone experiments were performed both in pure phosphate buffer and ethanol – phosphate buffer mixture. The effect of avarol – neurotransmitter interaction in ethanol – phosphate buffer mixture was observed in the voltammograms through change of peak–to–peak separation and midpoint potential. Similar interaction was observed with hydroquinone in buffer. Voltammograms of hydroquinone in ethanol were completely featurless indicating that phenolic hydroxyl group could not participate in redox process. This behavior was probably the consequence of formation of hydrogen bond between the hydroquinone's phenolic groups and the ethanol's hydroxyl groups.

(1) Gašić M. J, Sladić D, Tabaković I, Davidović A. The Avarol-avarone redox behaviour in acetonitril. Croat. Chem. Acta. 1985; 58 (4): 531-536. (2) Tabaković I, Davidović A, Muller W. E. G, Zahn R. K, Sladić D, Dogović N, Gašić M. J. Electrochemical reactivity of biologically active quinone/hydroquinone sesquiterpenoids on glassy carbon electrodes. Bioelectrochem. Bioenerg. 1987; 17: 567-577

Keywords: Avarol; Hydroquinone; Cyclic voltammetry

Back to programme

PS-43, session: Poster session

Successful Ti:Sapphire laser cell surgery of Phycomyces blakesleeanus cell wall

Tanja Pajić (1), Katarina Stevanović (1), Nataša Todorović (2), Aleksandar Krmpot (3), Mihailo Rabasović (3), Vladimir Lazović (3), Dejan Pantelić (3), Brana Jelenković (3), Miroslav Živić (1)

(1) University of Belgrade - Faculty of Biology, Serbia
(2) Institute for Biological Research, University of Belgrade, Serbia
(3) Institute of Physics, University of Belgrade, Serbia

Application of patch-clamp method for investigation of membrane ion channels of filamentous fungi is nontrivial task due to presence of chitinous cell wall. Complete removal of the wall patch is needed to make the membrane accessible to glass pipette. We use the model filamentous fungus organism, Phycomyces blakesleeanus which is undertaken to the cell surgery procedure by means of tightly focused femtosecond laser beam. The hyphae were grown on glass coverslips coated with collagen plasmolysed and imaged by homemade nonlinear laser scanning microscope by detecting two photon excitation fluorescence signal. Although intrinsic autofluorescence of chitin enables imaging of the cell wall the hyphae were stained by Calcofluor White dye prior to the imaging in order to improve signal to noise ratio. Ti:Sa laser, used for both imaging and surgery, was operating at 730nm, with 76MHz repetition rate and 160fs pulse duration. Carl Zeiss, EC Plan-NEOFLUAR, 401.3 oil immersion objective was used for tight focusing of the laser beam and for the collection of the fluorescence signal. A visible interference filter (415nm - 685nm) was placed in front of detector in order to remove scattered laser light. The successful cutting of cell wall could be achieved within the range of laser intensities and cutting speeds (dwell times). The hyphae were kept in azide throughout the experiment in order to block the regeneration of the cell wall. After the surgery, hyphae were slowly deplasmolysed to induce exit of a portion of the protoplast through the laser made incision in the cell wall.

Financing: This work was supported by Ministry of Education, Science and Technological Development, Republic of Serbia (grant no. III 45016).

Keywords: Cell surgery; Ti:Sapphire

Back to programme

PS-44, session: Poster session

New fluorescent probes for superresolution stimulated emission depletion microscopy based on 4-cyanocoumarin and nile blue fluorophores

Stane Pajk (1,2), Matej Novak (1), Hana Majaron (2), Boštjan Kokot (2), Janez Štrancar (2)

(1) University of Ljubljana, Faculty of pharmacy
(2) Jožef Stefan institute, Condensed matter physics, Laboratory of biophysics

Superresolution stimulated emission depletion (STED) microscopy has provided new insights and understanding of life on nanometre scale. To achieve such resolution fluorophores used in STED microscopy must possess certain properties such as low overlap of absorption and emission spectra, high brightness and above all high photostability. In recent years intense research was undertaken, with the goal of improving known fluorophores and finding new ones (1).

Our research was focused on two unexplored fluorophore scaffolds, 4-cyanocoumarin and nile blue, and their suitability as STED probes. Electron-withdrawing (EW) cyano group at position 4 of the coumarin provided significant red-shift and increased Stokes shift compared to the parent coumarin. The absorption and emission spectra of nile blue derivatives are generally more red shifted compared to 4-cyanocoumarin derivatives. The distinctly different absorption of both classes of fluorophores could be the basis for two-color imaging STED microscopy.

Several probes for membrane labelling based 4-cyanocoumarin and nile blue scaffolds were synthesized. Nile blue derivatives were tested under STED conditions and provided bright signal and excellent photostability. 4-Cyanocoumarin derivatives were not yet tested under STED conditions.

(1) Sednev MV, Belov VN, Hell SW. Fluorescent dyes with large Stokes shifts for super-resolution optical microscopy of biological objects: a review. Methods and Applications in Fluorescence. 2015;3(4): 2004.

Financing: European Union's Horizon 2020 research and innovation programme under grant agreement No. 686098

Keywords: fluorophore; STED microscopy

Back to programme

PS-45, session: Poster session

Characterization of pH dependence of conformational properties of AsLOV2 domain

Martina Petrencakova (1), Daniel Jancura (1,2), Erik Sedlak (2)

(1) Departmet of Biophysics, P. J. Šafárik University, Jesenná 5, 04154 Košice, Slovakia
(2) Center for Interdisciplinary Biosciences, P.J. Šafárik University, Jesenná 5, 04154 Košice, Slovakia

Plants, algae, bacteria and fungi contain Light-Oxygen-Voltage (LOV) domains that function as blue light sensors and control cellular responses to light. LOV domains non-covalently bind flavin chromophore, that is reduced upon blue light illumination and create a covalent bond with nearby cysteine residue. Blue light also induce a conformational change that activates attached phototropins kinase. The photoreaction process is fully reversible in the dark. Thanks to its light induced conformational change LOV domains have potential widepread use in optogenetics, i.e. as molecular switch in cell signaling (1,2).

Here, we present our findings regarding thermodynamic properties of AsLOV2 from Avena sativa. For the purpose of this study we used 2 forms of AsLOV2 domain: native form and dark state form. In dark state form, photoreactive cysteine is replaced by alanine and it does not undergo a photoreaction upon blue light illumination. We studied the influence of pH and temperature on the structural response in secondary and tertiary structure by CD, fluorescence spectroscopy, UV-Vis spectroscopy and differential scanning calorimetry. In addition, we investigated the effect of pH and temperature on photoproduct formation and dark reversion kinetics of native AsLOV2 as well as on the production of the reactive singlet oxygen. Our results indicate that despite an ability to undergo significant ligh-induced conformational transition, AsLOV2 is resilient against changing solvent conditions.

(1) Christie et al., LOV (Light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): Binding sites for the chromophore flavin mononucleotide. Proc. Natl. Acad. Sci. USA, 1999, 96, 8779-8783.
(2) Harper et al., Disruption of the LOV-Jalpha helix interaction activates phototropin kinase activity. Biochemistry, 2004, 43, 16184-16192

Financing: Slovak grant agency VEGA1/0423/16, APVV-15-0069 and VVGS-pf-2017-278.

Keywords: AsLOV2; pH dependence; thermodynamic properties

Back to programme

PS-46, session: Poster session

Integrated optical Mach-Zehnder interferometer biosensor and its improvement

Dániel Petrovszki (1), Sándor Valkai (1), László Fábián (1), András Dér (1)

(1) Biological Research Centre, Hungarian Academy of Sciences

Label-free optical biosensors are suitable to analyze biological samples in a fast and precise way. Thus, they are gaining ground in point-of-care diagnostics. During the research that was carried out earlier (1), the integrated optical structure used was made utilizing direct laser writing technique. The task of the device was to detect bacteria via evanescent waves in various body fluids. Recently, using this method (1), a detection limit indicative of the pathogen concentration in urine (10^6 cfu/ml) was achieved. The aim of the ongoing research is to make the interferometric system more sensitive by increasing the size of the sensing region and the number of detectable molecules in it. Based on these, the biosensor was prepared in a miniaturized form, and a microelectrode system was also added. Light propagation properties relevant to the optimization of the size of the waveguide were examined by simulation methods, thus a better waveguide construction was developed. Moreover, the applicability of various methods was studied in order to extend the sensing region. Using a microelectrode system, the phenomenon of dielectrophoresis was also employed to increase the analyte concentration in the evanescent space. In order to model these, the microelectrodes attracting the model objects of various sizes were successfully created.

(1) Mathesz, A., Valkai, S., Újvárosy, A., Aekbote, B., Sipos, O., Stercz, B., Dér, A. (2015). Integrated optical biosensor for rapid detection of bacteria. Optofluidics, Microfluidics and Nanofluidics. 2(1), 15-21. Available from: doi:10.1515/optof-2015-0002.

Financing: Bionics Innovation Center

Keywords: Biosensors; Integrated Optics; Mach-Zehnder Interferometer; Evanescent field

Back to programme

PS-47, session: Poster session

The role of Flightless-I (Fli-I) in the organisation of actin cytoskeleton

Réka Pintér (1), Péter Gaszler (1), Tamás Huber (1), Andrea Teréz Vig (1), Mónika Ágnes Tóth (1), Beáta Bugyi (1)

(1) University of Pécs, Hungary

Flightless-I (Fli-I) is a relatively newly identified protein, which alloys leucine-rich repeats (LRR) and gelsolin-homology domains (GH1-6). GH family proteins are involved in actin cytoskeletal rearrangements. Flightless-I is expressed widespread in tissues, mostly in skeletal, myocardial and nerve cells. Fli-I presents in the nucleus where it acts as a hormone-regulated nuclear receptor coactivator. In the presence of serum, Fli-I can translocate from the nucleus to the cytoplasm, where it plays a key role in cell migration, which is thought to be linked to its negative influences on wound healing and tissue regeneration. However, the underlying molecular mechanisms are not revealed, yet.

Our aim was to investigate the activities of Flightless-I underlying its cytoplasmic functions. We studied the interactions of different, recombinantly produced Fli-I constructs with actin, in vitro. According to our results, Fli-I interacts with both actin monomers and polymers. Through these interactions, it facilitates the nucleation of actin monomers and as a capping protein it inhibits polymer growth. Unlike gelsolin, the actin activities of Fli-I are not calcium dependent, which can be explained by the lack of the conservation of typesII calcium-binding sites between the two proteins. Importantly, our results suggest that the small actin-binding protein; profilin allows Fli-I to block actin polymer growth, but it inhibits its nucleation activities. In conclusion, our results suggest that in the cytoplasmic environment Flightless-I interferes with actin dynamics by capping polymer ends, which may explain its negative effects on cell migration, and thus wound healing and tissue regeneration.

Financing: “Supported BY the ÚNKP-17-4-III. New National Excellence Program of the Ministry of Human Capacities”

Keywords: actin cytoskeleton, gelsolin homologue domain, cell migration

Back to programme

PS-48, session: Poster session

Bioinformatics study of Curcumin anti-inflammatory properties

Vesna Postružnik (1), Eva Jovan (1), Timotej Brumec (1), Janja Belinc (1), Peter Marinko (2), Marko Šterk (2), Tina Zorec (3), Nataša Latinović Antić (4), Tadej Emeršič (5), Andrej Dobovišek (2), Maša Hojnik Niderl (6), Ales Fajmut (12)

(1) University of Maribor, Faculty of Health Sciences, Žitna ulica 15, 2000 Maribor
(2) University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška cesta 160, 2000 Maribor, Slovenia
(3) University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia
(4) University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška cesta 160, 2000 Maribor, Slovenia
(5) University of Ljubljana, Faculty of Medicine, Institute of Biophysics, Vrazov trg 2, 1000 Ljubljana, Slovenia
(6) Vitiva proizvodnja in storitve d.d., Nova vas pri Markovcih 98, 2281 Markovci, Slovenia

In recent years, curcumin has been recognized as a natural anti-inflammatory agent with its action similar to that of the nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the enzymes cyclooxygenase 1 and 2 (COX 1, 2) within the arachidonic acid (AA) metabolic pathway. Here we are reviewing and presenting all known effects of curcumin on the key enzymes of the AA metabolic pathway, and, in another presentation these effects are simulated by the mathematical model. According to several experimental studies, curcumin exhibits direct and indirect effect on the enzymes of the AA metabolic pathway. It acts as a direct competitive reversible inhibitor of the enzymes COX 1 and 2, 5- lipoxygenase (5-LOX) and prostaglandin E 2 synthase (PGES). The strength of the inhibition is evaluated by comparison of parameters K i and IC 50 for curcumin and other pharmacological inhibitors of these enzymes. Indirect effect of curcumin is reflected in the concentration dependent decrease in the mRNA expression of enzymes COX-2, 5-LOX and phospholipase A 2 (PLA 2). In this context the mathematical expressions, describing the enzyme expression vs. curcumin concentration will be presented. These effects of curcumin most likely originate from the inhibition of unidentified targets within the nuclear factor B (NF-B) signalling pathway. The work presented here is a part of the ongoing project PKP 2018 involving students of Bioinformatics, Physics and Chemistry and a partner company Vitiva d.o.o. The investment is co-financed by the Republic of Slovenia and the European Union under the Cohesion Fund EKP 2014-2020.

Financing: Republic of Slovenia and the European Union under the Cohesion Fund EKP 2014-2020.

Keywords: Arachidonic acid; eicosanoids; nuclear factor; curcumin; inflammation

Back to programme

PS-49, session: Poster session

Solving the mystery of disappearing CH2 group. A spectroscopic case study of mouse thymidylate synthase complexes.

Małgorzata Prokopowicz (1)

(1) Interdisciplinary Doctoral Studies in Natural Sciences and Mathematics, College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, ul. Banacha 2C, 02-097 Warsaw; 2. Biophysics Division, Institute of Experimental Physics,

Thymidylate synthase (TS) is an essential enzyme in the thymidylate salvage pathway. Due to its role, it has been recognized as a target in anticancer, antiviral and antiparasitic therapy (1). The reaction catalyzed by TS involves irreversible methylation of C(5) in 2'-deoxyuridine-5'-monophosphate with simultaneous methylene group transfer from mTHF (N 5,10- methylenetetrahydrofolate) (2). Results of crystallographic studies with mouse TS (mTS) showed that N 4 -OH-dCMP (NOH), mTS inhibitor, interacts with the enzyme and mTHF, provoking an abortive reaction that led to formations of the NOH-TS covalent complex. Moreover, unlike FdUMP, NOH caused transfer of the mTHF methylene group to so far unknown destination (3). Examination of the mechanisms of NOH and FdUMP interaction with mTS should benefit in clarification of this phenomenon and enrich our knowledge of intermolecular interactions. A spectroscopic approach involving UV absorption, fluorescence spectroscopy as well as time-correlated single photon counting (TCSPC), is applied to solve the mystery.

Financing: Studies funded by the National Science Centre, Poland: 2017/25/N/NZ1/0-1535. Biophysics Division, Institute of Experimental Physics, Faculty of Physics, University of Warsaw and Nencki Institute of Experimental Biology, Polish Academy of Sciences

Keywords: thymidylate synthase complexes; absorption and fluorescence spectropscopy; TCSPC

Back to programme

PS-50, session: Poster session

Label-free electrochemical and acoustic aptasensor for Jurkat cells detection as a potential diagnostic tool for leukemia

Lenka Bábelová (1,2), Marianna Eliášová Sohová (1), Alexandra Poturnayová (1,2), Monika Buríková (3), Jozef Bizík (3), Tibor Hianik (1)

(1) Department of Nuclear Physics and Biophysics, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia
(2) Institute of Animal Biochemistry and Genetics, Centre of Biosciences SAS, Dúbravská cesta 9, 840 05 Bratislava, Slovakia
(3) Cancer Research Institute, Biomedical Research Center SAS, Dúbravská cesta 9, 845 05 Bratislava, Slovakia

Electrochemical impedance spectroscopy (EIS) and thickness shear mode acoustic method (TSM) have been used for development of the aptamer-based biosensor for detection leukemic Jurkat cells. Thiolated DNA aptamers specific to the cancer marker protein tyrosine kinase-7 (PTK7) have been chemisorbed on a gold surface. Redox probe $[Fe(CN)_6]^{3-/4-}$ has been used for monitoring changes in charge transfer resistance, R_{ct}, in EIS experiments. R ct increased with increasing the concentration of Jurkat cells. TSM allowed label-free detection based on decrease of resonant frequency following addition of the cells. We obtained high sensitivity of Jurkat cells determination with limit of detection (LOD) 105±10 and 463±50 cells/mL for electrochemical and acoustic sensor, respectively. Small non-specific interactions have been observed for control U266 cells which can be particularly due to the interaction of the aptamers with lipid part of the biomembranes.

Financing: This work was supported by Slovak Research and Development Agency, APVV (project No. APVV-14-0267) and Science Grant Agency VEGA 1/0152/15.

Keywords: DNA aptamer; Electrochemical impedance spectroscopy; Jurkat cells; PTK-7 receptor

Back to programme

PS-51, session: Poster session

Characterization of spatio-temporal Ca2+ activity in human lens epithelial cells after mechanical stimulation

Sofija Andjelic (1), Marko Gosak (2,3), Dajana Gojić (4), Marko Hawlina (4)

(1) Eye Clinic, University Medical Centre, Ljubljana Očesna Klinika, Univrzitetni klinični center, Ljubljana
(2) Institute of Physiology, Faculty of Medicine, University of Maribor, Slovenia
(3) Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor, Slovenia
(4) Eye Clinic, University Medical Centre, Ljubljana

Cataracts are opacities of the lens and are the most common cause of vision impairments. The purpose of this study is to explore and identify intra- and inter-cellular calcium (Ca 2+) signaling in human lens epithelial cells (LECs) upon local mechanical stimulation, to understand better the role of Ca 2+ in intercellular communication related to cataract formation. The anterior lens capsule (aLC: basement membrane and associated LECs) were obtained from cataract surgery. LECs were stained with Fura-2 dye, the fluorescence of which was imaged to monitor spatio-temporal changes in cytosolic free Ca 2+ concentrations in response to localized, micropipette induced mechanical stimulation. Analysis of the Ca 2+ signaling from postoperative aLCs showed that the Ca 2+ signal spreads radially outwards. Ca2+ waves propagate faster in LC from more developed cataracts than in LC from less severe ones. Moreover, the relative amplitudes of Ca2+ transients were found to be always decreasing with increasing distance from stimulation point, but the amplitudes were found to be significantly lower in LC associated with higher degrees of cataract pathology. On the contrary, the durations of Ca2+ transients we found to be shorter in LC from less developed cataracts, which goes essentially on account of a faster Ca2+ decay rate. No significant differences were observed with regard to the type of the cataract. The modifications of Ca 2+ homeostasis in LECs, which are associated with different degrees of cataract, affect Ca 2+ signaling upon the local mechanical stimulation. Impairment of Ca 2+ signaling might be the basis of cataract formation.

Financing: ARRS

Keywords: cataract; calcium signaling; intercellular communication; lens epithelial cells

Back to programme

PS-52, session: Poster session

Nanoscale spatial organization of ligand influences T-cell activation

Joschka Hellmeier (1), Viktoria Motsch (1), Lukas Velas (), Gerhard J Schütz (1), Eva Sevcsik (1)

(1) Institute of Applied Physics, TU Wien, Vienna, Austria

In the human immune system, the recognition of an antigen by the T-cell receptor (TCR) takes place within the contact area between the T-cell and the antigen-presenting cell (APC). It is thought that the nanoscale spatial distribution of proteins within this contact zone plays an essential role in the initiation of an immune response. Despite extensive studies, the molecular details of this process, in particular the structural requirements for TCR triggering and how nanoscale events are translated into T-cell activation, are still poorly understood.

Here, we use DNA origami decorated with TCR ligands anchored to a planar glass-supported lipid bilayer to assess the effects of local ligand density and arrangement on T-cell activation. Our experimental setup allows for the precise nanoscale arrangement of TCR ligands on the DNA origami scaffold, while at the same time permitting the re-organization of ligand and TCR during T-cell activation. Further, the spatial distribution of ligands can be tuned independently of ligand concentration. We used fluorescently labeled TCR-reactive single-chain antibody fragment (scF v) as stimulatory ligand that was placed on the DNA origami at one to 8 engineered capture sites in different layouts and densities. The activation of T-cells interfaced with the APC-mimicking surfaces was measured using a Ca 2 -sensitive dye and the effects of local ligand density and nanoscale ligand arrangement were assessed. We found the nanoscale organization of scF V to influence T-cell activation: At that at the same overall density of scF V, the threshold for T-cell activation decreased with increasing number of scF V per origami.

Financing: FWF

Keywords: T-cell; DNA origami; nanoscale organization

Back to programme

PS-53, session: Poster session

Mechanism of water transport through SGLT1 protein

Marko Sever (1)

(1) National Institute of Chemistry, Theory department D01

M. Sever F. Merzel SGLT1 is a membrane protein and a high-affinity Na+/glucose cotransporter that is involved in the transport of glucose and galactose across the luminal side of enterocytes in the small intestine (1). In addition to its capacity to transport substrates this protein also facititates the movement of water across the cell membrane. Using explicit molecular dynamics simulations of SGLT1 and two of its mutants - F453C and Q457C in addition to a double mutant (2), we account for the systematic coupling between the water movement and local protein dynamics. Water permeabilites are compared to the wild-type cotransporter by optically recording the changes in cell volume in response to an osmotic challenge in /Xenopus/ oocytes. We calculate various time dependent parameters describing local structure of the water channels, including thermodynamic, electrostatic and geometric aspects. We determine the correlations between these parameters, with the final goal of better elucidating the mechanism of water transport in SGLT1. The characteristic movements present in the system are identified with the use of the normal mode analysis. In addition to SGLT1, we also apply our methodology to analogous cotransporter system LeuT (3).

1. Lehmann A, Hornby PJ. Intestinal SGLT1 in metabolic health and disease. Am J Physiol Gastrointest Liver Physiol. 2016;310(11): p. G887-98.
2. Zeuthen T, Gorraitz E, Her K, Wright EM, Loo DDF. Structural and functional significance of water permeation through cotransporters. Proceedings of the National Academy of Sciences of the United States of America . 2016;113(44):E6887-E6894. doi:10.1073/pnas-.1613744113.
3. Penmatsa A, Gouaux E. How LeuT shapes our understanding of the mechanisms of sodium-coupled neurotransmitter transporters. The Journal of Physiology . 2014;592(Pt 5):863-869. doi:10.1113/jphysiol.2013.259051.

Keywords: Molecular dynamics simulation; SGLT1; Cotransporter

Back to programme

PS-54, session: Poster session

Characterization of the functional changes and the dark- and light-adapted reaction center states of Photosystem II core complex isolated from T. vulcanus

Gábor Sipka (1), Pavel Müller (2), Klaus Brettel (2), Melinda Magyar (1), Qingjun Zhu (3), Guangye Han (3), Petar Lambrev (1), Jian-Ren Shen (3,4), Győző Garab (1,5)

(1) Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
(2) Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette, France.
(3) Photosynthesis Research Center, the Chinese Academy of Sciences, Beijing, China
(4) Photosynthesis Research Center, Okayama University, Okayama, Japan
(5) Faculty of Science, University of Ostrava, Ostrava 1, Czech Republic

Photosystem II (PSII) is a multisubunit enzyme embedded in the lipid environment of the thylakoid membranes of plants, algae and cyanobacteria. Our recent chlorophyll- a fluorescence transient measurements, using single-turnover saturating flashes (STSFs), revealed the involvement of conformational changes in the dark-to-light transition of PSII and a previously unknown rate-limiting step (1). In diuron-inhibited PSII, capable of only one stable charge separation, the fluorescence maximum could only be reached by a train of STSFs and with sizeable waiting times ($\Delta\tau$) between consecutive flashes. In PSII core complexes from T. vulcanus , a minimum of 1 ms was found at 20 C. In this work, we performed nanosecond transient absorption spectroscopy measurements (2,3), detecting at 819 nm the photooxidation and re-reduction kinetics of the primary electron donor P 680 in diuron-treated PSII core complexes. We used double laser-flash excitations with variable between the flashes. The experiments showed a rapid (4 ns) recombination of P 680 + Phe – upon the second flash for all values tested. These data offer no explanation on the origin of rate-limiting steps in PSII. Hence, the dark-to-light transition of PSII most probably originates from light-induced reorganizations, e.g. dielectric relaxations, affecting the fluorescence yield of chlorophyll-a.

(1) Magyar M, Sipka G, Kovács L, Ughy B, Zhu Q, Han G, Špunda V, Lambrev PH, Shen J-R, Garab G (2018) Rate-limiting steps in the dark-to-light transition of Photosystem II - revealed by chlorophyll-a fluorescence induction. Scientific Reports 8 (1):2755. doi:10.1038/s41598-018-21195-2.
(2) Byrdin M, Thiagarajan V, Villette S, Espagne A, Brettel K (2009) Use of ruthenium dyes for subnanosecond detector fidelity testing in real time transient absorption. Rev Sci Instrum 80 (4). doi:10.1063/1.3117208.
(3) Müller P, Ignatz E, Kiontke S, Brettel K, Essen L-O (2018) Sub-nanosecond tryptophan radical deprotonation mediated by a protein-bound water cluster in class II DNA photolyases. Chemical Science 9 (5):1200-1212. doi:10.1039/C7SC03969G

Back to programme

PS-55, session: Poster session

Binding of lactoferrin to plasminogen is conformationally regulated

Rostislav Skrabana (1), Ondrej Cehlar (1), Vladimir Leksa (2)

(1) Institute of Neuroimmunology, Slovak Academy of Sciences
(2) Institute for Hygiene and Applied Immunology

Recently we have shown that human lactoferrin (LF) inhibits plasminogen (Plg) activation through blocking the interaction between Plg and urokinase-type plasminogen activator [1]. Plg is the precursor of the protease plasmin, which is involved in many physiological and pathophysiological processes. Inhibition of Plg activation by LF is achieved through a direct binding of N-terminal region of LF to kringle 5 of Plg. Surface plasmon resonance binding responses of LF-Plg interaction do not fit a simple 1:1 mechanism and are dependent on activation of LF after its immobilization on sensor chip surface. In this work we analyse the conformational peculiarities underlying observed binding behaviour of LF and Plg. We discuss conformational diversity of binding-competent LF and its possible conformational switch upon Plg binding.

[1] Zwirzitz, A., et al., Lactoferrin is a natural inhibitor of plasminogen activation. The Journal of biological chemistry, 2018. doi: 10.1074/jbc.RA118.003145

Keywords: lactoferrin; plasminogen; mechnism of interaction

Back to programme

PS-56, session: Poster session

Stress-related lipid droplets biogenesis in rat astrocytes

Tina Smolič (1), Petra Tavčar (1), Anemari Horvat (1,2), Robert Zorec (1,2), Nina Vardjan (1,2)

(1) Laboratory of Neuroendocrinology – Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia.
(2) Laboratory of Cell Engineering, Celica Biomedical, Tehnološki park 24, Slovenia

Astrocytes are an abundant subset of glial cells in the central nervous system (CNS) involved in many processes, including in the regulation of CNS metabolism. As opposed to glucose handling that is in astrocytes highly regulated by noradrenaline, mechanisms underlying regulation of lipid metabolism, in particular the biogenesis of lipid droplets (LDs) that tend to accumulate in astrocytes during pathological states, are largely unknown. LDs are dynamic storage organelles composed of phospholipid monolayer and a core of triglycerides and sterol esters. It is thought that LDs form in non-adipose cells in response to stress and may serve as energy reserve for beta-oxidation. In order to test, whether astrocytes form LDs during stress and whether stress affects the cytoplasmic mobility of LDs, we exposed astrocytes in culture and organotypic brain tissue slices for 24 h to nutrient deprivation, excess of fatty acids, or various adrenergic agonists. We labeled LDs with the fluorescent markers (Nile Red, BODIPY493/503) and monitored the mobility and biogenesis of LDs by confocal microscopy. Nutrient deprivation (exchanging growth medium with 10 mM glucose extracellular solution), addition of excess oleic acid, and stimulation with noradrenaline increased the amount of LDs in astrocytes over 2-fold, indicating biogenesis of LDs. The mobility of LDs in astrocytes was reduced under nutrient deprivation. In conclusion, biogenesis of LDs in astrocytes both in vitro and in situ is tightly regulated by extracellular stress-related stimuli, which may govern LDs formation also in vivo.

Financing: Javna agencija za raziskovalno dejavnost Republike Slovenije / Slovenian Research Agency

Keywords: Lipid droplets; Astrocytes; Lipid metabolism; Confocal microscopy

Back to programme

PS-57, session: Poster session

Investigating the mechanism of membrane disruption by NLP proteins

Tina Snoj (1)

(1) National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia.

Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are produced by several phytopathogenic bacteria, oomycetes and fungi. They trigger leaf necrosis and immunity-associated responses in dicot plants. Our group recently reported that glycosylinositol phosphorylceramides (GIPC), the most abundant class of plant sphingolipids, are receptor molecules for NLP binding to plasma membranes. Furthermore, structural studies unveiled conformational changes upon binding of NLPs to GIPC sugars and in combination with biochemical and biophyscal studies proposed a model of early steps of NLP membrane interaction (1). However, the exact mechanism of membrane disruption by the toxin action remains to be elucidated. By confocal microscopy, we are exploiting giant unilamellar vesicles (GUVs) as model systems to observe NLP action on membranes, composed of palmitoyloleoylphosphatidylcholine (POPC) and GIPC. Phytosterols are added to the lipid mixture to mimic the composition of plant plasma membrane. GUVs are only formed under strict conditions. Optimizing the electroformation protocol to our lipid mixture with a high percentage of negatively charged GIPC is still in process, but first results are very promising. Visual information that may be obtained after NLP – membrane interaction include changes in morphology and integrity of the vesicles and differential leakage of different-sized probes from the vesicles. It will contribute to understanding of the nature and mechanism of interaction of NLP proteins with lipid membranes, be it either via pore formation or mebrane lysis.

(1) Lenarčič T. et al. Eudicot plant-specific sphingolipids determine host selectivity of microbial NLP cytolysins. Science. 2017;358 (6369): 1431 – 1434. Available from: doi: 10.1126/science.aan6874.

Financing: ARRS

Keywords: NLP; GIPC; GUV; membrane interaction; confocal microscopy

Back to programme

PS-58, session: Poster session

Raman and SERS characterization of Iron Gall Inks

Alba Espina (1), Pavol Miškovský (1,2), Santiago Sánchez-Cortés (3), Zuzana Jurašeková (1,2)

(1) Department of Biophysics, Faculty of Science. UPJŠ in Košice, Slovakia
(2) CIB, TIP-UPJŠ, Košice, Slovakia
(3) IEM CSIC, Madrid, Spain

Iron Gall Inks (IGIs) were the most common writing material until the beginning of the 20 th century. They were produced by mixing aqueous solutions of gallnut extracts and iron(II) sulfate agglutinated all by gum arabic. However, specific recipes varied depending on the region, culture and/or historical moment. It is well known that many of them have a corrosive nature and, thus, tendency to undergo significant colour and brightness changes. Thus, the analysis of IGIs can reveal useful information not only about the origin, antiquity and authenticity of historical documents, but also about the optimal ways of paper-based artifacts restoration, preservation and conservation. Whereas the standard analytical methods based on chromatographic techniques are destructiveness, Raman micro-spectroscopy (RS) is an efficient non-invasive and in situ technique widely used for identification and characterization of different materials. In addition, SERS (Surface Enhanced Raman Scattering) spectroscopy can overcome the problems of RS when applied in the study of natural organic dyes and pigments because of both fluorescence quenching and large enhancement produced by the presence of nanostructured metal surfaces. In this work we have focused on the characterization of IGIs, model ink systems prepared in our laboratory according traditional recipes using gallic and tannic acids, by means of Raman and SERS spectroscopies.

Financing: Acknowledgements This work is supported by the APVV-15-0485 and VEGA 1/0929/16 grants.

Keywords: Raman spectroscopy, SERS spectroscopy, Iron Gall Inks, Cultural Heritage

Back to programme

PS-59, session: Poster session

Re-scan confocal microscope modified for anisotropy imaging – as a part of a differential polarization system

Gabor Steinbach (1), David Nagy (1), Gabor Sipka (2), Erik Manders (3), Gyozo Garab (1), Laszlo Zimanyi (1)

(1) Institute of Biophysics, Biological Research Centre, Hungarian Academy of Science, Szeged, Hungary
(2) Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Science, Szeged, Hungary
(3) Innovative Microscopy Lab, Swammerdam Institute, University of Amsterdam, Amsterdam, Netherlands Confocal.nl, Amsterdam, Netherlands

The elucidation of the molecular architecture of complex, highly organized molecular macro-assemblies is crucial for basic biological research. The differential polarization attachment for LSMs constructed in the BRC is a useful technique that is based on high frequency modulation/demodulation units. Increasing the resolution and the quality of imaging of fluorescent microscopes is a fundamental task. The recently constructed high resolution imaging technique, Re-scan Confocal Microscopy (RCM) increases the lateral resolution by a factor of 1.4, by using a second scanner for the sCMOS camera detector, which provides a significantly better signal-to-noise ratio (compared to PMTs). With a modification of our RCM, we enabled this system for 2D and 3D microscopic mapping of the anisotropy of samples via measuring fluorescence-detected linear dichroism (FDLD) of their fluorophores. The modulation of the excitation laser is based on a liquid crystal (LC) retarder synchronized with the acquisition. The RCM and the LC are controlled by our external software. For demonstration, we show the FDLD of a plant cell wall sample with Congo Red staining.

Financing: GINOP-2.3.3-15

Keywords: fluorescence detected linear dichroism, DP-LSM, RCM

Back to programme

PS-60, session: Poster session

Osmotically activated anionic current in Phycomyces blakesleeanus, biophysically similar to VRAC, is not sensitive to classic VRAC blockers

Katarina Stevanović (1), Strahinja Križak (2), Nataša Todorović (3), Miroslav Živić (1)

(1) University of Belgrade, Faculty of Biology
(2) Medigroup, Belgrade, Serbia
(3) Institute for Biological Research, University of Belgrade, Serbia

We have recently described ORIC, osmotically activated anionic current in Phycomyces b lakesleeanus cytoplasmic droplet membrane obtained from sporangiophore. ORIC is outwardly rectifying, fast inactivating instantaneous current. It bares a close resemblance to VRAC (volume regulated anionic current), in biophysical properties as well as in the GTP-dependency of activation in the absence of osmotic stimuli. This is the only known current with properties of VRAC outside of Animal Kingdom. Here, using patch-clamp technique in the whole cell configuration, we have tested ORIC sensitivity to blockers that are known to potently reduce VRAC. We have shown previously that DIDS (4, 4'-diisothiocyanatostilbene-2, 2'- disulfonic acid) is ineffective in blocking ORIC. Other inhibitors of VRAC, niflumic acid and anthracene-9-carboxylic acid blocked ORIC albait only in ATP-dependent manner. Namely, the presence of 2mM ATP in the patch pipette prevented niflumic acid and anthracene-9-carboxylic acid block of ORIC. Only during prolonged recording in the presence of ATP, 0.5 mM niflumic acid had a significant effect on ORIC amplitude (p=0.004) that became more pronounced in time, eventually blocking 80% of ORIC. Considering that all tested blockers are effective on various animal and plant annionic transporters, their lack of effect on ORIC implies possibility of those compounds not acting directly on anionic channels but on some upstream signalling mechanisms that are different in filamentous fungi. In an attempt to test more specific VRAC blocker interfering with intracellular signaling that activates VRAC in animals, we used tamoxifen, and found that it is ineffective in inhibiting ORIC.

Financing: Ministry of Education, Science and Technological Development of the Republic of Serbia

Keywords: patch clamp electrophysiology; anionic current, filamentous fungi ion channel

Back to programme

PS-63, session: Poster session

Adhesion, unfolding forces, and molecular elasticity of fibronectin coatings: An atomic force microscopy study

Maria Sumarokova (1), Jagoba Iturri (1), José Luis Toca-Herrera (1)

(1) BOKU University of Natural Resources and Life Sciences, Vienna, Austria

Fibronectin is an extracellular matrix protein involved in cell adhesion, growth, or migration, among others. In many laboratories fibronectin is extensively employed in the shape of thin active coatings. This attends to its good performance in experiments involving specific cell attachment. In the present study, Atomic Force Microscopy (AFM) in Force Spectroscopy mode was employed to investigate the adhesion and mechanical properties of such fibronectin coatings, in the presence of non-specific interactions and upon varying factors: residence times (0, 1 and 2 s) and retracting rates (ranging between 0.1 and 10 m/s). Under some particular conditions, the unfolding of the different domains forming fibronectin could be also achieved. Thus, the stretching lengths matched the value of FN I (13.5 nm) for lowest pulling speeds, while for higher rates the measured values corresponded to the lengths of FN II (18 nm) and FN III (27 nm) domains. This investigation has answered and opened new questions about the mechanical stability and function of fibronectin coatings. The results have also raised theoretical questions about the difference between specific and nonspecific interactions to be addressed in future work.

Financing: Erasmus Iamonet Program; Austrian Science Fund (FWF), Grant Number: P29562-N28

Keywords: adhesion and unfolding forces; atomic force microscopy; fibronectin; unfolding length

Back to programme

PS-64, session: Poster session

Does the degree of labeling have an effect on antibody affinity?

Agnes Szabo (1), Timea Szatmari (2), Ildiko Radi (2), Laszlo Ujlaky-Nagy (1), Janos Szollosi (1,2), Peter Nagy (2)

(1) MTA-DE Cell Biology and Signaling Research Group, University of Debrecen, Egyetem square 1, Debrecen, Hungary
(2) Department of Biophysics and Cell Biology, University of Debrecen, Egyetem square 1, Debrecen, Hungary

Antibodies conjugated to fluorescent dyes are widely used for labeling cells. Since the degree of labeling (DOL) only gives the mean number of fluorescent dyes conjugated to an antibody, some of the antibodies contain fewer, while others contain more fluorescent dye/antibody. Monoclonal antibodies were conjugated with AlexaFluor647 or AlexaFluor546. Cell surface receptors were labeled with antibodies with different degrees of labeling (DOL ranging between 1 and 5). Fluorescence emission spectra were recorded in order to determine the relative brightness of the antibodies with different degrees of labeling. In parallel experiments the fluorescence intensity of cells labeled by the same antibodies was measured by flow cytometry. The receptor-bound antibodies were immunoprecipitated by protein G and the fluorescence anisotropy of the antibody stock solutions and the immunoprecipitated, bound antibodies was measured. The fluorescence intensity of the antibody stock solutions did not increase proportionally to the DOL suggesting that fluorophores undergo self-quenching. The intensity of the bound fraction did not change proportionally to the intensity of the antibody stock when plotted as a function of the DOL suggesting that the antibody affinity also decreases as a function of the DOL. Systematic differences were observed between AlexaFluor546 and AlexaFluor647 in this regard. Fluorescence anisotropy measurements confirmed these observations by revealing that the DOL of the cell-bound antibody fraction was typically lower than that of the stock. Model calculations confirmed that the distinct dependence of the quantum yield and the affinity on the DOL can lead to the different behavior of the two dyes.

Keywords: fluorescence antibody; DOL; fluorescence anisotropy; antibody affinity; quantum yield

Back to programme

PS-65, session: Poster session

Interplay between proteins and membrane cholesterol as revealed by in vitro evolution

Aleksandra Šakanović (1), Gregor Anderluh (1)

(1) National Institute of Chemistry, Department of Moleculat biology and nanobiotechnology

Biological membranes are essential components of all living cells. They consist of different types of lipids displaying diverse structural and biochemical variability. Among these lipids, cholesterol is one of the most important regulators of lipid organization in mammalian cell membranes. Moreover, through specific interactions, cholesterol controls the activity of a wide range of cellular and pathogen associated proteins. Many proteins, including different bacterial protein toxins, along with members of cholesterol dependent cytolysins (CDC) protein family, exhibit membrane binding in a cholesterol dependent manner. Identifying cholesterol recognition motifs is therefore critical for understanding physiological processes as well as the molecular mechanisms of bacterial pathogenesis.

The aim of this study is to identify the structural and biochemical characteristics of CDC proteins which are crucial for cholesterol recognition. As a model we used a membrane binding domain of perfringolysin O, a member of CDC. To study the initial binding of CDC toxins we employed a combination of directed protein evolution, high throughput DNA sequencing and model lipid membranes of different size and composition. Our results indicate that the recognition of membrane cholesterol might be carried out by several particular amino acids residues. Furthermore, the most abundant motifs after in vitro evolution using cholesterol containing model membranes consist of amino acid residues which are the same or chemically similar to the amino acids in the wild type of the toxin.

Back to programme

PS-66, session: Poster session

Minimum conditions for motility of a dimeric molecular motor

Andreja Šarlah (1)

(1) University of Ljubljana, Faculty of Mathematics and Physics

Linear motor proteins move along their tracks by performing a mechano-chemical cycle in which the conformational and binding affinity changes are coupled to the ATP hydrolysis cycle. However, the experiments on mutants with one inactive motor head suggest that the dimeric motor could be functional with a less complex mechanism.

Here we will theoretically discuss the minimal prerequisites for directed motility based on simpler schemes. Without any coupling between the catalytic site and binding domain, asymmetric unbinding of the trail/lead head and/or asymmetric binding in the forward/backward direction can be driven solely by an asymmetry in the force-induced unbinding rate, together with a conformation dependent internal tension of the dimeric motor. The energetic efficiency of such stepping is limited to approximately 20%. The low value provides a possible explanation for the universal presence of more complex mechanisms. However, the mechanism discussed here can additionally improve the stepping efficiency of molecular motors, especially at high forces.

Whereas motor proteins have evolved to work with a high efficiency, the design of artificial bipedal motors is still in its early stage and the focus is on making them move. In this respect, the discussed mechanism of asymmetric unbinding can provide a simple and viable mechanism.

Keywords: Molecular motors; Mechano-chemical models;

Back to programme

PS-68, session: Poster session

Cyclodextrin based supramolecular systems functionalized with adamantyl guanidines for gene delivery: preparation, characterization and testing the interaction with DNA

Adela Štimac (1), Matea Tokić (2), Ajasja Ljubetič (3), Tomislav Vuletić (4), Leo Frkanec (5), Marina Šekutor (5), Ruža Frkanec (5)

(1) University of Zagreb, Centre for Research and Knowledge Transfer in Biotechnology, Zagreb, Croatia
(2) University of Zagreb, Faculty of Science, Zagreb, Croatia
(3) National Institute of Chemistry, Ljubljana, Slovenia
(4) Institute of physics, Zagreb, Croatia
(5) Rudjer Bošković Institute, Zagreb, Croatia

The aim of the present study was preparation and characterization of supramolecular systems with adamantyl guanidine (AG) and probing of their interaction with DNA using fluorescence correlation spectroscopy (FCS). In the continuation of our study dealing with adamantyl guanidines and investigations of lipid-based nanovesicles as drug delivery systems (1,2) we prepared and characterized functionalized supramolecular systems of adamantyl guanidines AG 1-5 with phosphatidylcholine liposomes, liposomes incorporating amphiphilic -cyclodextrin (-CD) derivative and cyclodextrin vesicles composed only from the amphiphilic -CD derivative. Efficiency of incorporation of AG 1-5 into prepared supramolecular systems was measured spectrophotometrically, and the size and surface charge were determined by dynamic light scattering method. The incorporation of AG 1-5 did not affect the size of prepared supramolecular systems but surface charge was significantly changed. FCS was applied to examine the interactions between functionalized supramolecular systems and the Cy5-fluorescently labelled, double-stranded, 120 bp DNA (DNA120*). The obtained results have clearly demonstrated that the supramolecular systems with entrapped AG 1-4 present guanidinium groups on the surface of vesicles, which leads to interaction with DNA120* via guanidine-phosphate interaction. Preliminary results have shown that prepared functional supramolecular systems strongly bind DNA and as such could be used in gene delivery.

Šekutor M, Štimac A, Mlinarić Majerski K, Frkanec R. Synthesis and characterization of liposome incorporated adamantyl aminoguanidines. Org. Biomol. Chem. 2014;12(31): 6005-6013. Štimac A, Šekutor M, Mlinarić-Majerski K, Frkanec L, Frkanec R. Adamantane in Drug Delivery Systems and Surface Recognition. Molecules. 2017;22(2): 297-310.

Keywords: supramolecular systems, liposomes, vesicles, DNA, fluorescence correlation spectroscopy, FCS

Back to programme

PS-69, session: Poster session

Subclonal Structure of Tumors

Gergely Tibély (1)

(1) Department of Biological Physics, Eötvös University

Intratumor heterogeneity appears as a consequence of imperfect DNA replication during cell division, leading to a somatic evolutionary process. Williams et al. (1) argue that tumor evolution, apart from the driver mutations, can be well described by assuming a neutral evolution model, and give estimates for the mutation rate. Several questions remain, however, unanswered, such as the robustness of the results against measurement error, or the significance of cell death. We find that confining the tumor growth model into three dimensions leads to better fits to the empirical data. Also the estimated number of subclonal mutations is much larger than expected, based on the current assumptions on the human somatic mutation rate (1, 2). The elevated number of mutations must result from either a significantly higher mutation rate, or from frequent cell death, which is compensated by more cell divisions and elongates the cell lineages. We have developed tools to estimate the mutation rate and cell death rate of the tumor growth model using the frequency distribution of mutations in cancer data bases. Our preliminary results on synthetic data indicate that the real parameters can indeed be recovered, if the sequencing coverage is high enough.

(1) Williams M J, Werner B, Barnes C P, Graham T A, Sottoriva A. Identification of neutral tumor evolution across cancer types. Nat Genet 2016;48: 238-244. (2) Martincorena I, Campbell P J. Somatic mutation in cancer and normal cells. Science 2015;349: 1483-1489.

Keywords: cancer; intratumor heterogeneity; mutation; somatic evolution

Back to programme

PS-70, session: Poster session

Excitable and responsive orientational domains in liquid crystal microflows

Uroš Tkalec (1)

(1) University of Ljubljana, Faculty of Medicine

Nematic liquid crystals (NLCs) possess long-range orientational order of molecules that can be controlled by geometrical confinement, topological constraints and external fields. Past works have primarily focused on viscoelastic responses in static director fields and only recently hydrodynamics of liquid crystals has been characterised in microfluidic settings. In this work, we focus on optothermal manipulation of a flowing nematic medium that can result in excitable and tunable orientational domains with an interesting dynamic response. We show experimentally, theoretically and in numerical simulations how pressure-driven flow of a NLC in homeotropic microchannels interacts with laser- tweezers-induced distortions of an orientational order. The size, shape and birefringent colors of the nucleated domains can be tuned with flow modulation, temperature gradients and channel geometry. We believe that laser-tweezers-guided control over nematic microflows represents a convenient and versatile tool to drive complex fluid dynamics by optothermal effects.

[1] Sengupta A, Tkalec U, et al . Liquid crystal microfluidics for tunable flow shaping. Phys. Rev. Lett. 2013; 110, 048303.
[2] Sengupta A, Herminghaus S, Bahr C. Liquid crystal microfluidics: surface, elastic and viscous interactions at microscales. Liq. Cryst. Rev. 2014; 2, 73.
[3] Pieranski P, Godinho MH, Čopar S. Persistent quasi-planar nematic texture: Its properties and topological defects. Phys. Rev. E 2016; 94, 042706.
[4] Pieranski P, Hullin JP, Godinho MH. Rheotropism of the dowser texture. Eur. Phys. J. 2017, 40, 109.
[5] Giomi L, Kos Ž, Ravnik M, Sengupta A. Cross-talk between topological defects in different fields revealed by nematic microfluidics. PNAS 2017; 114, E5771.

Financing: Slovenian Research Agency, National Science Foundation U.S.A.

Keywords: Complex fluids, microfluidics, optothermal manipulation

Back to programme

PS-71, session: Poster session

Real-time probing the intracellular temperature distribution effect on NF- kB translocation in single living cells

Chien-Yuan Tsai (1), Long-Sheng Lu (1,2), Tzu-Sen Yang (3), bin-sian Li (4), Colley Mamadi M.S (1)

(1) Institute of Biomedical Materials and Engineering, Taipei Medical University, Taipei, ROC
(2) Department of Radiation Oncology, Taipei Medical University Hospital, Taipei, ROC
(3) Institute of biomedical Optomechatronics, Taipei Medical University, Taipei, ROC
(4) National Central University, Taoyuan, ROC

The NF-B is a protein complex that regulates DNA transcription. The NF-B activity can be induced by a variety of stimuli, including ultraviolet irradiation, cytokines, free radicals, etc. Incorrect regulation of NF-kB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection and improper immune development. Recent studies indicate that different temperatures can affect the transcription rate of NF-B. The transport occurred faster when the temperature was higher. However, few researchers have studied the relationship between temperature distribution and NF-B in single living cell. In this study, we use the micro-scale temperature probes to monitor temperature distribution in the single cell. The temperature probes use Rhodamine B coat on Polystyrene particles by bio-conjugation. The fluorescence intensity of rhodamine B will be weakened as temperature increase. In addition, we add Ti-6Al-4V powders as a heat source that can absorb the near-infrared (NIR)light and be converted into heat. We use this heat source to create a temperature gradient and observe the temperature probe fluorescence intensity distribution in the single living cell. The result that the temperature gradient does influence the distribution of fluorescent intensity of temperature probes. The closer to the heat source, the weaker the fluorescence intensity. This confirms that the temperature probes can measure the temperature distribution in the microenvironment. Furthermore, the temperature probes were delivered into the cell to investigate the correlation between temperature gradient and NF-B transcription inside the single living cell.

Keywords: NF-B;rhodamine B

Back to programme

PS-72, session: Poster session

The role of cyclic guanosine monophosphate (cGMP) on the Ca2+-contraction coupling in vascular smooth muscle cells

Tanja Vajs (1), Aleš Fajmut (1,2)

(1) University of Maribor, Faculty of Natural Sciences and Mathematics
(2) University of Maribor, Faculty of Health Sciences

We model the relaxing effect of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells (VSMC). cGMP is a second messenger downstream the nitric oxide (NO) production in the endothelium of arteries. NO concentration increases in response to shear stress and other stimuli, and acts as vasodilator. In vascular smooth muscle cells (VSMC) NO activates the enzyme soluble guanylate cyclase (sGC) that produces cGMP and the latter than affects a number of Ca 2+ -signal encoding mechanisms as well as the mechanisms of its decoding into force. cGMP potentiates OFF-mechanisms (SERCA pumps and Na + /Ca 2+ -exchangers) and inhibits ON-mechanisms (IP3-R channels) of the Ca 2+ -signal encoding process, it potentiates OFF-mechanisms (myosin light chain phosphatase (MLCP)) of the decoding process, and, moreover, it potentiates the ion-transport mechanisms across the plasma membrane (Na + /K + -ATPases, Ca 2+ -dependent K + channels, Ca 2+ -dependent Cl – channels). The predominant effect of cGMP on these transport mechanisms related with Ca 2+ signalling is the membrane hyperpolarisation and the consequent decrease in the conductance of voltage operated Ca 2+ channels (VOCC). All these and other complex encoding and decoding processes of Ca 2+ -contraction coupling are mathematically modelled by the dynamical system consisting of 21 first order differential equations containing more than 150 parameters. The final result of the model is a time dependent force development in VSMC as well as the magnitude of the steady force versus different cytosolic cGMP and/or NO concentrations and IP 3 as a trigger of Ca 2+ signalling. The analysis of the system is focused on the significance of individual mechanisms by which cGMP induces relaxation.

Financing: ARRS P1-0055

Keywords: Mathematical model; Vascular smooth muscle cell; Calcium signalling; Nitric oxide; Relaxation; Contraction

Back to programme

PS-73, session: Poster session

Homo- and Heteroassociations Drive Activation of ErbB3

Timea Varadi (1,2), Mario Brameshuber (1), Eva Sevcsik (1), Florian Baumgart (1), Gyula Batta (2), Tamas Kovacs (2), Rene Platzer (3), Magdalena Schneider (1), Johannes Huppa (3), Gerhard Schütz (1), Peter Nagy (2)

(1) Institute of Applied Physics, TU Wien, Vienna, Austria Department of Biophysics and Cell Biology
(2) Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
(3) Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectology and Immunology, Medical University of Vienna, Vienna, Austria

The four ErbB receptors (ErbB1-4) constitute a family of transmembrane proteins standing in the focus of interest of basic researchers and clinicians. ErbB1 and ErbB4 can be considered to be full-fledged receptors for EGF-like and heregulin-type ligands, respectively, since they contain fully functional ligand binding and tyrosine kinase domains. On the other hand, ErbB2 and ErbB3 harbor only half of the activity required for full activation with ErbB2 lacking an activating soluble ligand and ErbB3 having a not fully functional kinase domain. However, ErbB2/3 heterodimers formed upon binding of heregulin to ErbB3 constitute the most potent oncogenic unit capable of strong activation of both the MAPK and PI3K pathways. Here we used a single molecule imaging technique named "thinning out clusters while conserving stoichiometry of labeling" (TOCCSL) to measure the homoassociation of ErbB3 in the presence or absence of ErbB2 coexpression and its heterodimerization with ErbB2 in quiescent and heregulin-stimulated cells. Our results show that monomeric, inactive ErbB3 undergoes heregulin-induced homodimerization both in the absence or presence of ErbB2 coexpression. Heregulin also induced the formation of ErbB2/3 heterodimers. Pertuzumab, an antibody binding to the dimerization arm of ErbB2, blocked heregulin-induced heterodimerization, while it did not block the effect of the growth factor on homodimers of ErbB2, and even increased the fractional presence of ErbB3 homodimers in unstimulated cells. The results imply that a dynamic equilibrium exists between constitutive and ligand-induced dimers that not only differ in their stoichiometry, but also in the distance between subunits.

Keywords: receptor tyrosine kinases, ErbB2, ErbB3, single molecule fluorescence microscopy, fluorescence recovery after photobleaching

Back to programme

PS-74, session: Poster session

Supported and tethered lipid bilayers as model systems for T cell activation studies

Lukas Velas (1), Georg Mörtl (1), Dennis Michalak (2), Mario Brameshuber (1), Mathias Lösche (2), Gerhard Schütz (2)

(1) Institute of Applied Physics, Vienna University of Technology, Karlsplatz 13, 1040 Wien, Austria
(2) Physics Department and Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States

1 Institute of Applied Physics, Vienna University of Technology, Karlsplatz 13, 1040 Wien, Austria 2 Physics Department and Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213, United States velas@iap.tuwien.ac.at T cell activation is triggered upon binding of the T cell receptor (TCR) to the peptide loaded Major histocompatibility complex (pMHC) presented on the plasma membrane of an antigen presenting cell (APC). In order to avoid complex geometry in the cell to cell contact and to allow for total internal reflection fluorescence (TIRF) microscopy, supported lipid bilayers (SLBs) with anchored proteins (pMHCs and adhesion molecules ICAM) have been widely used as model system that mimick the surface of an APC. However, SLBs can contain defects which lead to unspecific activation of the T cells even in the absence of antigen. In contrast, tethered lipid bilayers have been shown to be defect free and hence would be a suitable platform for T cell activation studies. [1] Here, we use fluorescence microscopy methods such as single molecule tracking and fluorescence recovery after photobleaching (FRAP) to study the quality of the SLBs in terms of protein mobility. Furthermore, activation of T cells was studied on SLBs containing different surface densities of pMHC by measurement of intracellular calcium levels. These results were then compared to the results obtained on a tethered lipid bilayers.

BUDVYTYTE, Rima, et al. Structure and properties of tethered bilayer lipid membranes with unsaturated anchor molecules. Langmuir, 2013, 29.27: 8645-8656.

Keywords: Lukas

Back to programme

PS-75, session: Poster session

Gray level Co-occurrence matrix analysis in medical investigation: 2D images of the aspiny neurons from the human neostriatum

Velicko Vranes (1), Nabojsa Milosevic (1), Damian Stojic (1)

(1) Laboratory for image analysis, Institute of Biophysics, Medical faculty, University of Belgrade, Serbia

Introduction: Human neostriatum consists of two types of neurons, spiny and aspiny, according to the spine distribution (1) and density (2). Further classification recognizes three groups of aspiny neurons (3), of which group IV corresponds to cholinergic interneurons (3), important for the integration of cortical and thalamic signals by intermixed striatal microcircuits. The goal of this study analyze different morphometric properties of neurons digital images, from both core of the dorsal lamina.

Material and Methods: Micrographs of the vertical projections of the aspiny neurons are quantified by two computational and five textural parameters describing nonlinear and statistical properties of binary and grayscale image of the entire neuron.

Results: Six of seven parameters showed standard ($p < 0.05$) or very high statistical differences ($p < 0.01$ or $p < 0.001$) between neurons from caudate nucleus and putamen.

Conclusions: This study demonstrate significant differences in morphometric parameters, and confirms the hypothesis that neurons of the same function but different topologies, perform different levels of the same function (3).

(1) Yelnik J, François C, Percheron G, Tandé D. Morphological taxonomy of the neurons of the primate striatum. J Comp Neurol. 1991; 313: 273–294.
(2) Leontovich TA. (1998) Large neostriatal neurons in humans and their possible role in neuronal networks. Neurosci Behav Physiol. 1998; 28: 252–259.
(3) Krstonošić B. Morphological analysis of 2D projections of neurons from the human neostriatum (Ph.D. thesis), Medical faculty, Univesity of Novi Sad, Serbia, Balkans, 2013.

Financing: Instituto Tecnológico de Santo Domingo (INTEC), Santo Domingo, Republica Dominicana

Keywords: Human neostriatum, aspiny neurons, morphometric properties, GLCM textural parameters

Back to programme

PS-76, session: Poster session

Unravelling the role of microtubules on cytomechanics by nanoindentation

Andreas Weber (1), Jagoba Iturri (1), José Luis Toca-Herrera (1)

(1) University of Natural Resources and Life Sciences Vienna, Department of Nanobiotechnology, Institute of Biophysics.

Eukaryotic cells are complex systems that interact in manifold ways, not only limited to chemical or biochemical stimuli. They can also communicate via feeling, transducing and exerting forces (1). Therefore, mechanical properties of cells gained increased interest in the field of biophysics over recent years. How the interplay of different cellular components is defining apparent mechanical properties of cells is not yet fully understood. One main finding in the field of cell mechanics is that the organism's properties are mostly related to the nucleus (and cortex), membrane (and glycocalyx) and cytoskeleton (2). The latter is a complicated meshwork made of proteins with three main components: actin filaments, microtubules and intermediary filaments. The aim of this study is to assess the impact microtubules have on the mechanical properties of endothelial cells.

This was done by combined atomic force microscopy (AFM) with fluorescence microscopy (FM). The AFM was used for nanoindentation to quantify cell mechanical properties like Young's Modulus, relaxation time, adhesive work and rupture events during retracting. Results showed that depolymerization of microtubules with colchicine does indeed lead to significant changes in nearly all the studied properties over the time.

(1) Jonas O, Duschl C. Force propagation and force generation in cells. Cytoskeleton. 2010; 67(9):555-563. Available from: doi:10.1002/cm.20466.
(2) Fletcher D. A. Cell mechanics and the cytoskeleton. Nature. 2010; 463(7280): 485-492. Available from: doi:10.1038/nature08908

Keywords: Cell mechanics; Atomic Force Microscopy; Cytoskeleton

Back to programme

PS-77, session: Poster session

STED-compatible co-staining of t-tubules and proteins in fixed cardiac myocytes

Alexandra Zahradnikova, Jr. (1), Simona Kazmerova (2), Katarina Mackova (1), Ivan Zahradnik (1), Alexandra Zahradnikova (1)

(1) Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences
(2) Faculty of Natural Sciences, Comenius University in Bratislava

Proper localization of proteins of calcium signaling relative to the sarcolemma is important for understanding processes of excitation-contraction coupling in cardiac myocytes. We have tested selected methods of sarcolemma staining for their compatibility with subsequent immunostaining of ryanodine receptors, junctophillin, inositol trisphosphate receptors and Cav1.2 channels in isolated rat ventricular myocytes. We compared the STED-compatible fluorescent conjugates Texas Red and Oregon Green 488 of wheat germ agglutinin (WGA), a lectin that binds non-specifically to glycosylated membrane proteins, with the fixable lipophilic dye mCling-ATTO647N for staining the sarcolemma. Fixed and permeabilized cells were immunostained using the respective primary antibodies and DyLight 488 and 549-labeled secondary antibodies. Leica TCS SP8 STED3X confocal microscope was used to visualize cells during specimen preparation. The mCling probe provided excellent staining of live cells and was well retained in the membrane after fixation. However, despite fixation, permeabilization resulted in partial or even full loss of the mCling signal from t-tubules. On the other hand, staining of the sarcolemma with WGA, which required prolonged incubation (>2 h), resulted in partial internalization of WGA in juvenile myocytes. Prefixation of myocytes prevented WGA internalization even during overnight incubation with WGA conjugates. As a result, selective staining of the sarcolemma including t-tubules was achieved in the whole volume of myocytes. WGA staining was fully retained after permeabilization and immunostaining. Support: VEGA-2/0143/17, APVV-15-0302, ITMS-26230120006

Financing: Vedecká grantová agentúra, Agentúra na podporu výskumu a vývoja, Výskumná agentúra

Keywords: Protein localization; Sarcolemma; Immunolabeling; Cardiac myocytes; Confocal microscopy

Back to programme

PS-78, session: Poster session

Ultrafast kinetics of the intramolecular energy migration in NADH

Zsuzsanna Heiner (1), Thomas Roland (2), Jérémie Leonard (2), Stefan Haacke (2), Géza I. Groma (1)

(1) Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences Szeged
(2) University of Strasbourg, CNRS, Institut de Physique et Chimie des Matériaux de Strasbourg, UMR7504, F-67000 Strasbourg, France

Early observations indicate that in the folded conformation of the coenzyme NADH an effective energy transfer occurs from the excited adenine ring towards the nicotinamide group. Here we show that applying ultrafast absorption kinetics measurements the detailed kinetics of this process can be explored. The ratio of the molecules in the folded/unfolded conformational states was controlled by gradually adding methanol to the aqueous solution of NADH. A detailed compartmental analysis of the absorption kinetics traces revealed that in the unfolded conformation no energy transfer takes place, and the excited adenine returns to its ground state in a 230 fs process. In high contrast with this, in the folded molecules an extremely rapid (70 fs) energy transfer occurs, ensuring the high efficiency. Surprisingly, this high rate can be described well by a simple Förster mechanism, despite to the unusually low (<1 nm) inter-ring distance. The sudden transfer results in a both vibrationally and electronically excited state of the nicotinamide group. The vibrational relaxation is completed in a 1.7 ps process, followed by the electronic relaxation of 650 ps.

Keywords: ultrafast kinetics

Back to programme

PS-79, session: Poster session

Urinary pteridines as a potential tool for diagnose and prognosis of the ovarian tumor

Milan Zvarík (1)

(1) Department of Nuclear Physics and Biophysics Faculty of Mathematics, Physics and Computer Science Comenius University Mlynska dolina F1, 842 48 Bratislava Slovakia

Proper diagnosis of ovarian lesions is extremely important to differentiate between benign and malignant disease. In this work, we focused on determining urine pteridines as new potentional biomarkers of cancer disease. Levels of neopterin, biopterin, and pterin were assessed by fluorescence analysis of human urine after HPLC separation. We have revealed that the neopterin and pterin levels in the urine from patients with malignant ovarian tumors were significantly higher than those for the patients with benign tumors. Levels of urine biopterin in both groups remained unchanged. For discrimination of malignant and benign patients based on pteridine levels were determined the optimal cut-off value using receiver operating characteristic (ROC) curve analysis. For neopterin and pterin the diagnostic test specificity of 77.1 % and sensitivity of 68.0% resp. 52 % was reached. However, the highest diagnostic potential seems to be in the ratio of neopterin and biopterin (84.3 % specificity and 76.0 % sensitivity). We compared urine pteridine levels in patients with difference degree of tumor differentiation (grade) and stage of disease. We found statistically significant elevated levels of neopterin and pterine in patients with high grade ovarian tumor. Univariate analysis of survival shows product-limit estimates of progression free (PFS) and overall survival (OS) for patients according to preoperative neopterin concentrations in urine. Patients exhibiting high neopterin levels showed poor PFS and OS. Our results indicated the potential of urinary pteridines in precancer diagnosis and may provide useful information on the disease's stage of progression.

Financing: This study was supported by grants VEGA 1/0136/18, APVV-15-0119 and ITMS: 26240120027.

Keywords: Chromatography, fluorescence, pteridines, urine, ovarian tumor

Back to programme

PS-80, session: Poster session

Biophysical Modeling of Wave Propagation Phenomena: Experimental Determination of PWV in Viscous Fluid-filled Elastic Tubes in a Gravitation Field

Dejan Žikić (1), Bojana Stojadinović (1), Zorica Nestorović (1)

(1) Faculty of Medicine University of Belgrade Serbia

Biophysical understanding of arterial hemodynamics is highly important for medical diagnosis and investigation of the cause of cardiovascular disease. Pulse wave velocity (PWV) is influenced by the mechanical properties of the arterial wall and contains very important information on the state of the cardiovascular system. In humans like in most animals, blood flow through the blood vessels is affected of the gravitational field. In the vertical position of the body, the blood flow through the carotid artery against the direction of the gravitational force, while in the aorta the blood flows in the same direction with the gravitational force field.

For our study, we developed a biophysical model of the cardiovascular system that simulates the blood flow in the upright position and investigated the influence of gravity on PWV in fluid-filled elastic tube of different viscosity.

The results of the measurement of the biophysical model show the influence of gravity on PWV. The pulse waves propagating in the direction of the gravitational field have a higher velocity than the pulse waves propagating in the opposite direction. The results also show the relationship between PWV and viscosity. Gravity does not affect the shape of PWV depending on viscosity, but only on the value of PWV. The dependence of the PWV on viscosity in the gravitational field has the same shape obtained by measuring PWV in a horizontal position without the influence of gravity (1).

(1) Stojadinović et al. Effect of viscosity on the wave propagation. Journal of Biomechanics. 2015; 48(15):3969-3974 doi.org/10.1016/j.jbiomech.2015.09.016

Financing: The Ministry of Education, Science and Technological Development of the Republic of Serbia

Keywords: Pulse wave velocity, gravity, arterial blood flow

Back to programme

PS-81, session: Poster session

Se(0)-nanoparticles formation by fungus Phycomyces blakesleeanus

Milan Žižić (1), Goran Branković (1), Danica Bajuk-Bogdanović (2), Ivanka Rodić (1), Joanna Zakrzewska (3)

(1) Institute for Multidisciplinary Research, University of Belgrade
(2) Faculty of Physical Chemistry, University of Belgrade
(3) Institute of General and Physical Chemistry

Selenite (Se4+) is the most toxic form of Se, and its reduction to elementary Se nanopoarticles (SeNPs) is of great importance for living organisms. SeNPs were recognized recently, to have high antibacterial and anticancer properties, with low toxicity (1) and a number of applications due to its extraordinary conducting and catalytic properties (1). So far, only a few fungal species were documented to reduce Se4+ to elementary Se. In this study, the capability of the fungus P. blakesleeanus for SeNPs formation was examined. Gradual change of the mycelium color to red was a first sign of Se4+ reduction. Presence of SeNPs, as spherical nanoforms, was proved by SEM-EDS (scanning-electron microscopy with energy-dispersive X-ray spectroscopy). Although, the term 'nanoparticles' should be limited to particles with one of dimension up to 1 m, biogenic SeNPs less than 100 nm were found rarely (2). Here, the size of SeNPs formed by P. blakesleeanus mycelim was assessed as 1-95 nm, with an average value of 57 nm, by the light scattering measurements. Formed nanoparticles could appear in several structural orders. Results of Raman spectroscopy revealed single-chain SeNPs structure.

1. Wadhwani, S. A., Shedbalkar, U. U., Singh, R. Chopade, B. A. (2016). Biogenic selenium nanoparticles: current status and future prospects. Appl. Microbiol. Biotechnol. 100:2555–2566.
2.Dwivedi S, Alkhedhairy A, Ahamed M, Musarrat J (2013) Biomimetic synthesis of selenium nanospheres by bacterial strain JS-11 and its role as a biosensor for nanotoxicity assessment: a novel Se-bioassay. PLoS One 8:1–10.

Keywords: Selenite, SEM-EDS, Light scattering, Raman spectroscopy

Back to programme

PS-82, session: Poster session

A generic force-modulated mechanism for multivalent binding of fimbriated bacteria

Erik Reimhult (1)

(1) University of Natural Resources and Life Sciences, Vienna

Many bacteria interact with surfaces via long (m) and thin (nm) tethers called fimbriae or pili. For Escherichia coli (E. coli), type I fimbriae are implicated in the formation of pathogenic biofilms in the urinary tract via both specific and non-specific bonds. Colonization of abiotic surfaces such as catheters using nonspecific bonds have severe impact on hospital care and human suffering. We applied newly developed digital phase contrast holographic microscopy to map bacteria 3D trajectories. This technique can be used to measure position, size and orientation with high spatial and time resolution. We show that fimbriated E. coli form loose bonds and, irrespective of binding specificity, undergo a rolling motion regulated by the number of fimbriae forming bonds with the surface. This motion slows down and eventually stops in response to increased flow. The loss of translational motion is caused by a shear force-induced shift of the bacteria towards the interface, leading to additional binding of shorter fimbria and thereby increasing the binding valency. Our data suggest that fimbriae allow bacteria to explore different surface niches, responding both to the affinity and number of available contact points, as well as to flow. In contrast to other force-controlled binding mechanisms, e.g. catch-bonds, force modulation of the binding valency is generic; it is not limited to a specific interaction, but merely dependent on general properties such as the number, length distribution and mechanical properties of the tethers forming bonds.

Financing: EU-H2020-MSC, ERC

Keywords: bacteria adhesion; fimbriae; pili; force-modulation; holography; multivalency

Back to programme

PS-83, session: Poster session

Effects of shear stress on a lab-on-a-chip endothelial model

András Kincses (1)

(1) Biophysics Institute, Biological Research Centre, Hungarian Academy of Science

Lab-on-a-chip (LOC) models play important role to learn about the physiological function and transport mechanisms of the blood-brain barrier (BBB). The acquired information could be used to understand the pathologies and improve drug delivery. In our system all the crucial parameters of cell-culture based BBB models can be monitored (Trans-Endothelial Electric Resistance (TEER) measurement, permeability assays, visual confirmation of cell growth, immunostaining). In addition, we have the possibility to connect peristaltic and syringe pumps to the LOC device to mimic blood flow. The impact of the shear stress is monitored by an automated flow control and TEER measurement setup. The fast effects of the flow are presented with the TEER results, while the long term effects are presented with both TEER measurements and permeability assays.

Keywords: blood-brain barrier; shear stress; lab-on-a-chip

Back to programme

PS-84, session: Poster session

Bioinformatics study of the pharmacokinetic parameters of different curcumin formulations with enhanced oral bioavailability

Timotej Brumec (1), Janja Belinc (1), Eva Jovan (1), Peter Marinko (2), Vesna Postružnik (1), Marko Šterk (2), Tina Zorec (3), Nataša Antić Latinović (2), Tadej Emeršič (4), Andrej Dobovišek (2), Maša Hojnik Niderl (5), Aleš Fajmut (2)

(1) University of Maribor, Faculty of Health Sciences, Žitna ulica 15, 2000 Maribor, Slovenia
(2) University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška cesta 160, 2000 Maribor, Slovenia
(3) University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia
(4) University of Ljubljana, Faculty of Medicine, Institute of Biophysics, Vrazov trg 2, 1000 Ljubljana, Slovenia
(5) Vitiva proizvodnja in storitve d.d., Nova vas pri Markovcih 98, 2281 Markovci, Slovenia

Over the last decade, several curcumin formulations with enhanced bioavailability appeared on the market and many of them are commercialized as the best curcumin products with the highest bioavailability. Usually, the ratio of the area under the curve (AUC) within the time dependent pharmacokinetic measurement of the free curcumin blood concentration for the same dose of curcumin in formulated and unformulated form is used for this purpose. This information is very inaccurate due to high variations and errors in the measured data of free curcumin concentration in the blood for the unformulated form, which lies just at the edge of the detection limit of HPLC. This is due to poor absorption after oral dosing and a high rate of metabolic inactivation of curcumin. The purpose of our work is to present a systematic and consistent comparison of the pharmacokinetic data of different curcumin formulations, in which only the comparable pharmacokinetic parameters and values will be considered. This is often not the case. To be able to compare as much as possible data, we will fit the measured data with the two-store pharmacokinetic model, where possible, and compare also these data. In this way we will reveal the best published curcumin formulation on the market.

Financing: The work presented here is a part of the ongoing project PKP 2018 involving students of Bioinformatics, Physics and Chemistry and a partner company Vitiva d.o.o. The investment is co-financed by the Republic of Slovenia and the EU under the Cohesion Fund EKP 2014-2020.

Keywords: curcumin; enzyme inhibition; transcription factor; arachidonic acid; inflammation

Back to programme

PS-85, Session: Poster session

Characterization of physicochemical properties of DPPC membranes doped with 5-n-alkylresorcinols

Patrycja Zawilska (1), Katarzyna Cieslik-Boczula (2)

(1) Laboratory of Lipids and Liposomes, University of Wroclaw, Poland, and Laboratory of Biophysics, Condensed Matter Physics, Jožef Stefan Institute
(2) Faculty of Chemistry, University of Wroclaw

5-n-alk(en)ylresorcinols (known as resorcinolic lipids, ARs) are naturally occurring compounds, which belong to the group of non-isoprenoid phenolic lipids. Due to their strong amphiphilic character, these compounds can incorporate into erythrocyte and liposome membranes and alter their properties and functions. ARs showed a stabilizing effect on phospholipid membranes by making the bilayers less permeable to small solutes, and ARs-doped liposomes are more resistant to osmotic stress.
We report here an investigation of the influence of long-chain homologues of 5-n-alkylresorcinols (from C15:0 to C25:0) on dipalmitoylphosphatidylcholine (DPPC) membranes using Laurdan fluorescence and attenuated total reflectance-infrared spectroscopy (ATR-IR). The chain length- and concentration-dependent changes in DPPC:AR liposomes were investigated as a function of temperature.
Our data suggest strong interaction between ARs homologs with DPPC membranes. We have shown that incorporation of ARs lead to a shift in the gel-liquid crystalline phase transition of ARs-mixed bilayer towards higher temperatures. Additionally, with a further increase in the alkyl-chain length and concentration of doped compounds, we observed lower cooperativity of the chain-melting phase transition which is sign of increase stiffness within the lipid acyl chains. Furthermore, ARs decrease the hydration of the phospholipid headgroups. We also showed that -OH groups of ARs molecules form hydrogen bonds with the phosphate groups of DPPC in dry conditions, which may explain the stabilizing effect of ARs on phospholipid membrane.

Keywords: lipid membrane; ATR-IR; fluorescence spectroscopy

Back to programme

PS-86, Session: Poster session

Uncontrolled nanoparticle-labeling lead to misinterpreted STED images of exposure studies

Boštjan Kokot (1), Hana Majaron (1), Janez Štrancar (1)

(1) Laboratory for Biophysics, Condensed Matter Physics, J. Stefan Institute, Slovenia

Nanotoxicological assessment of nanoparticles frequently relies on nanomaterial localization within living cells. A popular method for the latter is fluorescence microscopy, which requires stable labelling of nanoparticles with fluorescent probe via surface functionalization. Issues such as desorption of fluorescent probe, poor free probe removal and changed morphology of nanomaterial may lead to biased or misinterpreted localization, nanotoxicity assessment and biophysical phenomena description. Labeling process should therefore be suitably verified to ensure that final nanomaterial is same or close to pristine and that the free probe was effectively removed. We demonstrate three possible outcomes when labeling process was poorly controlled that lead to false conclusions, using super-resolution stimulated emission depletion (STED) microscopy. Nanomaterial was incubated with LA4 mouse lung epithelial cells for approximately 48 hours prior imaging. For nanomaterial where free probe was insufficiently removed we show that localization conclusions are biased. When the functionalization process is not properly verified, final nanomaterial has wrong surface charge. This leads to very much different effect on cells than that of pristine nanomaterial. Lastly the nanomaterial that is over-sonicated is broken down in small sharp pieces which cause cytoskeleton destruction and consequently membrane destabilization. This could not be seen under confocal microscopy mode but was only seen when using STED microscopy depletion beam, resulting in an optical tweezer-like effect, where cell membrane was dragged across the image.

Keywords: STED microscopy; toxicology; live cell imaging; super-resolution microscopy; TiO2 nanoparticles; nanoparticles; cellular biophysics

Back to programme

PS-87, Session: Poster session

Temperature stabilization of STED microscopy

Jaka Močivnik (1), Janez Štrancar (2)

(1) Institut "Jožef Stefan", Jamova cesta 39, 1000 Ljubljana, Slovenija

While STED microscopy is one of the most precise microscopies in the world, it also demands very specific conditions, including extremely stable ambient tempreature. The consequences of temperature swings can be seen as uneven and non-linear stretching and shrinking of metal parts, which has tremendous influence on geometry of a microscope. Dinamic temperature swings causes drifting of a microscope in XY and XZ plane, which makes it difficult for repeated measurements.
With temperature stabilization we are trying to hold microscope's body, working table, lenses and the sample at higher temperature, which should not exceed the temperature limit, as it could cause deforming or even dying of the sample.
To measure temperature gradient between various parts of a microscope and ambient we used highly-precise temperature sensors, which we applied to different parts of a microscope to observe temperature flow through metal parts. Based on measurements we could determine geometry of thermal losses to guide us while attaching heating elements over the microscope.
To stabilize the temperature we used Thorlabs HT10K heating elements, which we systematically applied over the microscope, where the number of heating elements depends on shape and size of a microscope, swings of ambient temperature and time scale.
Temperature regulation is performed with PID regulator, where a locked loop allows us to correct the temperature with approximately 0.1K resolution, which is accurate enough for optimal STED microscopy.

Keywords: STED; temperature stabilisation

Back to programme

PS-88, Session: Poster session

Data processing and analysis of multimodal (super-resolution) hyperspectral fluorescence lifetime imaging to provide better insight into complex biological systems

Rok Podlipec (1), Iztok Urbancic (1), Zoran Arsov (1), Janez Štrancar (1)

(1) Laboratory for Biophysics, Condensed Matter Physics, J. Stefan Institute, Slovenia

Multimode fluorescence microscopy has become a valuable tool to study physiology and interactions of complex biological systems, where multiple spectroscopic parameters are studied simultaneously (1,2). The development and implementation of superresolution and nonlinear microscopy methods in the last years has enabled identification of microenvironment structures on even smaller scales. In order to gather as much resolution/sensitivity of the high-content imaging, correlations of the multimodal data have to be searched in proper way. Various complex cellular systems are being investigated by our new superresolution time-resolved multiphoton hyperspectral fluorescence lifetime imaging microscope rather lacking in multimodal data processing to identify possible correlations. By combining and upgrading home-built program for fluorescence spectral analysis (FMS+, LBF) and commercial FLIM data analysis program (SPCImage, Becker&Hickl) various photophysical parameters with known resolution such as fluorescence emission peak (λ_{avg}), fluorescence emission width (w), average fluorescence lifetime (τ_{avg}), ratio of bi-exponential lifetime decays useful for FRET (τ_1/τ_2), etc. can now be presented within user-defined multidimensional plots seeking for potential correlations and thus gathering more insight into the examined complex biological systems.

(1) C Blum, Y. Cesa, M Escalante, V. Subramaniam, Multimode microscopy: spectral and lifetime imaging, J. R. Soc. Interface 2009; (6) S35-S43.
(2) Q Pian, R Yao, N Sinsuebphon, X. Intes, Compressive hyperspectral time-resolved wide-field fluorescence lifetime imaging, Nature Photonics 2017; (11) 411-415.

Keywords: Fluorescence microscopy; multimodal imaging, data analysis

Back to programme

PS-89, Session: Poster session

Accurate identification of microorganisms by de novo sequencing

A. Hozić (1), J. Diminić (2), A. Štajduhar (3), M. Cindrić (1)

(1) Ruder Boskovic Institute, Division of Molecular Medicine, Bijenicka 54, 10000 Zagreb, Croatia
(2) Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10 000 Zagreb, Croatia
(3) Croatian Institute for Brain Researh, School of Medicine, University of Zagreb, Šalata 12, 10000 Zagreb, Croatia

Majority of currently used LC-MS/MS techniques for protein identification is based on database matching of non-derivatized peptide signals recorded in positive ion mode. Although widely used, such an approach does not always provide satisfactory sequence coverage to unambiguously identify a protein sample.
Protein de novo sequencing concept using CAF-/CAF+ reagent (chemically activated fragmentation negative/chemically activated fragmentation positive) enables fast, highly accurate, reliable and easy to use identification of microorganisms down to the species and subspecies level. CAF-/CAF+ (5-formylbenzene-1,3-disulfonic acid*) is a chemical reagent for the derivatization of peptide samples prior to analysis by tandem mass spectrometry (MS/MS) that enables gathering of both positive and negative peptide ions datasets. Peptide sequences are read from both positive and negative MS/MS spectra and matched against the NCBInr database by developed software named ProteinReader.
We present results that show identification of microorganisms down to the subspecies level using either MALDI- or ESI-TOF technique.

*EP2710380 A1, US 8647880

Keywords: Mass spectrometry; microorganism; de novo sequencing

Back to programme

4 Organizing and scientific committe

Local organizing Committee

Janez Štrancar (Slo)
Maja Garvas (Slo)
Hana Majaron (Slo)
Maja Tišler (Slo)
Uroš Tkalec (Slo)
Monika Koren (Slo)
Neža Golmajer Zima (Slo)
Petra Čotar (Slo)
Primož Peterlin (Slo)

Scientific Committee

Pavle Andjus (Serbia)
Mario Brameshuber (Austria)
Mátyus László (Hungary)
Carlo Musio (Italy)
Matej Praprotnik (Slovenia)
Mauro Dalla Serra (Italy)
Eva Sevcsik (Austria)
Janez Štrancar (Slovenia)
Cristiano Viappiani (Italy)
Tomislav Vuletić (Croatia)
Alexandra Zahradníková (Slovakia)
László Zimányi (Hungary)
Miroslav Živić (Serbia)