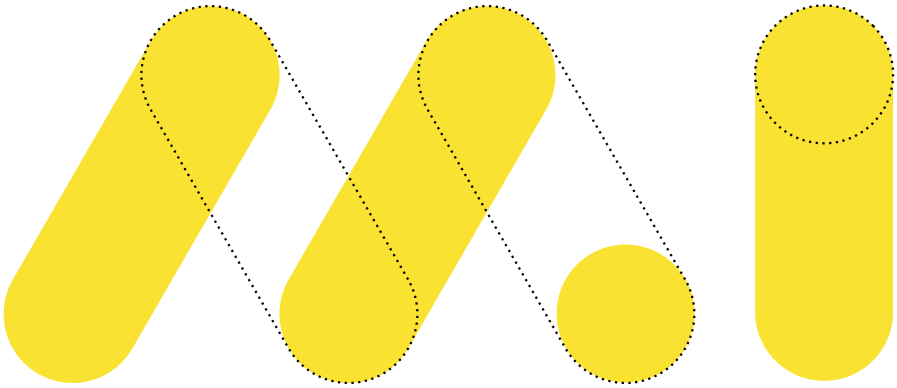

MOLECULAR INTERACTIONS

14·11·2018 ⋮ 15·11·2018

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

Minisymposium 2018
MOLECULAR INTERACTIONS

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Welcome

to the 7th Mini Symposium organized by the Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry. This year's topic, Molecular Interactions, is quickly gaining importance in both academic and industrial discussions because of possibilities of establishing molecular understanding of a variety of cell processes that are important for basic science and drug development. We organize these meetings in order to share our knowledge, experience and expertise in a regional context, as well as hold a timely overview on the state-of-the-art molecular interactions approaches.

We have assembled a selection of fifteen extraordinary speakers, including guests from abroad. We wish you all a pleasant and fruitful meeting. We also hope you take the advantage of the many sights during your stay in Ljubljana.

Dr. Gregor Anderluh and Dr. Marjetka Podobnik

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Sponsors

Thank you for your support.

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Lipid-binding proteins from the aegeroysin family as potential bioinsectidices

Abstracts

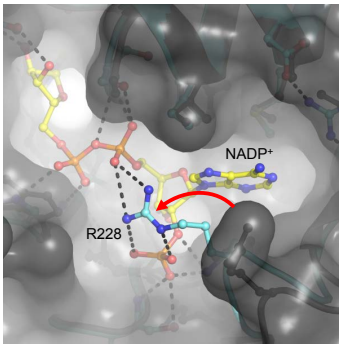
13:05
Plenary talk

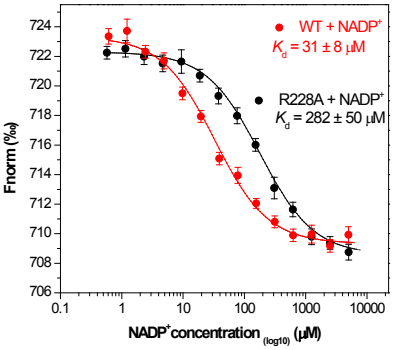
Structural study on the plant aldehyde dehydrogenase superfamily combined with a ligand binding analysis using MST

David Kopečný[1], Martina Kopečná[1], Radka Končitíková[1], Armelle Vigouroux[2], Eva Hájková[1], Pierre Briozzo[2], Klaus von Schwartzenberg[3], Marek Šebela[1] and Solange Moréra[4]

[1]Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University (Czech Republic); [2]Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech (France); [3]Biozentrum Klein Flottbek und Botanischer Garten, Universität Hamburg (Germany); [4]Institute for Integrative Biology of the Cell, CNRS-CEA-Univ. Paris-Sud, Université Paris-Saclay (France)

Aldehyde dehydrogenases (ALDHs) comprise a protein superfamily of $NAD(P)^+$-dependent enzymes (EC 1.2.1.-) and catalyze irreversible oxidation of aldehydes to carboxylic acids. ALDHs play a crucial role in detoxifying aldehydes produced by various metabolic pathways and during various stress conditions such as salinity, heat, cold and drought. Aldehydes are highly reactive molecules and toxic at higher concentrations. The superfamily of plant ALDHs currently contains 13 distinct families. In previous years we kinetically and structurally characterized four ALDH2, one ALDH7 and three ALDH10 family members from maize (*Zea mays*), which represents one of the most important crops worldwide, as well as unique ALDH21 in moss *Physcomitrella patens*, which is an important evolutionary model representing a transition step from water to land. Results on ALDH members from family 2, 10, 12 and 21 will presented using data obtained by X-ray crystallographic analysis, site-directed mutagenesis, enzyme kinetics, nano DSF and MST.





Understanding the protein synthesis machinery by coupling positional sequencing and nano-imaging

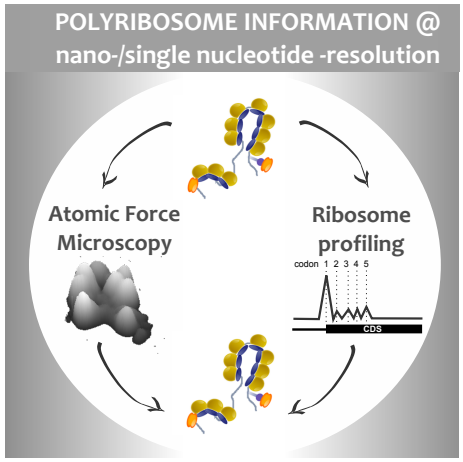
13:30
Plenary talk

Gabriella Viero
Laboratory of Translational Architectomics, Institute of Biophysics CNR (Italy)

In cells, groups of ribosomes form the so-called polyribosome, which simultaneously translates a single mRNA. As such, polyribosomes are among the biggest cytoplasmic machines and integrated platforms of molecules, 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 as rich as that of transcription?

We are trying to answer these questions dissecting the organization of polyribosomes with a multi-level approach by integrating imaging data (AFM) and positional information about ribosome footprints along the mRNA (Ribo-Seq). Our work illustrates a novel interdisciplinary approach connecting sequencing and imaging data for better understanding the role of ribosome biology in motor neuron diseases such as SMA and ALS.



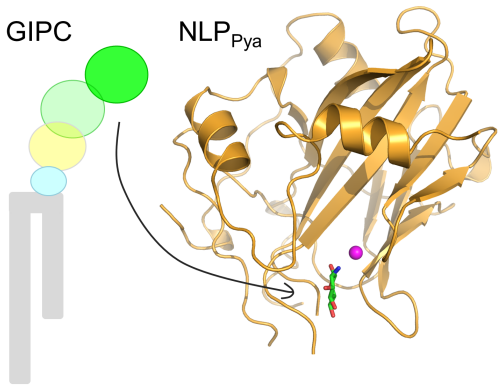
13:55

Unfolding the mechanism of NLP-membrane interaction

Katja Pirc[1], Tea Lenarčič[1], Vesna Hodnik[1,2], Tina Snoj[1], Marjetka Podobnik[1] and Gregor Anderluh[1]

[1]Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry (Slovenia); [2]Department of Biology, Biotechnical Faculty, University of Ljubljana (Slovenia)

Nep1-like proteins (NLPs), secreted by phytopathogenic microorganisms, cause necrotic lesions of plant tissue and facilitate eudicot plant infection, but are not active against monocots. Recently, we identified glycosylinositol phosphorylceramides (GIPCs), a major class of plant sphingolipids, as target molecules for NLP's association with plant plasma membrane. NLP from oomycete *Pythium aphanidermatum* (NLP_{Pya}) undergoes structural changes after binding to the terminal hexose moiety of GIPC. We proposed a model of early steps of NLP_{Pya}-membrane interaction, however, the exact mechanism of membrane disruption by the toxin action remains to be elucidated. Identification of NLP's membrane receptor enabled exploitation of various GIPCs-containing lipid model systems. The data obtained by liposome sedimentation assay, surface plasmon resonance, dynamic light scattering and giant unilamellar vesicles (GUVs) imaging contribute to our understanding of the nature and mechanism of NLP-membrane association, by either via pore-forming mechanism or other type of membrane integrity disruption.



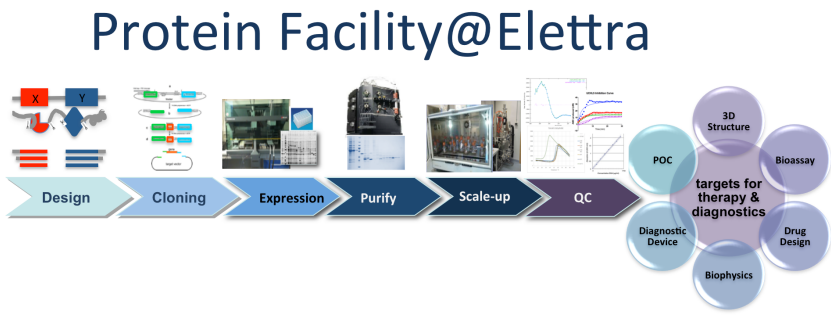
The Protein Facility of Elettra: a tool for research and development in drug discovery

14:10

Paola Storici

Protein Facility, Structural Biology Laboratory, Elettra - Sincrotrone Trieste S.C.p.A. (Italy)

At the Elettra synchrotron in Trieste we implemented a protein production facility opened to internal/external researcher from academia and industry to provide recombinant proteins for functional and structural studies. The lab aims to facilitate the process to get recombinant proteins suitable for analysis with synchrotron radiation techniques (X-ray diffraction, SAXS, FTIR). The laboratory is also offers tutoring and knowledge transfer to less experienced researchers such as young postdocs and PhD students. A number of different scientific projects are running in parallel and the principal interest is on protein targets for drug discovery with focus on cancer and neurodegenerative diseases. We have expertise on druggable protein families such as kinases and deubiquitinases. In addition, our pipeline encompasses between different types of protein such as membrane proteins, proteases as well as nanobodies. The protein facility supports in average 30 projects a year, divided between collaborative research projects, industrial services and tutoring activities.



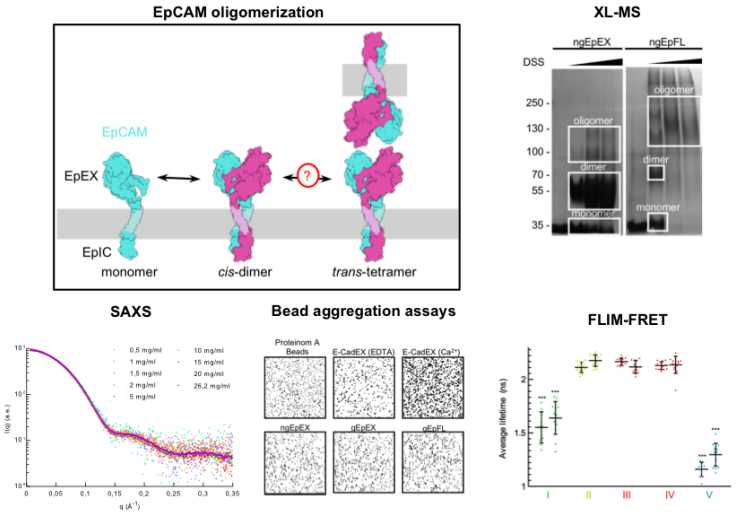
Analysis of EpCAM oligomerization as a case of studying weak, transient, homophilic interactions (which were not there at all)

14:25

Aljaž Gaber[1], Miha Pavšič[1] and Brigita Lenarčič[1,2]

[1]Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana (Slovenia); [2]Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute (Slovenia)

Epithelial Cell Adhesion Molecule (EpCAM) is an established carcinoma tumor marker, which plays a key role in proliferation, differentiation and adhesion processes in stem and epithelial cells. It was first established as a calcium independent cell-cell adhesion molecule. It was postulated that inter-cellular adhesion unit, a trans-tetramer, is formed by two cis-dimers on the opposing cells. While cis-dimers are stable complexes, trans-tetramers were believed to be connected by a much weaker and transient interactions, at least when analyzed in solution. We investigated homo-oligomerization utilizing SAXS, XL-MS, bead aggregations assays and FLIM-FRET. Our study provides a comprehensive and clear evidence that EpCAM indeed does not function as a homophilic cell-cell adhesion molecule, as it has been postulated for more than two decades. More than that, the insight obtained from our experiments also help in research of other weak, transient and/or homophilic interactions.

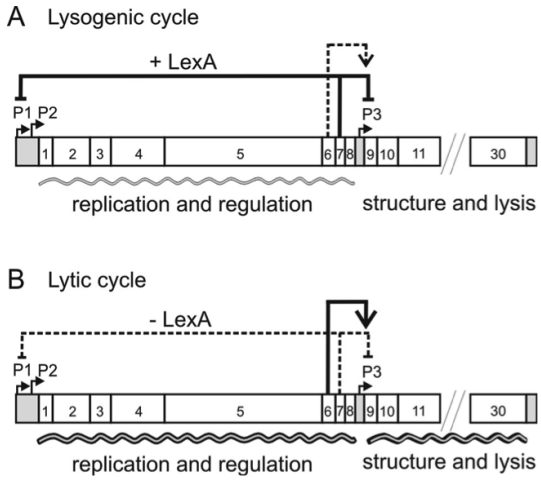


Molecular basis of bacteriophage GIL01-host interaction

14:40

Matej Butala
Department of Biology, Biotechnical Faculty, University of Ljubljana (Slovenia)

Temperate bacteriophages have a great impact on bacterial evolution, leading to the rearrangement of bacterial genomes and acquisition of potent virulence determinants. The Tectivirus phage GIL01 infects the insect pathogen *Bacillus thuringiensis* and can establish a stable lysogenic state inside the cell, where it resides as a 15-kbp extrachromosomal linear replicon. Lysogeny is stably perpetuated over generations until the host cell experiences genomic stress and DNA damage, and this is the inducer for GIL01 resurrection. I will decode protein-protein, protein-DNA interactions and the environmental stimuli that enable bacteriophage GIL01 to alternate between the lytic and the lysogenic lifecycle.



Development and biophysical characterization of novel catalytic inhibitors of human DNA topoisomerase IIα

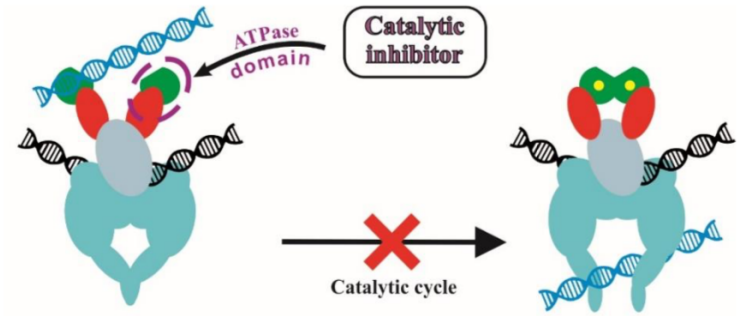
14:55

Kaja Bergant[1], Matej Janežič[2] and Andrej Perdih[1]

[1]Theory Department, National Institute of Chemistry (Slovenia); [2]Structural Bioinformatics Team, Division of Structural and Synthetic Biology, Center for Life Science Technologies, RIKEN (Japan)

Human topoisomerase IIα catalyzes topological changes of the DNA molecule and is a validated anticancer target. Due to its complex catalytic cycle, compounds that inhibit this enzyme can be divided into two groups; topoisomerase poisons and catalytic inhibitors. Due to severe side effects such as cardiotoxicity and induction of secondary malignancies of the known topoisomerase poisons already used in clinical practice novel inhibition paradigms are being actively explored.

Our group is designing novel catalytical inhibitors, that bind to the ATP binding site of the enzyme. In our work we are using structural data coupled with tools of computational chemistry to select favorable candidates and then check in the *in vitro* assays if selected compounds are inhibitors of the target enzyme. Subsequently we use various biochemical and biophysical assays to determine their mechanism of action. In our presentation the development of catalytic inhibitors of topo IIα will be outlined focusing on the utilization of Microscale thermophoresis (MST) and Surface plasmon resonance (SPR) techniques to confirm their binding to the ATPase domain of the enzyme.



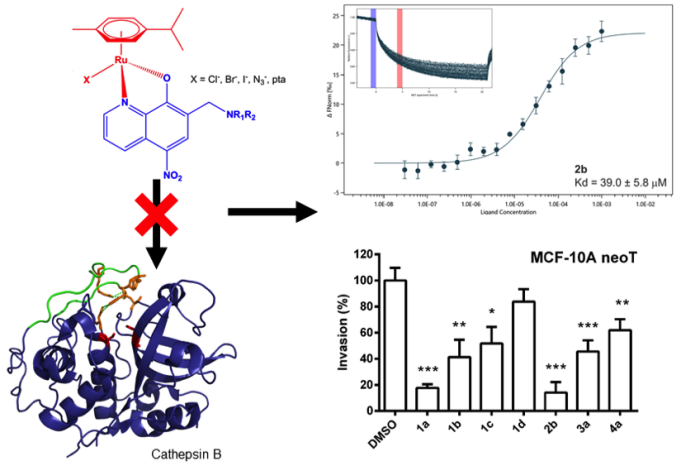
Ruthenium complexes with nitroxoline and its derivatives as novel inhibitors of cathepsin B activity in tumor progression

15:10

Ana Mitrović[1,2], Jakob Kljun[3], Izidor Sosič[2], Matija Uršič[3], Stanislav Gobec[2], Iztok Turel[3] and Janko Kos[1,2]

[1]Department of Biotechnology, Jožef Stefan Institute (Slovenia); [2]Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana (Slovenia); [3]Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana (Slovenia)

Lysosomal cysteine peptidase cathepsin B is increased in numerous pathological processes, where it is importantly involved in tumor progression and metastasis. Its increased activity can be regulated by exogenous inhibitors. Therefore, due to high pharmacological relevance cathepsin B represents a good target for development of new antitumor agents. In the present study we combined findings from our previous work, where on one hand, we identified antimicrobial agent nitroxoline as potent selective reversible inhibitor of cathepsin B activity and develop its numerous derivatives, and on the other hand, we identified clioquinol-ruthenium complex as potent cathepsin B inhibitor. Therefore, here we prepared new ruthenium complexes with nitroxoline and its derivatives and evaluated them as inhibitors of cathepsin B using enzyme kinetics and microscale thermophoresis. Furthermore, we showed that by inhibition of cathepsin B activity, new complexes can impair processes of tumor progression in *in vitro* cell based functional assays in low non-cytotoxic concentrations.



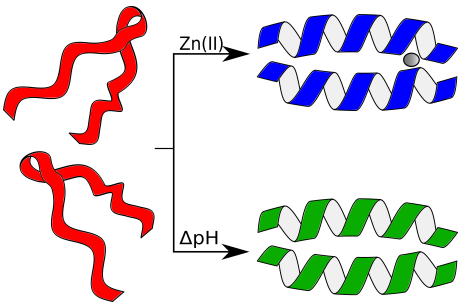
16:00

Metal-site design for controlling the assembly of coiled-coil dimers

Jana Aupič[1], Fabio Lapenta[1] and Roman Jerala[1,2]

[1]Department of Synthetic Biology and Immunology, National Institute of Chemistry (Slovenia); [2]EN-FIST Centre of Excellence (Slovenia)

Conformational change of proteins in response to chemical or physical signals is the underlying principle of many regulatory and transport mechanisms in biological systems. *De novo* protein design represents an exciting opportunity to explore the conformational space unexplored by nature and develop smart bio-inspired materials for diverse purposes. The design of molecular machines requires the ability to regulate the assembly and disassembly of building modules. We designed a metal-binding site into coiled-coil building modules in order to engineer a peptide-based conformational switch called SwitCCh that assembles into a homodimeric coiled-coil in response to the addition of Zn(II) ions or low pH. The addition of Zn(II) promoted formation of a parallel homodimer with an increase in thermal stability by more than 30 °C. The peptide could be reversibly cycled between the coiled-coil and random conformation. Furthermore, the SwitCCh peptide was orthogonal to the previously developed coiled-coil dimer set, indicating it could be used for regulated self-assembly of coiled-coil based nanostructures and materials.



Quartz crystal microbalance in molecular interactions studies and sensing

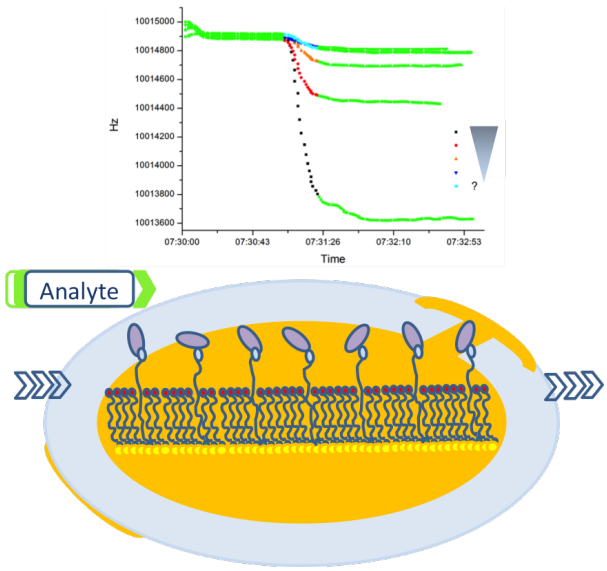
16:15

Simon Caserman

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

Quartz crystal microbalance (QCM) is a piezoelectric resonator. Binding of analyte to immobilized ligand on the resonator results in decrease of resonance frequency. The change is proportional to the bound mass. This enables a label free real-time detection of interactions between larger binding partners like protein molecules, DNA and others. QCM enables detection of mass changes in a broad range. Therefore the sensor surface can be functionalized for cell cultivation and detection of cellular interaction with macromolecules. Stiffness of the bound mass further affects the signal enabling detection of changes in viscoelastic properties of resonator-associated mass. Viscoelastic changes may arise from cellular responses to different agents binding to the surface receptors and/or compromising the integrity of cellular membrane.

QCM operates within larger volumes compared to SPR making it less sensitive to the clogging of the microfluidic system. We took advantage of this to develop sensor for in-line monitoring of the component of interest in a bioprocess fluid.



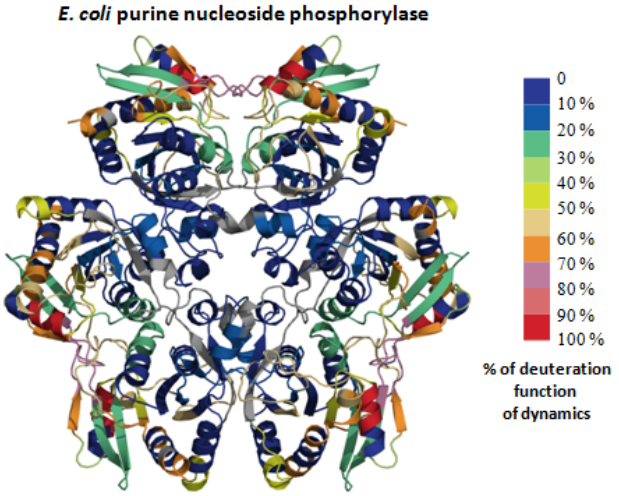
Mapping protein interactions by Hydrogen Deuterium eXchange Mass Spectrometry (HDX MS)

16:30

Saša Kazazić

Laboratory for Mass Spectrometry and Functional Proteomics, Ruđer Bošković Institute (Croatia)

In solution, proteins exist in many conformations dominated by the lowest free energy folded form. Because so many functional aspects of proteins are tied to higher order structural changes, it is just as important to investigate and characterize these structural changes as it is to understand general features of the protein structure. Hydrogen deuterium exchange (HDX), which probes for local dynamics of polypeptide chains, is a labeling approach to study mechanisms of interactions between proteins, protein folding, allosteric regulation, impact of post-translational modifications and other ligand binding to conformational dynamics of the protein structure. In this presentation I will show practical steps carried out in typical continuous HDX experiment and illustrate how HDX was utilized to characterize active site conformation dynamics of *E. coli* purine nucleoside phosphorylase (*E.coli* PNP) and interaction of dipeptidyl peptidase III (DPP III) ortholgs with tynorphin inhibitor.



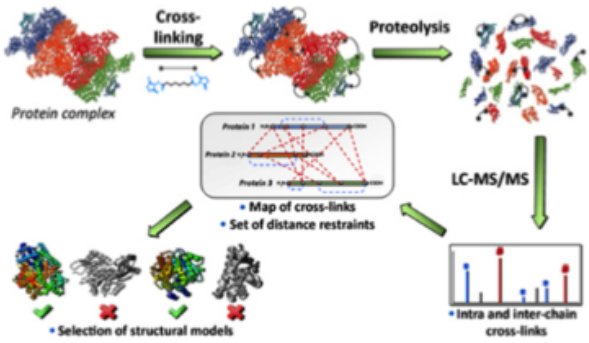
Study of interactions between proteins by chemical cross-linking

16:45

Igor Križaj

Department of Molecular and Biomedical Sciences, Jožef Stefan Institute (Slovenia)

Interactions between proteins represent a part of the interactome, the whole set of molecular interactions in a particular cell. Protein-protein interactions fundamentally influence biochemical processes. I my talk I will present the application of chemical cross-linking methodology to characterization of the physical interactions among protein molecules. With some of our examples, I will illustrate discovering of binding proteins, membrane and soluble receptors of secreted phospholipases A_2 (sPLA$_2$), and mapping of the protein-protein interaction sites using this approach.



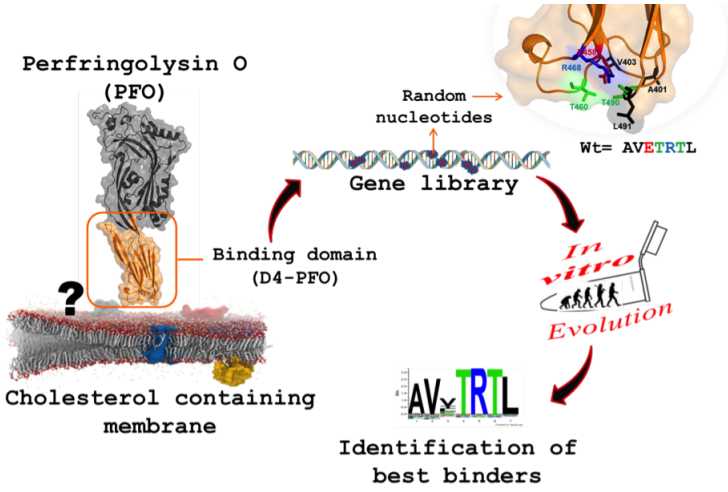
17:00

Study of cholesterol specificity by *in vitro* evolution

Aleksandra Šakanović and Gregor Anderluh
Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry (Slovenia)

Biological membranes function as platforms for the attachment of numerous proteins that are involved in many biological processes. The binding of proteins to biomembranes depends on structural and biochemical properties of proteins as well as lipid environment. Despite the importance of protein-biomembrane interactions in cell physiology, the exact determinants mediating specific lipid-protein recognition are poorly understood. The complexity of binding mechanisms raises the need for development of new high-throughput approaches for studying the specificity of lipid recognition.

Cholesterol, the main lipid in mammalian cell membranes, is recognized by many different proteins including members of cholesterol dependent cytolysins (CDC) family. To study cholesterol specificity, we use the binding domain of perfringolysin O, member of CDC and *in vitro* evolution by ribosome display combined with next generation DNA sequencing and model lipid membranes of different composition. Such approach will provide insights into the cholesterol recognition mechanism.



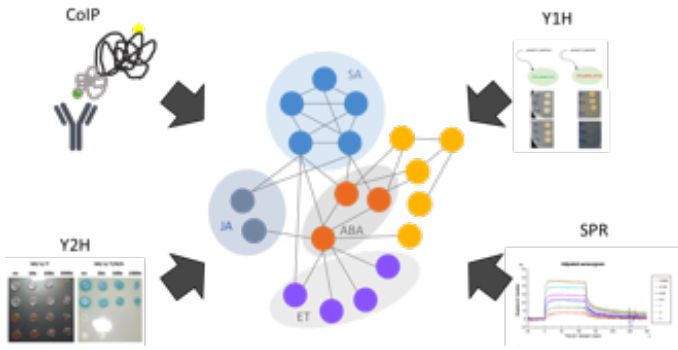
Exploring potato-PVY interaction using systems biology approach

17:15

Ana Coll[1], Špela Tomaž[1,2], Ion Gutierrez Aguirre[1], Ajda Taler-Verčič[2,3], Aleksandra Usenik[2], Vesna Hodnik[4], Dušan Turk[2,3], Maja Ravnikar[1] and Kristina Gruden[1]

[1]Department for Biotechnology and Systems Biology, National Institute of Biology (Slovenia); [2]Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute (Slovenia); [3]Centre of excellence for Integrated Approaches in Chemistry and Biology of Proteins, CIPKeBiP (Slovenia); [4]Infrastructural Centre for analysis of molecular interactions, Biotechnical Faculty, University of Ljubljana (Slovenia)

Plants have evolved complex immune responses in order to fight pathogen infections. In this study we are using a systems biology approach to unravel the mechanisms involved in the interaction between potato and potato virus Y (PVY), one of its major pathogen causing severe global crop losses. Integration of modelling and biological data enabled us to identify novel regulators of potato defence response. We are further evaluating the role of these novel players with functional genomics. We first studied the interactions between potato immune signalling proteins with each other and also with viral proteins using yeast two-hybrid (Y2H) system. Potential interactions are, afterwards, confirmed by *in planta* co-immunoprecipitation (CoIP) assays and surface plasmon resonance (SPR) experiments. We are also investigating protein-DNA interactions using yeast one-hybrid (Y1H) system to identify transcription factors controlling the expression of potato defence response genes. The results will contribute to ensure new efficient crop breeding strategies.



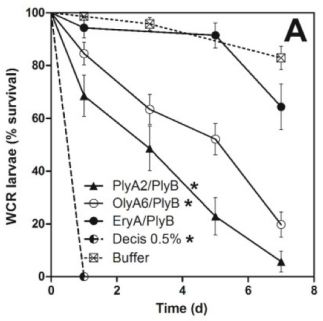
17:30

Lipid-binding proteins from the aegeroysin family as potential bioinsectidices

Kristina Sepčić
Department of Biology, Biotechnical Faculty, University of Ljubljana (Slovenia)

Aegerolysins are low molecular proteins found in several eukaryotic and bacterial taxa. Their common feature is the ability to bind different lipids and lipid derivatives, as well as biological and artificial lipid membranes. For example, some aegerolysins can target sphingomyelin/cholesterol membrane nanodomains. Furthermore, aegerolysins from the fungal genus *Pleurotus* preferentially bind to ceramide phosphoethanolamine, which is the major membrane sphingolipid of invertebrates (particularly insects and molluscs). Moreover, the genomes of *Pleurotus* mushrooms have nucleotide sequences that encode proteins with membrane-attack complex/ perforin (MACPF) domain. In the presence of a protein with a MACPF domain, *Pleurotus* aegerolysins can function as bi-component lytic complexes for target cell membranes.

Due to their specific interaction with ceramide phosphoethanolamine, cytolytic complexes based on *Pleurotus*-derived aegerolysins could represent a novel promising class of biopesticides for controlling important plant pests like western corn rootworm (WCR) and Colorado potato beetle.



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