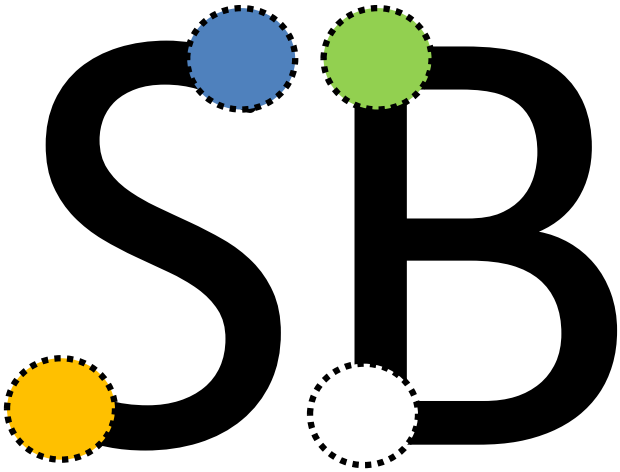
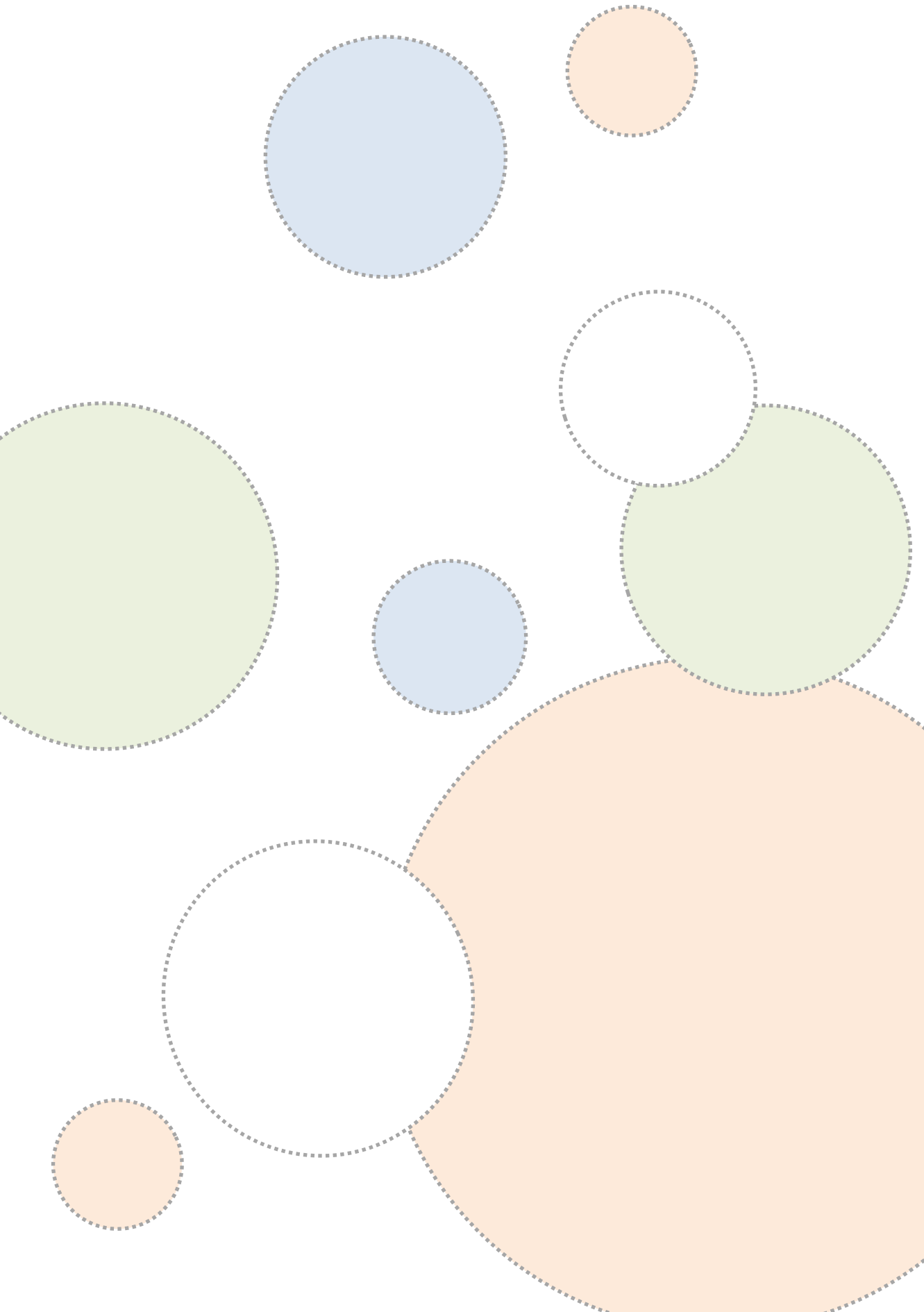


MINISYMPOSIUM 2022

28.09.2022

“GIVE STRUCTURAL BIOLOGY TO YOUNG PEOPLE”



September 28th, 2022

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

Minisymposium 2022

Give structural biology to young people

Book of abstracts

Organizer

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

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Technical editors

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Issued by

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

Ljubljana, 2022

Electronic version only

Kataložni zapis o publikaciji (CIP) pripravili v
Narodni in univerzitetni knjižnici v Ljubljani

COBISS.SI-ID 121862659
ISBN 978-961-6104-80-7 (PDF)

Dobrodošli

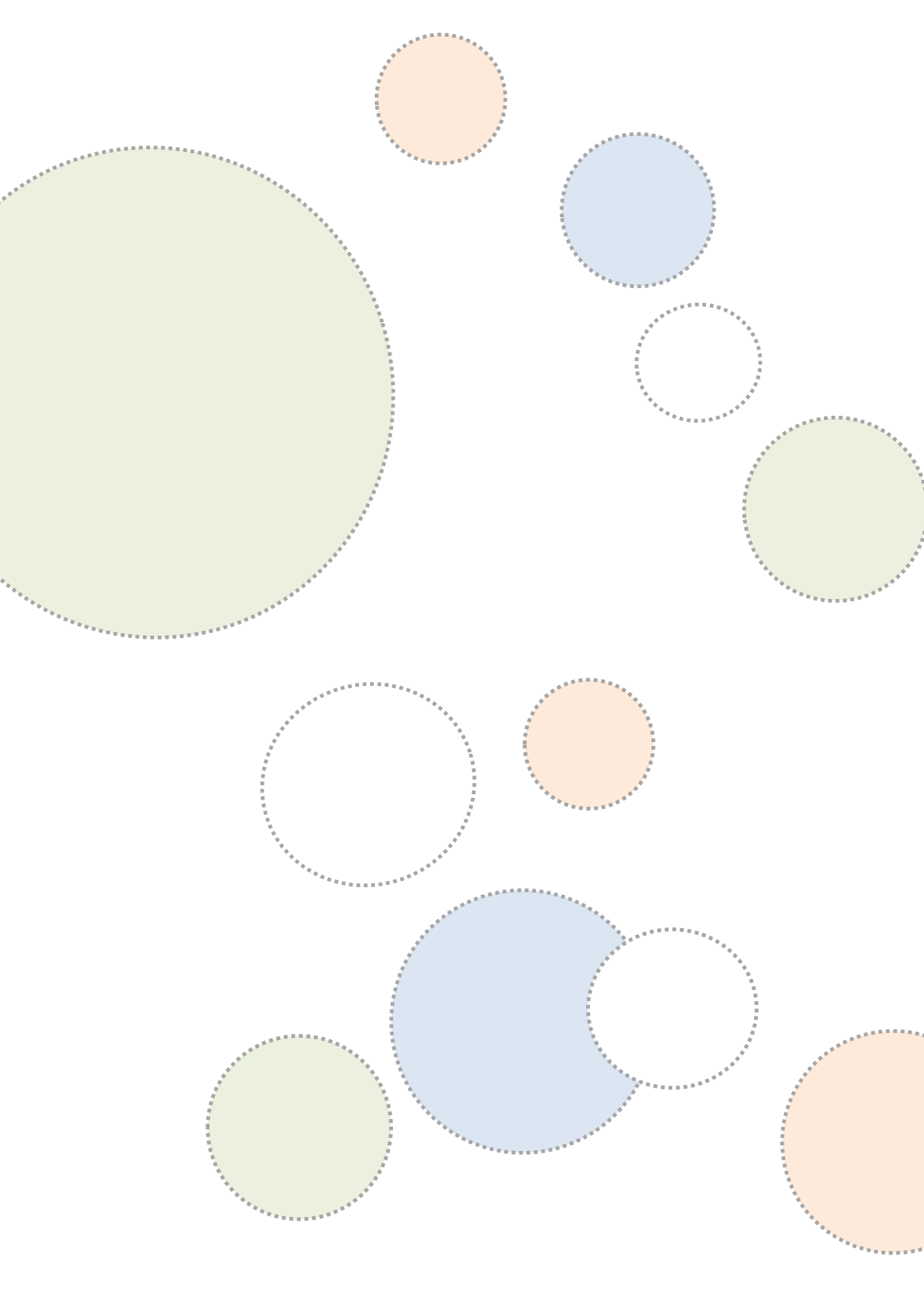
na 9. Mini simpoziju Odseka za Molekularno Biologijo in Nanobiotehnologijo Kemijskega inštituta! Po premoru zaradi pandemije smo se zopet vrnili, tokrat v hibridnem načinu, da omogočimo udeležbo čim večjemu številu poslušalcev.
Cilj naših mini simpozijev je deliti vznemirljive znanstvene rezultate, izkušnje in strokovno znanje ter razpravljati o najsodobnejših pristopih za proučevanje struktur bioloških molekul in njihovih interakcij. Letošnji simpozij nosi naslov „Podarimo strukturno biologijo mladim“ in v skladu s tem smo pripravili program, ki vključuje predavanja nadobudnih mladih raziskovalcev. Za odlične raziskovalne rezultate je potrebno trdo delo in sodelovanje znanstvenikov vseh generacij, vsekakor pa moramo še posebej spodbujati in podpirati mlade raziskovalce, da nadaljujejo in nadgrajujejo dediščino svojih mentorjev. Vsem želim uspešno in prijetno srečanje! Zelo se ga veselim in tudi vseh, ki bodo še sledila!

izr. prof. dr.
Marjetka Podobnik

Welcome

to the 9th Minisymposium organized by the Department of Molecular Biology and Nanobiotechnology at National Institute of Chemistry! After a hiatus due to the pandemics, we are finally back, this time in a hybrid mode, to enable as many attendees as possible.
The goal of our minisymposium is to share exciting scientific results, experience and expertise, as well as to discuss state-of-the-art approaches in studying structures of biological molecules and their interactions. This years' minisymposium is entitled "Give structural biology to young people" and we have prepared a program including talks from enthusiastic young researchers. As much as successful research is based on hand-in-hand hard work of scientists of all generations, we need to nourish and support young researches to continue and upgrade the legacy of their mentors. I wish everybody a fruitful and enjoyable meeting! I am very much looking forward to it and to many more to come!

Assoc. Prof. Dr.
Marjetka Podobnik



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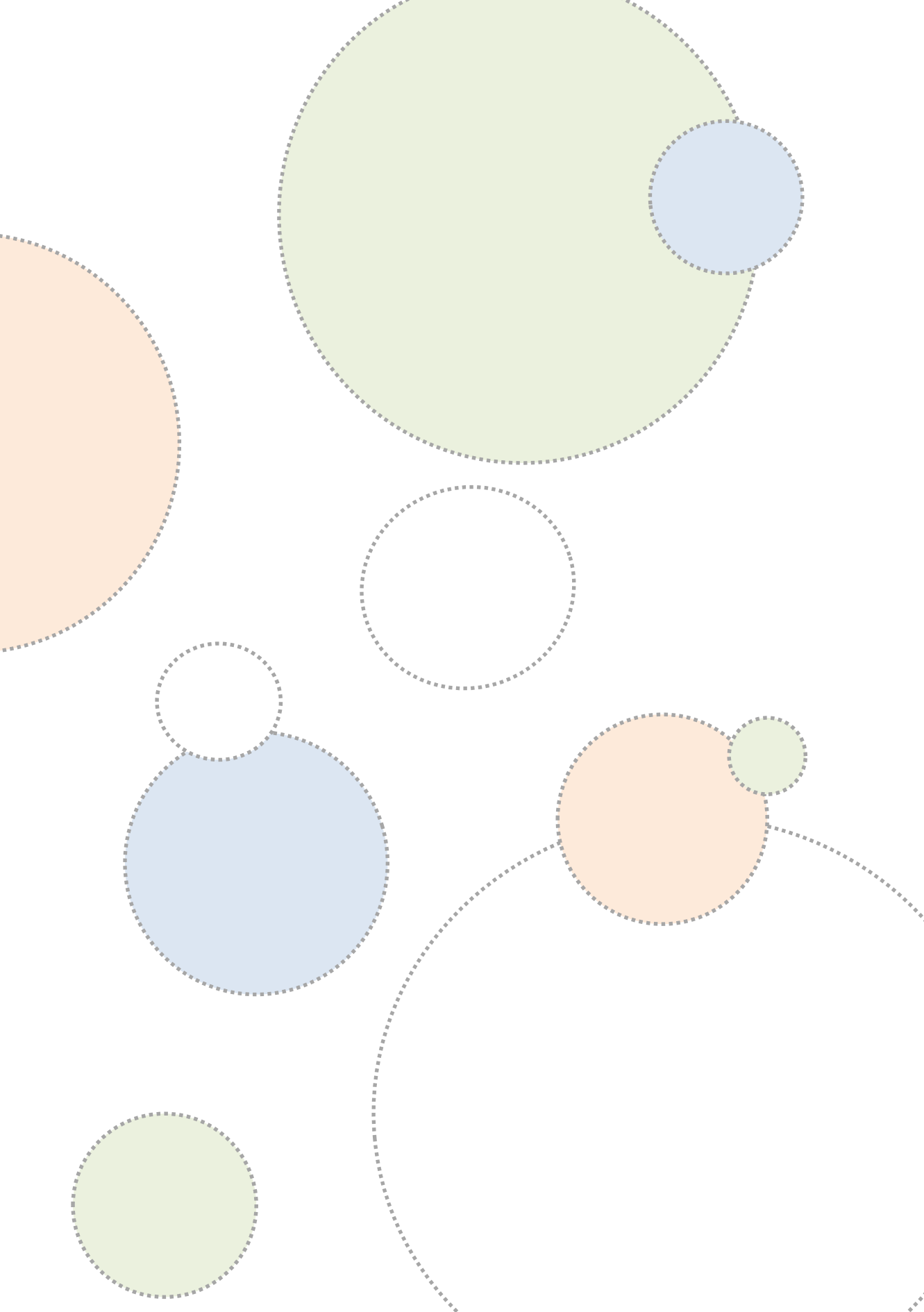


Schedule

9:00 - 9:05	**Marjetka Podobnik** *Welcome address by the head of the Department for Molecular Biology and Nanobiotechnology*
9:05 - 9:30	**Gašper Šolinc** *Structural studies of cnidarian pore-forming toxins, actinoporins, using cryo-EM*
9:30 - 9:55	**Ajasja Ljubetič** *Cryo-EM structure of a track for a completely de novo designed random protein roller*
9:55 - 10:20	**Martina Lenarčič Živkovič** *NMR insights into a G-quadruplex formation in the 5'-UTR region of the RANKL gene*
10:20 - 10:55	*tea/coffee, refreshments*
10:55 - 11:20	**Jure Loboda** *Rigidity and flexibility of structure govern specificity and promiscuity of cysteine cathepsins*
11:20 - 11:45	**Anemari Horvat** *Measurements of (sub)cellular Ca^{2+} and cAMP dynamics in single astrocytes using confocal microscopy*
11:45 - 13:00	*lunch*
13:00 - 13:15	**Žiga Strmšek** *Using small-angle X-ray scatering to determine the shape of decorated coiled-coil protein origami*

13:15 - 13:30 **Matic Kisovec**
The cryo-transmission electron microscopy facility at the National Institute of Chemistry

13:30 - 13:45 **Dorian Dolanc**
Regulation of L-lactate metabolism via GPCR

13:45 - 14:00 **Neža Koritnik**
Cryo-EM studies of filamentous potyviruses and their virus-like particles

14:00 - 14:30 *tea/coffee, refreshments*

14:30 - 14:45 **Zala Živič**
Stalk domain of SARS-CoV-2 spike protein and its structural polymorphism

14:45 - 15:00 **Tea Sinožić**
Structural aspects of the mutations associated to Type B Kufs disease (CLN13)

15:00 - 15:15 **Kity Požek**
Structural and functional aspects of VaaMPIII-3: a disintegrin-like/cysteine-rich protein from the venom of the nose-horned viper

15:15 - 15:30 **Anastasija Panevska**
The unique story of OlyA6: From specific lipid binding to potential biopesticide

15:30 - 15:45 **Marjetka Podobnik**
Closing remarks by the head of the Department for Molecular Biology and Nanobiotechnology

Abstracts

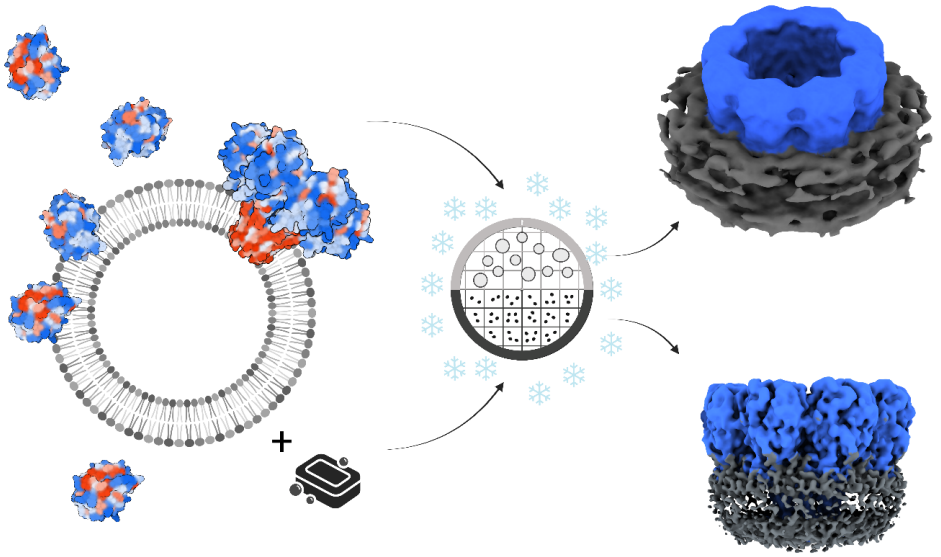
9:05

Structural studies of cnidarian pore-forming toxins, actinoporins, using cryo-EM

Gašper Šolinc[1], Marija Srnko[1], Ana Crnković[1], Gregor Anderluh[1], Marjetka Podobnik[1]

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

Pore-forming toxins are a diverse group of proteins that form pores in lipid membranes. The final pore complex usually consists of circularly arranged protomers of one or more protein species, crossing the membrane with their hydrophobic regions. In some cases, as in actinoporins, lipids are not only the medium in which the pores float but also a building block of the pore. These relatively large protein-lipid complexes are ideally suited for cryo-EM, a powerful and versatile tool in structural biology. In our work, we use the single particle analysis approach to study both solubilized actinoporin pores and pores embedded in lipid membranes.



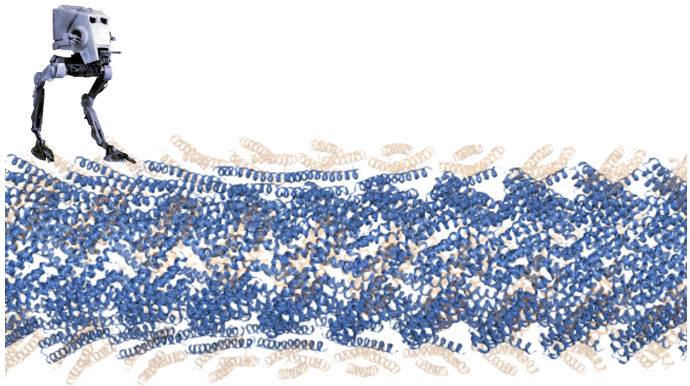
Cryo-EM structure of a track for a completely *de novo* designed random protein roller

9:30

Ajasja Ljubetič*[1,2], Hao Shen*[1], Eric Lynch*[1], David Baker[1,3]

[1]Department of Biochemistry and Institute for Protein Design, University of Washington (USA);
[2]Department of Synthetic Biology and Immunology, National Institute of Chemistry (Slovenia);
[3]Howard Hughes Medical Institute, University of Washington (USA)

Powered protein walkers such as kinesin, dynein or myosin are responsible for most movements within the cell. *De novo* design of static monomeric and oligomeric protein structures has advanced tremendously; however large dynamic protein robots have not yet been designed. I will present the design and characterization of a random protein walker that can diffuse along micrometer long fibers. This represents a scaffold for future powered molecular robots. In particular, I will focus on the track. We have determined the structure of the helical track using Cryo-EM and a partial brute-force search over helical parameters.



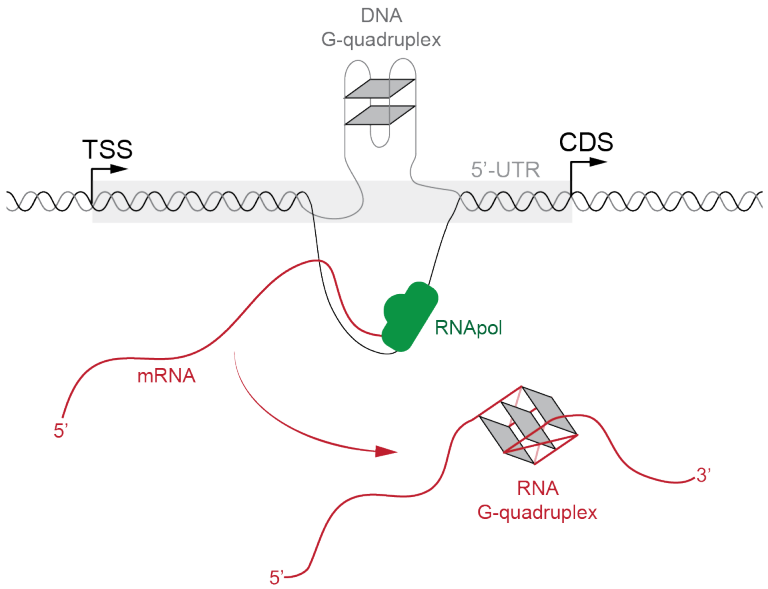
NMR insights into a G-quadruplex formation in the 5'-UTR region of the *RANKL* gene

9:55

Martina Lenarčič Živković[1], Lara Rems*[2], Janez Plavec*[1,2,3]

[1]Slovenian NMR Centre, National Institute of Chemistry (Slovenia); [2]Faculty of chemistry and chemical technology, University of Ljubljana (Slovenia); [3]EN-FIST Centre of Excellence (Slovenia)

NMR is one of the most powerful spectroscopic techniques in structural biology, allowing the structures of nucleic acids to be determined at atomic resolution under near-physiological conditions. In addition to the well-known double helix, nucleic acids can adopt various non-canonical structures, such as G-quadruplexes formed by G-rich sequences, which may play an important role in cell regulation. Our work focuses on the G-rich sequence from the 5'-UTR region of the *RANKL* gene, whose excessive activity may influence the occurrence of osteoporosis. NMR study reveals the formation of G-quadruplexes at the DNA and RNA levels and suggests their influence on the regulation of the *RANKL* gene.



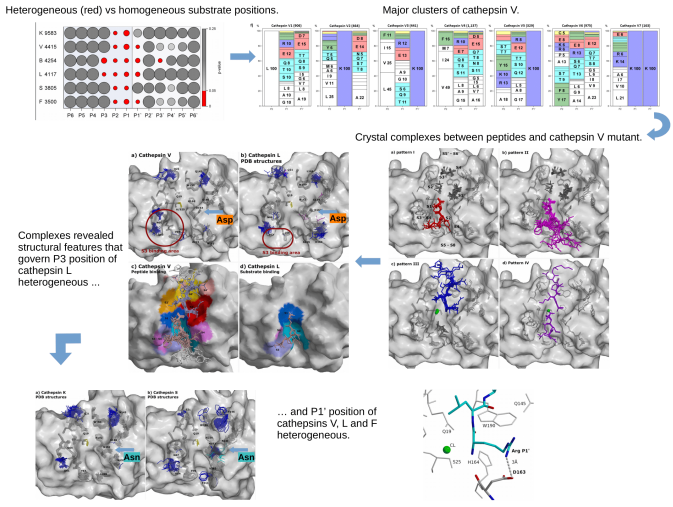
Rigidity and flexibility of structure govern specificity and promiscuity of cysteine cathepsins

10:55

Jure Loboda*[1,3], Livija Tušar*[1,2], Francis Impens*[4], Piotr Sosnowski[2], Emmy Van Quickelberghe[4], Robert Vidmar[1], Hans Demol[4], Koen Sedeyn[5], Xavier Saelens[5], Matej Vizovišek[1], Marko Mihelič[1], Marko Fonović[1], Jaka Horvat[6], Gregor Kosec[6], Boris Turk[1,7], Kris Gevaert**[4], Dušan Turk**[1,2]

[1]Department for Biochemistry and Molecular and Structural Biology, Jožef Stefan Institute (Slovenia); [2]Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP) (Slovenia); [3]The Jožef Stefan International Postgraduate School (Slovenia); [4]VIB-UGent Center for Medical Biotechnology and UGent Department of Biomolecular Medicine, Technologiepark-Zwijnaarde (Belgium); [5]VIB-UGent Center for Medical Biotechnology, Department for Biochemistry and Microbiology, Ghent University (Belgium); [6]Acies Bio d.o.o. (Slovenia); [7]Faculty of Chemistry, University of Ljubljana (Slovenia)

Statistical analysis of large-scale proteomics data prompted us to address the elusive specificity of cysteine cathepsins. Thirty peptidyl sequences, representing a variety of all seven clusters of cathepsin V substrates, were synthesized. Structural analysis showed that the heterogeneous positions (substrate positions with non-normal residue distribution) bind to structurally restraint regions, whereas homogeneous positions (normal distribution) exploit structural variability of the protease. Taken together, the combination of cathepsin specificity and promiscuity is explained by the restraining of specific substrate-binding interactions resembling the lock and key mechanism, complemented by the induced fit and conformational variability of promiscuous parts of the binding region.



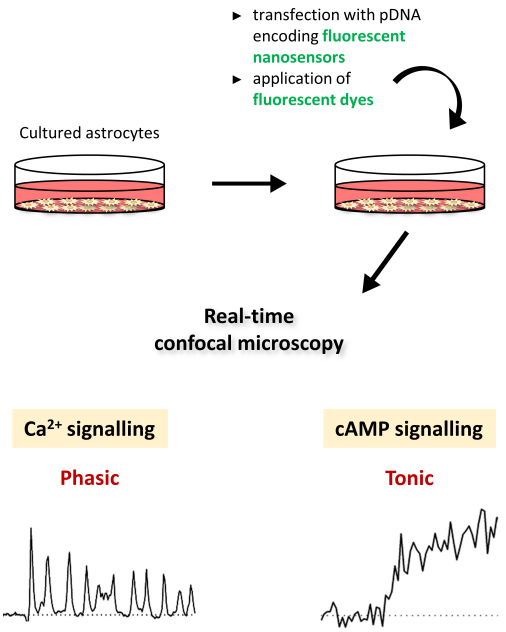
Measurements of (sub)cellular Ca^{2+} and cAMP dynamics in single astrocytes using confocal microscopy

11:20

Anemari Horvat[1,2], Samo Pirnat[1,2], Dorian Dolanc[1], Matjaž Stenovec[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 (Slovenia); [2]Laboratory of Cell Engineering, Celica Biomedical (Slovenia)

Advances in optical microscopy, together with constantly evolving tools and applications are making important contributions to our understanding of cell physiology including the dynamics of intracellular signalling mechanisms. Ca^{2+} and cAMP are important intracellular signals regulating cellular processes. Using real-time confocal microscopy in combination with genetically encoded fluorescent nanosensors and fluorescent dyes, we studied (sub)cellular dynamics of Ca^{2+} and cAMP signalling in single astrocytes, abundant and heterogeneous neuroglial cells. We found distinct temporal properties of Ca^{2+} and cAMP signalling (phasic Ca^{2+} and ∽10-fold faster tonic cAMP response) and subcellular distribution of cAMP levels in astrocytes, which may explain why Ca^{2+} and cAMP signals regulate astroglial cellular processes with distinct temporal dynamics.



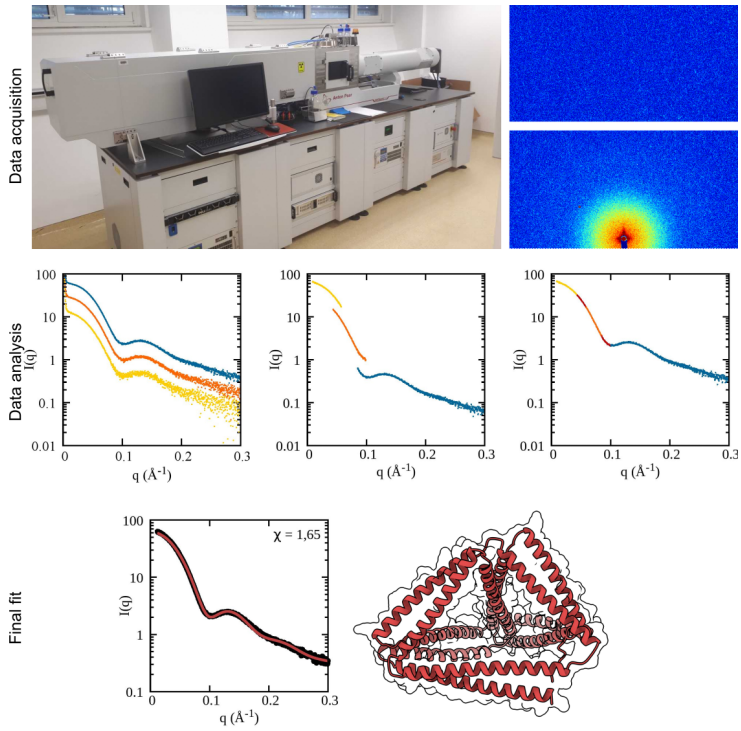
Using small-angle X-ray scatering to determine the shape of decorated coiled-coil protein origami

13:00

Žiga Strmšek[1,2], Anja Tušar[1], Neža Pavko[1], Jana Aupič[1], Fabio Lapenta[1], Roman Jerala[1,2]

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

Coiled-coil protein origami (CCPO) is a *de novo*, rationally designed type of protein folds, composed by concatenating basic building modules, coiled-coils (CC) into a single polypeptide chain, that form characteristic polyheadral shapes. CCPO have unique characteristics, such as lack of traditional hydrophobic core, which has been substituted by a hydrophilic cavity; therefore structural determination by methods, such as X-ray crystalography, or cryoEM prove challenging, therefore alternative approaches had to be investigated. Small-angle X-ray scattering (SAXS) has been proven to be a good method for CCPO shape determination, which coupled with in-house SAXS at NIC offers high throughput and robust pipeline, thus aiding further CCPO development.



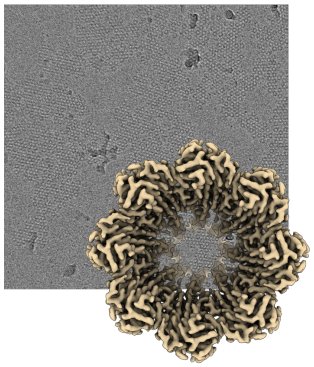
13:15

The cryo-transmission electron microscopy facility at the National Institute of Chemistry

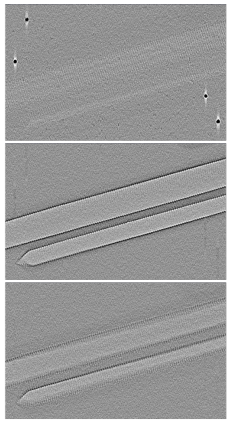
Matic Kisovec[1], Marjetka Podobnik[1]

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

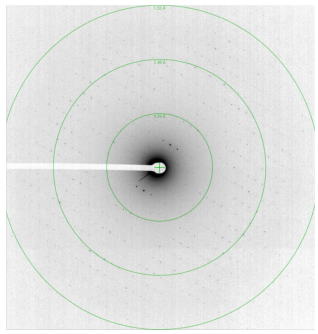
The cryo-EM facility at the National Institute of Chemistry was established in 2019 and remains the only one in the region. It includes the 200 kV Glacios cryo-transmission electron microscope, which can perform single particle analysis (SPA), tomography (cryo-ET) and microcrystal electron diffraction (MicroED). Samples are vitrified by plunge-freezing (Vitrobot), and the Falcon 3 detector enables acquisition of high-quality data. High performance computing (HPC) infrastructure is available for data storage and analysis. The cryo-EM facility is part of the Centre for Molecular Interactions and Structural Biology within the department D11 and is open to internal and external users.



Single Particle Analysis



Cryo-electron Tomography



Microcrystal Electron Diffraction

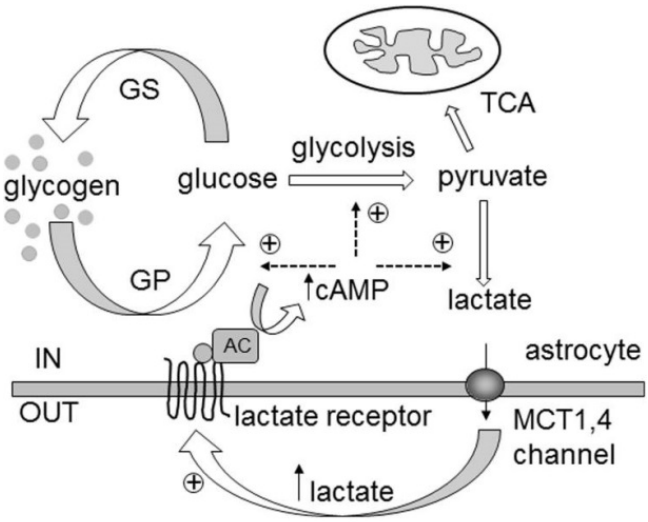
Regulation of L-lactate metabolism via GPCR

13:30

D. Dolanc[1], T. M. Zorec[2,3], Z. Smole[1], A. Maver[1], A. Horvat[1,3], T. Pillaiyar[4], S. Trkov Bobnar[3], N. Vardjan[1,3], M. Kreft[2,3,5], H. H. Chowdhury[1,3], R. Zorec[1,3]

[1]Laboratory of Neuroendocrinology, Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana (Slovenia); [2]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana (Slovenia); [3]Laboratory of Cell Engineering, Celica Biomedical (Slovenia); [4]Pharmaceutical/Medicinal Chemistry and Tübingen Center for Academic Drug Discovery, Institute of Pharmacy, Eberhard Karls University Tübingen (Germany); [5]Department of Biology, Biotechnical Faculty, University of Ljubljana (Slovenia)

Aerobic glycolysis represents the conversion of D-glucose to L-lactate despite the presence of oxygen. We investigated how L-lactate, acting as a fuel and a signal, regulates aerobic glycolysis. L-lactate signals via the activation of L-lactate-sensitive receptors such as the G-protein coupled receptor GPR81 or yet-unidentified plasma membrane receptor(s). We used fluorescence microscopy and FRET (fluorescence resonance energy transfer) nanosensors to see if agonists known to target GPR81 and other receptors determined by bioinformatics cause changes in cytosolic L-lactate in cells with knocked-out GPCR genes and wild type cells. The results revealed a new orphan GPCR that mediates L-lactate synthesis.



(Vardjan et al., 2018)

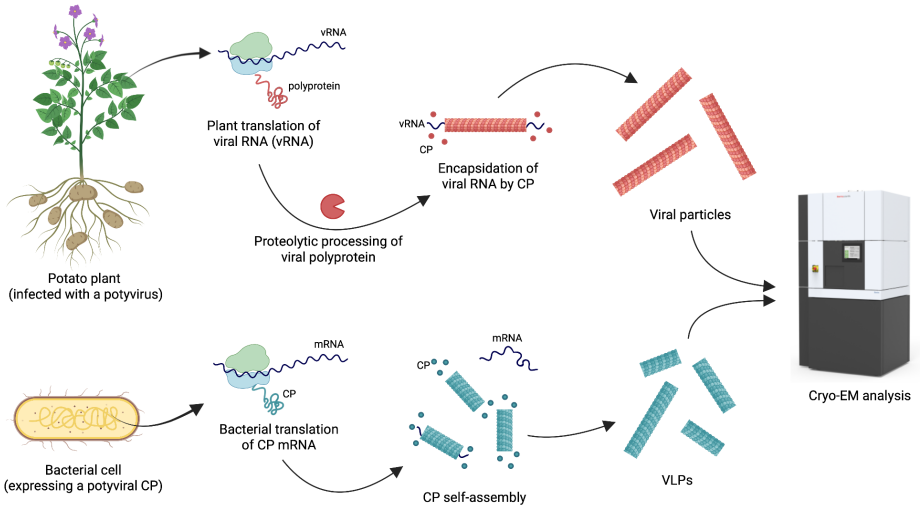
13:45

Cryo-EM studies of filamentous potyviruses and their virus-like particles

Neža Koritnik[1], Andreja Kežar[1], Luka Kavčič[1], Magda Tušek-Žnidarič[2], Swarnalok De[3], Maija Pollari[3], Kristiina Mäkinen[3], Marjetka Podobnik[1]

[1]Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry (Slovenia); [2]Department of Biotechnology and Systems Biology, National Institute of Biology (Slovenia); [3]Department of Microbiology and Viikki Plant Science Centre, University of Helsinki (Finland)

Potyvirus is the largest genus of plant RNA-viruses that infect a wide range of crops, causing significant economic losses worldwide. There are 183 potyviral species known to date and their virions are filamentous and flexible particles, consisting of an RNA-genome encapsidated by multiple copies of helically arranged coat protein (CP). Our research group uses cryo-EM to study potato virus Y (PVY), potato virus A (PVA) and their filamentous virus-like particles (VLPs). VLPs are produced upon expression and self-assembly of potyviral CP in bacterial cells. Despite the high similarity of the amino acid sequences of CP^{PVY} and CP^{PVA}, we found important differences in the quaternary structure of both, viral particles and VLPs.



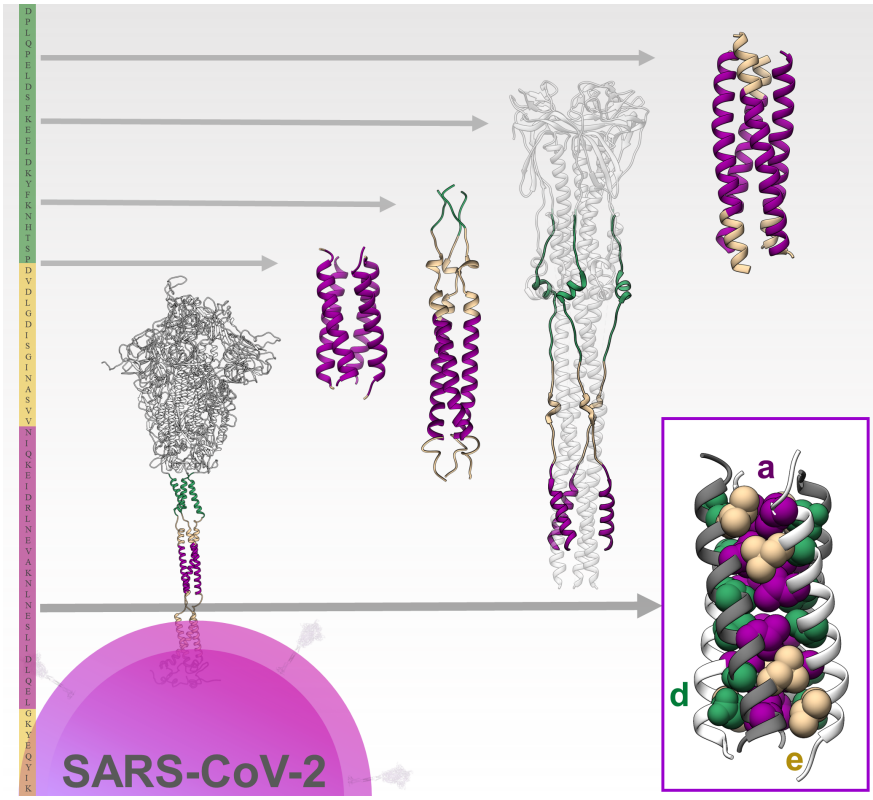
Stalk domain of SARS-CoV-2 spike protein and its structural polymorphism

14:30

Zala Živič[1], Žiga Strmšek[2,3], Marko Novinec[1], Jurij Lah[1], San Hadži[1,2]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana (Slovenia); [2]Department of Synthetic Biology and Immunology, National Institute of Chemistry (Slovenia); [3]EN-FIST Centre of Excellence (Slovenia)

Spike trimer plays a key role in SARS-CoV-2 infection and vaccine development. It consists of a globular head and a flexible stalk domain. While the head has been extensively studied, properties of the adjoining stalk are poorly understood. We characterized the coiled-coil formation and thermodynamic stability of the stalk domain and its segments. We found that the N-terminal segment of stalk remains disordered in solution, while the C-terminal stalk segment forms a trimeric coiled-coil in solution, which becomes significantly stabilized in the context of full-length stalk. Its crystal structure reveals a novel antiparallel tetramer coiled-coil with unusual hydrophobic core packing.



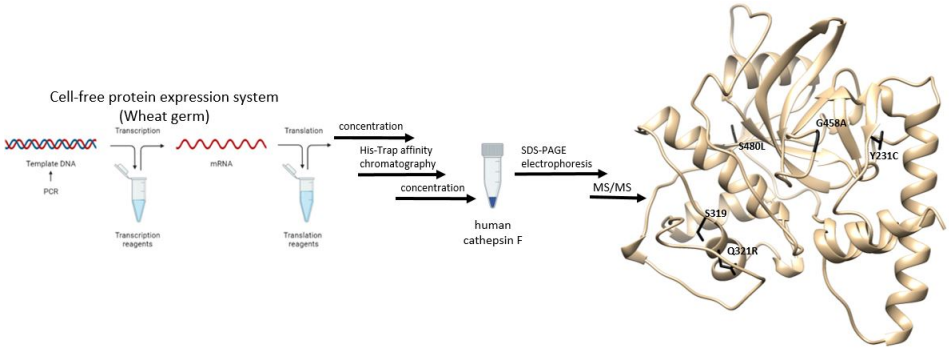
14:45

Structural aspects of the mutations associated to Type B Kufs disease (CLN13)

Tea Sinožić[1,2], Veronika Stoka[1,3]

[1]Department of Biochemistry and Molecular and Structural Biology, Jožef Stefan Institute (Slovenia); [2]PhD Program in Biomedicine, Faculty of Medicine, University of Ljubljana (Slovenia); [3]Jožef Stefan International Postgraduate School (Slovenia)

Cathepsin F is a lysosomal cysteine protease with some unique features. However, due to the difficulties of producing a recombinant protein in sufficient quantities, the enzyme is still poorly characterized. Therefore, a sequence-based approach was crucial to assess its suitability from cloning to 3D structure determination. On the available 3D structure of the human cathepsin F mature form, we showed CLN13 mutations have a destabilizing effect on the structure, confirming their detrimental effect on the enzyme's function. Furthermore, using a novel cell-free protein expression system, we expressed wild type human cathepsin F in sufficient amounts for its further characterization.



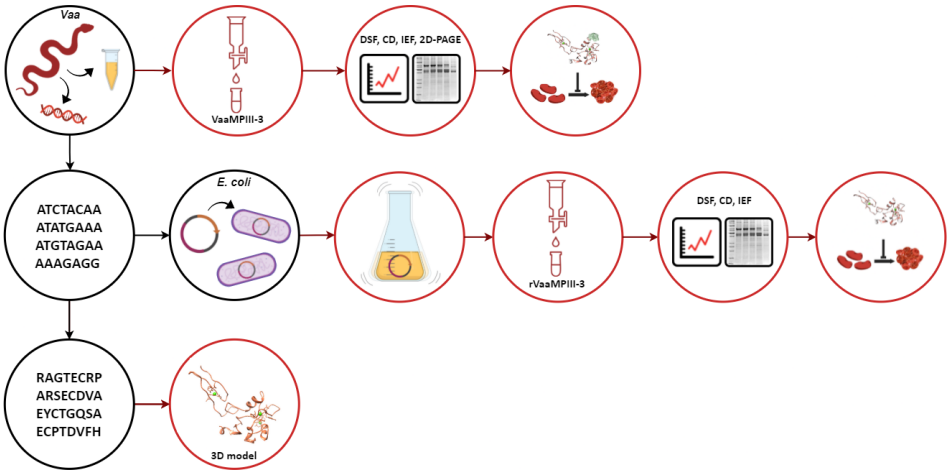
Structural and functional aspects of VaaMPIII-3: a disintegrin-like/cysteine-rich protein from the venom of the nose-horned viper

15:00

Kity Požek[1], Adrijana Leonardi[1], Igor Križaj[1]

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

Disintegrin-like/cysteine-rich (DC) proteins, haemostatically active toxins found in viperid venoms, were originally interpreted as proteolytic products of the P- III class of snake venom metalloproteinases (SVMPs). However, the recently discovered DC protein VaaMPIII-3 from the venom of the nose-horned viper (*Vipera a. ammodytes, Vaa*) was found to be encoded per se by a P- III SVMP-like gene lacking the entire MP-coding region and part of the disintegrin-like domain. This defined a new subclass of SVMPs called P-IIIe. We purified VaaMPIII-3 from the venom and determined its biochemical and some functional properties. We have constructed a 3D homology model of VaaMPIII-3 to predict its additional traits. We will present our latest findings.



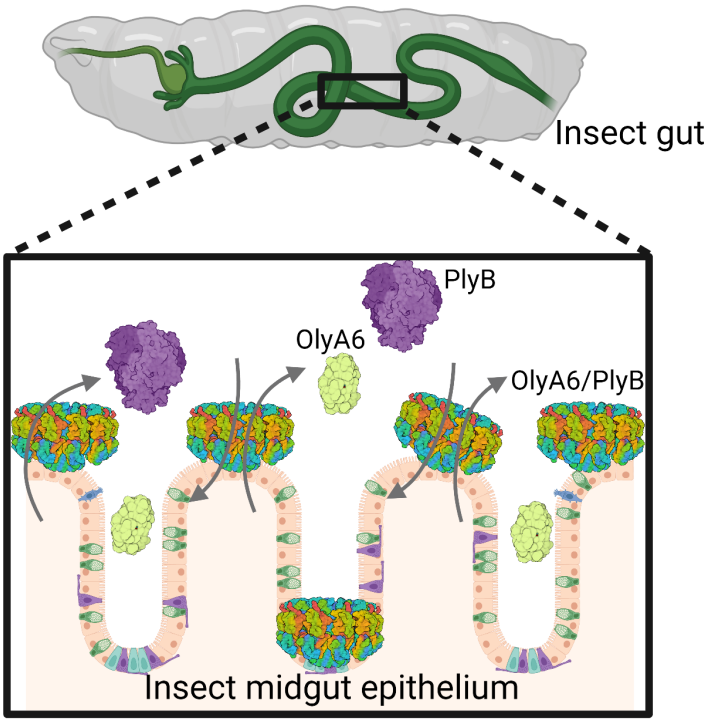
15:15

The unique story of OlyA6: From specific lipid binding to potential biopesticide

Anastasija Panevska[1], Matej Milijaš Jotić[1], Ioan Iacovache[2], Špela Modic[3], Jaka Razinger[3], Rok Kostanjšek[1], Kristina Sepčić[1]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana (Slovenia); [2]Institute of Anatomy, University of Bern (Switzerland); [3]Agricultural Institute of Slovenia (Slovenia)

Ostreolysin A6 (OlyA6), an aegerolysin from the fungal genus *Pleurotus*, interacts strongly with membranes containing ceramide phosphoethanolamine (CPE), the major lipid component in insects. Together with a 59-kDa protein partner with a MACPF domain, pleurotolysin B (PlyB), OlyA6 forms transmembrane pores and has potent insecticidal activity against selected pests. Using cryo-electron microscopy, we investigated the molecular mechanism of insecticidal activity of the OlyA6/PlyB complex in insects. The results strongly suggest that the molecular mechanism of OlyA6/PlyB is based on specific interactions of OlyA6 with CPE and the formation of transmembrane pores in the presence of PlyB in the insect midgut.



September 28th, 2022

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