12th Meeting of the Slovenian Biochemical Society with International Participation

12. srečanje Slovenskega biokemijskega društva z mednarodno udeležbo

Book of Abstracts
Zbornik povzetkov

Electronic version

Bled, 20-23 September 2017

Organisation: The 12th Meeting of the Slovenian Biochemical Society with International Participation is organised by University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia and the Slovenian Biochemical Society

University *of Ljubljana*
Faculty *of Medicine*
Institute of Biochemistry

12th Meeting of the Slovenian Biochemical Society with International Participation
12. srečanje Slovenskega biokemijskega društva z mednarodno udeležbo
Edited by: Katja Goričar, Petra Hudler
Published by: Slovenian Biochemical Society
Reviewed by: Marko Goličnik, Ana Plemenitaš
Cover Design by: Petra Hudler (based on Molecule Atomium, Vecteezy.com)
Organised by: University of Ljubljana, Faculty of Medicine, Institute of Biochemistry and the Slovenian Biochemical Society
Electronic version: *Available at: http://bled2017.sbd.si/en/conference-programme.html*
Electronic format*: PDF*
Available from*: 5. 10. 2017*

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID=292021504
ISBN 978-961-93879-5-5 (pdf)

Table of contents

Foreword

It is an honour and pleasure to welcome you at the 12th Meeting of the Slovenian Biochemical Society with International Participation in Bled, Slovenia, which is organised by the Slovenian Biochemical Society together with the University of Ljubljana, Faculty of Medicine, Institute of Biochemistry. The organisers are pleased that the Croatian Society of Biochemistry and Molecular Biology, Hungarian Biochemical Society and Serbian Biochemical Society joined us and will participate at the meeting with the FEBS3+ session. We are specially honoured by the presence of the Secretary General of the FEBS, Prof. Václav Pačes, who will chair this session. The programme covers the most important areas of biochemistry and molecular biology and renowned international and Slovenian lecturers will present new discoveries in the following sessions: cell signalling and membrane biochemistry, biotechnology and bionanotechnology, protein structures and functions, protein interactions and networks, functional genomics and systems medicine, molecular basis of the disease and systems biology. New technologies and innovations in biochemistry will be presented by sponsors of the meeting. Established researchers and also those at the beginning of their career, together with graduate and undergraduate students will have the opportunity to present the results of their research with short oral presentations or in poster sessions.

We trust that the meeting with more than 200 registered participants will also provide the opportunity for fruitful discussions and exchange ideas and experiences as well as for establishing new scientific collaborations and connections with the industry.

In addition to vibrant scientific programme, we invite you to participate at the round table: Ethics in biomedical research and regulatory acts in Slovenia, which is organised on Friday afternoon, and to enjoy in social events. Get together and wine tasting will be in the Concordia Hall the first evening and the Conference Dinner will be on Friday in the Grand Hotel Toplice. Guided tour in Bled and Bled Castle will be organised for our international guests on Thursday afternoon.

We wish you all a fruitful and enjoyable meeting and a pleasant staying in Bled.

Sincerely,

Damjana Rozman

Chair of the Scientific Committee

Ana Plemenitaš

Chair of the Organising Committee

Committees

Organising Committee:

Ana Plemenitaš, Chair
Marko Goličnik, Co-Chair
Aljoša Bavec
Nataša Debeljak
Katja Goričar
Petra Hudler
Metka Lenassi
Tomaž Marš
Katarina Trebušak Podkrajšek

Scientific Committee:

Damjana Rozman, Chair
Gregor Anderluh
Tatjana Avšič-Županc
Andrej Cör
Vita Dolžan
Kristina Gruden
Simon Horvat
Roman Jerala
Radovan Komel
Janko Kos
Igor Križaj
Tamara Lah Turnšek
Tea Lanišnik Rižner
Brigita Lenarčič
Gregor Majdič
Uroš Petrovič
Sergej Pirkmajer
Nataša Poklar Ulrih
Boris Rogelj
Maja Rupnik
Mateja Salobir
Jure Stojan
Katarina Trebušak Podkrajšek
Boris Turk

General Information

Conference Venue
Bled, Hotel Golf, Cankarjeva cesta 4, 4260 Bled, Slovenia.

Registration and Information Desk
The registration desk will be located in the lobby of the Hotel Golf. The certificate of attendance will be issued at the registration desk.

Registration and information desk opening hours:
Wednesday, 20 September 2017, 08:30–18:00
Thursday, 21 September 2017, 08:00–18:00
Friday, 22 September 2017, 08:00–17:00
Saturday, 23 September 2017, 08:30–12:00

Name Badges
All participants are kindly requested to wear badges at all times during the conference.

Presentation Preview and Deposition
Speakers are kindly requested to deliver their presentations to the computer technician in the symposium hall at least half an hour before the start of the session.

Poster Display
Poster session I with Get together & wine tasting will be held on Wednesday, 20 September 2017 from 18:45 to 21:00 in the Concordia Hall of the Hotel Golf.
Poster session II with beer tasting will be held on Thursday, 21 September 2017 from 18:45 to 21:00 in the Concordia Hall of the Hotel Golf.
Poster Session I presenters are kindly asked to mount their posters during 10:00 and 12:00 on Wednesday, 20 September 2017 and remove them on Thursday, 21 September 2017 before 10:00.
Poster Session II presenters are kindly asked to mount their posters during 10:00 and 12:00 on Thursday, 21 September 2017 and remove them on Friday, 22 September 2017 before 10:00.
Presenters should look-up the numbers assigned to their posters in the program book and pin up their posters on the display boards with the corresponding number. Material for mounting the posters will be available at the venue. Presenters are responsible for setting and removing their posters.
Authors are kindly asked to stand by their poster for the duration of the session.

Commercial Exhibitions
All sponsors exhibiting at the symposium will have their desks ready in the lobby of the Hotel Golf by Wednesday, 20 September 2017 at 07:00. All tables will be assigned in advance.

Social Programme

Social programme includes Get together & wine tasting on Wednesday, 20 September 2017, beer tasting on Thursday, 21 September 2017 and Conference Dinner on Friday, 22 September 2017.

Guided tour in Bled and Bled Castle will be organised for our international guests on Thursday afternoon.

Coffee breaks and Lunch

All participants are invited to refreshments during the coffee breaks, which will be held in the lobby of the Hotel Golf. Lunch will be available for all participants, except undergraduate students, in the Dining room of the Hotel Golf.

Programme outline

Wednesday 20	Thursday 21	Friday 22	Saturday 23
08:30–09:45 Registration	08:30–10:15 **Protein structure and function**	08:30–10:15 **Molecular basis of disease I**	
09:45–10:15 **Opening ceremony**			09:00–10:45 **Systems biology**
10:15–11:00 **Opening Lecture**	10:15–10:45 Coffee break	10:15–10:45 Coffee break	10:45–11:15 Coffee break
11:00–12:45 **Cell signalling and membranes**	10:45–12:30 **Protein interactions and networks**	10:45–12:30 **Technologies and innovations in biochemistry**	11:15–12:00 **Closing lecture**
12:45–14:15 Lunch	12:30–14:00 Lunch	12:30–14:15 Lunch	12:00–12:30 **Closing ceremony with poster awards**
14:15–16:00 **Biotechnology and bionanotechnology**	14:00–15:45 **Functional genomics and systems medicine**	14:15–16:00 **Molecular basis of disease II**	
16:00–16:30 Coffee break	15:45–16:15 Coffee break	16:00–16:30 Coffee break	
16:30–18:45 **FEBS3+**	16:15–18:15 **SBD National Assembly**	16:30–18:30 **Round table: Ethics in biomedical research and regulatory acts in Slovenia**	
18:45–21:00 Poster session I with Get together & wine tasting	18:45–21:00 Poster session II with beer tasting	20:00 Conference dinner	

Programme

Wednesday, September 20 *(Jupiter Hall, Hotel Golf)*	
08:30–09:45	**Registration**
09:45–10:15	**Opening ceremony**
10:15–11:00	**Opening lecture** (Chair: Janko Kos)
	Borut Štrukelj, University of Ljubljana, Slovenia: Recombinant probiotics: an alternative approach to modern biological medicines
11:00–12:45	**Session I: Cell signalling and membranes** (Chairs: Gregor Anderluh, Tamara Lah Turnšek)
11:00–11:30	**Nathalie Reuter**, University of Bergen, Norway: How do soluble proteins bind to biological membranes? Answers from molecular simulations and experiments
11:30–12:00	**Marjetka Podobnik**, National Institute of Chemistry, Slovenia: Structural perfection of biological nanopores and what can be learned from them
12:00–12:15	**Sergej Pirkmajer**, University of Ljubljana, Slovenia: Early vertebrate origin and diversification of FXYDs and other small transmembrane regulators of cellular ion transport
12:15–12:30	**Iva Hafner Bratkovič**, National Institute of Chemistry, Slovenia: The mechanisms of autoinhibition and activation of NLRP3 inflammasome
12:30–12:45	**Pia Pužar Dominkuš**, University of Ljubljana, Slovenia: Characterization of particles formed during staining of exosomes with the fluorescent dye PKH26
12:45–14:15	Lunch

14:15–16:00	**Session II: Biotechnology and bionanotechnology** (Chairs: Roman Jerala, Nataša Poklar Ulrih)
14:15–14:45	**Rolf Müller**, Helmholtz Centre for Infection Research, Germany: Antibiotic discovery from bacteria: technologies and innovative compounds
14:45–15:15	**Mojca Benčina**, National Institute of Chemistry, Slovenia: New insights into activation mechanism of TLR9
15:15–15:30	**Marko Petek**, National Institute of Biology, Slovenia: Development of RNAi-based pesticides targeting Colorado potato beetle
15:30–15:45	**Tina Lebar**, National Institute of Chemistry, Slovenia: Tunable coiled-coil interaction toolbox for engineering mammalian cells
15:45–16:00	**Katja Škrlec**, Jožef Stefan Institute, Slovenia: Surface display of evasins and BPC-157 on recombinant lactic acid bacteria for treatment of inflammatory bowel disease
16:00–16:30	Coffee break
16:30–18:45	**Session III: FEBS 3+** (Chairs: Václav Pačes, Janko Kos)
16:30–16:45	**Václav Pačes**, FEBS Secretary General: FEBS Activities and Opportunities
16:45–17:15	**Lapanje awardee lecture: Boris Rogelj**, Jožef Stefan Institute, Slovenia: Pathogenic mechanisms of RNA binding proteins in ALS and FTLD
17:15–17:45	**Igor Weber**, Ruđer Bošković Institute, Croatia: Formins orchestrate the cortical actin cytoskeleton in amoeboid cell migration and large-scale endocytosis
17:45–18:15	**Duško Blagojević**, University of Belgrade, Serbia: The role of redox congeners in physiological receptor-mediated regulation of contractile activity
18:15–18:45	**Mihály Kovács**, Eötvös Loránd University, Hungary: RecQ helicases: Motorized lifeguards in the gene pool
18:45–21:00	**Poster session I with Get together & wine tasting** *(Concordia Hall, Hotel Golf)*

Thursday, September 21 *(Jupiter Hall, Hotel Golf)*	
08:30–10:15	**Session IV: Protein structure and function** (Chairs: Boris Turk, Brigita Lenarčič)
08:30–09:00	**Remy Loris**, Vrije Universitet Brussel, Belgium: Regulation of persistence via intrinsic disorder
09:00–09:30	**Dušan Turk**, Jožef Stefan Institute, Slovenia**:** The mechanism behind the selection of two different cleavage sites in NAG-NAM polymer
09:30–09:45	**Tea Lenarčič**, National Institute of Chemistry, Slovenia: Structural insight into the mechanism of NLP toxicity
09:45–10:00	**Aljaž Gaber**, University of Ljubljana, Slovenia: Is Epithelial Cell Adhesion Molecule (EpCAM) really a cell adhesion molecule?
10:00–10:15	**Aleksandra Usenik**, Jožef Stefan Institute, Slovenia: The CWB2 cell wall-anchoring module is revealed by the crystal structures of the *Clostridium difficile* cell wall proteins Cwp8 and Cwp6
10:15–10:45	Coffee break
10:45–12:30	**Session V: Protein interactions and networks** (Chairs: Igor Križaj, Jure Stojan)
10:45–11:15	**Juan J. Calvete**, The Spanish National Research Council (CSIC), Spain: A bright future for integrative omics in venom research
11:15–11:45	**Stanislav Gobec**, University of Ljubljana, Slovenia: Development of new inhibitors of butyrylcholinesterase and monoamine oxidases for treatment of neurodegenerative diseases
11:45–12:00	**San Hadži**, University of Ljubljana, Slovenia: Avidity-mediated specificity in the regulation of the bacterial operon
12:00–12:15	**Toni Petan**, Jožef Stefan Institute, Slovenia: Lipid droplets are antioxidant and pro-survival organelles that protect Ras-driven breast cancer cells from nutrient and lipotoxic stress

12:15–12:30	**Mateja Manček Keber**, National Institute of Chemistry, Slovenia: Extracellular vesicle-mediated transfer of proinflammatory myddosomal signalling complex in Waldenstrom's macroglobulinemia
12:30–14:00	Lunch break
14:00–15:45	**Session VI: Functional genomics and systems medicine** (Chairs: Damjana Rozman, Sergej Pirkmajer)
14:00–14:30	**Werner Müller**, University of Manchester, UK: SysmedIBD, Systems Medicine of Inflammatory Bowel Disease
14:30–15:00	**Ulrich Zanger**, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Germany: Inflammation and the negative acute phase response of the liver: consequences for drug metabolism and possible role of microRNAs
15:00–15:15	**Jernej Kovač**, University Medical Centre Ljubljana, Slovenia: The structure and characteristics of a whole human genome sequencing data: Application in human disease genetic biomarkers discovery
15:15–15:30	**Katarina Uršič**, University of Ljubljana, Slovenia; Methylation and expression of *COMT* and *RELN* in suicide victims: comparison of brain tissue and blood
15:30–15:45	**Klementina Fon Tacer**, St. Jude Children's Research Hospital, USA: Melanoma antigen genes (MAGEs) at the crossroad of spermatogenesis and cancer: metabolic regulation of male germ cell differentiation
15:45–16:15	Coffee break
16:15–18:15	**SBD National Assembly**
18:45–21:00	**Poster session II with beer tasting** *(Concordia Hall, Hotel Golf)*

Friday, September 22 *(Jupiter Hall, Hotel Golf)*	
08:30–10:15	**Session VII: Molecular basis of disease I** (Chairs: Tea Lanišnik Rižner, Andrej Cör)
08:30–09:00	**Cornelis van Noorden**, University of Amsterdam, The Netherlands: Prolonged survival of *IDH1*-mutated glioblastoma patients but not of *IDH1/2*-mutated AML patients by reduced NADPH production capacity
09:00–09:30	**Tanja Čufer**, University Clinic Golnik, Slovenia: Contribution of tumour markers to personalized therapy
09:30–09:45	**Ivana Jovčevska**, University of Ljubljana, Slovenia: Nanobody-based anti-proteomics in glioma research and targeting
09:45–10:00	**Tamara Knific**, University of Ljubljana, Slovenia: Altered levels of phosphatidylcholines in endometrial cancer
10:00–10:15	**Ana Mitrović**, Jožef Stefan Institute, Slovenia: Higher levels of cathepsins B and X promote epithelial-mesenchymal transition of tumour cells
10:15–10:45	Coffee break
10:45–12:30	**Session VIII: Technologies and innovations in biochemistry** (Chairs: Katarina Trebušak Podkrajšek, Mateja Salobir)
10:45–11:15	**Oliver Goldenberg**, Illumina: Next-generation sequencing for studying biology
11:15–11:45	**Dominik Gaser**, Lek Pharmaceuticals, Slovenia: Development of biosimilars
11:45–12:15	**Jakub Nowak**, NanoTemper Technologies, Poland: MicroScale Thermophoresis and nanoDSF as advanced methods in life science research
12:15–12:30	**Uršula Prosenc Zmrzljak**, Labena, Slovenia: Bio-Rad's state of the art solutions to help you find answers: from DNA translation to single cell sequencing
12:30–14:15	Lunch

14:15–16:00	**Session IX: Molecular basis of disease II** (Chairs: Vita Dolžan, Boris Rogelj)
14:15–14:45	**Tomaž Curk**, University of Ljubljana, Slovenia: Global insights into condition-specific alternative splicing using ENCODE data
14:45–15:15	**Janez Plavec**, National Institute of Chemistry, Slovenia: NMR structural studies of DNA beyond double-stranded helix
15:15–15:30	**Ana Bajc Česnik**, Jožef Stefan Institute, Slovenia: Paraspeckle-like structures formed on the scaffold of the *C9orf72* hexanucleotide G4C2 intranuclear RNA repeats
15:30–15:45	**Theodora Katsila**, University of Patras, Greece: Multi-omics towards decision making in precision medicine
15:45–16:00	**Jana Ferdin**, University of Ljubljana, Slovenia: Viral protein Nef and human miRNAs are putative plasma biomarkers of HIV-1 reservoirs in infected individuals with undetectable viral RNA
16:00–16:30	Coffee break
16:30–18:30	**Round table: Ethics in biomedical research and regulatory acts in Slovenia** (Chairs: Radovan Komel, Gregor Majdič)
16:30–17:00	**Urh Grošelj**, University Medical Centre Ljubljana, Slovenia: The 20th anniversary of the Oviedo Convention — its impact on the biomedical research and how to move forward
17:00–17:30	**Tina Arič**, Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection: Legislative frameworks of animal protection in research
17:30–18:30	**Open discussion**
20:00	**Conference dinner** *(Panorama hall, Grand Hotel Toplice)*

Saturday, September 23 *(Jupiter Hall, Hotel Golf)*	
09:00–10:45	**Session X: Systems biology** (Chairs: Kristina Gruden, Uroš Petrovič)
09:00–09:30	**Mojca Mattiazzi Ušaj**, University of Toronto, Canada: Exploring the endocytic pathway on a global scale
09:30–10:00	**Marek Mutwil**, Max Planck Institute of Molecular Plant Physiology, Germany: Analysis of diurnal gene expression of major plant clades
10:00–10:15	**Špela Baebler**, National Institute of Biology, Slovenia: Network modelling of integrated prior knowledge and time-stamped gene expression data of potato immune signalling
10:15–10:30	**Maja Križnik**, National Institute of Biology, Slovenia: Small RNAs regulatory networks — linking developmental and immune signalling in potato
10:30–10:45	**Tanja Cvitanović**, University of Ljubljana, Slovenia: LiverSex: First computational model to investigate an interplay between sex hormones and liver
10:45–11:15	**Coffee break**
11:15–12:00	**Closing lecture** (Chair: Damjana Rozman)
	Henning Hermjakob, EBI/EMBL, UK: Visualisation concepts in the Reactome database of biomolecular pathways
12:00–12:30	**Closing ceremony with poster awards**

Abstracts of lectures

L1 Opening Lecture

Recombinant probiotics: an alternative approach to modern biological medicines

Borut Štrukelj[1,2], Aleš Berlec[2]

[1]*University of Ljubljana, Faculty of Pharmacy, Slovenia*
[2]*Jožef Stefan Institute, Slovenia*

Microbial surface display is an emerging technology with numerous potential applications in various fields of biotechnology. The gut microbiota significantly contributes to immune function, digestion, metabolism, gut development and physiology. Lactic Acid Bacteria (LAB) have been intensively used for the construction of effective mucosal delivery vehicles and could be used in therapy, with most applications aimed at the delivery of therapeutic protein to human mucosal surfaces. The high therapeutic potential of recombinant LAB represents surface attachment of affinity molecules, such as antibodies or single-chain variable fragments which can target pathogens, toxins or inflammatory mediators. We have successfully developed and patented the general platform for the surface expression of various proteins. The display of two types of affinity molecule, the B domain and the TNFα-binding affibody were achieved on the surface of *Lactococcus lactis*. We clearly show that such recombinant LAB are able to bind the patologically-overexpressed TNF-α in the biopts, obtained from the surgical treatment of the patients with inflammatory bowel disease. Another application of recombinant probiotics is mucosal vaccination. Delivery of antigens for the purpose of vaccination represents the most abundant application with numerous successful demonstrations of the efficacy. We successfully express a hepatitis A virus antigen in *Lactococcus lactis* and *Escherichia coli*. After oral administration, mice animal model developed specific IgG antibodies against hepatitis A viral antigen. In order to monitor the delivery and physiological conditions in the lumen of gastrointestinal tract, we expressed the infrared fluorescent protein IRFP713 in *Lactococcus lactis*, *Lactobacillus plantarum* and *Escherichia coli*. *In vivo* imaging of orally administered lactic acid bacteria (LAB) and commensal bacteria in mice is shown to provide information on the spatial and temporal distribution of bacteria in the gastrointestinal tract. The bacteria can be detected and monitored using bioluminescence or near-infrared fluorescence.
To avoid the use of genetically-modified LAB, we developed an alternative approach by using a non-covalent surface display of proteins by fusing them to LysM cell-wall binding domain. Three cytokine binders in fusion with LysM repeats were expressed in *L. lactis*. The fusion proteins were directed to the growth medium that was used for coating of *Lb. salivarius* with cytokine binders. IL-17-, IL-23- and TNFα-cytokine binders were simultaneously displayed on the surface of *Lb. salivarius* and were shown to bind all three cytokines. By this, we enabled the implementation of non-GMO alternative LAB in the advanced food and pharmaceutical industry.

L2

How do soluble proteins bind to biological membranes? Answers from molecular simulations and experiments

Nathalie Reuter

University of Bergen, Norway

With remarkable spatial and temporal specificities, peripheral membrane proteins bind to the surface of biological membranes where they participate in a myriad of intra-cellular and extra-cellular processes. The current model for membrane association mechanism of amphitropic proteins consists of an electrostatically-driven approach most often followed by the intercalation of hydrophobic side chains into the lipid bilayer. The prototypical interfacial binding site (IBS) is thus described as containing patches of basic amino acids and hydrophobic amino acids such as those with aromatic or aliphatic groups. Unfortunately the lack of structural data on protein-membrane interfaces has limited the characterization of the interfacial regions of peripheral membrane proteins.
Using bioinformatics and molecular simulations we seek to obtain a more detailed picture of protein-lipid interactions at such interfaces. I will present the result of our work on a bacterial phospholipase C (BtPI-PLC). We earlier showed that several tyrosine residues of BtPI-PLC engage in cation-pi interactions with phosphatidylcholine lipids, and postulated it as a mechanism explaining BtPI-PLC's affinity for PC-containing vesicles. Further we evaluated that the highest affinity of BtPI-PLC toward slightly anionic SUVs is the result of weak electrostatic contributions from basic residues, particularly from one key basic residue. Finally, the balance between weak nonspecific electrostatics and cation-pi interactions ensures that the protein will interact preferentially with anionic vesicles containing large amounts of PC lipids. Statistics on the distribution of basic amino acids in a data set of membrane-binding domains reveals that weak electrostatics, as observed for BtPI-PLC, might be a less unusual mechanism for peripheral membrane binding than is generally thought.

L3

Structural perfection of biological nano-pores and what can be learned from them

Marjetka Podobnik

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

Pore-forming proteins (PFPs) are widespread in nature and have important physiological roles in attack and defense mechanisms. They are expressed by organisms from all kingdoms of life to form pores in lipid membranes of target cells to cause killing or other undesired effects. In most cases they are secreted as water-soluble monomers, which upon binding to target lipid membranes oligomerize and form transmembrane pores. They can be classified as either α- or β-PFPs, based on the secondary structure of the transmembrane region of the pore. PFPs can form either small pores with diameters of 1–5 nm, or much larger pores of 10–30 nm. In this lecture I will present our recent studies on β-PFPs forming either large pores (listeriolysin O (LLO) from pathogenic bacterium *Listeria monocytogenes* and a member of a family of cholesterol dependent cytolysins) or small pores (lysenin, a member of aerolysin family from an earthworm *Eisenia fetida*). Using various biochemical, biophysical and structural approaches we try to understand detailed structural features of these proteins at the monomeric, intermediate-oligomeric as well as final transmembrane pore forms, and consequently their mechanism of action. The small-pore forming protein lysenin makes stable and compact pores of a perfect tube-like structure. In contrast, large pores built by LLO, while still showing perfection in structural details, exhibit high plasticity resulting in formation of membrane defects of various shapes and sizes. Our studies provide insights into general features of these nanopores, protein-protein, protein-lipid interactions and mechanism of pore assembly. This is important for understanding of their biological roles, as well as using PFPs as prime candidates for various applications in medicine and nanotechnology.

L4

Antibiotic discovery from bacteria: technologies, compounds and biosyntheses

Rolf Müller

Helmholtz Centre for Infection Research, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Germany

Microorganisms are accepted as a valuable source for chemical biology tools and drug leads. An outstanding group of microorganisms is represented by the ubiquitous myxobacteria, a largely underexploited resource for natural products (NP). Analyzing the few myxobacterial genomes known to date leads to the conclusion that they contain up to 50 respective biosynthetic gene loci per strain indicating the enormous genomic potential for the production of NPs. However, only few compounds are typically known per microbial isolate. In a comprehensive MS-based study we have addressed the question of compound diversity in correlation with phylogenetic diversity and found clear and measurable evidence that new compounds are most likely to be found in currently uncharacterized microbial genera and families.

Once novel microbial NPs are identified, their potential as antibiotic drug leads is analyzed by defining pharmaceutical properties and potential resistance mechanisms of pathogens against the compound. Target identification, which is usually a complex and rather unpredictable biochemical research endeavour, has become essential for drug development for numerous reasons including the possibility to rationally optimize lead compounds based on their molecular structure-target-complex. During the last decades genomics also became an integral part of NP drug research and allows not only for directed approaches to discover new natural products: target identification might be achieved by studying self-resistance mechanisms within the producer strains or, alternatively, by defining the molecular basis of resistance in pathogens by whole genome sequencing of evolved bacterial resistance.

The presentation will cover some examples of technologies and their application to identify novel antibiotics, their biosynthesis, compound and yield optimisation as well as target identification of microbial NPs.

L5

New insights into activation mechanism of TLR9

Mojca Benčina, Jelka Pohar, Roman Jerala

National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia

Receptor TLR9 belongs to a family of innate immunity receptors and is important for detecting bacterial and viral infections. TLR9 binds unmethylated CpG motif of single stranded DNA and triggers cascade of events that lead to pro-inflammatory cytokine production and activation of adaptive immunity. Although a crystal structure of TLR9 with ligand is known, it does not adequately explain activation of TLR9. We identified that the human TLR9 requires for its activation oligodeoxyribonucleotides (ODN) that contains at least two CpG motifs with one CG dinucleotide positioned adjacent to 5'-T; on the other hand, only one CpG motif within synthetic agonist is sufficient for activation of mouse TLR9. In light of these data, we identified the region within TLR9 that determines sequence-species specificity. A single-stranded DNA longer than 20 nucleotides is required for a robust TLR9 response; however, we showed that immune response activation is augmented by short CpG-containing oligodeoxyribonucleotides (sODN), which by themselves do not induce TLR9 activation. Furthermore, sODNs augment TLR9 activation by mammalian genomic DNA indicating the role of short DNA degradation products in the endosomes in response to infection or in autoimmune disease, particularly at limiting concentrations of ODNs.

L6 Lapanje awardee lecture

Pathogenic mechanisms of RNA binding proteins in ALS and FTLD

Boris Rogelj[1,2,3]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*Biomedical Research Institute BRIS, Slovenia*

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are devastating neurodegenerative diseases that form two ends of a complex disease spectrum. In 95% of all ALS and 60% of FTLD patients there is a pathognomonic cytoplasmic misaccumulation and aggregation of a nuclear RNA binding protein TDP-43, thus defining the major part of the disease spectrum as TDP-43 proteinopathies. Importantly, TDP-43 proteinopathy is also observed in other neurodegenerative diseases including about 60% of Alzheimer's disease. As only a very small percent of aggregation is caused by TDP-43 mutations, the main questions in the field is what mechanistic perturbations make wildtype TDP-43 aggregate in these diseases. To this end we have studied the nuclear transport and RNA binding properties of TDP-43 and functionally related protein FUS. Identification of the disease-associated expansions of the intronic hexanucleotide repeat GGGGCC (G4C2) in the *C9orf72* gene further substantiates the case for RNA involvement. *C9orf72* hexanucleotide repeat expansion mutation (HREM) has turned out to be the single most common genetic cause of ALS and FTLD and importantly also leads to TDP-43 proteinopathy. We have shown that HREM may enable the formation of complex DNA and RNA structures, changes in RNA transcription and processing and formation of toxic RNA foci, which may sequester and inactivate RNA binding proteins. Additionally, the transcribed expanded repeats from both directions can undergo repeat-associated non-ATG-initiated (RAN) translation resulting in accumulation and aggregation of a series of toxic dipeptide repeat proteins.

L7

Formins orchestrate the cortical actin cytoskeleton in amoeboid cell migration and large-scale endocytosis

Igor Weber[1], Jan Faix[2], Vedrana Filić[1]

[1]*Ruđer Bošković Institute, Division of Molecular Biology, Croatia*
[2]*Hannover Medical School, Institute for Biophysical Chemistry, Germany*

Directed locomotion toward the food source and active internalization of nutrients represent defining traits of free-living single-celled eukaryotic organisms. Cells of the protist *Dictyostelium discoideum* represent a suitable model to study regulation of the actin cytoskeleton during amoeboid cell migration and large-scale endocytosis: phagocytosis and macropinocytosis. *Dictyostelium* cells are capable of translocating one cell length and recycling their fluid content in less than a minute, using the basic constituents and regulatory mechanisms common to the actin cytoskeleton of all eukaryotes. For example, actin polymerases from the formin family nucleate and elongate linear actin filaments and, together with the Arp2/3 complex, represent the major promoters of actin assembly in *Dictyostelium*. In the first part of the talk, I will present the main mechanism of *de novo* generation of actin filaments at the back of polarized cells mediated by Diaphanous-related formin A (ForA). Further stabilization of the posterior actin cortex is accomplished by actin crosslinkers and IQGAP-related proteins, and their localization and activity is regulated by small GTPases and phosphoinositide lipids. Next, I will show how the activity of another Diaphanous-related formin from *Dictyostelium*, formin G (ForG), underlies actin assembly in large-scale endocytosis. ForG localizes to endocytic cups, efficiently elongates actin filaments in the presence of profilin, and the diminished actin content in the cups of ForG-null mutants is associated with a strongly impaired endocytosis. Interestingly, ForG is directly regulated in large-scale endocytosis by small GTPases RasB and RasG, which are highly related to the human proto-oncogene KRas. Taken together, the presented data will illustrate highly diversified functional roles that structurally similar proteins from the formin family play in the regulation of actin-driven processes.

L8

The role of redox congeners in physiological receptor-mediated regulation of contractile activity

Duško Blagojević

University of Belgrade, Institute for Biological Research "Siniša Stanković", Serbia

Different classes of small redox congeners, mainly reactive oxygen, nitrogen and sulphur species (ROS, RNS and RSS, respectively) that are produced in cells create a redox signalling network characterized by reversibility and a short-lasting activity. These molecules are capable of diffusing through tissue to provide a local discrete set of physiological regulatory mechanisms which are especially important in fine-tuning smooth muscle contractility and small vessel vascular tone. Our experiments on the isolated uterus showed that redox congeners inhibit uterine contractile activity through interactions with different receptors. We observed that hydrogen peroxide expressed its effects by interaction with voltage dependent potassium channels, despite the interaction of hydrogen sulphide with chloride ion channels. The link between local oxygen demands, the energetic state and physiological regulation is mediated by complex redox interactions whereby the redox state triggers different output signals, ranging from ATP to superoxide and NO. It seems that the main mechanisms of physiological redox regulation in contractile smooth muscles involve the cytosolic ROS/RNS production/elimination ratio, GSH/GSSG balance and redox sensitive mitochondrial K_{ATP} channels. Phase transitions of redox congeners are partially achieved by enzymatic antioxidant defense activity that controls their homeostatic levels and the presence of dominant congener. This in turn, according to the chemical characteristics and properties of individual redox congener, determines the target molecules and the subsequent extent and magnitude of their physiological impact.

L9

RecQ helicases: Motorized lifeguards in the gene pool

Gabor Harami[1], Yeonee Seol[2], Veronika Ferencziova[1], Mate Martina[1], Keir C. Neuman[2], Mihály Kovács[1]

[1]*Eötvös Loránd University, Department of Biochemistry, ELTE-MTA "Momentum" Motor Enzymology Research Group, Hungary*
[2]*National Institutes of Health, National Heart, Lung and Blood Institute, Laboratory of Molecular Biophysics, Hungary*

Homologous recombination (HR) is a ubiquitous and efficient mechanism for potentially error-free repair of DNA double-strand breaks, the most dangerous form of DNA damage. However, HR must be controlled because excessive HR can cause fatal genome rearrangements. Bacterial and eukaryotic RecQ-family helicases exert a broad range of pro- and antirecombinogenic activities, acting on a variety of HR-intermediate DNA structures. Our single-molecule and ensemble biophysical experiments and *in vivo* HR precision assays have recently revealed unexpected complex DNA-restructuring activities of *E. coli* RecQ helicase, raising the concept that the enzyme is capable of multiple means of DNA processing that are dependent of the geometry of the DNA substrate encountered. We found these mechanisms to be conserved but functionally fine-tuned in Bloom's syndrome (RecQ-family) helicase, a key enzyme responsible for HR control in humans. We provide a mechanistic model of helicase structural architecture supporting a versatile, DNA structure-dependent enzymatic behavior that has been optimized for multiple processes preserving genome integrity.

L10

Regulation of persistence via intrinsic disorder

Remy Loris[1,2], Abel Garcia-Pino[3], Ariel Talavera[1,2], San Hadži[4], Jurij Lah[4]

[1]*Vrije Universiteit Brussel, Belgium*
[2]*Vlaams Instituut voor Biotechnologie, Belgium*
[3]*Université Libre de Bruxelles, Belgium*
[4]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*

Persistence is a phenomenon where bacteria are temporarily tolerant to antibiotics or other external stresses without having acquired inheritable resistance. Recent data suggest a key role of bacterial toxin-antitoxin modules in the establishment of the persistence state. In the past years, we unraveled the interplay between protein function and its regulation at the levels of activity and transcription for several unrelated families of toxin-antitoxin modules, and discovered the importance of intrinsic disorder in this regulatory process. Using the Phd/Doc module from bacteriophage P1 as a model system, we showed that Doc inhibits translation via phosphorylation of EF-Tu. Transcription regulation functions through conditional co-operativity, which allows for maximal repression at about 1:1 ratios of Doc and Phd. Tuning of the conformational ensemble of the partly disordered antitoxin Phd induces co-operativity in protein DNA interactions. Two dimers of Phd can only bind simultaneously to two sites on the operator by restricting the conformational ensemble of their C-terminal domain. The entropic cost involved leads to significant negative co-operativity, which is relieved by Doc, which folds the C-terminal domain of Phd and bridges the two Phd dimers on the operator.
Other, unrelated TA modules such as GraTA from *Pseudomonas putida* and HigBA from *Vibrio cholerae* also employ intrinsic disorder to keep the ratio between toxin and antitoxin in check. In the case of HigBA, the intrinsically disordered N-terminus of the antitoxin HigA increases binding of HigA to its operator, while sequestering of this region by the toxin HigB prevents operator binding. In the GraTA module, the intrinsically disordered segment is located on the toxin, and prevents the toxin-antitoxin complex from interacting with the operator. Thus, different TA modules have evolved different molecular mechanisms by which an intrinsically disordered protein segment can modulate DNA binding activity.

L11

The mechanism behind the selection of two different cleavage sites in NAG-NAM polymer

Marko Mihelič[1,2], Kristina Vlahoviček-Kahlina[3], Miha Renko[1,2], Stephane Mesnage[4], Andreja Doberšek[1], Sara Pintar[1], Ajda Taler Verčič[1], Andreja Jakas[3], Dušan Turk[1,2]

[1]*Jožef Stefan Institute, Slovenia*
[2]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Slovenia*
[3]*Ruđer Boškovic Institute, Division of Organic Chemistry, Croatia*
[4]*University of Sheffield, Krebs Institute, UK*

Peptidoglycan is a giant molecule forming the cell wall that surrounds bacterial cells. It is composed of alternating N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) residues connected with β-(1-4)-glycosidic bonds and cross-linked with short polypeptide chains. Due to the increasing antibiotic resistance against drugs targeting peptidoglycan synthesis, studies of enzymes involved in degradation of peptidoglycan such as N-acetyl-glucosaminidases may expose new, valuable drug targets. The scientific challenge addressed here is how the muramidases lysozymes, likely the most studied enzyme ever, and bacterial N-acetyl-glucosaminidases discriminate between two different in sequence yet chemically equivalent glycosidic bonds in the same NAG-NAM polymers. In spite of more than fifty years of structural studies of lysozymes it is still not known how the enzyme selects the bond to be cleaved. Using macromolecular crystallography, chemical synthesis and molecular modelling this study explains how these two groups of enzymes based on an equivalent structural core exhibit difference in selectivity. The crystal structures of *Staphylococcus aureus (S. aureus)* N-acetyl-glucosaminidase Autolysin E (AtlE) alone and in complex with fragments of peptidoglycan revealed that N-acetyl-glucosaminidases and muramidases approach the substrate at alternate glycosidic bond positions from the opposite sides. The recognition pocket for NAM residues in the active site of N-acetyl-glucosaminidases can make them a suitable drug target.

L12

A bright future for integrative omics in venom research

Juan J. Calvete

The Spanish National Research Council (CSIC), Instituto de Biomedicina de Valencia, Valencia, Spain

Venoms are integrated phenotypes that evolved independently in, and are used for predatory and defensive purposes by, a wide phylogenetic range of organisms. The same principles that contribute to the evolutionary success of venoms, contribute to making the study of venoms of great interest in such diverse fields as evolutionary ecology and biotechnology. Evolution is profoundly contingent, and nature also reinvents itself continuosly. Changes in a complex phenotypic trait, such as venom, reflect the influences of prior evolutionary history, chance events, and selection. Reconstructing the natural history of venoms requires the integration of different levels of knowledge into a meaningful and comprehensive evolutionary framework for separating stochastic changes from adaptive evolution. The application of omics technologies and other disciplines have contributed to a qualitative and quantitative advance in the road map towards this goal.

In this paper I will discuss recent significant developments and applications in the field of snake venom research, specifically the emergence of top-down proteomic applications that allow achieving compositional resolution at the level of the protein species present in the venom, and the absolute quantification of the venom proteome using hybrid element and molecular mass spectrometry.

Unveiling the spatial and temporal distribution of venom variation within and between species is the key for understanding the evolutionary processes and the ecological constraints that molded snake venoms to their present-day variability. A straightforward translational application of the body of knowledge gained through venomics is the analysis of the immune reactivity of antivenoms against venoms, a field coined ‘antivenomics’. The combination of third generation antivenomics and *in vivo* neutralisation tests constitute a powerful toolbox for evaluating an antivenom's preclinical efficacy.

L13

Development of new inhibitors of butyrylcholinesterase and monoamine oxidases for treatment of neurodegenerative diseases

Urban Košak, Damijan Knez, Boris Brus, Stanislav Gobec

University of Ljubljana, Faculty of Pharmacy, Slovenia

Alzheimer's disease (AD) is characterized by severe cholinergic deficit, which results in progressive and chronic deterioration of memory and cognitive functions. Acetylcholinesterase and butyrylcholinesterase (BChE) both contribute to the termination of cholinergic neurotransmission, however, BChE enzymatic activity increases with disease progression, thus classifying BChE as a viable therapeutic target in advanced AD.

We developed a selective and highly potent noncovalent BChE inhibitor [1], which was used as a starting point for the synthesis of a comprehensive series of close structural analogues. More than 100 compounds were synthesized resulting in improved inhibitors with nano to picomolar inhibition constants. The most promising inhibitor improved memory, cognitive functions, and learning abilities of mice in a model of the cholinergic deficit that characterizes AD, without producing acute cholinergic side effects. This inhibitor therefore provides a promising advanced lead compound for the alleviation of symptoms caused by cholinergic hypofunction in AD [2].

Limited clinical efficacy of current symptomatic treatment and minute effect on the progression of AD has shifted the research focus from targeting single enzyme or receptor towards multifunctional ligands. These ligands are able to interact with multiple pathological processes of AD, and have the potential to halt the progression or even to cure the disease. Therefore, our potent BChE inhibitors were used as starting points to develop a new series of multifunctional ligands [3]. In addition, nanomolar MAO-A and MAO-B inhibitors with piperidine scaffold were derived thereof, and they showed promising neuroprotective activity in 6-hydroxydopamine cell model of Parkinson's disease.

References:

1. J Med Chem (2014) *57*, 8167.
2. Sci Rep (2016) *6*, 39495.
3. Bioorg & Med Chem (2017) *25*, 633.

L14

SysmedIBD, Systems Medicine of Inflammatory Bowel Disease

Werner Müller

University of Manchester, Bill Ford Chair of Cellular Immunology for the SysmedIBD consortium, UK

SysmedIBD is an European funded FP7 project in the area of Systems Medicine (www.sysmedIBD.eu). It focuses on the detailed understanding of the dynamics of NF-kappa-B signalling cascade under normal and disease conditions. SysmedIBD uses various technological approaches from mathematical modelling, next generation sequencing, complex pathway analyses, cell culture systems, animal models and patient samples.
Here I will report on the generation of complex transgenic mouse models that allow measurements of NF-kappa-B signalling events *in vitro* and *in vivo*. I will show the dynamic properties of the NF-kappa-B dynamics in various cell types from the mice.
I will also show translation of the work initiated in mice to patients. For this we modified the transgenes used in mice to lentil viral vectors that allow to infect primary human blood derived cells and subsequently to readout of the NF-kappa-B signalling dynamics *in vitro*.
This approach allowed us to better classify patients with inflammatory bowel disease and adds to a better understanding to the underlying mechanism of inflammatory bowel disease.

The work leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2012-2017) under grant agreement n°305564 (http://cordis.europa.eu/fp7/home_en.html).

L15

Inflammation and the negative acute phase response of the liver: consequences for drug metabolism and possible role of microRNAs

Ulrich Zanger

Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Germany

During inflammation cytokines reprogram the liver to enter the acute phase response (APR) and produce large amounts of secretory APR proteins such as C-reactive protein (CRP) needed to combat the inflammation. Simultaneously genes encoding drug metabolizing enzymes and transporters (DMET) are strongly downregulated by mechanisms that are less well understood. We characterized the APR in human liver by genome-wide RNA and miRNA profiling of livers from donors with elevated versus normal levels of the inflammation marker C-reactive protein. The data show that the positive APR is accompanied by an equally pronounced negative APR, including many DMET genes. Human hepatocytes (primary cells and HepaRG cells) treated with cytokines, especially IL-6, reproduce these features. Using chemical inhibitors of signalling pathways and high-throughput transcriptomics and proteomics coupled with fuzzy-logic mathematical modelling we propose MAPK and PI3K signalling pathways to be more important than JAK/STAT pathway for gene downregulation. The model suggests further a central role of heterodimeric complexes between the orphan nuclear receptor RXRα and nuclear receptors that regulate drug and lipid metabolism (e.g. CAR, PXR), which we could confirmed by siRNA-mediated knockdown experiments. We further identified several microRNAs as significantly up- or downregulated during inflammatory conditions in the liver. In particular, miR-130b was shown to be upregulated in inflammation, to bind directly to the 3'UTRs of CYP2C9 and RXRα, and to downregulate proteins and CYP enzyme activities in hepatocytes. In summary, effective downregulation of drug metabolism genes during inflammation appears to involve MAPK/PI3K pathways, nuclear receptors and microRNAs. The extensive negative APR of the liver may help to save energy needed for the positive APR required to control inflammation.

L16

Prolonged survival of *IDH1*-mutated glioblastoma patients but not of *IDH1/2*-mutated AML patients by reduced NADPH production capacity

Remco Molenaar, Mohammed Khurshed, Vashendriya Hira, Cornelis van Noorden

University of Amsterdam, Academic Medical Center, Department of Medical Biology, The Netherlands

Isocitrate dehydrogenase 1 or 2 is mutated (*IDH1/2*mt) in various types of human cancer such as glioma and acute myeloid leukemia (AML). The mutation causes catalysis of a-ketoglutarate to the oncometabolite D-2-hydroxyglutarate with concomitant consumption of NADPH. Wild type IDH1 (*IDH1*wt) converts isocitrate and NADP into a-ketoglutarate and NADPH. Patients with *IDH1*mt glioblastoma (glioma stage IV) have prolonged survival of one year, whereas *IDH1/2*mt AML patients do not. We found that IDH1 in glioma is responsible for 65% of NADPH production capacity, whereas the pentose phosphate pathway is the major provider of NADPH in AML cells. We have shown *in vitro* that glioma cells heterozygous for *IDH1*mt show after exposure to ionizing radiation or DNA-damaging chemotherapy higher levels of reactive oxygen species, more DNA double-strand breaks and cell death compared with *IDH1*wt glioma cells. This was not the case in AML *IDH1/2*mt cells. Our results argue that altered oxidative stress responses are a mechanism of prolonged survival of *IDH1*mt glioma patients and not of *IDH1/2*mt AML patients. Moreover, we showed that the inhibitor of IDH1mt, AGI-5198, annihilates effects of the *IDH1*mt on oxidative stress in glioma cells. These findings imply that AGI-5198 counteracts effects of irradiation and chemotherapy on *IDH1*mt glioma tumours and concomitant administration of AGI-5198 and irradiation or chemotherapy should be avoided. Finally, we found that AML patients with *IDH1/2*mt gene should not be treated with an IDH1/2 inhibitor and the chemotherapeutic daunorubicin at the same time, because it worsen clinical outcome. The mechanism is different than in glioma. In AML the *IDH1/2*mt causes repression of the DNA damage response gene *ATM* via D-2-hydroxyglutarate. It is concluded that the IDH1/2 inhibitors should not be administered concomitantly with standard therapy to *IDH1*mt cancer patients.

L17

Contribution of tumour markers to personalized therapy

Tanja Čufer[1,2]

[1]*University Clinic Golnik, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Slovenia*

Tumour markers provide insight into the biological processes underlying the cancer, and give clinically useful information about an individual cancer patient in addition to anatomical stage of disease and clinical characteristics, such as performance status (PS). In general, tumour markers are specific molecules appearing in the blood or tissues at the occurrence of cancer. They have a diagnostic (marker specific for the cancer), prognostic (marker correlates with extent and natural history of disease) or predictive (marker correlates with response/resistance to therapy) value. A number of biomarkers have been established in oncology practice, such as serum CA 125 in ovarian cancer, serum AFP and HCG in germ-cell malignancies and tissue hormone receptors (HR) in breast cancer.

Advances made over the last 10 years in the understanding of molecular oncology resulted in discovery of multiple novel biomarkers that help us to understand each individual cancer biology and to personalize anti-cancer therapy better, nowadays. In addition to established serum and tissue biomarkers, multiple novel genetic and signal transduction biomarkers, so called driving oncogenes have been discovered. With the advent of targeted therapies directed at growth factor receptors or their tyrosine kinases, it is becoming increasingly important to have real-time data on oncogene drivers. Multiple novel technologies, such as circulating tumour cells (CTCs) detection and circulating tumour DNA (ctDNA) give us a possibility to quantify the expression of multiple cancer related genetic alterations in a single blood sample by a single test on a real-time basis. That gives clinicians a possibility to maximize a predictive value of a specific biomarker for each individual patient benefit. Most recently, the plasma detection of specific epidermal growth factor receptor (EGFR) mutations revolutionized treatment selection and results in advanced non-small-cell lung cancer. Incoming new technologies, such as next generation sequencing (NGS) will further improve our ability of early cancer detection, prognostic classification and prediction of response to anti-cancer therapy in each individual patient, thus improving overall cancer control in society.

L18

Global insights into condition-specific alternative splicing using ENCODE data

Martin Stražar[1], Jernej Ule[2], Tomaž Curk[1]

[1]*University of Ljubljana, Faculty of Computer and Information Science, Slovenia*
[2]*The Francis Crick Institute, UK*

RNA-sequencing assays provide nucleotide resolution in mapping multiple cis- and trans-acting factors that regulate alternative splicing. Large-scale sequencing studies include hundreds of experimental conditions (e.g. RNA-binding protein knockdowns), which opens new opportunities for comparative analysis of alternative splicing. A limitation of contemporary differential exon usage tests is that they require a reference condition to test against. This requirement increases the probability for an exon to be called differentially expressed in multiple conditions because it is a part of the cell's general response network or because of the choice of the reference condition.
We developed csDEX (condition-specific differential exon expression). The test does not require a reference condition and can discover changes in exon usage that are specific to a small subset of conditions. We applied csDEX on the ENCODE project large data set with 189 shRNA knockdown RNA-seq samples (conditions) of different RNA-binding proteins, including SRSF1, U2AF1/2, PTBP1, hnRNPs and TARDBP. The effects of RNA-protein binding on alternative splicing were validated by independent data sources on binding (eCLIP data from ENCODE) and by performing short sequence motif analysis.

L19

NMR structural studies of DNA beyond double-stranded helix

Janez Plavec[1,2,3]

[1]*National Institute of Chemistry, Slovenian NMR Centre, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*EN-FIST Centre of Excellence, Slovenia*

The most common secondary structure of DNA is the right-handed double helix. When DNA is involved in processes such as replication and transcription, its double helical structure is partially unwound into two strands. Switching to single strands under certain conditions leads to formation of higher-order DNA structures which can interfere or even stop replication possibly resulting in harmful mutations. G-quadruplexes and i-motifs are formed by G- and C-rich oligonucleotides, respectively.
Increase in the number of GGGGCC repeats located within the non-coding region of *C9orf72* gene is the most frequent genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Our NMR studies on DNA oligonucleotides with four repeat units demonstrated their folding into multiple G-quadruplex structures in the presence of K^+ ions.
Oligonucleotides containing GGGAGCG repeats are found in regulatory region of the *PLEKHG3* human gene in the 14th human chromosome related to autism. A two repeat oligonucleotide with the sequence d(GGGAGCGAGGGAGCG) folded into a well-defined symmetric dimer. In contrast to the expected G-quartet-based structure, where two quartets were supposed to sandwich a GCGC-quartet, we found a novel four-stranded structure of G-rich DNA that expands the current repertoire of known DNA structures. Fold-back loops adopted by G-tracts are a surprising element in a G-rich DNA structure considering that a GGG tract would be generally expected to be involved in G-quartets.

Acknowledgements: The author is grateful to the colleagues named in the cited papers from his laboratory at Slovenian NMR centre, especially Drs. Kocman, Lenarčič Živković, Marušič, Šket, Wang, Trajkovski, Podbevšek, Kotar and Brčić. This work was supported partly by the Slovenian Research Agency (ARRS, grants P1-0242 and J1-6733).

References:

1. Kocman V., *et. al.* (2017) Nat Commun 8, pp. 15355.
2. Tateishi-Karimata H., *et. al*. (2017) Nucleic Acids Res 45, pp. 7021-7030.
3. M. Gajarský M., *et. al*. (2017) J. Am Chem Soc 139, pp. 3591-3594.
4. P. Galer p., *et. al*. (2016) Angew. Chem Int Ed 55, pp. 1993-1997.
5. Kotar A., *et. al*. (2016) Angew. Chem Int Ed 55, pp. 12508-12511.
6. Podbevšek P., *et. al*. (2016) Nucleic Acids Res 44, pp. 917-925.
7. Brčić J., *et. al*. (2017) Biochem. Biophys Acta 1861, pp. 1237-1245.

Round table: Ethics in biomedical research and regulatory acts in Slovenia

L20

The 20th anniversary of the Oviedo Convention — its impact on the biomedical research and how to move forward

Urh Grošelj[1,2]

[1]*University Medical Centre Ljubljana, University Children's Hospital, Slovenia*
[2]*Republic of Slovenia National Medical Ethics Committee*

The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (also known as Convention on Human Rights and Biomedicine) was adopted in 1997 in Oviedo (Spain); one of its fathers was also Slovenian bioethician acad. prof. dr. Jože Trontelj. Today, the Convention is the only international legally binding instrument on the protection of human rights in the biomedical field. It also deals specifically with biomedical research, genetics and transplantation of organ and tissues; the provisions of the Convention are further elaborated and complemented by Additional Protocols on specific subjects, one of them also on research on biological materials of human origin (Rec(2004)4).
The Convention has had a vast impact on the field of biomedical research ethics, also being a regulatory basis for national legislations of the 29 European states that has signed and implemented it so far. Thus, it also directs the decision-making within the research ethics committees in great majority of Europe. However, at the same time, the scientific progress in the last 20 years was enormous, posing many new ethical dilemmas and directions which might not yet be sufficiently approached.
Aim of the round table is to assess the impact of the Oviedo Convention on the biomedical field in the last 20 years and to recognize the emerging ethical questions in the field with possible solutions.

L21

Exploring the endocytic pathway on a global scale

Mojca Mattiazzi Ušaj, Oren Kraus, Nil Sahin, Matej Ušaj, Helena Friesen, Charlie Boone, Brenda Andrews

University of Toronto, The Donnelly Centre, Canada

Endocytosis is a highly conserved fundamental cellular process that controls the lipid and protein composition of the plasma membrane, and the exchange of the majority of molecules between a cell and its environment. It serves as a link between many intracellular signalling pathways and is often disrupted in human diseases. Proper execution of endocytosis requires precise coordination of molecular events and depends on an intricate network of interacting proteins and membrane components. Despite significant effort and study, the labour-intensive nature of live cell imaging experiments has limited our understanding of endocytosis to a relatively small number of genes encoding primary components of the endocytic system. Large-scale, systematic approaches are required to understand how endocytosis is regulated and its relationships to other fundamental cellular processes.

We have combined the synthetic genetic array (SGA) analysis method with high-throughput live-cell real-time fluorescence microscopy and quantitative image analysis to query the *S. cerevisiae* genome for genetic factors required for proper spatial and temporal dynamics of the early endocytic events. We acquired more than 14,300 dual-channel fluorescence movies, for a total of more than 1.8 million images, and analyzed the dynamics of more than 19 million individual actin patches. Furthermore, in order to gain a global understanding of the function and molecular regulation of the endocytic pathway, we have combined these data on actin patch dynamics with our single-cell high-content screening data on basic morphology of early, intermediate, and late endocytic compartments. Our systematic and automated approach has identified a comprehensive set of genes that govern the formation and maintenance of the major endocytic compartments. Here, the image acquisition and analysis pipeline, results and ongoing efforts will be presented.

L22

Analysis of diurnal gene expression of major plant clades

Camilla Ferrari, Marek Mutwil

Max Planck Institute of Molecular Plant Physiology, Germany

The number of sequenced plant genomes is growing rapidly, and the resulting data and comparative analyses have revealed the appearance of biological pathways throughout 1600 million years of plant evolution. However, activity of the evolved biological pathways is also under strict transcriptional control, which might not be revealed by genomics. To address this, we are generating dense diurnal expression atlases of *Cyanophora paradoxa* (glaucophyte), *Porphyridium purpureum* (rhodophyte), *Klebsormidium flaccidium* (charophyte), *Physcomitrella patens* (bryophyte), *Selaginella moellendorffii* (lycophyte), and *Picea abies* (gymnosperm). Together with publicly available data for other plants, these atlases allow us to compare gene expression and daily scheduling of biological processes of ten major plant clades. We found that, despite one billion years of evolution, diurnal transcriptomes of plants are similar, but observed an extensive reprogramming of the transcriptomes in land plants. We also show how the scheduling of biological processes was reprogrammed and how genes that appeared at specific time in evolution are expressed during the diurnal cycle. To conclude, the comprehensive group of diurnal atlases, together with novel bioinformatical approaches, allow us to determine how the establishment of new traits such as multicellularity and terrestrialization, has affected gene expression.

L23 Closing Lecture

Visualisation concepts in the Reactome database of biomolecular pathways

Henning Hermjakob

European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI), UK

Reactome (http://reactome.org) is a free, open-source, curated and peer-reviewed knowledgebase of biomolecular pathways. Reactome models pathway space as a hierarchy of increasingly detailed pathways. While we provide a hierarchical pathway browser as a key element of the Reactome web interface, the relationships and connectivity between high level pathways were previously not represented well. In addition, options for re-use of the manually laid out low level pathway diagrams were limited, as they were only downloadable as PNG images. Following intensive User Experience testing by external users, we implemented a series of major visual enhancements, to make Reactome more interactive and user-friendly: 1: In the detailed pathway diagrams, sub-pathways are now visually highlighted through shaded boxes. 2: Detailed pathway diagrams are now downloadable as PowerPoint™ slides, with pathway elements rendered as connected PowerPoint™ objects, allowing scientists to edit, modify, and re-use them to present their own pathway-related research results in presentations and publications. 3: The relationships between high level nodes in the Reactome hierarchy, for example between Adaptive Immune System, Innate Immune System, and Cytokine Signalling in Immune System, are now visualised through textbook-style diagrams developed by a professional illustrator. However, these diagrams are not static PNG images, but dynamic SVG graphics, allowing fast zooming and navigation, clicking to link to sub-pathways, as well as overlay of aggregated pathway analysis results. Diagrams as well as their components are open data and have been released as a library re-useable for biomolecular visualisation by the scientific community.

SL1

Next-generation sequencing for studying biology

Oliver Goldenberg

Illumina

Next-generation sequencing (NGS) has changed the way one can study biology as well as biological functions. The talk will focus on the application of NGS to analyse cellular gene expression and gene regulation. Examples will be given on how this technology can be used to study individual cells, as well as tissues from a variety of species.

SL2

Development of biosimilars

Dominik Gaser

Head Cell & Molecular Biology
Lek Pharmaceuticals d.d., Slovenia

Biologics offer real hope for many unmet patient needs, particularly complex diseases such as cancer and autoimmune disorders. As the global population continues to age, demand for state-of-the-art biologics is increasing. This growing demand drives overall costs steadily up, putting ever more pressure on healthcare budgets. Rising prices are driven partly by rising complexity, but also by lack of competition. However, cost is not the end of the story. Many patients, who need these medicines the most, already have difficulties to access them, as cost-benefit analyses increasingly determine how healthcare resources are allocated.

Biosimilars are follow-on versions or successors to biologic medicines that have lost patent protection or exclusivity. They are approved via a stringent approval pathway in the highly regulated markets (US, Canada, EU, Japan and Australia).

Biosimilars have a highly analogous structure to their reference products and offer comparable quality, safety and efficacy at a more affordable price.

The “biosimilar concept” is gaining ground every day — as market experience with existing products grows and key stakeholders spread the word. More and more physicians/KOLs are becoming aware that biosimilars are high-quality, clinically proven alternatives to more costly existing treatments. In fact, the introduction of biosimilars has already led to increased competition and significant savings.

Sandoz has been developing biosimilars since 1996, and also enjoys extensive synergies with other Novartis divisions. Effective biosimilar development involves managing opposing needs: speed to market on the one hand, and cost-effectiveness on the other. At Sandoz, we see this as a two-step process: first, guaranteeing a high level of molecular similarity to the reference product, then driving commercial viability through appropriate clinical tests to ensure a broad clinical label.

At the heart of our approach is a concept known as Quality by Design: ensuring quality by designing manufacturing processes that ensure comparability with the original product. In other words, the product does not need to “be” the process — it is end quality that counts. This approach has been tried, tested, and proved to work in practice. Built in quality ensures safety and efficacy of every batch we produce.

SL3

MicroScale Thermophoresis and nanoDSF as advanced methods in life science research

Jakub Nowak

NanoTemper Technologies sp. z o.o., Krakow, Poland

The 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 analyzes the directed movement of molecules in optically generated microscopic temperature gradients. This thermophoretic movement is determined by the entropy of the hydration shell around the molecules. Almost all interactions and any biochemical process relating to a change in size, charge and conformation of molecules alters this hydration shell and is thus detectable by MST. Measurements can be performed with purified molecules as well as in close-to-native conditions (lysate, serum). The readout of the method is fluorescence, derived either from fluorescently labeled interaction partners (via chemical dyes or fluorescent fusion proteins) or from intrinsic protein fluorescence.
The presentation will cover biophysical backgrounds of the technology, highlighting benefits of the MicroScale Thermophoresis platform, followed by specific examples of its application in characterizing biomolecular interaction in various scenarios.
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 fluorescence detection, providing an unmatched scanning speed and data point density. This yields ultra-high resolution unfolding curves 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, and over a concentration range of 150 mg/ml down to 5 µg/ml. In addition, information on protein aggregation can be recorded in parallel, providing insight into colloidal stability of the sample. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control.
The presentation will cover biophysical concepts of the technique showing benefits of the nanoDSF technology platform, and will be followed by specific examples of nanoDSF applications towards various experimental systems.

SL4

Bio-Rad’s state of the art solutions to help you find answers: from DNA translation to single cell sequencing

Uršula Prosenc Zmrzljak, Matjaž Rejec, Rok Košir

Labena d.o.o., Slovenia

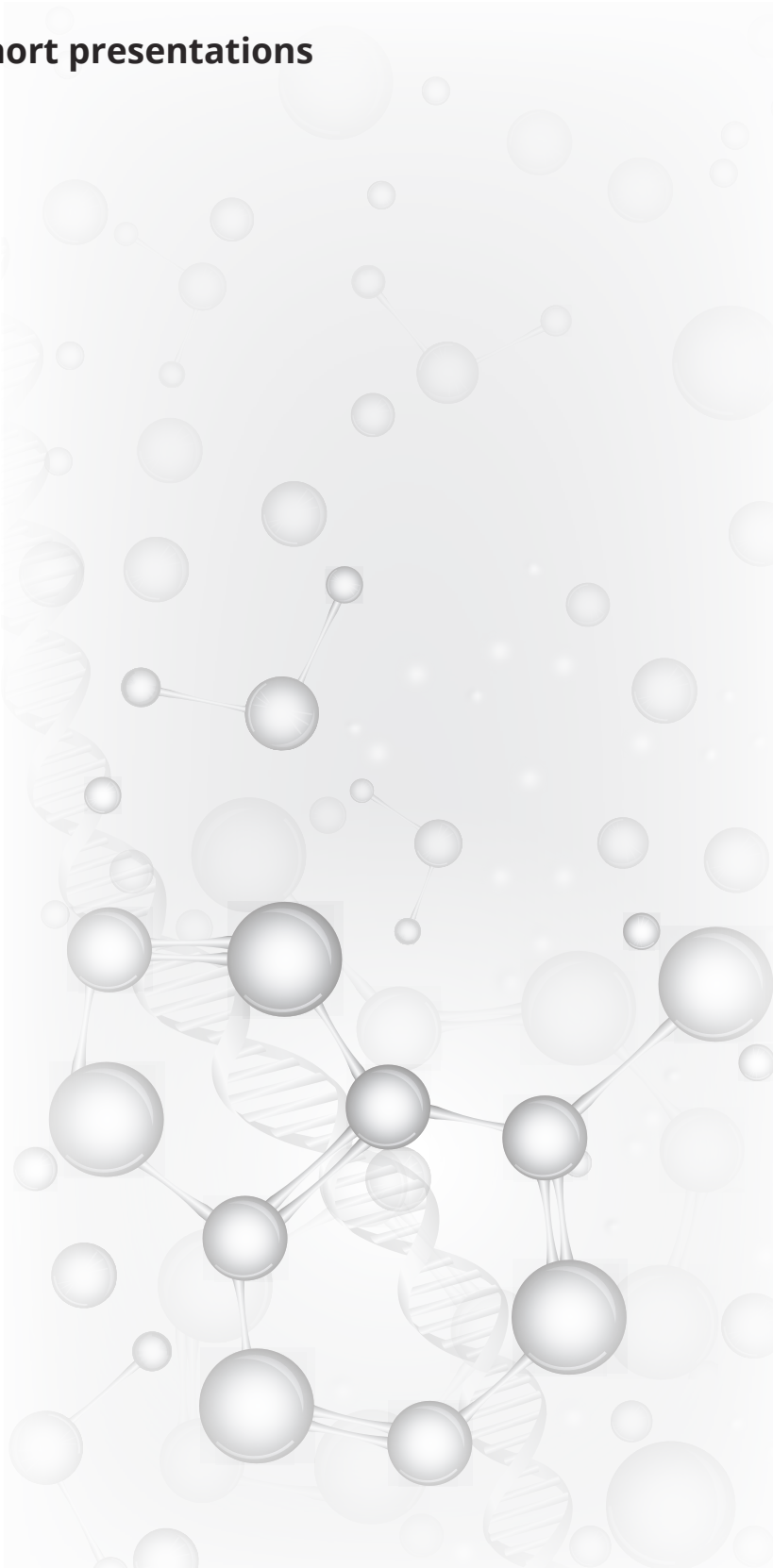
The central dogma of molecular biology has long been the focus of intense studies. These studies have brought us numerous methods that not only enabled us to identify and quantify nucleic acids and proteins but more importantly enabled us to answer questions relating to the regulation of DNA transcription and protein translation. However, certain parts of the central dogma remain shrouded in mystery even today.

For instance, current methods are failing to completely elucidate the specific steps of translation from mRNA to protein. With Bio-Rad’s V3 Western Workflow™, many scientists learned ways to obtain their best results of protein expression measurements with the help of visualisation of separated proteins, verification of protein transfer efficiency and validations of western Blots using total protein normalisation. In addition to this, Bio-Rad has pioneered the world of microfluidics to develop unique technologies for absolute quantification of nucleic acids and proteins. Just recently Albayrak *et. al.* (2017) used a proximity ligation assay (PLA) in parallel to a ddPCR assay to show that translational efficiency of mRNA to protein can be measured. The simplicity of the QX200 Droplet Digital PCR (ddPCR) system currently helps many scientists to reveal minute changes in nucleic acid expression in complex samples and more importantly provides a platform for development of other methodologies for absolute quantification of biological molecules as shown by Albayrak.

Another important application of microfluidics that Bio-Rad developed is the ddSEQ Single-Cell Isolator. A part of the Bio-Rad® Illumina® Single-Cell Sequencing Solution this platform brings Bio-Rad's Droplet Digital™ technology and Illumina's market-leading sequencing expertise to the single-cell level.

In conclusion, it is Bio-Rad’s aim to provide researchers with a wide array of state-of-the art techniques that will help them answer their questions accurately, easily and quickly.

Abstracts of short presentations



SP1

Early vertebrate origin and diversification of FXYDs and other small transmembrane regulators of cellular ion transport

Sergej Pirkmajer[1], Henriette Kirchner[2], Leonidas S. Lundell[2], Pavel V. Zelenin[3], Juleen R. Zierath[2,4], Kira S. Makarova[5], Yuri I. Wolf[5], Alexander V. Chibalin[4]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[2]*Karolinska Institutet, Department of Physiology and Pharmacology, Sweden*
[3]*Karolinska Institutet, Department of Neuroscience, Sweden*
[4]*Karolinska Institutet, Department of Molecular Medicine and Surgery, Sweden*
[5]*National Institutes of Health, National Center for Biotechnology Information, National Library of Medicine, USA*

Small transmembrane proteins play fundamental roles in regulation of ion transport in vertebrates. The most prominent among these proteins are members of the FXYD family (FXYD1-12), which regulate Na^+-K^+-ATPase, and phospholamban, sarcolipin, myoregulin, and DWORF, which regulate the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA). FXYDs and regulators of SERCA are present in fishes, as well as terrestrial vertebrates. However, uncertain evolutionary origins and phylogenetic relationships among these regulators of ion transport have led to inconsistencies in their classification across vertebrate species, thus hampering comparative studies of their functions.

We discovered that sea lamprey (*Petromyzon marinus*), a representative of extant jawless vertebrates (Cyclostomata), expresses an FXYD homologue. This finding strongly suggests that FXYDs predate the emergence of fishes and other jawed vertebrates (Gnathostomata). Using a combination of sequence-based phylogenetic analysis and conservation of local chromosome context, we determined that FXYDs markedly diversified in the lineages leading to cartilaginous fishes (Chondricthyes) and bony vertebrates (Euteleostomi). Diversification of SERCA regulators was much less extensive, indicating they operate under different evolutionary constraints. Finally, we found that FXYDs in extant vertebrates can be classified into 13 gene subfamilies, which do not always correspond to the established FXYD classification. We therefore propose a revised classification that is based on evolutionary history of FXYDs and that is consistent across vertebrate species.

Collectively, our results show that small transmembrane regulators of ion transport diversified early in the vertebrate lineage. Furthermore, our findings provide an improved framework for investigating physiological and pathophysiological functions of small transmembrane regulators of ion transport in health and disease.

SP2

The mechanisms of autoinhibition and activation of NLRP3 inflammasome

Iva Hafner Bratkovič[1,2], Duško Lainšček[1], Petra Sušjan[1], Kosta Cerović[3], Samo Roškar[3], Lucija Kadunc[1], Ana Tapia Abellan[4], Pablo Pelegrin[4], Roman Jerala[1,2]

[1]*National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia*
[2]*EN-FIST Centre of Excellence, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Uniform Second-level Master's Program of Medicine, Slovenia*
[4]*University of Murcia, University Clinical Hospital "Virgen de la Arrixaca", CIBERehd, Biomedical Research Institute of Murcia (IMIB-Arrixaca), Spain*

NLRP3 inflammasome is a multiprotein complex mediating inflammatory responses in a variety of common diseases, such as metabolic diseases and Alzheimer's disease. Upon stimulation with a plethora of diverse triggers NLRP3 oligomerizes and induces polymerization of adaptor ASC and self-activation of caspase-1. Caspase-1 activates proinflammatory cytokines IL-1β and IL-18 and induces pyroptosis. Despite the importance of NLRP3 in various pathologies the molecular mechanism of NLRP3 inflammasome initiation remains largely unknown.

To gain insight into early processes in NLRP3 inflammasome assembly, extensive mutagenesis of NLRP3 was performed. Using truncation mutagenesis we were able to define the minimal NLRP3 truncation variant (miniNLRP3), lacking LRR domain, which was fully responsive to various soluble and particulate instigators and phenocopied the full length NLRP3 in terms of sensitivity to inhibitors and the kinetics of activation. Cells expressing miniNLRP3 but not empty cells promoted peritonitis in NLRP3-deficient mice. We showed that further truncation led to the variant that still responded to NLRP3 inflammasome triggers but was unable to form an oligomer triggering functional inflammasome assembly. For NLRC4 inflammasome it was shown that a single activator bound to NAIP sensor can induce conformational change in NLRC4 molecule, which recruits naïve NLRC4 molecules in a prion-like polymerization. Using chimeric proteins we showed that there are striking differences in NLRP3 and NLRC4 inflammasome regulation and assembly.

The insight into the molecular mechanism of NLRP3 inflammasome activation is important for the design of specific NLRP3 inflammasome inhibitors for the development of novel therapies of NLRP3-associated diseases.

This study was supported by ARRS grants J3-5503, J3-6791, P4-0176 and STSM grant by COST action BM1406.

SP3

Characterization of particles formed during staining of exosomes with the fluorescent dye PKH26

Pia Pužar Dominkuš[1], Matjaž Stenovec[2,3], Simona Sitar[4], Eva Lasič[3], Robert Zorec[2,3], Ana Plemenitaš[1], Ema Žagar[4], Marko Kreft[2,3,5], Metka Lenassi[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*Celica BIOMEDICAL, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[4]*National Institute of Chemistry, Slovenia*
[5]*University of Ljubljana, Biotechnical Faculty, Slovenia*

Introduction: Extracellular vesicles (EVs) are small membrane particles, which transfer biomolecules between cells and have great potential as clinical diagnostic and prognostic tool. Their uptake by cells can be visualised by fluorescent lipid membrane dyes, like PKH26. The aim of this study was to assess if EV staining procedures induce fluorescent dye micelles and how these affect EVs cell uptake studies.
Methods: We characterized the particles formed during different staining procedures of small EVs (exosomes) based on ultracentrifugation, filtration, ultracentrifugation through a sucrose cushion, or separation on a sucrose gradient. We analysed the size, number density, surface area, and fluorescence intensity of particles in stained exosome samples and controls (only PKH26 dye) by confocal microscopy and asymmetric-flow field-flow fractionation connected to detectors. We additionally analysed the uptake of PKH26-stained exosomes and PKH26-micelles by rat astrocytes, using confocal microscopy.
Results: Our analysis showed that PKH26-micelles form during all four staining procedures in high numbers and are almost indistinguishable from PKH26-stained exosomes in their size, surface area and fluorescence intensity. In the case of ultracentrifugation-based staining, PKH26-stained exosomes represented only 11% of all particles in the mixture. Compared to control samples, the presence of exosomes even induces the formation of PKH26-micelles. Importantly, after the separation on sucrose gradient, contaminating PKH26-micelles were almost completely absent, at the expense of low exosome recovery. Uptake analysis showed that rat astrocytes uptake PKH26-stained exosomes and PKH26-micelles with similar efficiency.
Conclusion: Taken together, the sucrose gradient staining procedure should be used to effectively separate PKH26-micelles from PKH26-stained exosomes. All results of fluorescently labelled exosomes uptake should be interpreted with great caution.

SP4

Development of RNAi-based pesticides targeting Colorado potato beetle

Marko Petek, Kristina Gruden

National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia

In Colorado potato beetle (CPB), a serious potato pest, RNAi-mediated gene silencing is effectively triggered by ingestion of double stranded RNAs (dsRNAs). Properly designed dsRNAs can be used as environmentally acceptable insecticidal agents for the control of CPB. They can be expressed in plants from a transgene or applied to plants in the field by spraying. It is crucial that dsRNAs act specifically on the pest species and that the mechanism of its action is well understood.

The design and validation of novel RNAi pesticides targeting CPB will be presented. To select target genes, we have integrated CPB transcriptome annotations with gene expression data and publicly available *Drosophila melanogaster* and *Tribolium castaneum* RNAi screening datasets. Using an *in-silico* approach, we narrowed down the target list by excluding conserved nucleotide sequences with potential effect on other species. We have selected three target genes expressed in CPB guts, checked their expression levels in different beetle life stages and tissues, and designed dsRNAs for knockdown of these genes. We have tested the efficiency of designed dsRNAs in CPB feeding trials. To evaluate the efficiency of target gene knockdown and to gain insight into dsRNA's mechanism of action we measured gene expression in CPB guts in response to dsRNA.

SP5

Tunable coiled-coil interaction toolbox for engineering mammalian cells

Tina Lebar[1,2], Estera Merljak[1], Roman Jerala[1,2]

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

Modularity of intermolecular interactions is used by nature to assemble multimolecular complexes and guide versatile biological processes. Many different protein/peptide interaction domains with variable properties and size have previously been used to drive selected protein complex formation. Coiled-coil dimers are some of the most frequently used structural elements that underlie construction of molecular scaffolds, protein recognition, transcription and many other processes. The well-understood specificity of coiled-coil interaction has been used to design *de novo* building blocks for construction of nanostructures *in vitro*. Here we show that a rationally designed set of 6 pairs of heterospecific coiled-coil dimerization modules is highly orthogonal in mammalian cells. Moreover, we show that the interaction strength and stoichiometry of interacting partners can be tuned by selection of coiled-coil variants and by the number of concatenated coiled-coil modules. Fusions of coiled-coil modules were used to control protein localization and to drive transcriptional activation. Concatenation of orthogonal coiled-coil domains was used to construct the most potent CRISPR/dCas9- and TAL effector-based transcriptional activators known so far, as well as for amplification of light-inducible transcriptional regulation. The presented coiled-coil platform represents a toolbox of minimally disruptive modules to recruit and combine selected functional domains for many different applications in mammalian cells.

SP6

Surface display of evasins and BPC-157 on recombinant lactic acid bacteria for treatment of inflammatory bowel disease

Katja Škrlec[1], Borut Štrukelj[1,2], Aleš Berlec[1]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Pharmacy, Slovenia*

Lactic acid bacteria (LAB) are promising vectors for the delivery of active molecules to the mucosal tissue. The development of new approaches for the treatment of inflammatory bowel disease (IBD) is therefore of great interest. LAB could be engineered to bind chemokines on their surface or to deliver proinflammatory peptides, and thereby decrease intestinal inflammation. We used model LAB *Lactococcus lactis* for the surface display of chemokine binding proteins, produced by the brown tick *Rhipicephalus sanguineus*, named evasins. BPC-157 is a part of body protection compound protein and has been demonstrated to accelerate the healing of different wounds. Evasin genes were cloned into lactococcal surface display vector and over-expressed in *L. lactis* NZ9000 and NZ9000*ΔhtrA* in fusion with secretion signal and surface anchor. Secretion and surface display of BPC-157 has been achieved by fusing one or several copies of BPC-157 gene with: (1) basic membrane protein A (BmpA), (2) Usp45 secretion signal and AcmA surface anchor, or (3) Usp45 signal peptide for secretion to the growth medium. Expression of evasins and BPC-157 fusion proteins was confirmed by Western blot, and their surface localization by flow cytometry and whole cell ELISA. Evasin-displaying bacteria removed 15% to 90% of 11 different chemokines from the solution. The binding was dependent on bacterial concentration. Evasin-3 displaying *L. lactis* NZ9000*ΔhtrA* cells had superior chemokine binding ability and removed 88.0% of IL-1β-induced CXCL8 from the supernatant of Caco-2 epithelial cells. BPC-157 fusion proteins were purified with metal affinity chromatography and, upon trypsin digestion, with HPLC and determined with mass spectrometry. The quantity of delivered BPC-157 was determined with ELISA using custom antibody against BPC-157. Evasins- and BPC-157-producing probiotic bacteria should be useful as an innovative treatment of intestinal inflammations and will be tested in an animal model of IBD.

SP7

Structural insight into the mechanism of NLP toxicity

Tea Lenarčič[1], Vesna Hodnik[1,2], Marjetka Podobnik[1], Gregor Anderluh[1]

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

Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) are a group of proteins that are secreted by several phytopathogenic bacteria, fungi and oomycetes. They trigger leaf necrosis and immunity-associated responses in various dicotyledonous plants. Binding studies and X-ray crystallography revealed that NLP_{Pya} forms complexes with GIPC terminal hexose moieties glucosamine and mannosamine. By soaking NLP_{Pya} crystals in solutions containing either glucosamine or mannosamine, we solved the structures of sugar-bound NLP_{Pya} to 1.54 Å and 1.75 Å resolutions, respectively. Sugar binding induces several conformational changes within the toxin. Dislocation of loop L3 causes widening of the L2-L3-lined crevice and a 2.9 Å movement of Mg^{2+} towards the center of the protein relative to its position in apo-NLP_{Pya}, creating space for hexose recruitment between amino acid residues H101 and D158. Mutation of D158 to a smaller residue like alanine did not severely compromise necrotic activity, however, mutations to bulkier amino acid residues of diverse chemical properties, such as phenylalanine, leucine, glutamate, and lysine manifested in a decreased necrosis. Also, mutation of W155, which is conveniently placed in close proximity to hexose-binding site, into alanine, resulted in no detectable cytotoxin activity, most probably due to the loss of membrane anchoring capability upon mutation. Altogether, these results propose a model of early steps of NLP cytolysin action and might explain host selectivity due to steric limitations of GIPC glycoside modules binding into a newly generated cleft in NLP_{Pya} upon cell membrane attachment.

SP8

Is Epithelial Cell Adhesion Molecule (EpCAM) really a cell adhesion molecule?

Aljaž Gaber[1], Miha Pavšič[1], Seung Joong Kim[2], Robyn Kaake[3], Nevan Krogan[3], Andrej Šali[2], Brigita Lenarčič[1,4]

[1]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Department of Biochemistry, Slovenia*
[2]*University of California San Francisco, Department of Bioengineering and Therapeutic Sciences, California Institute for Quantitative Biosciences, Department of Pharmaceutical Chemistry, USA*
[3]*University of California San Francisco, Department of Cellular and Molecular Pharmacology, USA*
[4]*Jožef Stefan Institute, Department of Biochemistry, Molecular and Structural Biology, Slovenia*

Epithelial Cell Adhesion Molecule (EpCAM) was first discovered as a carcinoma antigen. Soon after, this type I transmembrane protein was described as a novel homophilic cell-cell adhesion molecule due to its localization in areas of cell-cell contacts and ability to promote cell aggregation. Its role in cell-cell adhesion was later found out to be more complex and seemingly contradicting: it interferes with classical, E-cadherin-mediated adherent junctions and contributes to the proper formation of tight junctions through direct interaction with claudin-7. The initial hypothesis of its ability to form independent intercellular connections was, until recently, never put to question.

EpCAM mediated cell-cell contacts are proposed to arise from *trans* (intercellular) interaction of two *cis* (intracellular) dimers on opposing cells. Here we present evidence on a molecular level that dimers do not interact in such manner. Despite our best efforts to capture and characterize EpCAM's *trans*-tetramers, SAXS analysis did not reveal the presence of any higher-than-dimer order oligomers of EpCAM's extracellular domains in solution. Further investigation of oligomerization with chemical crosslinking (CX-MS) also yielded no evidence of their existence. Lastly, bead aggregation assays, which are considered a standard method to study homophilic interactions of cell-cell adhesion molecules, confirmed our observation that EpCAM, on its own, is unable to mediate formation of cell-cell contacts.

Combined with results, recently published by other groups, which were also not able to confirm EpCAM's function as an adhesion molecule in cell adhesion experiments, our findings, at very least, suggest revisiting the question if EpCAM really is a homophilic cell-cell adhesion molecule, as it was now postulated for more than 20 years. We are currently exploring options to observe EpCAM's oligomeric state in cell membranes to gain further insight in proteins function in its native environment.

SP9

The CWB2 cell wall-anchoring module is revealed by the crystal structures of the *Clostridium difficile* cell wall proteins Cwp8 and Cwp6

Aleksandra Usenik[1], Miha Renko[1,2,3], Marko Mihelič[1,2], Nataša Lindič[1], Jure Borišek[4], Andrej Perdih[5], Gregor Pretnar[1,2], Uwe Müller[6,7], Dušan Turk[1,2]

[1]*Jožef Stefan Institute, Department of Biochemistry, Molecular and Structural Biology, Slovenia*
[2]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Slovenia*
[3]*MRC Laboratory of Molecular Biology, Protein and Nucleic Acid Chemistry, Slovenia*
[4]*National Institute of Chemistry, Department of Cheminformatics, Slovenia*
[5]*National Institute of Chemistry, Department of Computational Biochemistry and Drug Design, Slovenia*
[6]*Helmholtz Zentrum for Materials and Energy Berlin, Macromolecular Crystallography Group, Germany*
[7]*Lund University, Synchrotron Radiation Research, Department of Physics, Sweden*

In Gram-positive bacteria, the cell wall is a surface organelle composed of peptidoglycan, secondary polymers and various proteins that determine its structural and functional properties. The cell wall proteins include LPXTG-like proteins covalently anchored to the peptidoglycan and several types of proteins non-covalently attached via cell wall binding domains, which in certain cases form surface protein layers (S-layers). Of the two evolutionary conserved S-layer-anchoring modules composed of three tandem SLH or CWB2 domains (PF04122), the latter have so far eluded structural insight. In *Clostridium difficile* 630, one of the most important nosocomial pathogens, there are 29 cell wall proteins (CWPs), including the major S-layer precursor (SlpA), sharing the CWB2 module. The crystal structures of cell wall proteins Cwp8 and Cwp6 reveal multi-domain proteins, each with embedded triangular, disk-shaped trimer of Rossmann-fold CWB2 domains. Recently, it has been shown that CWB2 domains mediate non-covalent binding to the anionic polymer PSII. The structural and sequence analysis of the CWB2 modules predicts that PS-II binding site resides in the grooves at the upper side of the CWB2 disc. Additional structural insight into the molecular organisation of *C. difficile* cell wall shows partial structural similarity of the N-terminal parts of Cwp8 and SlpA, the most investigated S-layer protein. A comparison with previously reported AFM data of S-layers suggests that *C. difficile* S-layers are complex oligomeric structures, likely composed of several different proteins. Based on the activity of Cwp6, the structural alignment of its C-terminal domain with PDB entries, and the presence of an anomalous signal peak corresponding to Zn, we also conclude that Cwp6 is a zinc-dependent N-acetylmuramoyl-L-alanine amidase of Amidase_3 family (PF01520) that is involved in cell wall remodelling.

SP10

Avidity-mediated specificity in the regulation of the bacterial operon

San Hadži[1], Alexandra Vandervelde[2], Igor Drobnak[1], Remy Loris[2], Jurij Lah[1]

[1]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[2]*Vrije Universiteit Brussel, Department of Structural Biology, Belgium*

How can an intrinsically disordered bacterial repressor with low affinity and low specificity towards its operator sequence achieve efficient transcription regulation? One such example is CcdA antitoxin from *ccdBA* toxin-antitoxin module, which contributes to the antibiotic tolerance in bacteria. CcdA antitoxin is intrinsically disordered repressor unable to bind its operator due to low affinity of interaction. Transcription repression of the *ccdBA* operon is possible only in presence of stoichiometric amounts of CcdB toxin, which enables formation of A-B-A-B chains with high avidity. On the other hand, in excess of CcdB toxin repression is relieved and repressor chains disassociate into more stable B-A-B complex. The multiplicity of binding sites induces a digital on-off switch for transcription, regulated by the toxin:antitoxin ratio. In this system low affinity and specificity of the CcdA repressor for the operator are crucial for the functional regulation of transcription.

SP11

Lipid droplets are antioxidant and pro-survival organelles that protect Ras-driven breast cancer cells from nutrient and lipotoxic stress

Eva Jarc[1,2], Ana Kump[1,2], Petra Malavašič[1], Thomas O. Eichmann[3], Robert Zimmermann[3], Toni Petan[1]

[1]*Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*University of Graz, Institute of Molecular Biosciences, Austria*

The ability of cancer cells to survive stressful environmental conditions is crucial for tumour growth and metastasis. During stress, cancer cells adapt to alternative nutrient sources and modify their metabolism to match nutrient supply. Some of the most aggressive cancer cells, driven by the Ras oncogene, scavenge unsaturated fatty acids from their environment in order to cope with hypoxia and nutrient deprivation. The sources of unsaturated fatty acids, mechanisms of their uptake and the metabolic adaptations that enable fatty acid-induced cancer cell survival are poorly understood. Lipid droplets are newly recognized organelles that store neutral lipids and are central regulators of fatty acid metabolism. They accumulate in cancer cells, particularly in stressful conditions, and enable their survival by providing fatty acids for mitochondrial energy production. In this study, we asked whether lipid droplets induced by different unsaturated fatty acid species differ in their ability to support Ras-driven, triple-negative breast cancer cell survival during nutrient and lipotoxic stress. By targeting the rate-limiting enzyme in lipid droplet breakdown, adipose triglyceride lipase (ATGL), by inhibiting lipid droplet formation and by modulating the unsaturation levels of triglycerides stored in lipid droplets, we show that lipid droplets balance unsaturated fatty acid trafficking with cell survival mechanisms during stress. We found that lipid droplets protect sensitive ω-3 and ω-6 polyunsaturated fatty acids from oxidation by storing them in the form of inert triglycerides, while simultaneously providing fatty acids for mitochondrial energy production and redox homeostasis. Lipid droplets thus emerge as antioxidant and pro-survival organelles that protect cancer cells from stress and are attractive targets for novel therapeutic interventions in triple-negative breast cancer and likely other aggressive Ras-driven cancers.

SP12

Extracellular vesicle-mediated transfer of proinflammatory myddosomal signalling complex in Waldenstrom's macroglobulinemia

Mateja Manček Keber[1], Duško Lainšček[1], Mojca Benčina[1], Jiaji G. Chen[2], Rok Romih[3], Zachary R. Hunter[2], Steven P. Treon[2], Roman Jerala[1]

[1]*National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia*
[2]*Harvard Medical School, Department of Medicine, USA*
[3]University of Ljubljana, *Faculty of Medicine, Institute of Cell Biology, Slovenia*

A link between inflammatory signalling and cancer is particularly strong in Waldenström macroglobulinaemia (WM), a subtype of B-cell lymphoma, wherein almost all patients harbor an activating mutation in the innate immune signalling adaptor MyD88. $MyD88^{L265P}$ constitutively triggers myddosome assembly and activates the signalling pathway thus providing a survival signal to WM cells. We investigated whether mutated MyD88 affects nontumour cells in the tumour`s microenvironment. We showed that MyD88 is present in extracellular vesicles (EVs) shed from WM cells. Importantly, MyD88 transferred via EVs into the cytoplasm of the recipient cells, where it recruited the endogenous MyD88 thus augmenting the activation of the proinflammatory signalling. Internalization of the EVs containing MyD88 was observed *in vivo* with modification of the bone marrow microenvironment, where the main pathology of WM takes place. MyD88-loaded EVs were also detected in the bone marrow aspirates of WM patients thus establishing the physiological role of the EVs for transmission of $MyD88^{L265P}$ and shaping of the proinflammatory microenvironment. Our study identified a mechanism for the transmission of signalling components via EVs as a new paradigm of intercellular communication in WM.

SP13

The structure and characteristics of a whole human genome sequencing data: Application in human disease genetic biomarkers discovery

Jernej Kovač[1], Katarina Trebušak Podkrajšek[1,3], Tadej Battelino[2,3], Magdalena Avbelj Stefanija[2,3]

[1]*University Medical Centre Ljubljana, University Children's Hospital, Unit of Special Laboratory Diagnostics, Slovenia*
[2]*University Medical Centre Ljubljana, University Children's Hospital, Department of Endocrinology, Diabetes and Metabolic Disorders, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Slovenia*

The era of high-throughput sequencing has dramatically changed the diagnostic paradigm of human genetics. From targeted and diagnosis driven sequencing approach it has changed to high-throughput, big data, phenotype association supported analysis of human genome variation. The pinnacle of this revolution being affordable and fast whole human genome sequencing (WGS) generating per nucleotide resolution of a whole human genotype.
Recently, the WGS was utilized to analyse the genetic background of unexplained, maternally inherited, autosomal dominant form of precocious puberty. 4 family trios (father, mother, female sibling), where the disorder was maternally inherited by a sibling had whole genome sequenced. 12 human genomes were sequenced on Illumina HiSeq X10 system generating 112.5GB of data/sample and achieving coverage of 39X/sample on average. 740M reads/sample (99.8% of all reads) were successfully mapped to the reference genome. On average approximately 3.8M of SNVs, 845k of InDels, 570 CNVs and 1500 SVs were detected per sample.
Followed by in-house developed filtering pipeline, involving minor allele frequency filtering (based on the data from ExAc database, autosomal dominant inheritance model filtering, variant consequence and effect prediction algorithms, each family trio dataset was reduced to the potential candidate genes set. Reduced candidate genes sets from all four families were intersected using basic principles of set theory and common elements of all four datasets were evaluated.
A candidate gene, belonging to the family of regulative zinc-finger proteins, emerged as potential novel gene involved in the process of pubertal development in the human and is currently under the process of validation with bigger cohort and other affected family members, potentially followed by a functional validation.

SP14

Methylation and expression of *COMT* and *RELN* in suicide victims: comparison of brain tissue and blood

Katarina Uršič[1], Tomaž Zupanc[2], Radovan Komel[1], Alja Videtič Paska[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Institute of Forensic Medicine, Slovenia*

Suicide is a complex public health problem of global proportions. Suicidal behaviour is a result of interplay between hereditary and environmental factors, tied together by epigenetics. DNA methylation is the most common epigenetic modification in mammals and can result in altered mRNA expression.
Catechol-O-methyl transferase (COMT) is a catecholamine degrading enzyme. Reelin (RELN) is an extracellular matrix glycoprotein with dual role: it is involved in development of embryonic brain, while in adult brain it maintains synaptic plasticity and neurogenesis. Both genes have been previously associated with psychiatric disorders, but so far no such study has been made on Slovenian suicide victims.
The aim of our study was to examine promotor DNA methylation and mRNA expression in 22 suicide victims and 20 controls. Since methylation and expression are tissue specific, both experiments were carried out on blood and two brain tissues. Using NGS technology we sequenced a library, consisting of three amplicons within *COMT* and two amplicons within *RELN* promotor region. To analyse mRNA expression we used a two-step qPCR method.
Our results have shown some differences in methylation between suicide victims and controls. The mirror effect between different tissues has been confirmed by observing a comparable degree of methylation between blood and brain.

SP15

Melanoma antigen genes (MAGEs) at the crossroad of spermatogenesis and cancer: metabolic regulation of male germ cell differentiation

Klementina Fon Tacer[1], Marhiah C. Montoya[2], Melissa J. Oatley[3], Tessa Lord[3], Jon M. Oatley[3], P. Ryan Potts[1]

[1]*St. Jude Children's Research Hospital, Department of Cell and Molecular Biology, USA*
[2]*University of Rochester Medical Center, Department of Pediatrics, USA*
[3]*Washington State University, College of Veterinary Medicine, Centre for Reproductive Biology, USA*

MAGE genes were initially discovered as cancer testis antigens (CTAs), genes that are normally restricted to expression in testis, but are aberrantly activated in cancer. The intriguing association between spermatogenesis and tumorigenesis has been known for over 100 years and intensively pursued for cancer immunotherapy. However, the physiological function of CTAs and how they contribute to oncogenesis still remain understudied. The MAGE family comprises approximately 40 genes in mouse and human, of which the majority are CTAs. To determine the physiological role and molecular function of MAGE CTAs, we first mapped the expression of all MAGEs in 25 human and 55 mouse tissues. We found that MAGE CTAs are restricted to germ cells in the testis and importantly that different MAGE CTAs are enriched in distinct stages of germ cell development, from spermatogonia to haploid spermatids, suggesting that they play unique roles in all stages of spermatogenesis. Functional studies revealed that depletion of MAGE-B4, -B16 and -A genes disrupts the proportion of stem cells in spermatogonial culture and importantly impairs repopulation efficiency after transplantation in mice. Further, we generated a mouse model where 6 out of 8 MAGE-A genes are deleted which resulted in a 30% decrease in male fertility, additionally suggesting the important role of MAGE-A CTAs in spermatogenesis. On a molecular level, we have discovered that MAGE-A3/6 regulates AMPK stability, mTOR signalling and confer resistance to glycolysis inhibition to promote oncogenesis in human cancer cells. Intriguingly, MAGE-As expression in the testis coincides with the metabolic switch of differentiating germ cell suggesting their function in the metabolic adaptation of male germ cells during differentiation. In all, our study reveals a critical role of MAGE CTAs in germ cell development that may provide unique mechanistic insight into germ cell metabolism and the oncogenic potential of MAGE CTA regulated processes.

SP16

Nanobody-based anti-proteomics in glioma research and targeting

Radovan Komel, Ivana Jovčevska, Marko Vidak, Neja Zupanec, Mirjana Liović, Alja Zottel

University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia

Glioblastoma multiforme (GBM) is the most frequent and aggressive primary malignancy of the central nervous system. Patients who receive the best currently available treatment survive on average 15 months after being diagnosed. Namely, surgery usually fails to remove the most malignant cells that exhibit stem-like properties, are resistant to chemo- and radio-therapy and are thus responsible for the rapid relapse of the tumour in the brain regions essential for the survival of patient. A number of proteins have already been proposed as possible GBM stem cell biomarkers; however their ability to differentiate between the normal neuronal stem cells (NSC) and the GBM stem cells (GSC) remains poor.

In order to identify new, more specific GSC biomarker candidates, we applied a differential anti-proteomic approach with nanobodies (Nbs) derived from camelid heavy-chain-only antibodies after alpaca immunization with GSCs/GSC lysate. Differential immunoaffinity screening of the phage-displayed Nb library on the protein extracts from GSC vs. NSC, revealed eight Nbs that target the following intracellular antigens: TRIM28, ACTB/NUCL, VIM, NAP1L1, TUFM, DPYSL2, CRMP1, and ALYREF. In parallel, meta-analysis of GBM transcriptome data from two unrelated datasets from the GEO database exposed two genes (*F2* and *S1*) for cell surface proteins that perfectly comply criteria for GSC markers and were furthermore validated on the protein expression and GBM proteomic levels. Further evidence of their biological potential has come across the identification of the corresponding Nbs from the existing Nb library. Majority of the new proposed GBM/GSC biomarkers proved to be significantly upregulated in the GBM tissue and/or GSC, and, using RNA seq. data from TCGA, some of them were suggested not just as glioma-specific but also as glioma class predictive biomarkers.

References:

1. Jovčevska I., *et. al.* (2017) Oncotarget.
2. Vidak M., *et. al.* (2017) Molecular Cancer, submitted.
3. Jovčevska I., *et. al.* (2014) PLoS One.

SP17

Altered levels of phosphatidylcholines in endometrial cancer

Katja Vouk[1*], Tamara Knific[1*], Špela Smrkolj[2], Jerzy Adamski[3], Tea Lanišnik Rižner[1*]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*University Medical Centre Ljubljana, Department of Obstetrics and Gynecology, Slovenia*
[3]*Helmholtz Zentrum München, Genome Analysis Centre, Institute of Experimental Genetics, Germany*
*contributed equally

Endometrial cancer (EC) is the fourth most common gynecological cancer but single biomarkers or panel of biomarkers that could accurately predict the presence or the extent of this disease, especially in asymptomatic high risk women, are still missing [1]. Since altered metabolism is one of the hallmarks of cancer [2] we adapted a targeted metabolomics approach to evaluate metabolomics changes in EC patients compared to controls.

With electrospray ionization-tandem mass spectrometry we quantified 163 individual metabolites in plasma samples from 61 EC patients and 65 controls. In addition to single metabolites we evaluated 10731 metabolite ratios and 22 variables according to the metabolic subgroup classification. Only three single metabolites (PCaa C40:1, PCaa C42:5, PCaa C42:6) were significantly decreased in EC patients compared to controls ($P < 0.01$). Among metabolite ratios 341 showed significant difference ($P < 0.01$) where about a quarter of ratios also included phosphatidylcholines as a numerator or denominator. With backward step-wise logistic regression selection procedure we selected three metabolite ratios and constructed a diagnostic model that separates patients with EC from controls with an AUC of 0.837, sensitivity of 85.3% and specificity of 69.2%. We also constructed four prognostic models to stratify patients with versus patients without deep myometrial invasion (AUC = 0.844–0.857, sensitivity of 75.0–81.3% and specificity of 72.7–97.7%) and one model to stratify patients with versus patients without lymphovascular invasion (AUC = 0.935, sensitivity of 88.9% and specificity of 84.3%). These models have good diagnostic and excellent prognostic characteristics and have to be validated in a larger group of patients. The changed levels of phosphatidylcholines imply that this group of lipids have important role in EC. The underlining role of the reported alterations in EC remain to be fully elucidated.

References:

1. Lanišnik Rižner T. (2016) Expert Rev Mol Diagn 16 (12), pp. 1315-36.
2. Hanahan D. and Weinberg R. A. (2011) Cell 144 (5), pp. 646-74.

SP18

Higher levels of cathepsins B and X promote epithelial-mesenchymal transition of tumour cells

Ana Mitrović[1,2], Urša Pečar Fonović[2], Janko Kos[1,2]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Pharmacy, Slovenia*

Increased expression and activity of lysosomal cysteine carboxypeptidases cathepsins B and X are associated with a variety of pathological processes, including development and progression of cancer. In cancer, they have an important role in degradation of extracellular matrix, tumour invasion, migration, metastasis and angiogenesis. In addition, epithelial-mesenchymal transition (EMT) has been recognized as an important step during tumour progression, however the role of cathepsins B and X in this process is less known. Therefore, in the present study we investigated the involvement of cathepsins B and X in EMT. Using tumour cell lines differing in expression of epithelial and mesenchymal markers and cell morphology, we show that higher levels of cathepsins B and X promote EMT and are associated with mesenchymal-like cell phenotype. Additionally, a reverse process of mesenchymal to epithelial transition was triggered by their simultaneous knockdown. Among two cathepsins, cathepsin B appears to be stronger promotor of EMT. To gain insight into mechanistic role of cathepsins B and X in EMT, changes in their expression in transforming growth factor β1 (TGF-β1) signalling pathway, one of the key signalling mechanisms during EMT in cancer, were analyzed. While in epithelial breast adenocarcinoma cell line MCF7 cathepsin B expression and activity increased after triggering EMT with TGF-β1, no change in cathepsin X expression was observed. Our data thus define EMT as additional mechanism linking cathepsins B and X with tumour progression and add novel players that could serve as therapeutic targets to impair or revert EMT to less aggressive epithelial-like cell phenotype.

SP19

Paraspeckle-like structures formed on the scaffold of the *C9orf72* hexanucleotide G4C2 intranuclear RNA repeats

Simona Darovic[1], Sonja Prpar Mihevc[1], Ana Bajc Česnik[1], Maja Štalekar[1], Miha Modic[2], Marko Fonović[4], Boris Turk[4], Micha Drukker[2], Christopher E. Shaw[3], Boris Rogelj[1]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*Helmholtz Center Munich, German Research Center for Environmental Health, Institute of Stem Cell Research, Germany*
[3]*King's College London, Institute of Psychiatry, Department of Basic and Clinical Neuroscience, UK*
[4]*Jožef Stefan Institute, Department of Biochemistry and Molecular and Structural Biology, Slovenia*

Expansion of GGGGCC hexanucleotide repeat in the first intron of the *C9ORF72* gene is the most common pathogenic mutation in the families with autosomal dominant frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS) and FTD/ALS. The expanded repeat is transcribed from the sense and the antisense strand and forms intranuclear RNA foci. Here we show that the core paraspeckle proteins SFPQ, NONO and PSPC1 bind to $(G_4C_2)_n$ RNA *in vitro*, co-localise with intranuclear RNA foci in cells transfected with the expanded repeats and to a lesser extent in post-mortem brain tissue. Paraspeckles are nuclear structures with a putative involvement in the gene expression levels and specific RNA retention. They form on a backbone of the long non-coding RNA NEAT1. We demonstrated that the presence of $(G_4C_2)_n$ RNA foci increased the number of SFPQ stained subnuclear bodies. However, only a small fraction of the foci co-localised with paraspeckle platform NEAT1, indicating that $(G_4C_2)_n$ RNA foci lead to nuclear remodelling. Thus, our results suggest that $(G_4C_2)_n$ RNA may lead to the formation of paraspeckle-like structures, which may compete with NEAT1 for the associated proteins and modulate nuclear compartmentalization of the paraspeckle-bound RNAs.

SP20

Multi-omics towards decision making in precision medicine

Theodora Katsila, George P. Patrinos

University of Patras, Department of Pharmacy, Greece

Inter-individual variability has been a major hurdle to optimize disease management. Precision medicine holds promise for improving health and healthcare via tailor-made therapeutic strategies. Herein, we outline the paradigm of pharmacometabolomics-guided pharmacogenomics. We merge pharmacometabolomic and pharmacogenomic data (to address the interplay of genomic and environmental influences) with information technologies to facilitate data analysis as well as sense and decision-making on the basis of synergy between artificial and human intelligence. Humans can detect patterns, which computer algorithms may fail to do so, whereas data-intensive and cognitively complex settings and processes limit human ability. We propose that better-informed, rapid and cost-effective multi-omics studies coupled to information technologies allow for data reproducibility and robustness in genotype-to-phenotype correlations.

SP21

Viral protein Nef and human miRNAs are putative plasma biomarkers of HIV-1 reservoirs in infected individuals with undetectable viral RNA

Jana Ferdin[1,2], Katja Goričar[1], Vita Dolžan[1], Ana Plemenitaš[1], Jeffrey N. Martin[3], Boris M. Peterlin[2], Steven G. Deeks[2], Metka Lenassi[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*University of California San Francisco, Department of Medicine, USA*
[3]*University of California San Francisco, Department of Epidemiology & Biostatistics, USA*

Introduction: Antiretroviral therapy (ART) reduces plasma HIV RNA levels to below the limit of detection in most infected individuals, however virus persists in cellular reservoirs and contributes to diverse disorders. HIV protein Nef is a multifunctional virulence factor that perturbs host intracellular signalling pathways, also affecting miRNAs expression. Nef and miRNAs can both be secreted with exosomes and were previously detected in plasma of HIV-infected individuals. In this study we explored if Nef or miRNAs levels are disrupted in plasma and/or plasma exosomes of aviremic HIV-infected individuals and if their levels correlate with patient's clinical status.
Methods: Levels of Nef and miRNAs (miRNA-150-5p, -223-3p) were measured with ELISA and qPCR, respectively. In total, 134 subjects from the SCOPE cohort were included: 26 uninfected, 28 viremic (non-controllers) and 80 aviremic (38 ART-suppressed (sART), 42 elite controllers (EC)). All subjects were characterized with respect to age, gender, ethnicity, HIV status (CD4+ and CD8+ counts, HIV RNA level) and ART regimen. Statistical analysis was performed using IBM SPSS Statistics (v19.0). All subjects provided informed consent and the parent study was approved by the UCSF Committee on Human Research.
Results: Plasma Nef was detected in 23 non-controllers (Mdn = 11.6 ng/ml), 18 sART (47%; 8.3 ng/ml) and 22 EC (52%; 8.8 ng/ml). Nef levels positively correlated with HIV RNA levels (P = 0.048) in non-controllers and negatively correlated with NNRTI-based ART (P = 0.005) in suppressed subjects. miR-150-5p was more abundantly expressed in plasma exosomes, whereas miR-223-3p was more abundant in whole plasma in HIV-infected individuals. In general, miRNA levels were higher in aviremic subjects compared to non-controllers.
Conclusion: Nef is present in plasma of half of aviremic HIV-infected individuals and correlates with ART-regimen. HIV infection affects human miRNAs expression and packaging for extracellular release.

SP22

Network modelling of integrated prior knowledge and time-stamped gene expression data of potato immune signalling

Špela Baebler[1], Živa Ramšak[1], Anna Coll[1], Tjaša Stare[1], Oren Tzfadia[1,2,3], Kristina Gruden[1]

[1]*National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia*
[2]*VIB, Department of Plant Systems Biology, Belgium*
[3]*Ghent University, Department of Plant Biotechnology and Bioinformatics, Belgium*

Plant-pathogen interaction causes reprogramming of a complex signalling network, resulting in gene activity and metabolic changes. Understanding the mechanisms and dynamics involved is crucial for the development of efficient crop protection strategies and agricultural practices. The aim of our study was to efficiently use different existing knowledge sources and experimental datasets to create large knowledge network and in next step, through network modelling, generate new hypotheses on mechanisms of plant immune signalling.

We built our approach on manually constructed plant immune signalling model and complemented it with the information from the publically available high-throughput experimental datasets, namely on protein-protein interactions, transcriptional regulation and miRNAs regulation. Additionally, datasets describing the interaction between viral and plant components were included. The resulting large knowledge network was translated from the model plant *Arabidopsis* to potato based on orthologues relationship. We have further superimposed this knowledge network with the experimental data inferred networks generated from our time series transcriptomics data on potato-*Potato virus Y* interaction using an ensemble of three different algorithms. Additionally, we have improved our plant immune signalling model by inferring several novel connections between its components. The most interesting finding, however, came from the shortest path analysis searching for novel cross-talks between different hormonal signalling modules. Using this approach we found that the expression of the *NPR1* gene should be modulated by the ethylene. Indeed, we confirmed this hypothesis by wetlab experiments and thus identified one string of molecular events supporting studies which showed ethylene potential to modulate plant immune response.

Taken together, we here show that network modelling is a powerful tool for unravelling molecular mechanisms in complex systems.

SP23

Small RNAs regulatory networks — linking developmental and immune signalling in potato

Maja Križnik[1,2], Marko Petek[1], David Dobnik[1], Živa Ramšak[1], Špela Baebler[1], Stephan Pollmann[3], Jan Kreuze[4], Jana Žel[1], Kristina Gruden[1]

[1]*National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*Universidad Politécnica de Madrid (UPM)-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Centro de Biotecnología y Genómica de Plantas, Spain*
[4]*International Potato Center, Global Program of Integrated Crop and Systems Research, Peru*

Potato virus Y (PVY) is the most economically important potato viral pathogen worldwide. We aimed at unraveling the roles of small RNAs (sRNAs) in the complex immune signalling network controlling the establishment of tolerant response of potato cv. Désirée to the virus. We identified and quantified the endogenous miRNAs and phasiRNAs as well as virus-derived sRNAs. We constructed a sRNA regulatory network connecting sRNAs and their targets identified to link sRNA level responses to physiological processes. We discovered an interesting novel sRNAs-gibberellin (GA) regulatory circuit being activated as early as 3 days post inoculation, before viral multiplication can be detected. Increased levels of miR167 and phasiRNA931 were reflected in decreased levels of transcripts involved in GA biosynthesis. Moreover, decreased concentration of GA confirmed this regulation. We have additionally showed that this regulation is salicylic acid dependent as the response of sRNA network was attenuated in salicylic acid-depleted transgenic counterpart NahG-Désirée expressing severe disease symptoms. Moreover, we found that differentially expressed miR6022, which targets leucine-rich-repeat receptor-like kinases, likely links GA signalling with immune responses. Besides downregulation of GA signalling, regulation of several other parts of sRNA network in tolerant Désirée revealed striking similarities to responses observed in mutualistic symbiotic interactions. The intertwining of different regulatory networks revealed here shows how developmental signalling, symptomology and stress signalling are balanced.

SP24

LiverSex: First computational model to investigate an interplay between sex hormones and liver

Tanja Cvitanović[1], Žiga Urlep[1], Miha Moškon[2], Miha Mraz[2], Damjana Rozman[1]

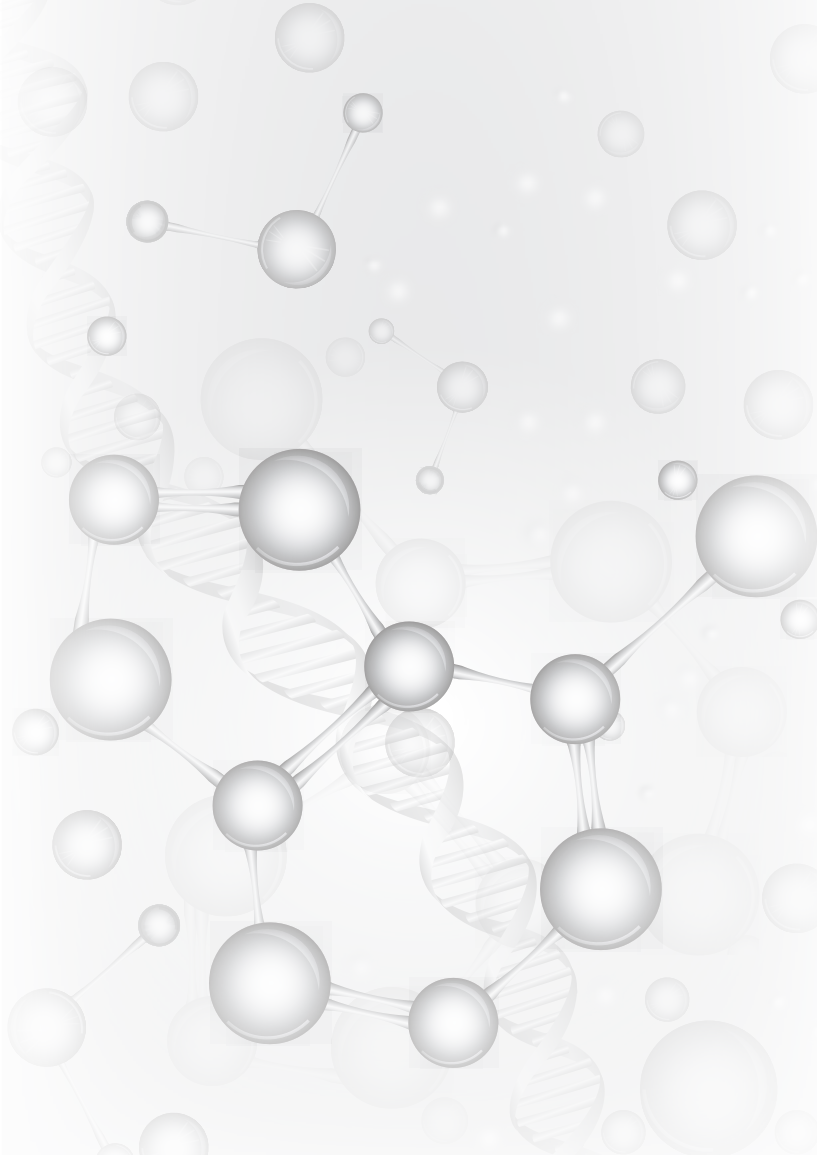
[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Centre for Functional Genomics and Bio-chips, Slovenia*
[2]*University of Ljubljana, Faculty of Computer and Information Science, Slovenia*

SteatoNet is an intertissue human metabolic computational model focused on understanding the complex metabolic disorders such a non-alcoholic fatty liver disease (NAFLD). *SteatoNet* accounts for interactions of the liver with peripheral tissues and includes metabolic, gene regulatory and signal transduction pathways. Due to its object-oriented nature, it can be easily adapted to liver-associated pathologies. Moreover, it can be used to perform dynamical simulations even in the case of sparse experimental data (Naik *et. al.*, *PLOS Comput.Biol.* 2014; Cvitanović *et. al.*, *Hepatology* 2017).
In our recent work, mouse models with conditional *Cyp51* liver knockout were produced to investigate the consequences of the disrupted hepatic cholesterol synthesis on the whole body homeostasis (Lorbek *et. al.*, *SciRep.* 2015; Urlep *et. al.*, *SciRep.* 2017). Liver is a sexually dimorphic organ (especially on the gene expression level) and gender-based differences were discovered also in the experimental *Cyp51* liver knockout responses. *LiverSex* presents a *SteatoNet* adaptation to differentiate between genders. Literature and expert based knowledge were used for its reconstruction. *LiverSex* allows us to simulate different sex hormone ratios in the blood and observe their relations with cholesterol synthesis and regulatory nodes in female and male livers. To the best of our knowledge, *LiverSex* represents the first gender-based liver metabolic model. The model has a potential to be developed further into diagnostic, target identification, predictive and analytical tool. It can potentially predict the network effects of polymorphisms associated with liver-related pathologies.

Poster abstracts

Poster Session I

Session	Posters
Cell signalling and membranes	PI-1 to PI-8
Biotechnology and bionanotechnology	PI-9 to PI-25
FEBS3+ session	PI-26 to PI-34
Protein structure and function	PI-35 to PI-49
Protein interactions and networks	PI-50 to PI-56



PI-1

The role of cystatin C in inflammasome activation and endotoxemia

Monika Biasizzo[1], Mojca Trstenjak-Prebanda[1], Janja Završnik[1], Anders Grubb[2], Boris Turk[1], Nataša Kopitar-Jerala[1]

[1]*Jožef Stefan Institute, Department of Biochemistry, Molecular and Structural Biology, Slovenia*
[2]*Lund University, Division of Clinical Chemistry and Pharmacology, Sweden*

Innate immune system is responsible for rapid recognition and elimination of microbial pathogens and damaged cells. Pattern-recognition receptors, such as TLR receptors and NLR receptors, recognize pathogen-associated molecular pattern, PAMP and trigger downstream signalling pathways that result in the expression of inflammatory genes to fight infection. Activation of NLR receptors triggers inflammasome assembly that mediates caspase-1-dependent processing of inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18. Cystatin C is a potent cysteine protease inhibitor that plays an important role in various biological processes including cancer, cardiovascular diseases and neurodegenerative diseases. It is ubiquitously expressed and secreted from various cell types and is abundant in body fluids.

In the present study we demonstrated that cystatin C-deficient mice were significantly more sensitive to the lethal LPS-induced sepsis. We demonstrated that pro-caspase-11 and pro-IL-1β are up-regulated in cystatin C-deficient bone marrow-derived macrophages (BMDMs) upon LPS stimulation. We examined the role of cystatin C deficiency in inflammasome activation and release of pro-inflammatory cytokines upon Nlrp3 inflammasome activation in BMDMs and showed that cystatin C-deficient BMDMs secrete significantly higher amounts of pro-inflammatory cytokine IL-1β due to increased caspase-1 and -11 activation upon Nlrp3 inflammasome activation. Our results revealed that cystatin C-deficient mice BMDMs have increased cathepsin activity in the cytosol during upon the activation of Nlrp3 inflammasome. Upon LPS stimulation cystatin C was secreted from the cells and the lack of cystatin C resulted in the increased destabilization of mitochondrial membrane potential and mitochondrial superoxide generation.

Collectively, our study demonstrates that the LPS-induced sepsis in cystatin C-deficient mice is dependent on increased caspase-11 expression and activation.

PI-2

Nucleosides block AICAR-induced AMPK activation in cultured skeletal muscle and cancer cells

Klemen Dolinar[1], Mojca Pavlin[2,3], Alexander V. Chibalin[4], Sergej Pirkmajer[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Laboratory for Molecular Neurobiology, Slovenia*
[2]*University of Ljubljana, Faculty of Electrical Engineering, Group for nano and biotechnological applications, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Biophysics, Slovenia*
[4]*Karolinska Institutet, Integrative Physiology, Department of Molecular Medicine and Surgery, Sweden*

AMP-activated protein kinase (AMPK) is a cellular energy sensor that is activated during energy stress. Once activated, AMPK stimulates energy producing catabolic pathways and inhibits energy consuming anabolic pathways and cell proliferation. These effects make AMPK an attractive target for the treatment of type 2 diabetes and cancer. The most commonly used pharmacological AMPK activator is AICAR. AICAR is an adenosine analogue and is taken up by the cells via nucleoside transporters. Once inside the cell, AICAR enters nucleoside metabolic pathways. It has been shown that nucleosides can inhibit AICAR uptake. This observation raises a question about the efficiency of AICAR in cells cultured in media containing nucleosides.

We investigated whether nucleosides in cell culture media can affect the ability of AICAR to activate AMPK, stimulate glucose uptake and inhibit cell proliferation. We also investigated whether nucleosides affect the expression of genes for transporters and enzymes involved in transport and metabolism of AICAR. Experiments were performed on skeletal muscle cells and breast cancer cells. Nucleosides inhibited AICAR-induced activation of AMPK in both skeletal muscle and breast cancer cells. In addition, nucleosides inhibited AICAR-stimulated glucose uptake in skeletal muscle cells and AICAR-inhibited proliferation of cancer cells. Nucleosides increased expression of one of the transporters for AICAR but had no effect on the expression of enzymes involved in the metabolism of AICAR.

In conclusion, we show that nucleosides in cell culture media can affect the effects of AICAR. Nucleosides could also affect the effects of other nucleoside-based drugs. As media composition can affect the response of cells to pharmacological substances, it should be considered during the design of the experiment as well as during the interpretation of the results.

PI-3

Effect of innervation on the expression of subunits of Na^+/K^+-ATPase and its regulators from the FXYD family

Vid Jan[1], Katarina Miš[1], Matej Podbregar[1,2], Tomaž Marš[1], Alexander V. Chibalin[3], Sergej Pirkmajer[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[2]*University Medical Centre Ljubljana, Clinical Department for Anesthesiology and Surgical Intensive Care, Slovenia*
[3]*Karolinska Institutet, Integrative Physiology, Department of Molecular Medicine and Surgery, Sweden*

Na^+/K^+-ATPase (NKA) is a P-type ATPase, which transports 2 K^+ into the cell and 3 Na^+ from the cell against their concentration gradients. NKA is a heterodimer that comprises the catalytic α-subunit (isoforms α_{1-4}) and the glycoprotein β-subunit (isoforms β_{1-3}). The α_2-subunit, which is the predominant α-isoform in skeletal muscle, is particularly important for maintaining ion homeostasis, while the α_1-subunit performs housekeeping functions under resting conditions. The predominant β-isoforms in skeletal muscle are β_1 and β_2. FXYDs are a family of small transmembrane proteins that regulate NKA. Among these, FXYD1 is the most important regulator of NKA in skeletal muscles, while FXYD5 also likely plays a role, especially in periods of inactivity. Whether and how innervation affects expression of NKA subunits and its FXYDs during myogenesis has not been established.

We studied a possible role of innervation in the expression of NKA subunits and FXYD regulators using a co-culture of human myotubes and segments of rat embryonic spinal cord. Innervation induced further development of cultured myotubes, which developed cross-striations and started to contract spontaneously. After three weeks of co-culture mRNA levels for NKA α_1-, α_2-, β_1- and β_3-subunits as well as FXYD1 and FXYD5 remained unaltered between innervated and non-innervated myotubes. Conversely, innervation led to 3-fold increase in expression of the NKA α_3-subunit and to more than 100-fold increase in expression of the NKA β_2-subunit.

Taken together, our results show that innervation of cultured human myotubes has an isoform-specific effects on the expression of NKA subunits. Increased expression of the α_3-subunit and β_2-subunit might be due to effects of innervation *per se* and/or due to innervation-induced differentiation of cultured myotubes.

This study was supported by ARRS (P3-0043, J7-8276 and Young researcher programme).

PI-4

Lipid droplets are involved in eicosanoid generation and protection against nutrient stress in breast cancer cells

Eva Jarc[1,2], Ana Kump[1,2], Petra Malavašič[1], Anja Pucer Janež[1], Vesna Brglez[1], Thomas O. Eichmann[3], Robert Zimmermann[3], Toni Petan[1]

[1]*Institut Jožef Stefan, Department of of Molecular and Biomedical Sciences, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*University of Graz, Institute of Molecular Biosciences, Austria*

Lipid droplets (LDs) are dynamic organelles composed of a neutral lipid core of triacylglycerols (TAG) and cholesterol esters surrounded by a phospholipid monolayer embedded with proteins. LDs are present in most cells, but they accumulate in various cancers and are important for the trafficking of exogenous and endogenous fatty acids (FAs). Secreted phospholipase A_2 ($sPLA_2$) enzymes release unsaturated FAs from plasma membranes of breast cancer (BC) cells and induce the formation of LDs, which are enriched with polyunsaturated FAs (PUFAs) and enable cell survival during nutrient stress. LD breakdown is crucial for protection against starvation-induced cell death, but its molecular mechanism is not clear. The rate limiting enzyme in LD breakdown is the TAG lipase adipose triglyceride lipase (ATGL). Recent studies have shown that ATGL is involved in the transfer of FAs from LDs to mitochondria, but it also mediates the synthesis of eicosanoids, pro-inflammatory and pro-tumourigenic lipid mediators. The aim of this study was to examine the role of ATGL in supplying LD-derived (PU)FAs for cell survival during stress and for the synthesis of eicosanoids in BC cells. We found that ATGL depletion reduced TAG lipolysis, LD breakdown and cell survival during prolonged serum deprivation, but, surprisingly, it was not necessary for $sPLA_2$- and unsaturated FA-induced cell survival. Conversely, we found that ATGL depletion led to a significant suppression of $sPLA_2$- and arachidonic acid-induced prostaglandin E_2 synthesis in BC cells. Our results suggest that TAG lipolysis is not necessary for LD-induced cell survival during starvation, but reveal a novel role for LDs and ATGL in enabling the production of pro-tumourigenic eicosanoid signalling molecules. Thus, LDs are metabolic and signalling hubs that modulate cancer cell growth and survival and are emerging targets for the development of new therapeutic strategies for breast cancer.

PI-5

Optimizing the isolation of extracellular vesicles from *Saccharomyces cerevisiae* culture

Tilen Konte[1], Špela Petelin[1], Simona Sitar[2], Samo Hudoklin[3], Ema Žagar[2], Peter Veranič[3], Ana Plemenitaš[1], Metka Lenassi[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*National Institute of Chemistry, Laboratory for Polymer Chemistry and Technology, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Cell Biology, Slovenia*

Introduction: An increasing body of scientific data point to chronic microbial infections as potential risk factors in neurodegenerative diseases. For the transport of molecules across the cell wall, fungi use extracellular vesicles (EVs), which may also act as virulence factors in fungal infectivity and pathogenicity. It has been proposed, that EVs secreted during infection could mediate immune-response modulation and host cell damage, however, the literature on fungal EVs isolation and characterization is scarce. Our aim was to optimize the method for isolation of EVs on the model *S. cerevisiae* culture for the future use of this method in studies of the possible role of EVs from selected polyextremotolerant fungi (model opportunistic pathogens) in neurodegenerative diseases.
Methods: The *S. cerevisiae* cultures were inoculated at different ODs and grown overnight or until the specific OD was reached. The ratio of dead versus live cells was determined before the isolation of EVs. With first centrifugations, cells were removed and supernatant was concentrated using ultrafiltration spin columns. The concentrate was ultracentrifuged twice at 100.000 x g for 1 hour and the pellet was resuspended in DPBS. Transmission electron microscopy (TEM) was used to confirm the presence of EVs and inspect their morphology. The concentration and size distribution of EVs was determined with Asymmetric-flow field-flow fractionation (AF4), coupled to a multidetection system (UV-MALS).
Results: We could not detect any measurable amount of EVs after isolation from the culture grown from OD 0.2 to 0.6. EVs were detected only after the cultures were grown overnight. With inoculations between ODs 0.015 and 0.1 and overnight growth, we were able to keep the amount of dead cells below 20%. TEM images confirmed the presence of vesicle-like spherical cup-shaped particles. The concentration of EVs, as measured by AF4, ranged from 10^{11} to 10^{12} particles/mL. Isolated EVs had diameters of 40 to 180 nm, with the average of 154 nm.
Conclusions: With the optimized protocol for EVs isolation from *S. cerevisiae*, we are able to harvest concentrations of vesicles comparable to isolations from human cell lines, but the average diameter of *S. cerevisiae* EVs is smaller than human EVs.

PI-6

Preconditioning with metformin attenuates ischemia-induced activation of HIF-1 in cultured human myotubes

Urška Matkovič[1], Nives Škorja[1], Katarina Miš[1], Matej Podbregar[1,2], Tomaž Marš[1], Sergej Pirkmajer[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[2]*University Medical Centre Ljubljana, Clinical Department for Anaesthesiology and Surgical Intensive Care, Slovenia*

Preconditioning with metformin, the most frequently used oral antidiabetic drug, protects heart and brain against ischemia-reperfusion injury. Whether metformin might also protect skeletal muscle has not been established. We determined whether metformin modulates ischemia-induced responses in cultured human myotubes

Human myotubes were pretreated with metformin for 3 days and then exposed to artificial ischemia (serum starvation, glucose-free medium, 0.1% O_2) for 2 hours. We assessed ischemia-induced activation of the hypoxia-inducible factor-1 (HIF-1), a heterodimeric (HIF-1α/HIF-1β) transcription factor that plays a major role in regulation of oxygen homeostasis. Pretreatment with metformin markedly decreased expression of HIF-1α, the oxygen-sensitive subunit of HIF-1, in ischemic myotubes. Expression of HIF-1β, the constitutive subunit of HIF-1, remained unaltered. Ischemia-induced up-regulation of vascular endothelial growth factor (VEGF) and phosphoglycerate kinase 1 (PGK1), downstream target genes of HIF-1, was blocked by the metformin pretreatment.

We also assessed effects of metformin on the the mTOR pathway, which is involved in regulation of HIF-1α expression. We measured phosphorylation of the AMP-activated protein kinase (AMPK, Thr172), a negative mTOR regulator, as well as phosphorylation of positive mTOR regulators ERK1/2 (Thr 202/204) and Akt (Ser 473). In ischemic myotubes metformin increased phosphorylation of the AMPK and decreased phosphorylation of Akt and ERK1/2, which would tend to suppress the mTOR pathway. Consistent with suppression of mTOR, metformin reduced phosphorylation of the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1, Thr 37/46), a major downstream effector of mTOR.

Taken together, our results show that pretreatment with metformin attenuates ischemia-induced activation of HIF-1. The underlying mechanism likely involves suppression of the mTOR pathway.

PI-7

Approaching the elucidation of the protein-membrane lipid interactions through *in vitro* evolution

Aleksandra Šakanović, Marjetka Podobnik, Gregor Anderluh

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

Biological membranes are essential component of all living cells. Through interactions with different molecules, they are enabling the course of many biological processes and shape the physiology of cells. Binding of proteins to membrane lipids regulates the structure, dynamics and morphology of cell membranes. During initial binding to biological membranes some proteins are able to specifically recognize a particular component of the membrane. For instance, cholesterol, an essential component of mammalian cell membranes, is recognized by many different bacterial protein toxins, including members of cholesterol dependent cytolysins (CDC) protein family. The precise mechanism of initial attachment and cholesterol recognition of CDC toxins is yet unknown.

The aim of this study is to select protein domains which could be used for specific targeting of some lipids in eukaryotic membranes. The membrane-binding domain of perfringolysin O, a member of CDC, was used for directed evolution. Ribosome display combined with high-throughput DNA sequencing was used to estimate the role of particular amino acids in cholesterol recognition mechanism. Conducted analysis of the frequencies of amino acids in the sequenced gene library before and after the selection procedure points toward the possibility that the recognition of the membrane cholesterol might be carried out by several particular amino acids residues. Furthermore, the most abundant motifs after selection with 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 in five of the seven randomized positions. On the contrary, no selection of protein motif occurred when model membrane system with atypical sterol compositions was used.

PI-8

Cytotoxicity of oximes tested as antidotes in organophosphorus compound poisoning

Antonio Zandona, Tamara Zorbaz, Zrinka Kovarik, Maja Katalinić

Institute for Medical Research and Occupational Health, Croatia

Organophosphorus compounds (OPs) belong to a large group of compounds that include nerve agents, pesticides, industrially fire-resistant hydraulic fluids, coolants and lubricants. OPs cause irreversible inhibition of enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), thereby preventing the degradation of acetylcholine, essential in neurotransmission, which consequently leads to cholinergic crisis and death. Only compounds known as oximes have shown the ability to reactivate inhibited ChEs and are therefore used as antidotes in cases of OP poisoning. Development of more efficient oximes is currently an ongoing endeavor worldwide. However, little is known about possible effects on the cellular level of new oximes as well as about the adverse effects they could possibly induce. To improve the selection of a lead candidate for preclinical antidote development, we performed a set of cell-based assays with several oximes showing desirable ChE reactivation kinetics. We monitored cell viability and induction of reactive oxygen species (ROS) in various cell lines upon exposure to the selected oximes. Several of the newly-developed oximes significantly influenced cell viability and induced ROS changes in concentrations relevant for reactivation studies ($IC_{50} \leq 300$ μM). The results were compared to the results obtained for oximes HI-6 and 2-PAM, currently used in practice, which did not show any cytotoxic effect or ROS induction within the studied concentration range (≤ 800 μM and 400 μM, respectively). Although the exact mechanism of the observed effects of the tested oximes is not clear and will be in the focus of our future research, such an unwanted effect on cells presents a major drawback to their further development as potential antidotes.

Acknowledgments: This work was supported by the Croatian Science Foundation grant no. 4307.

PI-9

Plasmids for inducible dual protein expression and CRISPR/Cas9 genetic modification in *Lactococcus lactis*

Aleš Berlec[1], Janja Kocjan[1], Maria Olenic[1], Katja Škrlec[1], Borut Štrukelj[1,2]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Pharmacy, Slovenia*

Lactic acid bacteria (LAB) have received considerable attention in recent years due to their long history of safe usage in food, and their health-promoting effects as probiotics. Recombinant LAB have been engineered for therapy, with most applications aimed at the delivery of antigens or biologicals to the human intestine. *Lactococcus lactis* is a model lactic acid bacterium with relatively well established techniques for genetic modification. However, novel and improved techniques are still needed.

Nisin-controlled expression system is used for inducible expression of proteins in *L. lactis*. In the current research pNZDual plasmid with two nisin promoters and multiple cloning sites (MCSs) has been prepared from plasmid pNZ8148. Alternatively, pNZPolycist plasmid has been constructed with a single nisin promoter and two MCSs, separated by ribosome binding site (RBS), enabling translation from polycistronic mRNA. The expression efficacy was tested with two model proteins, infrared fluorescent protein (IRFP) and human IgG-binding DARPin. Genes for both model proteins were cloned in all possible combinations. The expression levels of both proteins with pNZDual were similar, albeit lower than that achieved with a single protein expression plasmid pNZ8148. Expression of model proteins with pNZPolycist resulted in higher expression of model gene that was cloned immediately downstream of the promoter.

Inducible dual protein expression system was adapted for the development of a single-plasmid CRISPR/Cas9 system. Thereby, pNZCRISPR plasmid was constructed, in which one of the nisin promoters was used to drive Cas9 expression and the other sgRNA transcription. Different sgRNAs were cloned into pNZCRISPR and their efficacy was confirmed by observing the changes in bacterial growth and survival.

The present study introduces novel techniques for advanced genetic modification of LAB *Lactococcus lactis.*

PI-10

ALF: A novel genome walking method for detection of unknown/unauthorised genetically modified organisms (UGMOs)

Alexandra Bogožalec Košir[1,2], Alfred J. Arulandhu[3,4], Marleen M. Voorhuijzen[3], Hongmei Xiao[5], Rico Hagelaar[3], Martijn Staats[3], Adalberto Costessi[6], Jana Žel[1], Esther J. Kok[3], Jeroen P. van Dijk[3]

[1]*National Institute of Biology, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*RIKILT Wageningen UR, The Netherlands*
[4]*Wageningen UR, Food Quality and Design Group, The Netherlands*
[5]*Nanjing Agricultural University, College of Food Science and Technology, China*
[6]*BaseClear, The Netherlands*

With the increased use of authorized genetically modified organisms (GMOs) in industrial countries, especially in feed products, the possibility of intentional or unintentional presence of unauthorized or unknown GMOs (UGMOs) is also on the rise. A number of strategies have been developed that focus on the detection of UGMOs in the recent years. However, there is a lack of specific detection methods, due to the absence of detailed sequence information and reference materials. In our research, a novel genome walking approach was developed, called ALF: Amplification of Linearly-enriched Fragments. With coupling of ALF to next generation sequencing (NGS) we aim for simultaneous detection and identification of all GMOs, including UGMOs, in one sample, in a single analysis. The ALF approach was assessed on a mixture made of DNA extracts from four reference materials, in an uneven distribution, mimicking a real life situation. The complete insert and genomic flanking regions were known for three of the included GMO events, while for the GMO event MON15985 only partial sequence information was available. Since the organisation of elements was known, this GMO served as a model for a UGMO. Sequences matching with the known organisation of elements were successfully identified, serving as proof of principle for ALF as new UGMO detection strategy. Additionally, this study provides a first outline of an automated, web-based NGS data analysis pipeline for the identification of UGMOs containing known GM elements.

PI-11

Combining different analytical methods for best characterization of viral vectors for gene therapies

David Dobnik, Polona Kogovšek, Magda Tušek Žnidarič, Denis Kutnjak, Maja Ravnikar

National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia

Viral vectors have emerged as safe and effective delivery vehicles for clinical gene therapy. Manufacturing of clinical grade vectors requires knowledge of the complex methods needed to generate, purify and characterize viral vectors. One of the most problematic steps is the characterization of viral vectors in different production phases during process development. Molecular methods such as quantitative real-time PCR (qPCR) and digital PCR (dPCR) enable relative and absolute quantification, respectively, of target DNA or RNA. qPCR is, due to its capability to detect nucleic acids in traces, widely used for detection and relative quantification of various targets, from viral nucleic acids, to residual DNA. We have coupled qPCR with high throughput DNA extraction system to enable fast and accurate quantification of the host cell DNA. Our experiments have shown that dPCR is less sensitive to impurities originating from sample matrix, and is the method of choice for direct virus quantification in process development (up- or down-stream samples). We have shown that dPCR is accurate, robust and repeatable in qualified or validated assays (coefficient of variation usually below 10%). It is especially reliable for absolute quantification of rare nucleic acid targets of interest in the presence of high background of other targets. We introduced MinION sequencing technology in for purpose of whole genome sequencing and confirmation of the uniformity of virus/vector sequence. To complement results of molecular methods, we use electron microscopy (EM) for direct observation and counting of virus particles. With EM analysis we evaluated viral structure, presence of full and empty/damaged/broken capsids and presence of impurities in various intermediate samples. Performance of different methods for characterization of viral vectors with will be presented in light of different sample matrices and the combinations of methods used.

PI-12

Engineering a pH-responsive pore-forming protein listeriolysin O

Matic Kisovec, Saša Rezelj, Primož Knap, Miša Mojca Cajnko, Marjetka Podobnik, Gregor Anderluh

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

Listeriolysin O (LLO) is a cytolysin capable of forming pores in cholesterol-rich lipid membranes of host cells. It is one of the main virulence factors of an intracellular food-borne pathogen bacteria *Listeria monocytogenes*. *L. monocytogenes* causes listeriosis which is a serious disease of immunocompromised patients, elderly citizens, small children and pregnant women. In worst cases it causes meningoencephalitis and death. Pore-forming toxins have recently been successfully engineered and used in different nanobiotechnological applications such as DNA sequencing, bio-sensing and drug delivery. With protein engineering it is possible to change specificity, improve efficiency and change biochemical properties to attain better suited proteins for use in biotechnology.

pH-dependent stability of LLO is controlled by the so called pH sensor. This triad of acidic amino acid residues can cause premature unfurling of transmembrane regions at neutral pH and temperatures above 30 °C. Can we make LLOs pore-forming activity also pH-dependent?

Based on the known structure of LLO monomer we mutated the tyrosine 406 to alanine, as it is located in the interface of two domains and close to the pH sensor. We used molecular dynamics simulations to check for any effects of such a mutation and noticed significant differences in pKa values of the residues from the pH sensor. The Y406A mutant has similar pore-forming activity as the wild-type LLO at low pH (~ 5.7) but its permeabilising activity diminishes quickly above pH 6.5.

Such an engineered variant could be used in nanobiotechnological applications such as controlled release of substances in delivery scenario or as a sensor of environmental pH. We showed that the Y406A mutant can, in a pH-regulated manner, release fluorescent marker from artificial liposomes and *in vitro* perforate Caco-2 cells.

PI-13

Comparison of two deglycosylation methods for N-glycan analysis of therapeutic proteins

Tina Kljun, Mojca Beseničar, Tilen Praper

Lek d.d., BTDM Mengeš, Biofarmacevtika, Razvojna analitika, Slovenia

A majority of biopharmaceutical drugs are glycoproteins (monoclonal antibodies, fusion proteins, blood proteins...). Glycosylation is one of the most important post-translational modifications, it has a great impact on solubility, folding, stability, pharmacokinetics, pharmacodynamics and safety (e.g. immunogenicity). Due to its various functional implications, glycosylation represents a Critical Quality Attribute (CQA) and therefore requires close monitoring during bioprocess development and routine manufacturing.
A classical approach to N-glycan analysis is to remove N-linked glycans from the protein prior to analysis using an enzyme N-glycosidase F. Once released, the glycans are derivatized with a fluorophore and separated chromatographically. Enzymatic deglycosylation step is the most critical step during the entire glycan preparation as the total removal of N-glycans is essential for reliable results.
In our study we have tested the deglycosylation of several monoclonal antibodies (mAbs) and fusion proteins. We have compared two different techniques for deglycosylation — the classical one (in-solution deglycosylation) and high-throughput method (deglycosylation on solid surface). The efficiency of both methods was verified and compared with capillary electrophoresis and by glycan mapping.

PI-14

Cholesterol-dependent binding to archaeosomes by cytolysin listeriolysin O

Mirijam Kozorog[1], Tomaž Švigelj[1], Saša Rezelj[1], Nataša Poklar-Ulrih[2], Gregor Anderluh[1]

[1]*National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology, Slovenia*
[2]*University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Slovenia*

Listeriolysin O (LLO) is the main virulence factor of the Gram-positive pathogenic bacteria *Listeria monocytogenes*. The bacteria causes listeriosis, which is animal and human disease that starts with the ingestion of contaminated foods. LLO belongs to a group of cholesterol-dependent cytolysins (CDCs) that need cholesterol for efficient membrane binding and subsequent pore-formation. CDCs form large pores that allow release of encapsulated substances or entry of various molecules into the cells. Liposomes made from archaeal lipids, termed archaeosomes, may represent an interesting delivery system due to their high stability. In order to allow interactions with CDCs we have included cholesterol in archeosomes. Inclusion of cholesterol increased their thermal stability. CDCs LLO and its homologe perfringolysin O (PFO) binded cholesterol-rich archaeosomes and also retained their pore-forming activity. Interestingly, we also observed specific binding of LLO to archaeosomes even in the absence of cholesterol, which was not observed before. These results indicate a potential use of CDC-archaeosomes as a delivery system in various nanobiotechnological applications.

PI-15

Peptide ligands to the Fc portion of immunoglobulin G for use in affinity chromatography

Nika Kruljec[1], Peter Molek[1], Borut Štrukelj[1,2], Tomaž Bratkovič[1]

[1]*University of Ljubljana, Faculty of Pharmacy, Slovenia*
[2]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*

In the last decades, affinity chromatography based on bacterial immunoglobulin (Ig)-binding proteins has been adopted as the initial capture step of choice in therapeutic antibody purification process. However, this approach has some misgivings related to relatively high costs, limited ligand stability at column cleaning and sanitization conditions, and concern over the product contamination with leached ligands. Short peptides represent potential alternative affinity ligands to natural immunoglobulin-binding proteins as they display higher stability and are less expensive to produce. Furthermore, the expected lower affinity for immunoglobulins should allow for elution under milder conditions, preserving antibody structure and function. The aim of our research was to identify novel short peptide ligands for the Fc region of human IgGs.
We have screened three commercially available phage display libraries of random cyclic and linear peptides for binding to the human Fc region in solution using an optimized biopanning approach. Five selected non-homologous linear peptides were shown to specifically interact with different subclasses of immunoglobulins as verified by a set of phage ELISA assays. Individual phage-displayed peptides were able to recognize specific subclasses of IgG. The highest-affinity peptide (12L-19Fc), which competed for Fc binding with protein A, was subjected to mutagenesis studies. The results revealed the minimal binding motif and the minimal length of the peptide ligand. Binding characteristics of the minimized peptide were further analysed using SPR biosensor. The peptide was immobilized onto a stationary phase to produce affinity matrix and its binding characteristics were evaluated. The details will be disclosed at the meeting.

PI-16

Advancing plant synthetic biology by developing Plant X-tender for the assembly, delivery and expression of multigene constructs in plants

Tjaša Lukan[1], Fabian Machens[2], Anna Coll[1], Špela Baebler[1], Katrin Messerschmidt[2], Kristina Gruden[1]

[1]*National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia*
[2]*University of Potsdam, Cell2Fab Research Unit, Germany*

Cloning multiple DNA fragments for delivery of several genes of interest into the plant genome is one of the main technological challenges in plant synthetic biology. Despite several modular assembly methods developed in recent years, the plant biotechnology community has not widely adopted them yet, due to the lack of structured plant biological parts libraries, appropriate vectors and software tools. Here we present Plant X-tender, an extension of the AssemblX, consisting of a set of plant expression vectors, protocols for most efficient cloning into the novel vectors and a set of genetic parts for plant expression. The novel vector set covers different backbones and selection markers to allow full design flexibility. We have included *ccd*B counterselection, thereby allowing the transfer of multigene constructs into the novel vectors in a straightforward and highly efficient way. Vectors are available as empty backbones and are fully flexible regarding the orientation of expression cassettes and addition of linkers between them, if required. In addition, we have extended the plant grammar implemented in GenoCAD, facilitating easier design of complex constructs. We optimised the assembly and subcloning protocol by testing different commercial and non-commercial scar-less assembly approaches. Optimizing the amount of vector and insert and insert to vector molar ratio was found to be critical for higher cloning efficiencies. Plant X-tender was applicable even in combination with low efficient chemically competent or electrocompetent *E. coli*. We have further validated the developed procedure for plant protein expression by cloning two cassettes into the newly developed vectors and subsequently transferred them to *N. benthamiana* in a transient expression setup. Thereby we show that complex genetic constructs can be delivered into plant cells in a streamlined and highly efficient way. Our results will support faster introduction of synthetic biology into plant science.

PI-17

The spread of *Potato virus Y* is not restricted by the Ny-1 resistance-gene-mediated HR cell death

Tjaša Lukan, Katja Guček, Magda Tušek Žnidarič, Maruša Pompe Novak, Špela Baebler, Kristina Gruden

National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia

Potato virus Y (PVY) is a virus affecting cultivated potatoes and is responsible for significant losses in agriculture. Hypersensitive resistance (HR) is one of the main types of resistance to PVY, it restricts virus multiplication and spreading and induces formation of necrotic lesions. In potato cv. Rywal, HR is manifested as formation of necrotic lesions on inoculated leaves three days post inoculation (dpi). The exact function of the cell death process in resistance is still debated. In some plant-pathogen interactions, the cell death was envisioned as directly responsible for the restriction of pathogen growth by killing the cell to prevent pathogen multiplication.

The aim of the study was to investigate if Ny-1-mediated HR cell death, manifested as lesions, restricts virus multiplication and spreading. Therefore, spreading of PVY was studied in nontransgenic potato cv. Rywal and in transgenic potato cv. Rywal impaired in SA accumulation (Rywal NahG), where the spread of the virus is no longer restricted. The multiplication of PVY was followed from 1 to 11 dpi. Using confocal microscopy, we were unable to detect GFP-tagged PVY N605 isolatesion formation in both genotypes. From 4 dpi on, the unlimited spread of the PVY was observed in Rywal NahG plants, confirming the involvement of SA in the inhibition of the PVY spread. In cv. Rywal less abundant PVY multiplication was observed outside the lesions from 3 dpi on. With the light camera, we were able to determine presence of PVY outside the lesions. Our results have revealed that Ny-1 gene governed cell death is not limiting the PVY spread. We suggest that cell death is separate from the resistance mechanisms which lead to PVY restriction as already observed in some other biotrophic pathosystems.

PI-18

Nanobodies for structure determination and targeting of coiled-coil based protein origami cages

Andreja Majerle[1], San Hadži[2,3], Jana Aupič[1], Helena Gradišar[1,4], Remy Loris[3], Roman Jerala[1,4]

[1]*National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Department of Chemistry and Biochemistry, Slovenia*
[3]*Vlaams Instituut voor Biotechnologie, Molecular Recognition Unit, Center for Structural Biology, Belgium*
[4]*EN-FIST Centre of Excellence, Slovenia*

Recently we designed and experimentally demonstrated the formation of polyhedral protein cages that can self-assemble *in vivo* [1, 2]. In addition to the demonstration of polyhedral cage formation in agreement with the designed shape using small-angle X-ray scattering (SAXS) analysis we started cristallization studies, where we use nanobodies as crystallization chaperones. A tetrahedron that self assembles from a single polypeptide chain comprising 12 concatenated coiled coil forming segments separated by flexible peptide hinges was used for the immunization for the production of nanobodies. Nanobodies specific for tetrahedron variant TET12SN were generalized, identified and tested for their binding affinity to tetrahedron or peptide segments with size-exclusion chromatography (SEC) with multi-angle light scattering, dynamic light-scattering, native polyacrylamide electrophoresis, and isotermal calorimetry. Several nanobodies, which bind with high affinity to the same side of the tetrahedron, have been identified. The best candidates were analyzed by SEC-SAXS and used for the crystallization trials. SAXS analysis demonstrated formation of complexes comprised of one molecule of tetrahedron and one molecule of nanobody (Nb 26) or two molecules of nanobodies (Nb 28 and Nb 30), where one of them apparently binds into the cavity of the tetrahedron. Crystallization studies with protein or its specific peptide pair are in progress. Use of nanobodies will help us not only to determine a tertiary structure of a tetrahedral protein cage, but also open possibilities for biotechnological or medical applications, where our designed polyhedra can be uses as protein cages for different cargo molecules.

References:

1. Gradišar H., *et. al.* (2013) Nature Chem Biol 9, pp. 362–6.
2. Ljubetič, *et. al.* in revision.

PI-19

Identification of inhibitors of NLP necrotic activity

Katja Pirc[1], Vesna Hodnik[1,2], Marjetka Podobnik[1], Anita Kotar[3], Boris Brus[4], Izidor Sosič[4], Stanislav Gobec[4], Janez Plavec[3], Gregor Anderluh[1]

[1]*National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology, Slovenia*
[2]*University of Ljubljana, Biotechnical Faculty, Slovenia*
[3]*National Institute of Chemistry, Slovenian NMR Center, Slovenia*
[4]*University of Ljubljana, Faculty of Pharmacy, Slovenia*

Proteins belonging to the family of NLPs ("Nep1 (Necrosis and Ethylene Inducing Peptide)-like proteins") are produced by bacteria, fungi and oomycetes and there are several hundred known representatives. The vast majority of species of microorganisms that secrete NLPs are plant pathogens. The mechanism(s) of NLPs toxic action remain to be resolved, although it appears to involve cell membrane disruption. The widespread presence of these proteins in plant pathogens makes a possible inhibitor very appreciated for crop protection against a variety of pathogenic organisms that secret NLPs like *Phytophthora infestans* that causes potato and tomato blight. In this study a surface plasmon resonance (SPR)-based approach was used to discover small molecular weight compounds that target NLP proteins. More than 500 unique and commercially mostly unavailable compounds from our in-house library (University of Ljubljana, Faculty of Pharmacy) were tested. We have identified several molecules that show binding to NLP proteins in micromolar range. Data obtained by STD-NMR and microscale thermophoresis (MST) independently confirmed binding of the most promising compound to NLP. The compounds that showed the highest affinity according to the SPR experiments also prevented necrotic activity of NLP in tobacco leaves. These results indicate that NLP proteins are promising target for further development of novel phytopharmaceutical compounds and strategies.

PI-20

Surface display of recombinant proteins through different anchors on *Lactococcus lactis*

Tina Vida Plavec[1], Aleš Berlec[2]

[1]*University of Ljubljana, Faculty of Pharmacy, Slovenia*
[2]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*

Display of recombinant proteins on the bacterial surface is an emerging research area with wide range of potential biotechnological applications. Because of its GRAS (generally recognized as safe) status, lactic acid bacterium *Lactococcus lactis* represents an attractive host for surface display and promising vector for *in situ* delivery of bioactive proteins. For that purpose new carrier proteins are being sought. The present study focused on comparison of divergent options of protein anchoring to bacterial surface and selection of the optimal carrier for surface display. As a benchmark for surface display, we used plasmid pSDBA3b consisting of Usp45 secretion signal, AcmA anchoring motif and model protein B-domain capable of biding Fc fragment of human IgG. Anchoring motif of AcmA was replaced with C-terminal parts of CluA containing LPxTG motif. Display of B-domain, achieved with LPxTG fusion proteins, was demonstrated on the surface of *Lactococcus lactis* by flow cytometry and whole cell ELISA; however it was inferior to that achieved with pSDBA3b. Alternative approach to surface display is binding via isopeptide bond formation. This was assessed through the following two protein/peptide pairs, SpyCatcher/SpyTag and SnoopCatcher/SnoopTag. Both showed efficient attachment of tagged B-domain to the cell surface of *Lactococccus lactis* displaying the catcher protein. Model protein B-domain was replaced with non-immunoglobulin binders adnectins against goat and rabbit immunoglobulins. Some binding capabilities of corresponding immunoglobulins were demonstrated with whole cell ELISA and flow cytometry; adnectin against rabbit immunoglobulins was superior to adnectin against goat immunoglobulins. We demonstrated effective use of different protein anchors which thus represent an alternative to established approaches for surface display on *Lactoccocus lactis*.

PI-21

Large scale membrane damages of listeriolysin O and applications in synthetic biology

Saša Rezelj, Apolonija Bedina Zavec, Gregor Anderluh

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

Membrane disruption is the result of activity of pore forming proteins. The largest protein pores known are formed by cholesterol dependent cytolysins (CDC). A CDC listeriolysin O (LLO) is a major virulence factor of bacteria *Listeria monocytogenes*. The exact mechanism of LLO pore forming activity and its biological role is yet unknown at the molecular level. We used fluorescent confocal microscopy and flow cytometry on giant unilamellar vesicles (GUV) to investigate LLO action on membranes. We observed that LLO forms arc shaped pores that propagate in time and cause large-scale membrane defects, which lead to destruction of GUVs. We propose that large-scale membrane disruption enables bacteria to escape host phagolysosome [1].
LLO's unique pore forming activity could be exploited in biotechnology, e.g. for release of large biological molecules from synthetic vesicles. Furthermore, we characterised pH dependent LLO mutant Y406A, which is active only at acidic pH environment and not at physiological pH [2]. With cell free expression we are producing LLO and its mutants inside GUVs, that are formed by water in oil transfer method. We were able to produce fluorescent fusion proteins inside these vesicles. We used archaeal lipids to form GUVs, which were bigger than 100 mm and stable for several days. In this way, we are developing specifically controllable artificial cells for applications in biotechnology.

References:

1. Ruan Y., *et. al.* (2016) PLoS Pathog 12(4), e1005597.
2. Kisovec M., *et. al.* (2017) Scientific Reports, 7, pp. 42231.

PI-22

The ultrasound assisted extraction of oleuropein from olive leaves *Olea europaea* L. and its stabilization by encapsulation

Domenico Cifà[1], Veronica Felti[1], Mihaela Skrt[2], Carla Di Mattia[1], Paola Pittia[1], Nataša Poklar Ulrih[2,3]

[1]*University of Teramo, Faculty of Bioscience and Technology for Food, Agriculture and Environment, Italy*
[2]*University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Slovenia*
[3]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CipKeBiP), Slovenia*

Oleuropein is one of the most abundant phenolic compound found in *Olea europea* leaves. This secoiridoid glycosidec compound has several pharmacological properties (antioxidant, antiinflamatory, antiatherogenic and antimicrobial effects) and recently it was shown that oleuropein inhibits growth and induces apoptosis in a variety of cancer cell lines [1] and could inhibit collagen fibril formation [2].
The aim of this study was to examine the ultrasound assisted extraction efficiency of oleuropein from olive leaves (*Olea europaea* L.), belonging to the olive variety *Istrska Belica*. The best conditions for the ultrasound assisted extraction were choosen for the prepration of the high oleuropein extract from olive leaves and further used for the encapsulation studies. The ultrasound assisted extraction gave 29% higher oleuropein content in comparison with conventional extraction method. The oleuropein extract from olive leaves was encapsulated into different matrixes (phospholipids, alginate and pectin hydrogels). The best encapsulation efficiency of high oleuropein content of olive leaves extract was obtained by using the proliposomes techniques.

References:

1. Yao J., *et. al.* (2014) J Pharmacol Sc 125, pp. 300–11.
2. Bharathy H., *et. al.* (2017) Int J Biol Macromol 101, pp. 179–86.

PI-23

New approaches to monitoring deamidation of IgG antibodies

Rok Sekirnik, Nuša Slabajna, Tilen Praper

Lek d. d., BTDM Mengeš, Biofarmacevtika, Razvojna analitika, Slovenia

Due to their high selectivity and capacity to be produced recombinantly with high titers, monoclonal antibodies have emerged as an important class of therapeutic agents in a wide range of disease areas. Although they are generally considered as stable molecules, IgGs are subject to a number of post-translational modifications, arising either during fermentation processes, or during formulation and storage.

In particular, IgGs are prone to deamidation and isomerization of asparagine, and to a lesser extent glutamine, which affect overall charge and potentially affect potency. Due to a small difference in mass caused by deamidation (1 Da) and chromatographic co-elution of deamidated variants, monitoring of deamidation using mass spectrometry approaches is challenging and time-consuming. A conserved sequence motif, generally called PENNYK, has been reported as the site of highest exposure to deamidation; a selectively cleaved peptide fragment containing the sequence can therefore be used as a reporter sequence for deamidation of IgGs.

Here we report the development of a HPLC method for separation of deamidated variants of PENNYK peptide and relative quantification of deamidation with fluorescence detection. The method is selective for the target sequence, has high sensitivity for detection of deamidated variants and is significantly shorter than currently available methods, thus allowing higher throughput for analysis. It can be applied during development of new IgG therapeutics, including formulation, and in routine quality control to monitor stability of drug product.

PI-24

Construction and production of active pernisine in *Streptomyces rimosus*

Marko Šnajder, Miha Bahun, Luka Kranjc, Hrvoje Petković, Nataša Poklar Ulrih

Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Pernisine is a thermostable protease originating from Archaea *Aeropyrum pernix* K1 with properties suitable for number of industry applications from medical field to cleaning industry [1, 2]. Unprocessed pernisine (propernisine) sequence consists of 430 amino acids residues (aa), composed from signal sequence (first 24 aa) and proregion from 24 to 92 aa. Propernisine produced in *E. coli* needs to be thermally activated (80 °C for 30 min) [3]. Using bioinformatics tool and template of homologous thermostable protease Tk-subtilisin, 3D models of unprocessed and mature pernisine lacking the proregion was designed. To test the enzymatic activity, we assembled constructs using pVFx vectors under *tet* promoter for production of pernisine variants in the extracellular media in *Streptomyces rimosus*. Our data revealed that pernisine lacking the first 92 aa was active whereas propernisine showed negligible activity, despite attempt to thermally activate propernisine (80 °C for 30 min). Moreover these date indicate that proregion is not important for folding of pernisine while Tk-subtilisin needed its proregion for proper folding. *S. rimosus* as the alternative expression system to *E. coli*, produced higher yield of pernisine (12 mg vs 10 mg per liter culture). Further biophysical characterization of recombinant pernisine from *S. rimosus* showed that pernisine retained similar characteristics as native one.

References:

1. Šnajder M., *et. al.* (2012). PLoS ONE 7, e39548.
2. Poklar U. N. and Vilfan T. European patent specification: EP 2 311 323 (B1), 2013-01-16.
3. Šnajder M., *et. al.* (2015) PLoS ONE 10, e0123288.

PI-25

QbD approach to development of analytical methods for biosimilars

Igor Kristian Štern, Tomaž Natek, Luka Brvar, Špela Gorjan Železnik, Tilen Praper

Lek, d. d. BTDM Mengeš, Slovenia

Biosimilars are biological drugs (biologics) approved via stringent regulatory pathways, following a loss of exclusivity of their originator reference drug products. Basis for their approval is demonstration of similarity (comparability): there should be no meaningful difference in efficacy, safety and overall quality between the biosimilar and the originator. To achieve this, it is essential to establish a proper control of the drug substance and drug product manufacturing process.
Here we present two analytical methods for the content and purity determination which were developed according to Quality by Design (QbD) principles. First, an Analytical Target Profile (ATP) was prepared where the requirements for both methods were defined. After a successful method development and after setting the suitable acceptance criteria, the method robustness was further evaluated. A risk based approach was used where the critical parameters of the methods were identified by risk assessment (RA), some of which were tested individually, whereas for some others, a software supported experimental design (DoE approach) was used. Based on the final results of robustness testing, both methods are robust and suitable for their intended purpose and can be validated for the release of drug substance and drug product.

PI-26

The budding yeast *Saccharomyces cerevisiae* as a model to study lipid membrane interactions of NLP proteins

Apolonija Bedina Zavec[1], Anja Porenta[1], Vesna Hodnik[2], Saša Rezelj[1], Katja Pirc[1], Tea Lenarčič[1], Marjetka Podobnik[1], Gregor Anderluh[1]

[1]*National Institute of Chemistry, Department for Molecular Biology and Nanobiotechnology, Slovenia*
[2]*University Ljubljana, Department of Biology, Slovenia*

Necrosis and ethylene-inducing peptide 1-like (NLP) proteins are produced by plant pathogenic bacteria, fungi and oomycetes. Many NLPs are virulence factors facilitating infection of Dicot host plants. The molecular mechanism of NLP-mediated cytolysis is not known. Glycosylinositol phosphorylceramides (GIPC), the major class of plant sphingolipids are probably receptors of NLPs. The budding yeast *Saccharomyces cerevisiae* is a widely used model organism, because of the ease of culturing and genetic manipulation. *S. cerevisiae* has only three classes of sphingolipids: inositol phosphorylceramide, mannosylinositol phosphorylceramide and mannosyldiinositol phosphorylceramide ($M(IP)_2C$). $M(IP)_2C$ is the most abundant complex sphingolipid in budding yeast and it is similar to series A GIPCs that are abundant in Dicot plants but not in Monocot plants. Our results have shown that cytotoxic NLPs bind to protoplasts of *S. cerevisiae* and exhibit cytotoxic activity, while the nontoxic NLPs don't bind to protoplasts and are not cytotoxic. NLPs don’t bind to intact yeasts, they only bind to yeast protoplasts. These results are consistent with the predicted mechanism of NLP-membrane interaction. Therefore, we propose *S. cerevisiae* as an appropriate model to study the NLP proteins.

PI-27

Biogenic amines are potent antioxidants in bulk oils

Alenka Levart[1], Denis Gutirea[2], Michael Murkovich[3], Nataša Poklar Ulrih[2], Blaž Cigić[2]

[1]*University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Slovenia*
[2]*University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Slovenia*
[3]*Graz University of Technology, Institute of Biochemistry, Austria*

Biogenic amines (BA) are found in large variety of fresh and processed foods. Their content in food is often undesired as they are indicator of microbial spoilage with unpleasant smell and some of them as histamine are involved in immune system disorders. On the other hand endogenously synthesized BA have many beneficial physiological functions as neuromodulators, neurotransmitters, growth factors and DNA-binding agents. Some BA also act as free radicals scavenger and inhibitors of lipoperoxidation *in vivo*.

We have analyzed whether biogenic amines in submilimolar range can delay the oxidation of lipids in vegetable oils. Spermine, spermidine and decarboxylated products of some amino acids were added into the highly reactive linseed and sunflower oils exposed to accelerated ageing.

BA showed potent and concentration dependent antioxidant activity. Polyamines, spermine and spermidine were the most efficient in stabilizing the oils, resulting in attenuated peroxidation of fatty acids and better stability of vitamin E in oils.

PI-28

The conserved D0 domain of flagellin is involved in the activation of Toll-like receptor 5 and the intracellular Naip-NLRC4 inflammasome

Vida Forstnerič[1,2], Karolina Ivičak-Kocjan[1], Tjaša Plaper[1], Roman Jerala[1,2], Mojca Benčina[1]

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

Flagellin is the main structural protein of bacterial flagella and a virulence factor of several human pathogens. Flagellin is recognized through a conserved patch within the D1 domain by the membrane-bound Toll-like receptor 5 (TLR5) and through a conserved hydrophobic motif within the C-terminal D0 domain by the intracellular inflammasome receptor Naip5/NLRC4. While it has been common perception that TLR5 and Naip5/NLRC4 recognize separate regions of the protein ligand, we show for the first time that the recognition sites in fact overlap, as a hydrophobic motif within the C-terminal D0 domain affects both Naip5/NLRC4 and TLR5 signalling. We further demonstrate the functional importance of amino acid residues within the C-terminal portion of flagellin as several point mutations in this area affected bacterial motility. Toll-like receptors form active signalling dimers upon ligand binding. A partial crystal structure of the receptor-ligand complex reveals primary binding of flagellin to TLR5 and additional, lower affinity interactions, which supposedly contribute to dimer formation. However, these interactions appear to be insufficient as they were not detectable in solution and flagellin from the crystal structure lacks the D0 domain and is thus unable to activate the receptor. We propose a functional role for the D0 domain in bridging two receptor monomers into an active signalling complex through the use of a dimeric flagellin construct in which we attempt to mimic the role of the D0 domain in physically bridging two receptor monomers. Together, our results implicate the role of a previously unrecognized region of flagellin in TLR5 activation and provide additional knowledge concerning the activation mechanism of the TLR5 receptor.

PI-29

Tumour microenvironment regulates glioblastoma progression

Tamara Lah Turnšek[1], Barbara Breznik[1,2], Mona Oliveira[2,3], Henning Ulrich[3], Cornelis J. van Noorden[4]

[1]*National Institute of Biology, Department of toxicology and Cancer Biology, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*University of Sao Paolo, Institute of Biochemistry, Brazil*
[4]*University of Amsterdam, Academic Medical Center, Department of Medical Biology, The Netherlands*

Glioblastoma (GBM) is the most lethal brain tumour with limited treatment options, due to their aggressive invasion and therapy resistance. GBM microenvironment includes stromal cells, as are glial cells (GC), fibroblasts (FB) macrophages (MF), endothelial cells (EC), and bone marrow/blood derived mesenchymal stem cells (MSC) and hematopoietic stem cells (HSC) that both home to GBM. Their interplay orchestrated by GBM stem-like cells (GSLC) plays a significant role in modulating tumour progression. On one hand, these cells comprise the GSLC niche, protecting GSLC from therapeutic cytotoxicity, and on the other hand, infiltrating HSC/MSC interact indirectly and directly with heterogeneous population of mature/differentiated GBM cells and regulate their malignancy. However, different GBM cells are differentially affected with respect to proliferation and invasion by stromal cells, as we have shown in the *in vitro* co-culture and *in vivo* zebra fish model of GBM lines U87 and U373 cells, with more or less expressed mesenchymal genotype characteristics, respectively, when co-cultured with MSCs (Breznik, 2017). In addition, bradykinin receptor B1R was pointed out as an important molecular switch simultaneously modulating cell fusion and invasion events, induced by MSC direct contact with GBM cells.
Migration of GSLC to/out of the niches and the GBM & MSC invasion into brain parenchyma are associated, possibly even dependent on selected proteases (Breznik, 2017). We focused on cysteine cathepsins (CTS), of which CTSB has been proven as the key pro-invasive protease. Out of other upregulated CTS, immunohistochemical data provide an evidence that CTSK and X (Verbovšek, 2016), are also related to GSLC niche, and should be further validated as therapeutic target in GBM patients. In conclusion, here we are addressing the interplay between intra tumour cell autonomous and inter-tumour cell non-autonomous heterogeneity, pointing on a high complexity of tumour microenvironment.

PI-30

Next generation *in vivo* self-assembling coiled-coil protein origami

Ajasja Ljubetič[1], Helena Gradišar[1,2], Fabio Lapenta[1], Andreja Majerle[1], Jana Aupič[1], Žiga Strmšek[1], Duško Lainšček[1], Iva Hafner-Bratkovič[1,2], Mojca Benčina[1,2], Roman Jerala[1,2]

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

Polypeptides are nature's most versatile programmable biopolymers that can self-assemble into complex tertiary structures encoded by the linear sequence of their building units. DNA nanotechnology re-purposed DNA for the rational *de novo* design of complex assemblies unseen in nature. A similar strategy can be applied to construct protein origami cages using coiled-coil (CC) dimers as building modules. The fold and shape of such constructs is defined by the topology rather than by the hydrophobic core as in globular natural proteins.

We have designed second generation protein origami cages, based on a toolbox of supercharged CC dimer building modules and a computational design platform, which enabled construction of protein origami cages composed of more than 700 amino acid residues.

Solution small-angle X-ray scattering, TEM imaging and biophysical analysis confirmed that the designed tetrahedron, square pyramid and triangular prism attain the correct/designed structure. The stability and folding kinetics of protein origamis are comparable to natural proteins. Moreover, second generation protein origamis were produced and self-assembled in bacteria without the need for refolding, as well as in mammalian cells and in animals, without causing inflammation or other adverse pathological effects.

The developed computational platform allows for automated design of arbitrary higher order polyhedral CC cages, limited only by the number of available CC modules. Together with the *in vivo* experiments the way to numerous future applications is opened.

PI-31

Characterisation of L1 ORF1p in mammalian cells

Mirjana Malnar[1], Vera Župunski[2], Tomaž Bratkovič[3], Miha Modic[4], Micha Drukker[4], Jernej Ule[5,6], Boris Rogelj[1,2,7]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*
[4]*Helmoltz Zentrum München, Institute of Stem Cell Research, Germany*
[5]*University College London, Institute of Neurology, UK*
[6]*The Francis Crick Institute, UK*
[7]*Biomedical Research Institute BRIS, Slovenia*

L1 is one of the mobile elements in the human genome — DNA sequences that are able to "jump" to different sites in genome. Mobile elements played an important role in the evolution of human genome and depending on target sites of their integration they can cause approximately 65 different diseases, mostly cancers. Approximately 45% of human genome is constituted of mobile elements that can be divided into three groups: DNA transposons, LTR (long terminal repeats) retrotransposons and non-LTR retrotransposons. The three groups differ in the way of transferring to new target sites in genome — DNA transposons are cut from their original site in genome and transferred to the new target site, while retrotransposons move by RNA intermediate. Reverse transcript of later is inserted to new target site. The only autonomous non-LTR retrotransposon in human genome is L1 — a 6kb long DNA sequence with two open reading frames encoding for L1 ORF1p and L1 ORF2p. The role of L1 ORF1p is still largely unknown. L1 ORF1p is a 40 kDa protein with RNA binding activity, however, the library of target RNA molecules is not yet known. In order to gain better understanding of the role of this protein during mobility of L1 retrotransposon and other mobile elements we aim to characterise the protein in mammalian cells and define its target RNA molecules. We used FlpIn system to establish inducible HEK 293T, HeLa and SH-SY5Y cell lines expressing L1 ORF1p and 2012Ep and NT-2 cells which express high levels of endogenous L1 ORF1p. Custom made anti-L1 ORF1p antibodies were used for immunolocalization of the protein, which was detected mostly in cytoplasmic clusters. The immunoprecipitation of the L1 ORF1p constructs containing either GFP or Flag tag was conducted in order to proceed with iClip experiment. Later experiment is used to detect protein-RNA interactions *in vivo* and therefore determine target RNA molecules of L1 ORF1p in chosen mammalian cell lines.

PI-32

Reactivation efficacy of new chiral *n*-substituted 2-hydroxyiminoacetamide reactivators of phosphylated cholinesterases

Nikola Maraković[1], Anamarija Knežević[2], Vladimir Vinković[2], Zrinka Kovarik[1], Goran Šinko[1]

[1]*Institute for Medical Research and Occupational Health, Croatia*
[2]*Ruđer Bošković Institute, Croatia*

Acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) play an important role in the neurotransmission and biotransformation of xenobiotics, respectively. Organophosphorus (OP) nerve agents used as warfare agent in armed conflicts and terrorist attacks act as an irreversible AChE inhibitors making covalent bond between its phosphorus atom and catalytic serine oxygen atom. The current therapy in cases of OP nerve agent poisoning includes the reactivation of AChE by standard quaternary pyridinium oximes. However, due to their permanent positive charge, these compounds do not cross the blood-brain barrier and thus cannot reactivate AChE in the central nervous system. We evaluated the reactivation efficacy of novel centrally acting chiral oxime reactivators I-IV designed using computational methods of molecular modelling and prepared by introducing a phenyl ring in the structure of a previously reported *N*-substituted 2-hydroxyiminoacetamide scaffold. The azide group in structure of oxime I enabled us to prepare more elaborate structures of oximes II, III, and IV by the well-known copper-catalysed azide-alkyne cycloaddition. Oximes were tested in both racemic and enantiomerically pure form for reactivation of AChE and BChE inhibited with OP nerve agents tabun, sarin, cyclosarin, and VX. Oximes III and IV were efficient reactivators of AChE inhibited with sarin, cyclosarin, and VX. Oxime IV being was the most efficient reactivator in case of AChE inhibited with VX, albeit less efficient than standard oxime 2-PAM. Oximes I-IV were efficient reactivators of BChE inhibited with sarin, cyclosarin, and VX. (*S*)-enantiomer of oxime III proved to be a promising reactivator of BChE inhibited with cyclosarin with almost three times higher efficiency than the reference oxime 2-PAM. Molecular docking studies confirmed that the oxime group of oxime III is at proper distance from the phosphorus atom conjugated to the catalytic serine residue of BChE which allows nucleophilic substitution of conjugated cyclosarin, but only after rotation around CH-NH bond or distinctive conformational change of the cyclosarin-BChE conjugate.

This work was supported by the Croatian Science Foundation (project 4307).

PI-33

Chromosome number and genome size of some *Centaurea* (Asteraceae) from Croatia

Olivera Politeo[1], Ivana Carev[1], Fatima Pustahija[2], Mirko Ruščić[3], Sonja Siljak-Yakovlev[4]

[1]*University of Split, Faculty of Chemistry and Technology, Depertment of Biochemistry, Croatia*
[2]*University of Sarajevo, Faculty of Forestry, Bosnia and Herzegovina*
[3]*Faculty of Natural Sciences, Croatia*
[4]*Université Paris-Sud, Ecologie Systématique et Evolution, Université Paris-Saclay, Centre Nationnal de la Recherche Scientifique (CNRS), France*

Genome size and chromosome number are characteristic species trait important in systematics, taxonomy and evaluation of biodiversity. The Adriatic coast of Croatia presents a unique area for biological and chemical research of plant material. Genus *Centaurea* (Asteraceae family) is already known for many medicinal plants that present a valuable source of potential medicines. In Croatia 79 *Centaurea* taxa have been listed, comprising 27 endemics. Previous chemical and biological observations of extracts and volatiles isolated from *Centaurea* species, showed differences in chemical composition and biological activity of volatile substances between different species. Some *Centaurea* species have many similarities in their morphological appearance, therefore the criteria of genome size and number of chromosomes attributes to plant material identification. Due to a high demand for a clear and authentic plant material identification in chemical research, genome size and number of chromosomes for six *Centaurea* species have made prior to chemical and biochemical research of isolated plant extracts. The genome size was estimated using flow cytometry. The chromosome number was determined using the standard karyological methods. The plant samples, seeds and leaves, were collected from wild growing natural populations of Croatia. The studied species present three basic chromosome numbers, x = 9, 10 and 11. Two ploidy levels were observed among investigated species. The nuclear DNA amounts (2C DNA) range from 1.70 to 3.57 pg. Using the Leitch's *et. al.* criteria all these taxa belong to the group of very small C-values. The results present the novel values for *C. cristata, C. calcitrapa,* and *C. rhenana.* These species will be the object of our future studies concerning the chemical composition of volatile and non-volatile plant extracts and their biological activity.

PI-34

Computational support for management of animal samples in research laboratories

Staš Hvala[1], Žiga Pušnik[1], Živa Rejc[2], Uršula Prosenc Zmrzljak[3], Kaja Blagotinšek[3], Žiga Urlep[3], Nejc Nadižar[1], Stanislav Moškon[4], Damjana Rozman[3], Miha Moškon[1]

[1]*University of Ljubljana, Faculty of Computer and Information Science, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Centre for Functional Genomics and Bio-Chips, Slovenia*
[4]*Osir d.o.o., Slovenia*

We present a design and implementation of an information system, which is meant be used within research laboratories, where a vast number of animal samples is collected every day. The obtained samples can be aliquoted and further processed (e.g. DNA or RNA can be isolated). Therefore, even a greater amount of data is gathered. Managing, tracking and analysis of all the samples and the data have become a big issue within the laboratory research environments. Consequently, a need for an information system, which would solve the problem of the poor and non-systematic organisation of the data, appeared. The system we invented supports various tasks. It is able to (1) serve as a database of the collected, analysed and stored samples, (2) manage the registered data and (3) prepare the acquired data to be computationally analysed. The system incorporates several experimental workflows ranging from the initial collection of samples and their aliquots to the subsequent biochemical (or other) analyses. The information system supports data import and export in CSV format, which makes it compatible with the majority of commercial software products for data management and analysis. It is designed with open-source tools and platforms. That makes it accessible to a wide scientific community. Additionally, its simplicity and flexibility make it even more user-friendly. It is easy to maintain and can be customized according to the users' demands. We strongly believe that the proposed system presents an excellent alternative to the popular general-purpose computational tools that are currently prevalent within research laboratories.

PI-35

Kinetic evaluation of PON1 interactions with pharmacologically active carbamates

Anita Bosak[1], Aljoša Bavec[2], Marko Goličnik[1,2], Goran Šinko[1], Zrinka Kovarik[1]

[1]*Institute for Medical Research and Occupational Health, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*

Paraoxonase 1 (PON1) is a calcium-dependent enzyme whose physiological substrate and physiological role are still the subject of many research efforts. The reduced catalytic activity of PON1 in humans has been demonstrated in many pathological conditions such as diabetes, chronic kidney or liver disease, hyperlipoproteinemia, Alzheimer's and thyroid diseases. Mammalian PON1 possesses arylesterase, lactonase and phosphotriestrase activity and it is able to hydrolyse a very broad spectrum of esters, like some xenobiotics, endogenous proantibiotics and lactones. The aim of this work was to determine whether carbamate esters affect a G2E6 variant of recombinant PON1, expressed and purified in the *Escherichia coli* bacterial system. We tested carbamates of different application fields; bambuterol in use as bronchodilator, carbofuran as pesticide, physostigmine as a drug for treating glaucoma and delayed gastric empting, and Ro(02-0683) in use for BChE phenotyping. The impact of carbamates on PON1 activity was expressed as an enzyme-inhibitor dissociation constant K_i, while $1/K_i$ represents the enzyme affinity towards the tested carbamate. Enzyme activity was measured spectrophotometrically at 412 nm at 25 °C in 50 mM TrisHCl buffer, pH 8.0, containing 1 mM $CaCl_2$, and using thiophenyacetate (TPA) as a substrate for PON1's arylesterase activity. Evaluation of the K_i constants demonstrated that all carbamates compete with TPA for binding to the active site of the enzyme. PON1 displayed almost the same affinity towards Ro(02-0683), bambuterol and physostigmine, while it was about five times higher towards carbofuran. Generally, affinity of PON1 towards carbamates was 2–10 times lower compared to that of TPA. In conclusion, carbamates could reduce the level of PON1 activity, which should be kept in mind especially in conditions characterized by reduced PON1 level.

Acknowledgments: Work was supported by HrZZ (Grant No.4307) and Croatian-Slovenian bilateral project 2016–2017.

PI-36

A better insight into cathepsin X prodomain

Bojan Doljak, Janko Kos

University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Biology, Slovenia

Cysteine proteases are synthesized *in vivo* with N-terminal prodomains that cover the active site cleft in an orientation that does not allow the hydrolysis of the prodomain's peptide bond, consequently preventing the access of substrate molecules to the active site. The 38 amino acids long prodomain of cathepsin X is the shortest prodomain among cysteine proteases of the papain family and it is the only prodomain not containing a helical structure (compared to prodomains of i.e. cathepsin B, L or F). The prodomain is also not homologous to any other protein sequence. It does not extend to the prodomain binding loop (PBL) instead it forms a disulfide bond between Cys10p and Cys31 of the active site. The inability of procathepsin X to autoprocess itself *in vitro* must therefore be a reasonable consequence of all the above. Little or no data exist on the *trans* inhibitory activity of the cathepsin X prodomain toward other cysteine proteases.

We have shown that procathepsin X could be activated *in vitro* without the presence of cathepsin L. Reducing agents (DTT, TCEP, L-cystein) and acidic conditions led to significant, although not complete activation of procathepsin X. On the other hand, classical *in vitro* activation with cathepsin L and subsequent column purification that is supposed to completely strip the prodomain off the mature enzyme, resulted in a mature form, that still needed the presence of 5 mM DTT. We also noted that a cleaved prodomain does not have to be full-length to act inhibitory on the cognate enzyme. Namely, preincubation of mature cathepsin X with a 14 aa long peptide corresponding to the very first N-terminal part of the prodomain, resulted in a 43% reduction of cathepsin X activity.

A better insight into the inhibitory role of cathepsin X prodomain would definitely contribute to the understanding of the behavior of (pro)cathepsin X both *in vitro* and *in vivo*.

PI-37

Synthesis and characterization of a novel allosteric effector of cathepsin K

Tjaša Goričan, Nejc Petek, Jurij Svete, Marko Novinec

University of Ljubljana, Faculty of Chemistry and Chemical Technology, Department of Chemistry and Biochemistry, Slovenia

Cathepsin K is a member of papain-like proteases, which are mostly known to degrade protein substrates in (endo)lysosomes. Cathepsin K also has additional unique functions among which the most important one is its involvement in the process of bone resorption. Since its elevated expression and activity are associated with numerous disseases, it represents an important target for drug development. It is already known that cathepsin K can be allosterically regulated not only by its natural effectors, glycosaminoglycans, but also by synthetic compounds that bind to its allosteric sites.
On the basis of the crystal structure of a known effector bound to cathepsin K we developed a new compound Su-Gly-O-Me and showed that it binds to the same allosteric site with higher affinity and in a novel binding mode. We synthesized this compound from glycine methyl ester and maleimide in one step and purified it by flash chromatography on silica with high yield. We also tested this newly developed compound by measuring the activity of cathepsin K using synthetic substrates and type I collagen which represents its most important natural substrate. We determined the kinetic mechanism of the compound and characterized it as a partial inhibitor which is consistent with known effectors. In the future, the developed synthetic procedure will be used to develop new compounds with higher affinity and specificity for cathepsin K.
Furthermore, using molecular dynamics simulations we predicted the pathway of allosteric signal propagation from the allosteric site to the active site of cathepsin K. We verified this predictions by alanine mutagenesis and identified several residues critical for transmission of allosteric signalling with the aim to eventulally characterize the whole allosteric mechanism of cathepsin K.

PI-38

Tail flexibility and rigidity of the central modular region as determinants of testican-2 function

Anja Kerš[1], Brigita Lenarčič[1,2], Miha Pavšič[1]

[1]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[2]*Jožef Stefan Institute, Department for Biochemistry, Molecular and Structural biology, Slovenia*

Testicans are modular 44 kDa proteoglycans of the extracellular matrix (ECM) of various tissues where they contribute to ECM integrity, exert various effects on cells like promotion of neurite outgrowth and cell migration, and regulate extracellular protease activity. Recently, they were also found to be implicated in progression of Alzheimer disease. Still, little is known on the structural aspects of testican biology which we are addressing using testican-2 as a representative member of the functionally redundant testican family (testican-1, -2 and, -3). Our hypothesis is that distinct parts of the testican molecule are disordered thereby giving testicans the sufficient structural plasticity to affect both cell behaviour and ECM organisation.
We are studying the structure-function relationship of testicans by various approaches, primarily by X-ray crystallography, small-angle X-ray scattering (SAXS), pull-down assays and cell culture experiments. SAXS results indicate that both N- and C-termini are disordered while the central part of the molecule, comprised of the follistatin-like domain, calcium binding domain and thyroglobulin type 1 domain, forms a more compact shape stabilized by calcium binding.
For the assessment of testican impact on cell migration *in vitro* wound healing assay was utilized. Here, closure of an artificially created gap in the presence of mitomycin C was monitored as a function of time. Preliminary results show that both testican-1 and -2 promote cell migration which is even more prominent when both testicans are present at the same time. This indicates that while the function of testicans seems redundant their mechanism of action is not entirely identical. Current efforts are directed towards elucidation of which regions of testican molecule are involved in creation of anchoring sites that are crucial for testican involvement in cell migration.

PI-39

Pseudomonas aeruginosa RahU protein interaction with bacterial or invertebrate characteristic lipids

Eva Kočar[1], Vesna Hodnik[1], Tea Lenarčič[2], Anastasija Panevska[1], Gregor Anderluh[2], Marjetka Podobnik[2], Peter Maček[1], Kristina Sepčić[1], Matej Butala[1]

[1]*University of Ljubljana, Biotehnical Faculty, Department of Biology, Slovenia*
[2]*National Institute of Chemistry, Slovenia*

Aegerolysin protein family (Pfam 06355) comprises over 300 homologous proteins from different kingdoms (bacteria, eucaryotes). They are mostly small (13–20 kDa) and β-structured proteins, whose biological role has not been identified yet. Data indicate that aegerolysins play a role in the interaction with their hosts. The opportunistic pathogen, *Pseudomonas aeruginosa*, carries a gene for the quorum-sensing-directed aegerolysin RahU. Recently it has been shown that RahU interacts with virulence factors, rhamnolipid bisurfactants and that aegerolysins specifically bind ceramide phosphoethanolamine (CPE), most represented sphingolipid in membranes of invertebrates. Here we determined the interaction of RahU with selected lipids. We substituted several RahU residues by alanine and applied sedimentation assays and surface plasmon resonance spectroscopy to show that RahU interacts with CPE-rich membranes composed of CPE, cholesterol, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) (40:30:30). In addition, we identified crucial residues for the RahU-CPE or RahU-rhamnolipid interaction. Our results add to the growing knowledge on aegerolysins and have a biotechnological potential.

PI-40

Serine protease homologue from the venom of the nose-horned viper is a promising new anticoagulant lead molecule

Zorica Latinović[1,2], Adrijana Leonardi[1], Jože Pungerčar[1], Cho Yeow Koh[3], R. Manjunatha Kini[3], Igor Križaj[1]

[1]*Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*National University of Singapore, Faculty of Science, Department of Biological Sciences, Singapore*

Most frequently used anticoagulant therapies, based on aspirin, heparin or warfarin, may induce severe complications. To overcome these limitations, new anticoagulants from natural sources have been intensively searched for. We report here the purification and characterization of a glycoprotein from the venom of the European most venomous snake, the nose-horned viper (*Vipera a. ammodytes*), which significantly prolonged the activated partial thromboplastin time (aPTT) in human plasma, indicating perturbations in the intrinsic pathway of blood coagulation. Amino acid sequence of this monomeric N-glycosylated protein of 34 kDa revealed it as a serine protease harbouring two mutations in its catalytic triad that renders it enzymatically inactive. Hence the name *Vipera a. ammodytes* serine protease homolog 1 or *Vaa*SPH-1.

Detailed analysis of the mechanism of blood coagulation by *Vaa*SPH-1 exposed that the molecule inhibited the activity of both tenase and a prothrombinase complexes. We demonstrated that the inhibition of the formation of complexes was due to the binding of *Vaa*SPH-1 to blood coagulation factors involved in these complexes. *Vaa*SPH-1 was also found to bind specifically to negatively charged phosphatidylserine, which is crucial for constitution of coagulation complexes on cellular membranes. Reflected by its marked effect on aPTT, we assume that the potent anticoagulant action of *Vaa*SPH-1 is mainly due to its binding to FIX/FIXa. FIXa, complexed to *Vaa*SPH-1, retained its full enzymatic activity towards chromogenic peptide substrates; therefore *Vaa*SPH-1 binds in the proximity of coagulation factor active site.

As a potent non-enzymatic and coagulation factor active site-independent inhibitor of blood coagulation process, *Vaa*SPH-1 is a unique natural molecule. As such it is very interesting for further characterization to design, based on its structure, a novel family of selective FIXa inhibitors to be used in anticoagulant therapy.

PI-41

Clostridium difficile surface proteins

Nataša Lindič[1], Aleksandra Usenik[1], Dušan Turk[1,2]

[1]*Jožef Stefan Institute, Department of Biochemistry, Molecular and Structural Biology, Slovenia*
[2]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Slovenia*

The incidence and severity of *Clostridium difficile* (*C. difficile*) infections, the leading cause of healthcare-associated diarrhea world-wide, is increasing. These infections often occur due to disruption of normal gut flora after antibiotic treatments of unrelated infections. The microbiota of the gut is then further damaged by the broad-spectrum antibiotics used to treat the *C. difficile* infection, often leading to a vicious circle of infection recurrences. Hence, novel, more specific therapeutics are needed to successfully fight against this detrimental pathogen. Interfering with bacterial adhesion to intestinal epithelial cells, the first step of the infection, is a potentially novel approach. The *C. difficile* cell is surrounded with a peptidoglycan wall and interspersed with protruding secondary polysaccharide ligands, serving as anchors for the outermost layer of the cell wall, the S-layer. While it has been established that the S-layer is composed of 29 cell wall proteins (Cwps), the mechanisms of its production and assembly are not known. To find new ways of action against *C. difficile*, we aim to investigate the process of adhesion at several critical points. First, to block the functions of the S-layer proteins, we are studying the structural basis of Cwp protein function and assembly. Second, to interfere with S-layer assembly, we are investigating the process of secretion of S-layer proteins, the synthesis of the polysaccharide ligand, and its binding to Cwp proteins. Finally, to block the interaction of the S-layer with intestinal cells, we aim to identify its host binding partner(s). These studies will form the basis for the development of new *C. difficile*-specific treatments targeting the initial, essential step of its pathogenesis.

PI-42

Cathepsin B activity regulation by a combination of evolutionarily conserved and group-specific elements

Marko Novinec, Gašper Marinšek

University of Ljubljana, Faculty of Chemistry and Chemical Technology, Department of Chemistry and Biochemistry, Slovenia

Allosteric communication in proteins is transmitted via evolutionarily conserved networks of coevolving residues termed protein sectors. Nevertheless, unique interactions of sector residues can result in modes of regulation specific to certain family members. Herein we describe a regulatory mechanism in cathepsin B based on specific interactions between sector and non-sector residues. This enzyme differs from other family members not only by containing the occluding loop which covers the active site, but also by a unique pattern of internal salt bridges that interconnect both subdomains and were proposed to be associated with its stability.

Our recent high resolution crystal structure of ligand-free cathepsin B showed that sector residue Arg21, located in a cavity on the back side of the molecule underneath the active site, adopts two different conformations. Subsequent experimental and computational experiments revealed that this residue plays a central role in regulating the catalytic activity of cathepsin B by modulating sector connectivity and by interacting with internal salt bridges. Its conformation was found to be controlled by nearby residues Glu19 and Glu53. Replacement of Glu19 with Ala resulted in increased catalytic rate (k_{cat}) towards synthetic substrates, whereas the Glu53Ala mutant had a 4-fold reduced value of k_{cat}.

Ala replacement of Arg21 or its interacting residues inside the cavity (Phe32, Glu36, Ser39, Asp40, Arg202) resulted in drastic (10 to 100-fold) reduction of k_{cat} values. The crystal structure of the Arg21Ala mutant showed that while the core catalytic machinery and the non-primed sites are intact, part of the primed site region is misfolded. Additional conformational changes were observed throughout the molecule. Despite this, the thermal stability of the Arg21Ala mutant was comparable to the wild-type. These results indicate that Arg21 not only regulates cathepsin B activity but also directs its folding into the native state.

PI-43

Interaction of aegerolysin proteins from the fungal genus *Pleurotus* with artificial lipid membranes

Anastasija Panevska[1], Vesna Hodnik[1], Maruša Novak[1], Eva Kočar[1], Matej Skočaj[2], Matej Butala[1], Peter Maček[1], Kristina Sepčić[1]

[1]*University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia*
[2]*Pulp and Paper Institute, Slovenia*

Aegerolysin protein family (Pfam 06355, InterProIPS009413) comprises over 350 mostly β-structured, small (15–20 kDa) proteins from different bacterial and eukaryotic taxa. Their common feature is the ability to bind different lipids and lipid derivatives, as well as biological and artificial lipid membranes. Our recent findings show that almost all bacterial and fungal aegerolysins specifically recognize ceramide phosphoethanolamine (CPE), the major membrane sphingolipid of several invertebrate classes. Moreover, in the presence of partner protein with a membrane-attack-complex/perforin (MACPF) domain, fungal aegerolysins from the genus *Pleurotus* can permeabilize lipid membranes through the formation of bi-component transmembrane pore complexes.

We have successfully isolated and characterized three recombinant *Pleurotus* aegerolysins, namely ostreolysin A6, pleurotolysin A2 and erylysin A, and their MACPF-protein partner pleurotolysin B. Using surface plasmon resonance we monitored the binding of these aegerolysins (with or without pleurotolysin B) to large unilamellar vesicles containing CPE. Furthermore, we determined the interaction of some soluble lipid derivatives with the selected aegerolysins. Lytic activity of fungal aegerolysins with their partner MACPF-protein was assessed on calcein-loaded small unilamellar vesicles containing CPE.

We show that all the tested aegerolysins specifically interact with CPE-containing artificial membranes, and that this interaction is stabilized in the presence of pleurotolysin B. Moreover, these membranes/vesicles were permeabilised with aegerolysins and pleurotolysin B only if containing CPE.

Our findings give base to explore the possible lytic effects of fungal aegerolysins and their MACPF-protein partner on biological membranes with similar lipid composition, and their possible biotechnological use as tools for selective control of invertebrate pests containing CPE in their membranes.

PI-44

Characteristics of *Spirosoma linguali* aegerolizyn

Jaka Snoj, Vesna Hodnik, Anastasija Panevska, Peter Maček, Kristina Sepčić, Matej Butala

University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia

Aegerolysin protein family (Pfam 06355) comprises over 300 homologous, small (13–20 kDa) and mostly β-structured proteins that are present in different kingdoms of life (bacteria, eucaryotes). Among bacteria that harbor genes for aegerolysins there are also strains of free-living and non-pathogenic *Spirosoma linguale*. These bacteria are very pleomorphic with a cell shape appearing as filaments, helices, rings and horseshoes. Interestingly, aegerolysin of *S. linguale* is encoded on an operon encompassing downstream of the aegerolysin gene also a gene for an approximately 70 kDa protein. Earlier studies showed that aegerolysins together with larger protein partners can form pores in biological membranes. Thus, we isolated recombinant *S. linguale* aegerolysin, and the larger protein whose gene is co-transcribed with aegerolysin, from the inclusion bodies in *E. coli*. We characterized the proteins and tested the binding of the proteins to artificial membranes using surface plasmon resonance.

PI-45

Structural studies of flexible filamentous virus and virus-like particles

Andreja Šink[1], Ion Gutierrez-Aguirre[2], Magda Tušek Žnidarič[2], Gregor Anderluh[1], Maja Ravnikar[2], Marjetka Podobnik[1]

[1]*National Institute of Chemistry, Department of Molecular Biology and Nanobiotechnology, Slovenia*
[2]*National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia*

Virus-like particles (VLPs) are structures of self-assembled virus coat proteins with highly related or identical structure to their corresponding native viruses. They appear in many different shapes and sizes, and due to their unique structural characteristics, they can be used in various fields of research like biology, biotechnology, medicine, biopharmacy, material science and microelectronics. Our work is focused on studying plant flexible filamentous virus-like particles. We have performed several experiments to determine their biochemical and biophysical characteristics. The filaments are 12 nm in diameter and up to 2 μm long. Secondary structure determined by circular dichroism shows majority of α-helices, which is in agreement with other flexible filamentous viruses. Our current goal is to determine three-dimensional structure of the virus and corresponding VLPs which will be beneficial in further development of nano-applications based on these flexible filamentous particles. Due to complexity of the sample, we are implementing various methods of structural biology into the study. Structural studies using cryo-electron microscopy of the virus and VLP filaments are in progress. Furthermore, we have constructed various coat protein mutants and have been able to heterologously express and purify one that is incapable of assembling into filaments, but rather forms smaller homogeneous oligomers. The secondary structure shows only minor differences in comparison to wild type coat protein, as expected based on protein truncation. These complexes probably represent disk-like structures, which are presumed to be intermediates in VLP/virion formation. We will attempt to determine structures of these disks using X-ray crystallography.

PI-46

Effect of surface composition on interaction of silver nanoparticles with serum transport proteins

Goran Šinko[1], Ivona Capjak[2], Ivana Vinković Vrček[1]

[1]*Institute for Medical Research and Occupational Health, Croatia*
[2]*Croatian Institute of Transfusion Medicine, Croatia*

Nanotechnology is gaining more and more significance in daily life by developing nano-based products. Due to specific physico-chemical properties nanoparticles (NPs) are used in many fields. Silver nanoparticles (AgNPs) belong to the most commercialized NPs due to biocidal activity against bacteria, fungi, and viruses. The use of NPs in medicine requires a molecular-level understanding of how NPs interact with macromolecules. It has been established that serum proteins present in blood will adsorb onto the surface of NPs, forming a "protein corona".
We used albumin and α1-acid glycoprotein (α1AGP) as model proteins. Albumin is low glycosylated protein (55% in serum) which binds water, hormones and xenobiotics. α1-acid glycoprotein is plasma glycosylated α-globulin (3% in serum) which binds basic and neutrally charged lipophilic compounds. Changes in protein secondary structure may affect protein carrier function. Therefore, native structure is important for normal function. Aim of this study was to understand how surface composition of AgNPs affects protein secondary structure due to formation of AgNP-protein complexes. We have prepared AgNPs with structurally diverse surface coatings: trisodium citrate (CIT), sodium bis(2-ethylhexyl) sulfosuccinate (AOT), cetyltrimethylammonium bromide (CTAB), poly(vinylpyrrolidone) (PVP), poly(L-lysine) (PLL), Brij-35 and Tween-20. Circular dichroism (CD) spectroscopy was used to evaluate differences in the absorption of left and right circularly polarized light to probe protein secondary structure. Proportion of the eight structural elements including α-helicity was calculated from experimental CD curves using BeStSel method. CD spectroscopy showed that exposure of BSA to cationic CTAB- and PLL-coated AgNPs did not perturb the secondary structure of BSA but reduction in α-helicity was observed (– 29%). In case of a1AGP, reduction in α-helicity was observed for cationic CTAB- and anionic AOT-coated AgNPs (– 70%).

PI-47

From genes to protein crystals: platform for high throughput protein production and structure determination

Ajda Taler-Verčič[1,2], Aleksandra Usenik[1], Nataša Lindič[1], Katarina Karničar[1,2], Sara Pintar[1], Jure Loboda[1], Marjeta Arnolj[1], Gregor Pretnar[2], Špela Tomaž[1], Dušan Turk[1,2]

[1]*Jožef Stefan Institute, Department of Biochemistry, Molecular and Structural Biology, Slovenia*
[2]*Centre of Excellence for Integrated approaches in chemistry and biology of proteins (CIPKeBiP), Slovenia*

Crystal structure determination consist of the following steps: molecular cloning, small scale protein production testing, large scale protein production, protein purification, protein characterization, protein crystallization and structure determination. To achieve high throughput process the procedures are standardized and parallelized whenever possible.

Molecular cloning: Ligation independent cloning (LIC) enables us to clone several genes at the same time into different expression plasmids. We are using 4 LIC plasmids which add different tag to the protein at the N-terminus. 24 genes, each into 4 different plasmids can be cloned simultaneously.

Small scale protein production testing: Successful clones are applied in small scale protein production test in 96 well format. The use of multichannel pipets and 96 well plates makes the process easy and fast. For gene expression the autoinduction media is used.

Large scale protein production**:** Selected positive clones are further used for large scale protein production.

Protein purification: Aktaxpress chromatography system is used for automated multistep protein purification of up to four protein samples in parallel. Ni affinity chromatography and size exclusion chromatography are used at the beginning of each purification. When needed additional purification steps are introduced (affinity or ion exchange). Protein tags are removed for crystallization.

Protein characterization: Differential scanning fluorimetry (DSF) and solubility tests are used for buffer and additive testing (96 well format testing).

Protein crystallization: Crystallization plates are prepared with the pipetting robot using several commercial screens. Automated imaging system records the plates by the predefined schedule and provides images for remote observation.

Crystal diffraction data collection: Crystals are tested on our in house rotating anode (X-ray). To work with high quality data we collect them at synchrotrons.

PI-48

Production of potato immune signalization and *Potato virus Y* proteins in *Escherichia coli* and crystallization of potato protein TGA2.1

Špela Tomaž[1,2], Ajda Taler-Verčič[2,3], Aleksandra Usenik[2], Anna Coll[1], Kristina Gruden[1], Dušan Turk[2,3]

[1]*National Institute of Biology, Slovenia*
[2]*Jožef Stefan Institute, Slovenia*
[3]*Centre of excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Slovenia*

Potato virus Y (PVY) is one of the most important potato crop pathogens. Different proteins respond to the infection of potato with PVY and cooperate in orchestration of the immune response. Studying protein interactions and their structural characteristics is crucial for understanding the processes, which occur during infection. In the scope of our study we analysed known interactions between selected PVY proteins and potato immune signalization proteins and attempted to produce them in *Escherichia coli*. We were able to produce 13 of 38 selected proteins. We chose to produce potato protein TGA2.1 on a larger scale. We biochemically characterised TGA2.1 with determination of its oligomeric state, its solubility in different buffers and by N-terminal sequencing. TGA2.1 was crystallized in order to determine its structure with X-ray crystallography.

PI-49

Salivary tick cystatin OmC2 targets two key lysosomal cathepsins, S and C, in human dendritic cells

Tina Zavašnik-Bergant[1], Robert Vidmar[1], Andreja Sekirnik[1], Marko Fonović[1], Jiří Salát[2], Lenka Grunclová[2], Petr Kopáček[2], Boris Turk[1,3,4]

[1]*Jožef Stefan Institute, Department of Biochemistry, Molecular and Structural Biology, Slovenia*
[2]*Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, Czech Republic*
[3]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Slovenia*
[4]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*

To ensure successful feeding tick saliva contains a number of inhibitory proteins that interfere with the host immune response and help to create a permissive environment for pathogen transmission. Among the potential targets of the salivary cystatins are two host cysteine proteases, cathepsin S, which is essential for antigen- and invariant chain-processing, and cathepsin C (dipeptidyl peptidase 1, DPP1), which plays a critical role in processing and activation of serine proteases. Here, the effect of salivary cystatin OmC2 from *Ornithodoros moubata* was studied using differentiated MUTZ-3 cells as a model of immature dendritic cells of the host skin. Following internalization, cystatin OmC2 was initially found to inhibit the activity of several cysteine cathepsins, as indicated by the decreased rates of degradation of fluorogenic peptide substrates. To identify targets, affinity chromatography was used to isolate His-tagged cystatin OmC2 together with the bound proteins from MUTZ-3 cells. Cathepsins S and C were identified in these complexes by mass spectrometry and confirmed by immunoblotting. Furthermore, reduced increase in the surface expression of MHC II and CD86, which are associated with the maturation of dendritic cells, was observed. In contrast, human inhibitor cystatin C, which is normally expressed and secreted by dendritic cells, did not affect the expression of CD86. It is proposed that internalization of salivary cystatin OmC2 by the host dendritic cells targets cathepsins S and C, thereby affecting their maturation.

PI-50

Characterization of erythropoietin and estrogen receptors in breast cancer cell lines by western, far-western and double blotting

Mateja Cigoj[1,2], Katja Čop[1,2], Blaž Lebar[1,2], Urška Kotnik[1,2], Barbora Fecková[3], Peter Solár[3], Radovan Komel[1], Nataša Debeljak[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*P.J. Šafárik University in Košice, Faculty of Science, Institute of Biology and Ecology, Laboratory of Cell Biology, Czech Republic*

Breast cancer systemic treatment selection is currently based on status of three receptors; estrogen (ER), progesterone (PR) and human epidermal growth factor (HER2) receptor. However, recent studies suggest involvement of erythropoietin receptor (EPOR) in tumour progression and worse treatment outcome [1]. Our recent study confirmed the correlation of EPOR expression with resistance to tamoxifen treatment [2]. Routine detection of EPOR in clinical samples is limited due to poor antibody specificity.
In aim to elucidate the mechanisms of EPOR induced tamoxifen resistance a model cell line with well characterized EPOR and ER status is required. The objective of our study was to determine expression of classic and alternative EPOR and ER receptors on protein level in different human and rat breast cancer cell lines. The expression has previously been evaluated on gene level [3]. Specific and reproducible western blot detection requires appropriate validation and normalization procedure. Antibody validation was assessed by selection of appropriate positive and negative controls. Normalization to different housekeeping proteins and total protein staining was considered.
In aim to develop specific and reproducible method for EPOR detection, far-western and double blotting were tested. Far-western blotting allows indirect detection of EPOR through the recognition of its interacting partner erythropoietin (EPO) by highly specific EPO primary antibodies [4]. Double blotting is a solution to nonspecific binding of secondary antibodies in immunoblotting procedures that furtherer increases the specificity of results.

References:

1. Larsson A. M., *et. al.* (2009) Clin Cancer Res, 15 (17), pp. 5552–9.
2. Ilkovičova L., *et. al.* (2017) International journal of oncology (in revision).
3. Trošt N., *et. al.* (2013) Int J Mol Med, 31 (3), pp. 717–25.
4. Fecková B., *et. al.* (2016) Oncology Letters, 12 (2), pp. 1575–80.

PI-51

Role of the potato ethylene response factor StERF49 in potato defence against PVY

Anna Coll[1], Tjaša Lukan[1], Hazel McLellan[2], Ana Lazar[1], David Dobnik[1], Paul R.J. Birch[2,3], Kristina Gruden[1]

[1]*National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia*
[2]*University of Dundee (at JHI), College of Life Science, Division of Plant Sciences, UK*
[3]*James Hutton Institute (JHI), Department of Cell and Molecular Sciences, UK*

Potato *(Solanum tuberosum* L*.)* is the world's most widely grown tuber crop and *Potato virus Y* (PVY) is one of the major potato pathogen causing severe crop loss in different areas worldwide. To understand the potato-PVY interaction we studied the role of ethylene response factor (*ERF*) genes from group IX since they have been defined as important elements in response to various biotic stresses. Based on previous transcriptomics experiments performed in our group we selected *StERF49* for further analyses. Expression patterns of the gene in potato cultivar exhibiting hypersensitive resistance (HR) to PVY suggested the importance of *StERF49* as a signalling component in potato defence response. With virus-induced gene silencing (VIGS) we demonstrated that PVY systemic spread is delayed in *StERF*49 silenced *Solanum venturii* plants. We additionally confirmed that the gene negatively regulates resistance to PVY using stable transgenic potato lines in which *StERF49* was knockdown by short hairpin RNAs (shRNAs). Considering the important role of *StERF49* in potato-PVY interaction we are studying the possible regulation mechanism of this gene by means of *in silico* promoter analysis and Y1H. In order to identify its interaction partners we used *StERF49* as a bait to screen a Y2H potato cDNA library. Our results show the importance of *StERF49* in potato defence response to PVY. Therefore, the data will contribute to a better understanding of the complex network of plant defence signalling pathways.

PI-52

Venomics of *Vipera ammodytes ammodytes*

Adrijana Leonardi, Jože Pungerčar, Tamara Sajevic, Igor Križaj

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

Snake venoms are complex mixtures of diverse proteins and peptides, and powerful weapons for pray capture and defense. Characterization of venom composition is required for understanding its pathophysiological action, identification of new bioactive compounds and improvement of effectiveness of antivenoms. The nose-horned viper, *Vipera a. ammodytes* (*Vaa*), is the most venomous European snake, whose venom provokes hematotoxic, neurotoxic, myotoxic and cardiotoxic disturbances in mammals. Combining venom gland transcriptomics and different proteomics technics, we explored, in the so-called venomics approach, the proteome and peptidome of *Vaa* venom. Crude *Vaa* venom was separated by two-dimensional gel electrophoresis into 208 distinct protein spots of 10–100 kDa. Each spot was subjected to in-gel trypsin digestion followed by LC-ESI-MS/MS analysis. Experimental data were searched against the NCBI non-redundant protein database of snake species, supplemented with the information obtained from our analysis of the *Vaa* venom gland transcriptome. Proteins were unambiguously identified in 178 spots, assigned to 15 protein families, seven of which are typical for viperid venoms: serine proteases, metalloproteinases (MPs), C-type lectin-like proteins (snaclecs), phospholipases A_2, cysteine-rich secretory proteins, *L*-amino acid oxidases and disintegrins. In parallel, raw *Vaa* venom was separated by gel filtration chromatography and fractions containing low molecular mass proteins and peptides further analyzed using RP-HPLC, followed by Edman sequencing and MS/MS analysis. In this way, we also identified disintegrins, vascular endothelial growth factor, natriuretic peptides, bradykinin-potentiating peptides and a tripeptide inhibitor of snake venom MPs. By defining the *Vaa* venom, we provided knowledge to explain, at the molecular level, clinical pictures induced by envenomation with this snake species. Our results will also aid in producing more effective *Vaa* antiserum.

PI-53

Identification of in vivo interaction partners of RNA-binding protein TDP-43

Julija Mazej, Sonja Prpar Mihevc, Boris Rogelj

Jožef Stefan Institute, Department of Biotechnology, Slovenia

TDP-43 protein was identified as a predominant component of the ubiquitinated neuronal inclusions deposited in cortical neurons in frontotemporal dementia (FTD) and in motor neurons in amyotrophic lateral sclerosis (ALS). In these diseases, commonly termed as TDP-43 proteinopathies, TDP-43 is redistributed from its predominantly nuclear location to ubiquitin-positive, cytoplasmic foci.

TDP-43 is ubiquitously expressed RNA/-DNA binding protein belonging to the heterogeneous nuclear ribonucleoprotein (hnRNP) family. It has been associated with important cellular functions ranging from gene expression regulation at the transcription and splicing level to mRNA transport and stabilization. To date, only a few TDP-43 interacting proteins have been identified, which makes further insight into both the normal and pathological functions of TDP-43 difficult. What it is known is that the glycine-rich C-terminus of TDP-43 can interact with several hnRNP proteins, namely hnRNPs A1, A2/B1, C1/C2, and A3. Apart from hnRNP proteins, TDP-43 was shown to interact with ATXN2, UBQLN2 and MATR3. To elucidate *in vivo* interaction network of TDP-43 and find its physiologically relevant proximity interaction partners we employed BioID method. In this approach TDP-43 is fused to biotin ligase and expressed in HEK293 cells. When supplied with biotin the promiscuous biotin ligase generates reactive biotinyl-AMP, which covalently reacts with primary amine residues of endogenous proteins in the range of approximately 10 nm. With subsequent biotin affinity capture we isolate biotinylated proteins and further identify them using mass spectrometry.

PI-54

The *Bacillus thuringiensis* temperate phage GIL01 factor that modulates lytic life cycle

Anja Pavlin[1], Miha Bahun[1], Vesna Hodnik[1], Ana Felicita Gobec[1], Douglas F. Browning[2], Nadine Fornelos[3], Matej Butala[1]

[1]*University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia*
[2]*University of Birmingham, School of Biosciences, Institute of Microbiology and Infection, UK*
[3]*The Broad Institute of MIT and Harvard, USA*

After injecting the phage genome into a bacterial host cell, temperate phages can enter a latent state inside the cell. During dormancy, viral functions needed for replication and packaging are tightly shut down by a transcriptional repressor. The *Bacillus thuringiensis* temperate phage GIL01 persists in the host cell as an independent linear replicon. While most temperate phages code for their own transcriptional repressor, the GIL01 lytic cycle is induced by the cellular response to DNA damage and more specifically, the LexA master transcription regulator itself. In order to ensure that the host is not lysed at each round of genomic stress, a second regulator is expected to uncouple the SOS response from phage induction. Here we isolated and characterized a GIL01-encoded factor that is key to lytic phase development. To determine its role in phage cycle regulation we applied beta galactosidase tests and used surface plasmon resonance spectrometry to analyze the protein-DNA interaction. We present an interesting interplay of host- and phage-derived regulators of the lytic cycle. Our results might help in understanding also the molecular mechanism behind latency in other infectious diseases.

PI-55

HNRNPH and its localisation to nuclear G4C2 foci of C9ORF72 amyotrophic lateral sclerosis

Sonja Prpar Mihevc[1], Valter Bergant[1,2], Julija Mazej[1,2], Boris Rogelj[1,2,3]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*Biomedical Research Institute BRIS, Slovenia*

Progressive motor neuron degeneration observed in amyotrophic lateral sclerosis (ALS) has been linked with abnormally extended GGGGCC (G_4C_2) repeat region in the first intron of the *C9ORFf72* gene. This repeat extension forms very stable secondary DNA and (or) RNA structures like G-quadruplexes (G-Q) or hairpins which can bind RNA-binding proteins (RBPs). Accumulation of such repeat containing motifs and their subsequent sequestration of RBPs results in toxic nuclear G_4C_2 RNA foci, disrupted RNA metabolism and decreased expression of C9ORF72 protein. Recently heterogeneous nuclear ribonucleoprotein H (HNRNPH) has been shown to bind G-quadruplexes in G_4C_2 RNA foci in C9ORF72 ALS tissues which disabled its cellular function as splicing regulator, leading to mis-splicing of its targets in patient brain.
In this work we studied HNRNPH and its dependence of quasi RNA recognition motifs (qRRM) for sequestration into pathological G_4C_2 foci and stress granules (SGs). HNRNPH has three qRRMs, each bearing two aromatic amino acids required for forming contacts with G-rich RNA. Substituting both aromatic residues with aliphatic amino acids within individual qRRM did not influence its binding to G_4C_2 foci. The colocalisation of HNRNPH with G_4C_2 foci was also observed with single deletions of qRMM1, qRRM2, and qRMM3 and with deletion of both N-terminal qRMMs. This indicates that qRRM3 alone is sufficient for anchoring HNRNPH to G_4C_2 foci and that HNRNPH bearing at least one of the N-terminal qRRM domains is also sufficient for colocalization in G_4C_2 foci. Apart from nuclear sequestration, HNRNPH is prone to cytoplasmic sequestration in SGs. During stress conditions qRRM2 and qRRM3 but not qRRM1 are sufficient for sequestration of HNRPH in SGs.

PI-56

Modelling condition-specific alternative splicing

Martin Stražar[1], Jernej Ule[2], Tomaž Curk[1]

[1]*University of Ljubljana, Faculty of Computer and Information Science, Slovenia*
[2]*The Francis Crick Institute, RNA Networks Laboratory, UK*

The role of most RNA-binding proteins (RBPs) in alternative splicing (AS) remains unclear. Next-generation sequencing (NGS) assays enable the search for the *splicing code*, a model that can relate multiple *cis*- and *trans*-acting factors to differential exon usage. Contemporary differential exon usage (DEU) statistical tests compare multiple experimental conditions (e.g. RBP knockdowns) to a single reference condition. The emergence of datasets including hundreds of experimental conditions calls for tailored models to detect condition-specific changes in AS and uncover RBP-specific regulation.
We design a novel statistical model, named condition-specific Differential Exon eXpression (csDEX), to discover changes in exon usage that occur only in a small subset of conditions. The package supports both read count- and Percent spliced-in (PSI)-based exon expression quantification. We test for splicing changes on a real-size public dataset with 189 shRNA knockdown samples of different RBPs (including SRSF1, U2AF1/2, PTBP1, hnRNPs, TARDBP) provided by the ENCODE project. We demonstrate the advantages of PSI-based quantification when seeking changes in AS, which are not due to gene expression. The precision of related methods is evaluated using the UCSC *knownAlt* annotation, where csDEX PSI-based model retrieves known AS events with highest precision (98%). The causal effect of RBP binding on AS is further validated by multiple independent data sources, such as RBP binding assays (eCLIP) and motif analysis. For TARDBP, the functional relevance was further verified by successfully retrieving cryptic exons known to be specifically TARDBP-regulated.
We provide the first statistical package for computationally efficient detection condition-specific AS changes in RNA-seq datasets with hundreds of experimental conditions. The predicted condition-specific changes in AS were verified by multiple independent data sources provide functionally relevant candidates.

Poster Session II

Session	Posters
Functional genomics	PI-57 to PI-58
Molecular basis of disease	PI-59 to PI-106
Systems biology	PI-107 to PI-110

PII-57

Computational support for diagnosis of Alzheimer's disease

Jure Fabjan[1,2], Luka Toni[3], Nejc Nadižar[1], Miha Moškon[3], Milica Gregorič Kramberger[2], Uršula Prosenc Zmrzljak[1], Damjana Rozman[1], Zvezdan Pirtošek[2,4]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Centre for Functional Genomic and Bio-chips, Slovenia*
[2]*University Medical Centre Ljubljana, Neurological clinic, Slovenia*
[3]*University of Ljubljana, Faculty of Computer Science and Informatics, Slovenia*
[4]*University of Ljubljana, Faculty of Medicine, Chair of Neurology, Slovenia*

The majority of cases of dementia can be attributed to Alzheimer's disease (AD) [1]. The biggest genetic risk factor for AD is Apolipoprotein E (ApoE) E4 allele, where the presence of the allele translates to an increased risk of AD [2]. Application of diagnostic tests in clinical setting carries the need of robust system for following patients' data. As of now there was no assessment of ApoE allele frequency performed in Slovene AD and healthy populations. Our aim is to perform the genotyping and at the same time develop an information system suitable for diagnostic laboratories. We present an implementation of an information system, which is a part of a larger project called INFOGEN. INFOGEN aspires to create a versatile information system suitable of holding sample data for laboratories involved in a wide variety of medical research topics. Our database is normalized, the data is stored anonymously and the interface enables both automatic and manual entry of data. The traceability of the samples and ease of inputting the data is accomplished by the use of QR codes. At the end of processing pipeline the report is generated for each sample. The developed information system represents an open-source alternative to commercially accessible data management and analysis tools. It enables a safe storage of data and the traceability of the samples during testing. The current database stores data belonging to already obtained samples as well as DNA quality tests and genotyping results.

Acknowledgements: This work was co-financed by the Republic of Slovenia and the European Union under the European Social Fund (project ID 25-13-3) by Slovenian Research Agency grants P1-0390, P2-0359 and the infrastructure grant ELIXIR.

References:

1. Hort J., *et. al.* (2010) Eur J Neurol 17(10), pp. 1236–48.
2. Reitz C., *et. al.* (2014) Biochem Pharmacol 88(4), pp. 640–51.

PII-58

Gene modificaton and therapeutic genome editing via extracellular vesicles delivery of CRISPR/Cas system

Duško Lainšček[1], Lucija Kadunc[1], Mateja Manček Keber[1], Roman Jerala[1,2]

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

The CRISPR/Cas system emerged as the highly potent tool for genome engineering and regulation of gene transcription. This novel gene editing tool consists of Cas9 enzyme, representing the scissors and RNA complex as the precise targeting component. TracrRNA and crRNA, an RNA complex, guides endonuclease Cas9 to target the desired genome site, which bears a short PAM sequence, that is recognized by the Cas9 protein. Target DNA is then cleaved and double stranded breaks of targeted DNA are repaired with cell repair mechanisms. Using CRISPR/Cas9 system we can make knockout models by introducing indel mutations or knock in models by codelivery of a donor DNA that reacts like a DNA template for the repair. Another important benefit of CRISPR/Cas system is highly efficient gene expression alteration. By using catalytically inactive endonuclease dCas9 that possesses no activity and acts only as a binding tool to DNA to recruit heterologous activation or repression domains we can regulate gene expression. One of the additional important features of CRISPR is that it can function in basically any type of cells or organism. However the efficiency of its delivery into cells, particularly for safe therapeutic *in vivo* applications, remains a major bottleneck. Extracellular vesicles, released by cells, can mediate the transfer of different functional molecules. We have shown the efficient packaging and delivery of the CRISPR/Cas system via extracellular vesicles to target cells. Extracellular vesicles can transfer the functional Cas9 or designed transcriptional regulator dCas9-VPR, enabling genome editing or regulating gene transcription. Delivery and robust genome editing and gene upregulation function was shown for cell lines, primary cells and in the animals. *In vivo* delivery of dCas9-VPR/sgRNA by using extracellular vesicles demonstrated therapeutic efficiency in a mouse model of liver damage, which opens the path towards therapeutic applications.

PII-59

The NA(F)LD induced hepatocellular carcinoma in liver specific Cyp51 knock-out mouse models

Kaja Blagotinšek[1], Martina Perše[2], Jera Jeruc[3], Gregor Lorbek[1], Žiga Urlep[1], Uršula Prosenc Zmrzljak[1], Damjana Rozman[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Centre for Functional Genomics and Bio-Chips, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Institute of Pathology, Medical Experimental Centre, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Pathology, Slovenia*

The most common malignant liver tumour of contemporary society is hepatocelullar carcinoma (HCC). Non-alcoholic (fatty) liver disease (NA(F)LD) as an increasing disorder of the liver has been recently identified as one of the important etiological cause of HCC development. The association between NA(F)LD and HCC is alarming due to the high prevalence of NA(F)LD and consequently of the globally rising incidence of HCC. The general risk of the NA(F)LD induced HCC is much higher in males. Interestingly, after the menopause the frequency of HCC in females becomes 1.5-fold higher compared to the males, possibly due to reduced sexual hormones and increased hepatocellular damage and liver inflammation.

To investigate the HCC occurrence due to NA(F)LD induced by the block in cholesterol synthesis we used two hepatocyte knockout mouse models of lanosterol 14α-demethylase (*Cyp51*). The first is the model where the floxed *Cyp51* gene is deleted in the hepatocytes by *Cre* recombinase under control of the albumin promoter (*Alb-Cre*) ($H^{Cyp51-/-}$). The second is the $H^{Cyp51\ doxy+/doxy+}$ mouse model which allows a time-dependent control of *Cyp51* deletion upon application of doxycycline.

Analysis of gene expression in hepatocytes as parenchymal cells that represent just over 50% of total cells in mouse liver was by qPCR while expression profiling by microarrays is in progress. Histopathology and immunohistochemistry were performed on liver sections.

Our current results show age-dependent development of NA(F)LD induced HCC in the $H^{Cyp51-/-}$ mouse model. First tumour development is observed in year 1 with a rising number at later age and depends on the sex with a female to male ratio of 2:1. The results of expression profiling with identification of biochemical pathways and networks involved in the progression from NA(F)LD to HCC and their biochemical validation will be discussed.

PII-60

Implementing genotyping for preemptive pharmacogenomics testing within the U-PGx project: The Slovenian experience

Tanja Blagus[1], Katja Goričar[1], Savica Soldat[1], Sara Redenšek[1], Lidija Konta[2], Vita Dolžan[1] for Ubiquitous Pharmacogenomics Consortium[3]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Pharmacogenetics Laboratory, Slovenia*
[2]*bio.logis Zentrum für Humangenetik, Germany*
[3]*Leiden University Medical Center, The Netherlands*

Introduction: The analysis of the existing published data and Dutch Pharmacogenetics Working Group (DPWG; https://www.pharmgkb.org/page/dpwg) guidelines performed by the U-PGx Consortium showed that actionable drug prescribing for 56 drugs is most strongly linked to 13 pharmacogenes (www.pharmgkb.org/cpic/pairs) [1]. One of the aims of the Horizon2020 project Ubiquitous Pharmacogenomics (U-PGx): Making actionable pharmacogenomic data and effective treatment optimization accessible to every European citizen (www.upgx.eu) was to implement genetic testing for a panel of pharmacogenes to support a clinical study on preemptive pharmacogenetics testing in seven European countries, including Slovenia.
Methods: 1. Identification of a panel of common functional variants within the 13 pharmacogenes with major influence on interpatient variability in drug response and existing evidence based treatment guidelines; 2. Setting up of the infrastructure and methodology for prospective genotyping for selected variants.
Results: In total 48 common functional SNPs within these genes along with the *CYP2D6* gene deletion and duplication were identified as targets for pharmacogenetics testing. Allele-specific PCR fluorescence-based endpoint genotyping (KASPar) was chosen as the genotyping method and SNPline (LCG Group) genotyping system was set up at the Pharmacogenetics Laboratory. The respective KASPar assays were designed and validated by LCG Group and Biologis and introduced and tested at the Pharmacogenetics Laboratory. XL-PCR amplification followed by SNP genotyping was used to detect *CYP2D6* gene deletion and duplication alleles.
Conclusion: We have successfully implemented the genotyping methodology and infrastructure for preemptive pharmacogenomics testing within the U-PGx Project.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 668353.

References:

1. van der Wouden C. H., *et. al.* (2017) Clin Pharmacol Ther 101(3), pp. 341–58.

PII-61

Peri-arteriolar glioblastoma stem-like cell niches express cysteine cathepsins B, K and X

Barbara Breznik[1,2], Vashendriya V.V. Hira[3,4], Mohammed Khurshed[3], Cornelis J.F. Van Noorden[3], Tamara Lah Turnšek[1,2]

[1]*National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*University of Amsterdam, Academic Medical Center, Department of Medical Biology, The Netherlands*
[4]*Barrow Neurological Institute, Barrow Brain Tumor Research Center, USA*

Glioblastoma (GBM) is the most lethal and common brain tumour due to malignant and therapy resistant fraction of GBM stem-like cells (GSLCs) that reside in tumour specific niches. Identification of proteins and their signalling pathways within the GSLC niches may have therapeutic value, because niche-residing GSLCs are therapy resistant and their removal from the niches may increase sensitivity to anti-cancer therapy. Invasion-associated proteases cysteine cathepsins (CTSs) B, K and X are possibly responsible for the remodelling of the niche stroma, in particular for the cleavage of chemotactic cytokine stromal-derived factor SDF-1α (Breznik, 2016), being co-localized with its receptor CXCR4 and GSLC markers in the niche (Hira 2016). Here, we determined the expression of CTSs in peri-arteriolar GSLC niches in serial sections of 25 human GBM samples using chromogenic immunohstochemistry. Moreover, we aimed to detect the activity of these CTSs in the area of GSLC niches using histochemical fluorescence method and specific substrates for CTSB and K (Van Noorden, 1987). Our results showed that peri-arteriolar GSLC niches, expressing GSLC markers CD133 and nestin, SDF-1α and smooth muscle actin (SMA) associated with muscle cells in the arterioles' wall, are present in GBM sections and express high levels of CTSB, K and X. However, these proteases were also expressed in other parts of GBM tissues, CTSB in particular at the invasive edges as demonstrated previously (Strojnik, 1999). Furthermore, we observed the activity of CTSB, but not that of CTSK, in the same GSLC niches, which were characterized by the expression of stem cell markers. Taken together, we proved that CTSs are present in GSLC niches around large arterioles. The next step is to unravel the mechanisms with respect to the role of CTSs in release of GSLCs out of their protective niches. We hypothesize that CTS activity regulate GSLC adhesion and migration out of the niche by cytokine SDF-1α cleavage (Hira, 2017).

PII-62

Comparison of two methods for detecting aminoacids in plasma: tandem mass spectrometry and ion-exchange chromatography

Vanja Čuk[1], Jernej Kovač[1], Urh Grošelj[2], Mojca Žerjav Tanšek[2], Jurij Trontelj[3], Barbka Repič Lampret[1]

[1]*University Medical Centre Ljubljana, University Children's Hospital, Unit of Special Laboratory Diagnostics, Slovenia*
[2]*University Medical Centre Ljubljana, University Children's Hospital, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Slovenia*
[3]*University of Ljubljana, Faculty of Pharmacy, Slovenia*

Amino acids represent an important biochemical marker for a group of inborn errors of metabolism called aminoacidopathies. Regular and accurate monitoring of blood amino acids is also very important for a successful management of patients, to evaluate their nutritional status and dietary compliance. Ion-exchange chromatography (IEC) with post-column ninhydrin derivatisation represented a gold standard in quantitation of amino acids in physiological samples. In recent years IEC method is being replaced by tandem mass spectrometry (MS/MS). Advantages of the later method are mainly shorter time of analysis and greater specificity. The main focus of our study was to assess the influence of measured values on clinical interpretation of the results.

We quantified amino acids in blood plasma of 123 children. The Bland-Altman test was used to compare agreement between methods. The expected limit of agreement that does not affect the clinical interpretation was set at 15%. Results showed that percent differences for 11 amino acids were below the set 15%, except for citrulline (31%), serine (19.5%) and tyrosine (19.0%). Accuracy and precision were also calculated. Accuracy was better for IEC method (RSD 3 – 13.5%) compared with RSD for the LC-MS/MS method (4.3–14.8%). Precision was calculated as relative error between measured values of control plasma and true values. The results were similar between methods. We set 15% acceptability of errors for both parameters, which were not exceeded. Correlation coefficient > 0.70 showed high degree of linear relationship between the two methods.

In conclusion we showed high degree of agreement between methods for most of amino acids compared. We can conclude that IEC method can be replaced with LC-MS/MS method, as average differences between methods do not influence clinical interpretation of the results. Although when monitoring tyrosine levels single method is preferred and new reference ranges should be calculated for citrulline.

PII-63

New molecular-genetic diagnosis for familial erythrocytosis in Slovenia

Tanja Kunej[1], Martina Fink[2], Tadej Pajič[2], Saša Anžej Doma[2], Peter Černelč[2], Radovan Komel[3], Irena Preložnik Zupan[2], Nataša Debeljak[3]

[1]*University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Slovenia*
[2]*University Medical Centre Ljubljana, Clinical Department of Haematology, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*

Erythrocytosis is blood disorders characterized by an increased mass of erythrocytes, hematocrit (Ht) and hemoglobin (Hb). It is caused by an inherited or acquired mutations or as a compensatory mechanism in some chronical disorders. Polycythemia vera (PV) is the most common acquired erythrocytosis caused by mutations in Janus kinase 2 (*JAK2*). Familial erythrocytosis (FE) is a rare congenital disorders with different genetic background [1]. Type 1 FE (ECYT1) is primary FE caused by mutation in erythropoietin receptor (*EPOR*). Other secondary FE are caused by several mutations of factors involved in oxygen sensing pathway leading to increased erythropoietin (EPO) production, such as Von Hippel-Lindau tumour suppressor (VHL, indicative for ECYT2), HIF prolyl hydroxylase 2 (PHD2, indicative for ECYT3) and hypoxia-inducible factor 2 alfa (HIF2A, synonym for Endothelial PAS domain protein 1 (EPAS1), indicative for ECYT4). Recent whole-genome sequencing identified 21 candidate genes and variants involved in FE [2].
The aim of our study was to analyse frequencies of *JAK2* negative FE and to extend diagnostic procedures by development of additional molecular-genetic tests.
Current diagnostic procedure in Slovenia enables exclusion of *JAK2* gene mutations, the cause of PV. *JAK2* negative patients are not tested further. Within our study we determined sequence variants of the *EPO, EPOR, VHL* and *EPAS1* gene associated with FE, now in process of integration into diagnostic procedures for FE in Slovenia [3]. *JAK2* negative patents with increased Ht (n = 81) were invited to collaborate in extensive questionnaire and genetic testing. Lifestyle and comorbidities were assessed to determine correlations with clinical pictures.
New diagnostic tests will enable to determine genetic variations and frequencies of FE in Slovenian population in correlation with clinical picture.

References:

1. Hussein K. (2012) Eur J Hum Genet.
2. Camps C., *et. al.* (2016) Haematol.
3. Vočanec D., *et. al.* (2017) In preparation.

PII-64

PREemptive Pharmacogenomic testing for Preventing Adverse drug REactions (PREPARE) study: The Slovenian experience

Vita Dolžan[1], Tanja Blagus[1], Sara Redenšek[1], Katja Goričar[1], Gregor Mlinšek[2], Antonija Poplas Susič[3], Barbara Mazej Poredoš[3], Živka Vivod Pečnik[3], Anja Zidanšek[3], Blanka Kores Plesničar[4], Polona Vidič Hudobivnik[5], Jasna Klen[2,5] for SLO-UPGx* and Ubiquitous Pharmacogenomics Consortium[6]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Pharmacogenetics Laboratory, Slovenia;* [2]*University Medical Centre Ljubljana, Slovenia;* [3]*Health Care Center Ljubljana, Slovenia;* [4]*University Psychiatric Clinic Ljubljana, Slovenia;* [5]*Health Care Center Kočevje, Slovenia;* [6]*Leiden University Medical Center, The Netherlands;* *SLO-UPGx: Vanja Peršič[2], Damjan Kovač[2], Barbara Vajdič Trampuž[2], Boštjan Bizjak[2], Angelika Marjetič Ulčakar[3], Nadja Khalil[3], Nika Šatej[3], Tea Stegne Ignjatovič[3], Zala Kumše[3], Andrej Škrinjar[3], Črt Zavrnik[3], Marko Saje[4], Tea Terzič[4], Arijana Turčin[4], Marko Vrbnjak[4]

Pre-emptive pharmacogenomic testing can lead to improved patient treatment outcomes, however its application to clinical practice is mostly limited to single drug-genes pairs [1]. The clinical study PREemptive Pharmacogenomic testing for Preventing Adverse drug REactions (PREPARE) within the Horizon2020 project Ubiquitous Pharmacogenomics (U-PGx) (www.upgx.eu) aims to establish, if implementation of PGx-guided drug prescribing for a panel od drug-pharmacogene pairs reduces drug-genotype associated adverse drug reactions (ADRs) in comparison to patients receiving standard of care treatment in seven European countries, including Slovenia. Within a prospective, block-randomized, controlled clinical study, pre-emptive genotyping is performed for a panel of 13 pharmacogenes with available treatment guidelines. Slovenia was randomized to start with PGx-guided prescribing (study arm) and will switch to standard of care (control arm) after 18 months. In total 1200 patients will be included. Currently, patients are recruited at the University Clinical Center Ljubljana and Health Care Center Ljubljana, to be followed by the University Psychiatric Clinic Ljubljana and Health Care Center Kočevje. Patients in routine care are invited in the study when first prescribed a drug with DPWG guideline. DNA samples from patients are genotyped for a panel of 48 genetic variants in 13 pharmacogenes. For study arm genotype data and DPWG guidelines are used by physicians to guide the dose and drug selection for the study drugs. For control arm, genotype data will be reported after the end of the study. All patients are followed-up for 12 weeks to assess the impact of preemptive genotyping on patient outcomes and cost-effectiveness. Within the U-PGx project and the PREPARE study, panel-based pre-emptive pharmacogenetic was successfully introduced in Slovenia.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 668353.

References: 1. van der Wouden C. H., *et. al.* (2017) Clin Pharmacol Ther 101(3), pp. 341–58.

PII-65

Immune checkpoints *PD-1* and *PD-L1* polymorphisms and chemotherapy outcome in malignant mesothelioma

Katja Goričar[1], Viljem Kovač[2], Vita Dolžan[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Pharmacogenetics Laboratory, Slovenia*
[2]*Institute of Oncology Ljubljana, Slovenia*

Introduction: Immune checkpoints are crucial modulators of a balanced physiological immune response. Tumours can exploit these mechanisms to acquire immune resistance, which promotes survival of cancer cells and tumour progression. Recently, therapeutic approaches inhibiting immune checkpoints programmed cell death 1 (PD-1) or programmed cell death 1 ligand 1 (PD-L1) have been successful in several tumours. Furthermore, platinum drugs were shown to affect immune response, and expression of PD-1 and PD-L1 was associated with resistance to cisplatin. Cisplatin-based chemotherapy is commonly used in malignant mesothelioma (MM), an aggressive cancer of pleura or peritoneum. Our aim was therefore to determine whether single nucleotide polymorphisms (SNPs) in genes for PD-1 and PD-L1 influence the outcome of cisplatin based chemotherapy in MM patients.
Methods: The study included 171 MM patients treated with gemcitabine/cisplatin or pemetrexed/cisplatin doublet chemotherapy. All were genotyped for six SNPs in genes coding for PD-1 (*PDCD1*) and PD-L1 (*CD274*). Cox and logistic regression were used to assess their influence on treatment outcome.
Results: CD274 rs4742098 (c.*2635A>G) was significantly associated with the outcome of gemcitabine/cisplatin chemotherapy: carriers of at least one polymorphic allele had significantly longer PFS compared to carriers of two wild-type alleles (9.1 vs 7.1 months, HR = 0.64, 95% CI = 0.43–0.94, *P* = 0.025), as well as significantly longer OS (21.5 vs 14.0 months, HR = 0.59, 95% CI = 0.39–0.91, *P* = 0.016). They were also more likely to achieve partial or complete response (OR = 2.19, 95% CI = 1.02–4.71, *P* = 0.045).
CD274 rs4742098 was also associated with increased risk of nausea or vomiting (*P* = 0.024) and alopecia (*P* = 0.036). *CD274* rs4143815 and *PDCD1* rs10204525 were also associated with increased risk of nausea or vomiting (*P* = 0.023 and *P* = 0.025, respectively).
Conclusion: In conclusion, genetic variability of immune checkpoints may influence response to gemcitabine/cisplatin chemotherapy in MM and may potentially contribute to a more personalized treatment approach.

PII-66

The effect of sulfasalazine and its metabolites on regulation of energy metabolism in skeletal muscle

Simona Hanželič[1], Vid Jan[1], Katarina Miš[1], Alexander V. Chibalin[2], Sergej Pirkmajer[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[2]*Karolinska Institutet, Department of Molecular Medicine and Surgery, Sweden*

AMP-activated protein kinase (AMPK) plays a key role in regulation of whole-body energy metabolism. AMPK activation in skeletal muscle, a major site of postprandial glucose uptake and insulin resistance in type 2 diabetes, increases glucose uptake and fatty acid oxidation. AMPK is therefore a promising pharmacological target to treat type 2 diabetes. Methotrexate, a widely used antirheumatic drug, inhibits clearance of ZMP, an AMP-mimetic and the active form of AMPK activator AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside), and promotes AMPK activation in skeletal muscle [1]. Sulfasalazine (SSZ), another widely used antirheumatic drug, also inhibits clearance of ZMP [2], indicating it might act as an AMPK activator.

We determined whether SSZ or its metabolites 5-aminosalicylic acid (5-ASA) and sulfapyridine activate AMPK in skeletal muscle. We treated rat L6 myotubes and primary human myotubes with 1 mM SSZ, 5-ASA or sulfapyridine. In L6 myotubes SSZ increased phosphorylation of the AMPK α-subunit (Thr172) as well as phosphorylation of acetyl-CoA carboxylase (ACC, Ser79), a downstream target of AMPK. Similarly, phosphorylation of ACC was increased in SSZ-treated human myotubes. We also examined whether therapeutic concentrations of SSZ might activate AMPK. Low concentrations of SSZ (50–300 μM) increased phosphorylation of AMPK and ACC in human myotubes. Increase in AMPK and ACC phosphorylation was also observed in L6 myotubes, although the difference did not reach statistical significance. Sulfapyridine and 5-ASA did not alter AMPK and ACC phosphorylation.

Collectively, our results show that SSZ acts as an AMPK activator, while its two metabolites, sulfapyridine and 5-ASA, do not. Our findings might have important implications for further development of clinically useful AMPK activators.

This study was supported by ARRS (P3-0043, J7-8276).

References:

1. Pirkmajer S., *et. al.* (2015) Diabetes 64(2), pp.360–9.
2. Baggott J. E., *et. al.* (1992) Biochem J 282 (Pt 1), pp.197–202.

PII-67

Trans-activation response element RNA is detectable in the plasma of a subset of aviremic HIV-1-infected patients

Anžej Hladnik[1], Jana Ferdin[1], Katja Goričar[1], Steven G. Deeks[2], Boris M. Peterlin[2], Ana Plemenitaš[1], Vita Dolžan[1], Metka Lenassi[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*University of California, San Francisco (UCSF), Department of Medicine, USA*

Introduction: Plasma trans-activation response element (TAR) RNA has been suggested as a possible biomarker for determining the HIV-1 reservoir size in infected individuals. TAR RNA is produced during non-processive transcription in HIV-1 productively infected and latent T cells. The aim of this study was to determine whether TAR RNA can be found in the blood plasma of aviremic HIV-1-infected patients and to examine whether TAR levels correlate to patient clinical characteristics.
Methods: This study accessed 55 plasma samples from the SCOPE cohort: 9 uninfected, 8 viremic (non-controllers) and 38 aviremic (19 ART-suppressed, 19 elite controllers) subjects. Subjects were characterized with respect to age, gender, ethnicity, HIV status (CD4+ and CD8+ counts, and HIV RNA level) and ART regimen. All subjects provided informed consent and the parent study was approved by the UCSF Committee on Human Research. For TAR RNA detection, RNA extracted from plasma and plasma exosomes was reverse transcribed, amplified by PCR with TAR-specific primers and analysed by gel electrophoresis. Statistical analysis was performed using IBM SPSS Statistics (v19.0).
Results: Our optimised PCR-based assay provided 100% specificity and 100% sensitivity for detection of TAR RNA. TAR RNA was present in clinical samples of 63% (24/38) of aviremic HIV-1-infected patients, who were either treated with antiretroviral therapy or were elite controllers. Of those, TAR RNA of exosomal origin was detected only in 37.5% (9/24). Statistical analysis of obtained data has shown that TAR RNA levels did not correlate with patient gender, age, CD4 levels, CD8 levels and treatment regime, but showed a statistically significant correlation with CD4/CD8 ratio ($P = 0.047$).
Conclusion: This study is the first to investigate plasma TAR RNA in a relatively large cohort of HIV-1 infected patients. Furthermore, we show that the TAR RNA molecules in the plasma of these aviremic patients are not limited to exosomes.

PII-68

Aldo-keto reductase 1C3 as a potential biomarker of serous ovarian cancer?

Marko Hojnik[1], Caroline Kreuzinger[2], Nataša Kenda[3], Špela Smrkolj[3], Snježana Frković Grazio[4], Dan Cacsire Castillo-Tong[2], Tea Lanišnik Rižner[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*Medical University Vienna, Department of Obstetrics and Gynecology, Austria*
[3]*University Medical Centre Ljubljana, Division of Gynecology and Obstetrics, Slovenia*
[4]*University Medical Centre Ljubljana, Division of Gynecology and Obstetrics, Department of Pathology, Slovenia*

Ovarian cancer is the sixth leading cause of cancer-related death in Europe, with poor 5-year survival rate and compared to other cancers the overall survival has not increased significantly in the last 40 years (Bast, 2011). The high mortality rate of ovarian cancer patients is partly due to the lack of effective screening and diagnosis methods and the development of resistance to all classes of chemotherapeutical agents (Armstrong *et. al.*, 2012). The mechanisms underlying the development of resistance have been intensively studied and include repair of DNA lesions, altered cellular transport of the drug, increased antioxidant production and reduction of apoptosis, among others.

The involvement of the AKR1C enzymes in the mechanisms of resistance to platinum and taxane based drugs have not been defined in detail; however the human AKR1C enzymes metabolize a variety of xenobiotics, including doxorubicin and oracin, which partially explains their involvement in drug resistance (Novotna *et. al.*, 2008). Increased AKR1C3 levels have been shown in ovarian cancer cell lines resistant to carboplatin and docetaxel, while to the best of our knowledge increased levels of AKR1C3 have not yet been reported in larger sets of ovarian cancer specimens.

The objective of this study is thus to examine AKR1C3 levels in serous ovarian cancer and to evaluate the potential association between AKR1C3 and drug resistance. We have collected 132 formalin-fixed, paraffin-embedded samples of serous ovarian cancer at the University Medical Centre Ljubljana, Slovenia and at the Medical University Vienna, Austria together with all relevant clinical information. Preliminary immunohistochemical staining with anti-AKR1C3 antibodies (Sigma-Aldrich, clone NP6.G6.A6) showed weak staining in nuclei and cytoplasm of epithelial cells. The whole set of samples will be stained automatically on immunostainer (BenchMark XT, Ventana Medical Systems). Scoring and results of the correlation studies will be presented.

PII-69

Computational support for management and analysis of human samples obtained from suicide victims

Marjeta Horvat[1], Žiga Pintar[2], Miha Moškon[2], Uršula Prosenc Zmrzljak[3], Damjana Rozman[3], Katarina Uršič[1], Alja Videtič Paska[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*University of Ljubljana, Faculty of Computer and Information Science, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Centre for Functional Genomics and Bio-Chips, Slovenia*

Suicide is one of the most common causes of death in the world and also in Slovenia, where approximately 500 people commit suicide every year. It is a very complex phenomenon, resulting from an interplay of several environmental and genetic factors. Researches on suicide are crucial as they help us to understand the genetic background of suicidal behaviour and thus to find potential treatments.

Suicidal behaviour is one of the research fields in the laboratory of the Institute of Biochemistry in Ljubljana, where numerous human samples, such as blood, cerebrospinal fluid, liquor and brain tissue, are collected every week. Storing this large amount of data in notebooks or Excel tables could cause loss of information as they do not provide orderliness, sufficient traceability and user-friendliness. Therefore, we decided to establish a new information system that would enable more efficient and organised storage of information about human samples obtained from suicide victims.

Our recently designed multi-purpose information system enables the storage of information about collected and/or analysed samples, managing and editing the data, searching for suitable samples for experiments, as well as statistical analyses and comparison between the samples. Furthermore, it is necessary to keep a record of stored original samples, extracted DNA and RNA samples, as well as results of performed analyses (such as gene expression analyses — qPCR, DNA methylation). For this reason, the information system supports data import and export in CSV format, which enables fast and reliable data entry and compatibility with the majority of commercial software products for data management and analysis. It was designed with open-source tools. A great advantage of the system is its accessibility to a targeted community of authorised researchers.

We believe that the system will contribute to better organisation of laboratory work also in other medical related research fields.

PII-70

First report of coexistence of neurofibromatosis type 1 and Klinefelter syndrome

Tinka Hovnik[1], Maruša Debeljak[1], Jernej Kovač[1], Sara Bertok[2], Katarina Trebušak Podkrajšek[1,3]

[1]*University Medical Centre Ljubljana, Children's Hospital Ljubljana, Unit for Special Laboratory Diagnostics, Slovenia*
[2]*University Medical Centre Ljubljana, Children's Hospital Ljubljana, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*

Introduction: We present two cases with coexistence of two distinct genetic conditions in one individual. Neurofibromatosis and Klinefelter syndrome are relatively frequent genetic disorders affecting 1/3000 and 1/1000 people, respectively. Both are clinically defined genetic disorders with neurobehavioral development and variable phenotypic outcome.
Methods and results: At first standard cytogenetic analysis, karyotipization on GTG banded metaphases from peripheral blood and fluorescence *in situ* hybridization were performed. Primary chromosomal aneuploidies 47,XXY were detected in both cases confirming the clinically suspected Klinefelter syndrome. Later on, following an unusual clinical presentation with typical signs of neurofibromatosis, a second genetic disorder was suspected. Therefore, next generation sequencing (NGS) of the neurofibromatosis related genes *NF1*, *NF2*, *SPRED1* was issued. In each case different heterozygous nonsense mutations in *NF1* gene, namely p.Arg461Ter (c.1381C>T) and p.Arg1362Ter (c.4084C>T) were found. Both mutations were already reported as pathological in patients with neurofibromatosis type 1.
Conclusion: The coexistence of two distinct genetic conditions is known to be a rare event. Nevertheless, we report two cases of a coexistence of neurofibromatosis type 1 and Klinefelter syndrome that according to our knowledge have not been reported yet.

PII-71

BUB1B polymorphisms contribute to gastric cancer susceptibility

Petra Hudler, Aida Zečkanović, Tadej Žlahtič, Marija Rogar, Radovan Komel

University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia

Gastric adenocarcinoma has been in decline in most developed countries in the last few decades. Nevertheless, this disease remains one of the most common causes of cancer-related death worldwide. The poor outcome has been attributed to late detection of the condition, aggressive pathogenesis and lack of symptoms during the early stages of tumour development. One of the most prominent global features of sporadic gastric cancers is genomic instability, which can be distinguished via two common footprints found in cancer genomes. The first, microsatellite instability (MSI), has been fairly well elucidated and attributed to defects in mismatch repair genes. Another type, chromosomal instability (CIN) is characterized by frequent chromosome missegregation and has been associated with poor patient prognosis. It has been hypothesized that reduced mitotic fidelity could contribute to cancer progression by increasing genetic diversity among tumour cells. In addition, CIN may be influenced by exposure to the cytotoxic drugs used for chemotherapy.

The primary aim of this study was to determine whether selected polymorphisms in mitotic kinase *BUB1B* and kinetochore proteins contribute to gastric cancer risk. A group of 520 patients diagnosed with gastric cancer and control subjects were included in the genotyping association study.

AA genotype of rs1801389 was more represented in gastric cancer patients than expected (P = 0.036). Under the recessive model, patients with AA genotype had 3-times higher odds for developing gastric cancer. Patients with GG genotypes of rs1565866 and rs2277559 had increased risk of developing a more aggressive diffuse form of gastric cancer. Survival of patients was associated with perineural invasion and lymph node involvement.

In conclusion, the study revealed significant associations of polymorphisms in mitotic gene *BUB1B* with gastric cancer risk and histopathological features.

PII-72

Yeast as a model for research of amiotrophic lateral sclerosis

Tajda Klobučar[1], Iztok Dogša[2], Blaž Jug[1], Boris Rogelj[3,4,5], Polona Jamnik[1]

[1]*University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Chair of Biotechnology, Microbiology and Food Safety, Slovenia*
[2]*University of Ljubljana, Department of Food Science and Technology, Chair of Microbiology, Slovenia*
[3]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[4]*Biomedical Research Institute BRIS, Slovenia*
[5]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*

The occurrence of neurodegenerative diseases in the past years is constantly growing. Although Alzheimer's and Parkinson's diseases probably get the majority of attention, researchers are also putting more effort in understanding less common neurodegenerative disease such as amiotrophic lateral sclerosis (ALS). All these disorders are known for their association with protein misfolding and aggregation. To be able to cure such disease we first have to understand their development and progression. The development of cellular and animal models that reproduce central aspects of neurodegeneration has been crucial for our understanding of these diseases. Yeast *Saccharomyces cerevisiae* has been recognized as a powerful tool to study the molecular basis of different neurodegenerative diseases, since several pathways associated with neurodegeneration are conserved between yeast and humans, such as protein quality control, apoptotic and necrotic cell death, protein folding, trafficking and secretion. In our study, yeast *S. cerevisiae* expressing human TDP-43 fused to GFP was used. After adding galactose as inductor and glucose as repressor of TDP-43 expression, yeast growth was monitored by measuring optical density, culturability and total cell number. Expression of TDP-43 was followed by measuring fluorescence of cell suspension ad formation of protein aggregates was detected by fluorescence microscope. Preliminary results showed that aggregation of TDP-43 in the yeast cells has occurred and the response of yeast to aggregation was dependent on the growth phase. Results present a good basis for further research of TDP-43 aggregation toxicity as well as modulation of toxicity by small molecules and extracts of natural origin.

PII-73

IDH1-mutated gliomas rely on anaplerosis of glutamate and lactate whereas *IDH1* wild-type gliomas rely on glycolysis and acetate anaplerosis

Mohammed Khurshed[1], Krissie Lenting[2], Remco Molenaar[1], William Leenders[2], Cornelis van Noorden[1]

[1]*University of Amsterdam, Academic Medical Center, Department of Medical Biology, The Netherlands*
[2]*Radboud University Medical Centre Nijmegen, Department of Pathology, The Netherlands*

Hotspot mutations in isocitrate dehydrogenase 1 ($IDH1^{MUT}$) initiate low grade glioma (LGG) and secondary glioblastoma and induce neomorphic activity that converts α-ketoglutarate (α-KG) to the oncometabolite *D*-2-hydroxyglutarate (*D*-2-HG). This causes metabolic rewiring that is not fully understood and *in vitro* studies have shown that $IDH1^{MUT}$ cancer cells rely on glutaminolysis, providing cells with α-KG via activities of glutaminase (GLS) and glutamate dehydrogenase (GLUD). We first show by *in silico* analysis of 269 *IDH1* wild-type ($IDH1^{WT}$) and 408 $IDH1^{MUT}$ gliomas, obtained from the The Cancer Genome Atlas (TCGA) database, that $IDH1^{WT}$ gliomas have high expression levels of genes encoding for enzymes that are involved in glycolysis and acetate anaplerosis. On the other hand, the tricarboxylic acid (TCA) cycle, rather than glycolytic lactate production, is the predominant metabolic pathway in $IDH1^{MUT}$ gliomas and is driven by lactate and glutamate anaplerosis to facilitate production of α-KG, and ultimately *D*-2-HG. $IDH1^{WT}$- and $IDH1^{MUT}$-related differences in expression were found in both LGG and glioblastoma. Furthermore, via *in situ* enzymatic activity mapping, we show in human gliomas and in xenocraft models that GLUD activity is increased and GLS activity is decreased in $IDH1^{MUT}$ glioma, indicating that $IDH1^{MUT}$ gliomas depend on glutamatolysis, rather than glutaminolysis. Finally, we confirmed the glutamate dependency of $IDH1^{MUT}$ gliomas by MRS-flux analysis. We show that $IDH1^{WT}$ gliomas have a typical Warburg phenotype and rely on acetate anaplerosis whereas $IDH1^{MUT}$ gliomas are glutamate and lactate dependent. This metabolic rewiring in $IDH1^{MUT}$ glioma, enables targeting of glutaminolysis rather than direct inhibition of $IDH1^{MUT}$ for therapy. It diminishes the supply of glutamate-derived α-KG and directly inhibits the production of *D*-2-HG and simultaneously worsen the redox status of the glioma cells by inhibiting NAD(P)H production by GLUD.

PII-74

Sulphasalazine does not promote AICAR-stimulated AMPK activation in cultured myotubes

Nejc Kikelj[1], Rebeka Komel[1], Vid Jan[1], Katarina Miš[1], Alexander Chibalin[2], Sergej Pirkmajer[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[2]*Karolinska Institutet, Department of Molecular Medicine and Surgery, Sweden*

Pharmacological activation of the AMP-activated protein kinase (AMPK) in skeletal muscle is a promising approach for treatment of type 2 diabetes. The search for clinically useful AMPK activators is therefore a major focus of current research. ZMP (AICAR-monophosphate) is an AMP-mimetic and a pharmacologically active form of the most commonly used AMPK activator AICAR. Methotrexate (MTX), a widely used antirheumatic drug, increases intracellular concentrations of ZMP by inhibiting its conversion to inosine monophosphate and enhances AICAR-stimulated AMPK activation in skeletal muscle. Sulfasalazine (SSZ), another widely used antirheumatic drug, also inhibits ZMP clearance. We explored whether SSZ enhances AICAR-stimulated AMPK activation in skeletal muscle.

We treated rat L6 myotubes, grown in nucleoside-free media, with SSZ, MTX and/or AICAR. Activation of AMPK was assessed by measuring phosphorylation of the AMPK α-subunit (Thr172) and phosphorylation of its downstream target acetyl-CoA carboxylase (ACC, Ser79). SSZ alone increased AMPK and ACC phosphorylation in dose-dependent manner. MTX markedly increased AICAR-stimulated phosphorylation of AMPK and ACC. Conversely, SSZ did not enhance AICAR-stimulated phosphorylation of AMPK and reduced AICAR-stimulated phosphorylation of ACC. Absence of nucleosides in cell culture media stimulates de novo purine synthesis, which might modulate effects of SSZ. To explore this possibility, we treated L6 myotubes with SSZ in the absence or presence of nucleosides. SSZ increased phosphorylation of ACC in nucleoside-free as well as nucleoside-containing medium.

Collectively, our results suggest that SSZ alone can activate AMPK in cultured myotubes, but does not enhance AICAR-stimulated AMPK activation. MTX and SSZ likely promote AMPK activation in skeletal muscle through different molecular pathways.

PII-75

Search for biomarkers of endometriosis with multiplex approach

Tamara Knific[1], Andrej Vogler[2], Manuela Gstöttner[3], René Wenzl[3], Dmytro Fishman[4,5], Hedi Peterson[4,5], Jaak Vilo[4,5], Tea Lanišnik Rižner[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*University Medical Centre Ljubljana, Department of Obstetrics and Gynecology, Slovenia*
[3]*Medical University Vienna, Department of Obstetrics and Gynecology, Austria*
[4]*University of Tartu, Institute of Computer Science, Estonia*
[5]*Quretec Ltd., Estonia*

Endometriosis is a complex gynecological disease, which comprises three different entities, ovarian, peritoneal and deep infiltrating endometriosis, with different etiologies and pathogeneses. The definitive diagnosis of endometriosis requires surgery, thus there is a great need for non-invasive diagnostic approaches. Although more than 100 potential biomarkers have been investigated, none of them has been translated into clinical practice [1].

We have searched for potential diagnostic biomarkers in plasma samples of 230 women that underwent laparoscopy at the University Medical Centre Ljubljana, Slovenia and at the Medical University Vienna, Austria. Our case group comprised 116 women with surgically and histologically verified endometriosis. Control group included 94 women with endometriosis-like symptoms without the presence of endometriosis but with various benign gynaecological diseases. We used a targeted multiplex proteomics approach to measure the concentrations of 40 cytokines and chemokines in plasma samples. Wilcoxon singed-rank test revealed that a single cytokine/chemokine does not differentiate patients with endometriosis from the control group of patients. Further stratification according to the type of endometriosis (i.e. ovarian, peritoneal, deep infiltrating and their combinations) identified seven potential biomarkers for peritoneal endometriosis (CCL21, CCL11, CCL26, CX3CL1, CCL1, IL-6, CCL3), two for peritoneal and ovarian endometriosis (CXCL11, CXCL12), one for peritoneal combined with deep infiltrating endometriosis (INF-γ) and two for all three types of endometriosis (CCL15, CXCL12) ($P < 0.05$). Further statistical modelling to develop a diagnostic algorithm that would include plasma levels of biomarkers combined with clinical data is currently ongoing.

References:

1. Lanišnik Rižner T. (2014) Expert Rev Mol Diagn 14 (3), pp. 365–85.

PII-76

The role of cystatins in non-canonical NLRP3 inflammasome activation

Mojca Trstenjak Prebanda[1], Monika Biasizzo[1], Katarina Maher[1], Janja Završnik[1], Veroniqie Brault[2], Yann Hérault[2], Boris Turk[1], Nataša Kopitar-Jerala[1]

[1]*Jožef Stefan Institute, Department of Biochemistry, Molecular and Structural Biology, Slovenia*
[2]*Université de Strasbourg, Institut de Génétique Biologie Moléculaire et Cellulaire, France*

Cystatins are reversible, tight-binding inhibitors cysteine cathepsins and legumain that exert various physiological functions. Type 1 cystatins, also called stefins, are intracellular proteins that are present in most cells (cystatin A and B). Type 2 cystatins are secreted proteins found in most body fluids (cystatin C, D, E/M, F, G, H, S, SA, and SN). The stefin B is encoded by *CSTB* on human chromosome 21q22.39 and the loss-of-function mutations in the stefin B gene were reported in patients with Unverricht-Lundborg disease (EPM1). Stefin B is found in cytoplasm, nucleus and mitochondria. In our study we used stefin B-deficient mice, as well as mice with an additional copy of stefin B gene, stefin B-trisomic mice and cystatin C-deficient mice and compared the signalling pathways upon induction of sepsis. Both, stefin B-deficient and cystatin C-deficient mice were significantly more sensitive to the lethal LPS-induced sepsis, due to increased caspase-11 expression. We demonstrated that the increased expression of caspase-11 in stefin B-deficent macrophages was not due to activation of TRIF pathway and type I interferon signalling, but to NF-κB activation. Upon Nlrp3 inflammasome activation, the amount of mitochondrial ROS in stefin B- and cystatin C-deficient macrophages was greater than that in wild-type macrophages. Treatment with LPS induced autophagy in wild-type but less in stefin B deficient macrophages, as shown by immunoblot and confocal microscopy analysis. Stefin B deficient macrophages also had less p62/SQSTM1 protein upon LPS stimulation than did wild-type macrophage that resulted in impaired mitophagy and increased ROS formation. In stefin B-trisomic macrophages, we determined diminished caspase-11 expression upon LPS stimulation; however, additional copy of stefin B gene did not protect animals form LPS induced sepsis.

PII-77

Effects of low intensity exercise on cardiac lipid metabolism of fructose fed male rats

Milan Kostić, Goran Koričanac, Snežana Tepavčević, Tijana Ćulafić, Snježana Romić, Jelena Stanišić, Marija Pantelić, Mojca Stojiljković

University of Belgrade, "Vinča" Institute of Nuclear Sciences, Laboratory for Molecular Biology and Endocrinology, Serbia

Metabolic syndrome is a growing health problem worldwide associated with modern day diet, rich in saturated fat and sugar. Increase in fructose consumption contributes to ectopic lipid storage which in turn is a risk factor for development of type 2 diabetes and cardiovascular disease. Defective substrate utilization in the heart caused by systemic derangements in metabolic syndrome leads to cardiac dysfunction. Exercise is shown to alleviate most of the symptoms related to this metabolic disorder. The aim of this study was to analyze the impact of low intensity exercise on molecular mechanism of cardiac free fatty acid (FFA) transport and metabolism and serum lipid profile of fructose fed male rats. Male Wistar rats were divided into control group and two groups that received 10% fructose for 9 weeks, one of which was additionally exposed to low intensity exercise. Concentration of circulating FFA, triacylglycerol (TAG), total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were measured as well as TAG content in heart tissue. Protein content of fatty acid translocase CD36, carnitine palmitoil transferase 1 (CPT1) and Lipin1 in cardiac lysate and CD36 content in plasma membrane were evaluated. High fructose diet elevated serum TAG and showed a trend of increase of total cholesterol (P = 0.066). Exercise didn't change TAG and total cholesterol, but FFA were decreased compared to control group. Although there was no change in protein expression of CD36 transporter, exercise raised the level of CD36 in plasma membrane compared to fructose group. Exercise increased protein expression of both CPT1, mitochondrial transporter of FFA, and Lipin1, enzyme involved in TAG synthesis, compared to control group. High fructose consumption changed serum lipid levels, whereas low intensity exercise had greater influence on increasing the transport and metabolism of FFA in the heart.

PII-78

Novel links between circadian clock and lipid metabolism in patient with multifactorial metabolic diseases and dyslipidemia

Urša Kovač[1], Caitlin J. Smith[2], Uršula Prosenc Zmrzljak[1], Bruce Bedell[2], Nancy Weathers[2], Laura L. Jeliffe-Pawlowski[3,5], Rebecca J. Baer[4,5], Urh Grošelj[6], Katarina Trebušak Podkrajšek[7,8], Tadej Battelino[6,9], Damjana Rozman[1], Kelli K. Ryckman[2]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Centre for Functional genomics and Bio-Chips, Slovenia;* [2]*University of Iowa, Department of Epidemiology, USA;* [3]*University of California San Francisco School of Medicine, Department of Epidemiology and Biostatistics, Division of Preventive Medicine and Public Health, USA;* [4]*University of California San Diego, Department of Pediatrics, USA;* [5]*California Department of Public Health, Genetic disease Screening Program, USA;* [6]*University Medical Centre Ljubljana, University Children's Hospital Ljubljana, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Slovenia;* [7]*University Medical Centre Ljubljana, University Children`s Hospital, Unit of Special Laboratory Diagnostics, Slovenia;* [8]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia;* [9]*University of Ljubljana, Faculty of Medicine, Slovenia*

Some multifactorial metabolic diseases, such as hypercholesterolemia, preterm delivery and obesity, have very little in common on the first glance; however, they all have a similar metabolic phenotype of dyslipidemia. By using appropriate statistical models to investigate these complex human disorders we can infer their genetic overlaps. Disorders of lipid metabolism and transport are responsible for the development of a large spectrum of pathologies, including the development of malignant tumours. We propose that the chosen multifactorial metabolic diseases share significant genetic overlaps also due to the similar metabolic phenotype. We aim to define networks of disease associated single nucleotide polymorphisms (SNPs) from circadian clock and lipid metabolism because it is already known that both processes are highly interconnected. Our results showed that *PNPLA3* and *TM6SF2* (but likely not *CYP51A1*) could represent potential markers for non-alcoholic fatty liver disease in patients with hypercholesterolemia. A correlation between the *PNPLA3* rs738409 polymorphism and *TM6SF2* rs58542926 polymorphism with hypercholesterolemia has been identified in 121 boys through Slovenian universal screening program for hyper-cholesterolemia if compared to male European individuals from 1000 Genomes project and European Americans from the EVS collection. Furthermore, 904 women having preterm delivery from prenatal screening program were genotyped using a custom genotyping platform of 72 SNPs covering genes from circadian clock and lipid metabolism. Six SNPs met the statistical threshold with Bonferroni correction from *APOE*, C*ELSR2-PSRC1-SORT1* gene cluster, *PER3*, *PNPLA3*, and *ABCA1*. Other SNPs mostly from circadian clock metabolism genes, *CLOCK*, *CRY2*, *PER3*, *BMAL*, *SIRT1* and *PER1* were close to the threshold after multiple correction. Additional work to strengthen the role of SNPs from lipid and circadian metabolism in pathogenesis of different multifactorial metabolic disease is in progress.

PII-79

Differential expression of small RNAs in Slovenian patients with amyotrophic lateral sclerosis and healthy controls

Anja Kovanda[1], Lea Leonardis[2], Janez Zidar[2], Blaž Koritnik[2], Leja Dolenc Grošelj[2], Stanka Ristić Kovačič [2], Tomaž Curk[3], Boris Rogelj[1]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University Medical Centre Ljubljana, Division of Neurology, Institute of Clinical Neurophysiology, Slovenia*
[3]*University of Ljubljana, Faculty of Computer and Information Science, Slovenia*

Aim: Our study aimed to identify, for the first time, small RNAs differentially expressed in muscle biopsy tissue of ALS patients vs. healthy age-matched controls, by using next generation small RNA sequencing (RNA Seq) and bioinformatics analysis.
Background: Small RNAS (including microRNAs (miRNAs) and snoRNAs are short non-coding RNAs that serve as important regulators of gene expression and can act both globally and in a tissue- or cell-type specific manner. In muscle, miRNAs are involved in various processes, such as myogenesis, muscle homeostasis, response to exercise, as well as muscle atrophy due to aging, immobility and muscular and neuro-muscular disorders. Because they can act as specific regulators, muscle miRNAs show promise for therapeutic use.
Results: A total of 11 ALS patients and 11 controls were included in the study. Small RNA Seq showed approx. 30 microRNA families/species and several other small RNAs to be differentially expressed between the patient and control groups. Bioinformatics revealed the differentially expressed small RNAs have several thousand potential protein targets, with some of the proteins being targeted by multiple miRNAs. Of the targets, approx. 40 have already been implicated in ALS pathology in neurological tissue or other known disorders involving muscle wasting, while several hundred other targets are known to be involved in muscle contraction, muscle organ development, skeletal muscle cell differentiation, muscle morphogenesis etc. The study represents an important first step in determining possible novel target approaches in slowing or stopping atrophy of muscle tissue in ALS.

PII-80

Different pathological stimuli induce the release of distinct extracellular vesicles from human microglia

Pia Pužar Dominkuš[1], Matjaž Stenovec[2], Jure Loboda[1], Simona Sitar[3], Nataša Resnik[4], Saša Trkov Bobnar[2], Eva Lasič[2], Ana Plemenitaš[1], B. Matija Peterlin[5], Peter Veranič[4], Marko Kreft[2], Ema Žagar[3], Metka Lenassi[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Laboratory of Neuroendocrinology-Molecular Cell Physiology, Slovenia*
[3]*National Institute of Chemistry, Department of Polymer Chemistry and Technology, Slovenia*
[4]*University of Ljubljana, Faculty of Medicine, Institute of Cellular Biology, Slovenia*
[5]*University of California San Francisco, Department of Medicine, USA*

Introduction: Microglia protect the central nervous system against injury or infection, but also promote neurodegeneration when activated improperly. Activated microglia may communicate with the environment by the release of extracellular vesicles (EVs). We here examined whether different pathological stimuli (ATP — a signal for brain lesion, Ca^{2+} ionophore ionomycin, and expression of HIV-1 protein Nef) evoke release of distinct EVs than in resting immortalized human microglia.
Methods: We analyzed morphology and molecular composition of EVs by transmission electron microscopy, asymmetric-flow field-flow fractionation connected to detectors, flow cytometry, nanoparticle tracking analysis and immunoblotting; and examined the properties of punctuated Nef.GFP in live cells by confocal microscopy.
Results: The average radius (R_{rms}) of EVs constitutively released from non-stimulated microglia (~5×10^7 EVs/10^6 cells) increased from 191 nm (after 24h incubation) to 365 nm (48h) and 445 nm (72h). After pulse increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), bigger (R_{rms} 338 nm (ATP), 422 nm (ionomycin)), but not more numerous EVs with specific protein composition, were released. Conversely, EVs released from Nef.GFP-expressing cells were more concentrated (up to 30x), smaller (R_{rms} 172 nm), floated on sucrose gradient in exosome fractions (immuno-positive for flotillin, Tsg101, annexin) and contained Nef.GFP to a small extent. Nef was also released with flotillin-positive EVs from HIV-1 infected microglia. In live cells, punctuated Nef.GFP comprised large, $[Ca^{2+}]_i$ independent, non-directional population that differed from the dextran- and LysoTracker-labelled vesicles; mobility of later was diminished in Nef.GFP-expressing cells in comparison to controls.
Conclusion: Microglia respond to diverse pathological stimuli by releasing specific (but still heterogeneous) EV populations, which could explain diverse functions of microglial EVs.

PII-81

Changes of skeletal muscle myokines in obesity and type 2 diabetes: from primary human myotubes to muscle tissue

Denisa Maderova[1], Timea Kurdiova[1], Miroslav Balaz[1], Marek Vician[2], Daniela Gasperikova[1], Vitazoslav Belan[3], Barbara Ukropcova[1], Jozef Ukropec[1]

[1]*Biomedical Research Center Slovak Academy of Sciences, Institute of Experimental Endocrinology, Slovak republic*
[2]*Comenius University, Faculty of Physical Education and Sports, Slovak republic*
[3]*University Hospital Bratislava, Department of Radiology, Slovak republic*
[4]*Comenius University, Faculty of Medicine, Institute of Pathophysiology, Slovak republic*

Introduction: Myokines are involved in regulation of energy metabolism, muscle functional and structural remodelling, including angiogenesis and inflammation. Their secretion can be modulated by physical (in)activity, inflammation, lipids, insulin resistance or hyperglycemia.
Aims: To investigate the regulation of chemokines (CC:MCP1; CXC:IL8,NAP2,GROα), their receptors (CCR2a; CXCR1/2) and macrophage markers (M1:CD86/40; M2:CD206/163) in skeletal muscle and primary muscle cells of donors with obesity, prediabetes and type 2 diabetes (T2D).
Methods: Donors (men, n = 97): lean controls; obese: normal glucose tolerant, prediabetics or T2D. Glucose tolerance (oGTT), whole-body insulin sensitivity (EHC), body composition (bioimpedance), intramyocellular & hepatic lipid content (^{1}H-MRS) were assessed and *m. vastus lateralis* samples taken (Bergstrom needle biopsy). Primary skeletal muscle cells were treated with palmitate or glucose. Myokines secretion into cell media (multiplex assay, Millipore) and gene expression (qPCR) were assessed.
Results: In vitro, gene expression and media content of myokines tended to decrease with obesity and T2D ($P > 0.05$). Palmitate treatment reduced MCP1 secretion in myotubes from obese and T2D donors and increased IL8 mRNA in obese compared to lean ($P < 0.05$). In muscle, prediabetes was associated with increase of IL8 ($P = 0.07$), GROα, NAP2 ($P < 0.01$) and their receptors mRNA ($P < 0.05$), T2D increased expression of macrophage markers ($P < 0.05$), MCP1 ($P < 0.01$) but not MCP1 receptor.
Conclusion: Increase of chemo-myokines in human skeletal muscle in prediabetes and T2D suggests a gradual activation of inflammatory state with a disease progression. Reciprocal changes in tissue and cells may suggest the existence of regulatory factors associated with the development of metabolic disease *in vivo*, and/or contribution of the other cell types to the myokines pool in skeletal muscle.

Grant support: APVV 15-0253, MZ-CR 16-33746A, EFSD New Horizons, VEGA 2/0191/15.

PII-82

Evidence b(i)ased medicine. Research about research

Tomaž Makovec

University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia

Which came first, mental illness or drugs? While in other areas of medicine this is a no-brainer, some argue that certain psychiatric conditions are created and classified because of the effects particular drugs have on the body, and not the other way around. In 1987, the second generation of selective serotonin reuptake inhibitors (SSRI) antidepressants emerged on the market with fluoxetine (Prozac) as the most popular one. Since then, depression has transitioned from acute to chronic illness and at that time, the mechanism of depression was discovered.
Depression is supposed to be a consequence of a »biochemical imbalance« in the brain: the lack of neurotransmiter serotonin in sinapses. SSRI inhibit the reuptake of serotonin by the presinaptic neuron. Such antidepressants lower the Ham-D score (Hamilton Rating Scale for Depression) in a statistical but not in a clinically significant manner, and no better than the old generation (tricyclic) antidepressants. But there is also another antidepressant that has a very different mode of action, proven by a French drug regulatory agency: Tianeptine is a selective serotonin reuptake *enhancer*. Instead of increasing the amount of serotonin in the sinapses it is supposed to decrease it. We would expect that tianeptine makes depression worse. But it doesn't. It lowers the Ham-D score by the same amount as SSRI, SNRI, NDRI antidepressants or even thyroid medications do. It simply doesn't matter obviously which medication is used. The effect on depression is the same. In reality depression (and many other mental disorders) cannot be boiled down to an excess or deficit of any particular chemical or even a suite of chemicals. “Biochemical imbalance is a part of last-century thinking. It's much more complicated than that,” said Joseph Coyle, the neuroscientist of the Harvard Medical School.
A brief explanation of these anomalies is presented here.

PII-83

Mutation analysis of *VHL* and *EPAS1* genes in patients with JAK2 negative erythrocytosis: development of a molecular-genetic test

Iva Mencin[1,2,3], Aleša Kristan[1,2,3], Martina Fink[4], Tadej Pajič[4], Saša Anžej Doma[4], Peter Černelč[4], Radovan Komel[1], Irena Preložnik Zupan[4], Tanja Kunej[2], Nataša Debeljak[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*
[2]*University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Slovenia*
[3]*University of Ljubljana, Biotechnical Faculty, Master study programme in Biotechnology, Slovenia*
[4]*University Medical Centre Ljubljana, Clinical Department of Haematology, Slovenia*

Erythrocytosis is a heterogeneous group of rare disorders defined by increased red blood cell (RBC) number, haemoglobin (Hb) and haematocrit (Ht). Secondary familial erythrocytosis (FE) is associated with variants in erythropoietin (*EPO*) gene and other genes involved in oxygen-sensing pathway, such as Von Hippel-Lindau tumour suppressor (*VHL*) and endothelial PAS domain (*EPAS1*). Hormone EPO is the main regulator of red blood cell production in bone marrow and its transcription is activated by *EPAS1* gene in low-oxygen conditions. In normoxia, EPAS1 is rapidly degraded by VHL protein [1]. Recently, sequence variants of the *EPO* gene associated with FE were identified and are in the process of integration into diagnostic procedures for FE in Slovenia [2]. The aim of the current study was to extend diagnostic procedures and develop additional diagnostic tests for *VHL* and *EPAS1* genes variants with potential effect on erythrocytosis. From Ensembl genomic browser and literature we screened *VHL* and *EPAS1* variants associated with FE. In the current version of the Ensembl browser 743 variations are present in the transcript *EPAS1*-001 and 1927 within transcript *VHL*-001. Literature search revealed 12 *EPAS* and 20 *VHL* missense substitutions associated with FE. In both genes, variants associated with FE are often located close to important protein-protein interacting sites or post-translational-modification sites, with effect on proteins stability and activity. *EPAS1* and *VHL* sequence analysis is currently under design. Control samples were already tested and validated, and mutation analysis on Slovenian patients with FE is undergoing. This study and diagnostic tests will enable more accurate and extended procedures for molecular genetic testing regarding FE.

References:

1. Lee F. S. and Percy M. J. (2010) Annu Rev Pathol 6, pp. 165–192.
2. Vočanec D., *et. al.* (2017) in preparation.

PII-84

Telomere length and arterial pulse wave velocity in children with hypercholesterolemia

Matej Mlinarič[1], Tine Tesovnik[1], Urh Grošelj[2], Jernej Kovač[1], Primož Kotnik[2,3], Tadej Battelino[2,3], Katarina Trebušak Podkrajšek[1,3]

[1]*University Medical Centre Ljubljana, University Children's Hospital, Unit of Special Laboratory Diagnostics, Slovenia*
[2]*University Medical Centre Ljubljana, University Children's Hospital, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Slovenia*

Telomeres are repetitive, non-coding DNA sequences (TTAGGG) at the end of chromosomes and their length is affected by several factors. In patients with hypercholesterolemia (HH), one of them is the prolonged exposure to oxidative stress associated with inflammation. High lypoprotein(a) (Lp(a)) concentration in HH patients is associated with impaired endothelial function and coronary artery calcification. A number of factors for risk assessment have been studied, including the arterial pulse wave velocity.

Our hypothesis were that children with HH and high Lp(a) have shorter telomere length (TL) and greater pulse wave velocity compared to children with HH and lower Lp(a).

The study included 58 children (aged 5–8 years at the begging of the study, 27 females) referred to the University Children's Hospital Ljubljana through the national screening program for HH. In 32 children Lp(a) values were < 100 mg/L in 26 they were > 500 mg/L. Relative TL was determined with modified Cawthon's method of monochrome multiplex quantitative real-time PCR (MMQPCR). To estimate arterial stiffness pulse wave velocity of the aorta was determined with Complior Analyse®.

Groups did not differ regarding age, gender ratio and total cholesterol, HDL, LDL and triglycerides. Relative TL was shorter in the group with a higher Lp(a) (P = 0.0011; unpaired t-test with Welch correction) were also hsCRP levels where higher (P = 0.0195; unpaired t-test with Welch correction). Pulse wave velocity tended to be lower in the group with Lp(a) below 100 mg/L, it however didn't reach statistical significance.

Children with HH and higher Lp(a) values have shorter relative TL and higher hsCRP levels comparing to those with lower Lp(a) levels. This is indicating that as early as in childhood, higher levels of the oxidative stress associated with inflammation due to high cholesterol levels are resulting in shorter telomeres; however, arterial stiffness at that stage was implied but not yet significant.

PII-85

Cytoplasmic phosphorylation of FUS impairs its nuclear localization

Simona Darovic[1], Marija Srnko[1,2], Helena Motaln[1], Boris Rogelj[1,2,3]

[1]*Jožef Stefan Institute, Slovenia*
[2]*Biomedical Research Institute BRIS, Slovenia*
[3]*University of Ljubljana, Faculty of Chemistry and Chemcal Technology, Slovenia*

Improper regulation of RNA-binding proteins (RBPs) including FUS protein of the FET family is known to cause various fatal neurodegenerative diseases like frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS), which are characterized by progressive neuron loss. Aberrant nucleocytoplasmic localization of various RNA-binding proteins, including FUS was associated with neurotoxicity. FUS immunoreactive cytoplasmic inclusions are found in 3% of familial ALS cases and 10–15% of FTLD cases [1]. Still the underlying pathological mechanisms leading to FUS mislocalization and aggregation in both diseases appear different. FUS is predominantly nuclear protein that possesses non-classical PY-type nuclear localization signal (NLS) at its extreme C-terminus. In ALS but not in FTLD, the mutations in the NLS of FUS are responsible for its impaired nuclear transport mediated by nuclear import receptor transportin 1 (TNPO1) [2]. We have recently reported on novel posttranslational modification — the phosphorylation of C-terminal tyrosine at position 526 in NLS of FUS. This abolished FUS interaction with TNPO1 and potentially impaired transport of C-terminal FUS fragment into the nucleus [3]. But, as proteins with molecular mass bellow 40 kDa can enter/exit nucleus also by passive diffusion, here our aim was to elaborate on phosphorylation state of Y526 in C-terminal fragment compared to full-length FUS and their exact nucleocytoplasmic localization. We show here that besides in sole C-terminal fragment of FUS, the Y526 undergoes phosphorylation also in full-length FUS in the cytoplasm. This cytoplasmic phosphorylation of Y526 may serve to fine tune the nucleocytoplasmic shuttling of FUS, to ensure that a small amount of FUS always remains present in the cytoplasm possibly for dendritic mRNA transport.

References:

1. Snowden J. S., *et. al.* (2011) Acta Neuropathol 122, pp. 99–110.
2. Dormann D., *et. al.* (2010) EMBO J 29, pp. 2841–57.
3. Darovic S., *et. al.* (2015) J Cell Sci 128, pp. 4151–9.

PII-86

Genetic analysis of congenital hypogonadotropic hypogonadism with targeted next generation sequencing

Tamara Obreza[1], Magdalena Avbelj Stefanija[1], Marija Pfeifer[2], Jernej Kovač[1], Tadej Battelino[1,3], Katarina Trebušak Podkrajšek[1,3]

[1]*University Medical Centre Ljubljana, University Children's Hospital, Slovenia*
[2]*University Medical Centre Ljubljana, Division of Internal Medicine, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Slovenia*

Congenital hypogonadotropic hypogonadism (HH) is a rare but clinically and genetically heterogeneous disease caused by pathogenic mutations in several genes leading to impaired production, secretion or action of gonadotropin-releasing hormone (GnRH), which is the master hormone regulating the reproductive axis. HH is characterized by an absence or delay of puberty and infertility. The association of congenital HH with hyposmia or anosmia is defined as Kallmann syndrome (KS). The aim of our study was to use next generation sequencing (NGS) in identification of causal mutations in the coding regions and exon-intron regions of 24 genes related to the development of congenital HH.

The study cohort included 11 participants aged between 16 and 67 years (10 males and one female) with clinical symptoms of HH. Seven males showed features of KS, three males had normosomic HH. The female had suspicion of KS. To identify potential disease causing variants the analysis of "clinical exome" (TruSight One, Illumina, USA) was performed on MiSeq NGS sequencer (Illumina, USA). Further evaluation of variants with Variant Studio 2.2 software (Illumina, USA) was restricted to those located in 24 genes related to congenital HH. The presences of candidate causative variants were confirmed by Sanger sequencing.

Seven causative variants in five genes (*PROK2, GNRHR, PROKR2, FGFR1* and *CHD7*) were detected in six out of 11 patients. Among them, three variants namely *PROK2* NM_001126128.1: c.171_172delTT (p.Ile57MetfsTer17), *FGFR1* NM_023110.2: c.196T>C (p.Typ66Arg) and *CHD7* NM_017780.3: c.5759A>G (p.Tyr1920Cys) have not yet been described in HGMD professional, dbSNP or ExAc databases.

NGS enables fast and reliable identification of causative variants in several genes related to HH simultaneously. Presented subject group with HH was genetically very diverse and the results expand the spectrum of mutations implicated in HH.

PII-87

The importance of OATP, OAT and ABC transporters in endometrial cancer

Renata Pavlič[1], Csilla Laczka[2], Maja Anko[1], Tea Lanišnik Rižner[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*Hungarian Academy of Sciences, Research Centre for Natural Sciences, Institute of Enzymology, Hungary*

Endometrial cancer (EC) is associated with increased actions of estrogens. In peripheral tissues estrogens are formed from inactive steroid precursors dehydroepiandrosterone sulfate (DHEA-S) and estrone sulfate (E1-S) by the actions of estrogen biosynthetic enzymes. Steroid precursors must be transported into cells by the organic anion-transporting polypeptides (OATP) or organic anion transporters (OAT) while their efflux is catalyzed by ATP-binding cassette (ABC) transporters. To date, 20 transporters are known to be involved in DHEA-S and E1-S transport and their roles in EC have not yet been examined.

Here, we confirmed the expression of 13 *SLCO, SLC* and *ABC* genes encoding OATP, OAT and ABC transporters in model cell lines of EC, Ishikawa, HEC1A, and control endometrial cell line HIEEC, and paired samples of EC and adjacent control tissue by qPCR. In Ishikawa the expression of *SLCO1B3, SLCO3A1, SLCO2B1, ABCC1* and *ABCC4* was significantly decreased and expression of *SLCO4C1, SLCO1C1, SLCO1A2, ABCG2, SLC51B* and *SLC51A* significantly increased compared to HEC1A. As *SLCO1B3, SLCO3A1* and *SLCO2B1* are known to be involved in DHEA-S and E1-S uptake their down-regulation supports lover estrogen formation in Ishikawa observed in our previous study [1]. These results are further supported by kinetic studies where we found significantly lover influx of E1-S into Ishikawa compared to HEC1A.

In EC tissue we found significant changes in expression of 10 transporters, where the highest down-regulation was observed for *ABCG2* (9.30-fold) and *SLC51B* (2.67-fold) and the highest up-regulation for *SLCO1A2* (2.21-fold) and *SLCO1B3* (1.87-fold). Since the first two genes are associated with the efflux and the latter two with the uptake of DHEA-S and E1-S into cells such observations support increased production of estrogens in EC.

These results suggest important roles of OATP and ABC transporters in EC thus further studies at the protein levels are in progress.

References:

1. Hevir-Kene N. and Lanišnik Rižner T. (2015) Chem Biol Interact 234, pp. 309–19.

PII-88

Zinc levels in seminal plasma as an additional tool in predicting boar semen quality after storage

Maja Zakošek Pipan[1], Janko Mrkun[1], Breda Jakovac Strajn[2], Katarina Pavšič Vrtač[2], Alenka Nemec Svete[3], Petra Zrimšek[4]

[1]*University of Ljubljana, Veterinary Faculty, Clinic for Reproduction and Horses, Slovenia*
[2]*University of Ljubljana, Veterinary Faculty, Institute for Hygiene and Pathology of Animal Nutrition, Slovenia*
[3]*University of Ljubljana, Veterinary Faculty, Clinic for Small Animal Medicine, Slovenia*
[4]*University of Ljubljana, Veterinary Faculty, Institute of Physiology, Pharmacology and Toxicology, Slovenia*

The aim of this study was to examine the association between concentration of Zn in fresh boar seminal plasma and oxidative stress biomarkers in fresh seminal plasma and seminal plasma after semen storage (superoxide dismutase (SOD), total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBARS)). We also examined the association between concentration of Zn in fresh boar seminal plasma and semen quality parameters after short-term storage.
Twenty ejaculates from 8 boars were collected. Concentration of Zn was determined in fresh boar seminal plasma by inductively coupled plasma mass spectrometry (ICP-MS). Semen analysis (motility, progressive motility, morphology and tail membrane integrity) was preformed and oxidative stress biomarkers (SOD, TAC, TBARS, 8-isoprostane) measured on the day of collection (day 0) and on day 3 (72h) of semen preservation. In between semen was stored at 15–17 °C at gentle agitation.
Zn significantly correlated with TAC on day 0 and day 3 ($P < 0.05$). No correlations were found between other oxidative stress biomarkers and Zn. Higher levels of Zn also better preserved mitochondrial function after three days of short-term storage ($P < 0.05$). Moreover, semen samples that, after 3 days of storage, fulfilled all criteria for semen characteristics (motility > 60%, progressive motility > 25%, normal morphology > 55%) had significantly higher Zn levels on the day of semen collection than those with at least one criteria not fulfilled ($P < 0.05$) following storage.
Higher levels of Zn in fresh boar seminal plasma were associated with higher total antioxidant capacity and accompanied with better preservation of semen quality characteristics after storage. Zn measured in fresh seminal plasma could be therefore used as an additional toll in predicting semen quality after storage based on the combination of three standard parameters: motility, progressive motility and morphology.

PII-89

New selective and reversible cathepsin X inhibitor impairs tumour cell migration and neurite outgrowth

Urša Pečar Fonović[1], Ana Mitrović[1], Damijan Knez[1], Tanja Jakoš[1], Anja Pišlar[1], Bojan Doljak[1], Jure Stojan[2], Stanislav Gobec[1], Janko Kos[1,3]

[1]*University of Ljubljana, Faculty of Pharmacy, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[3]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*

A cysteine peptidase cathepsin X is a carboxymonopeptidase found mainly in immune cells, where it regulates migration, adhesion, proliferation, maturation, phagocytosis and signal transduction. In the last 10 years several of its molecular targets were identified and characterized including β-chain of integrin receptors, g-enolase, chemokine CXCL-12, bradykinin, kallidin, huntingtin and profilin 1. Besides, cathepsin X is highly elevated in certain types of cancer, neurodegenerative disorders, inflammatory diseases and other and it became an object of interest as a possible therapeutic target. Till now, an epoxysuccinyl-based inhibitor AMS36 was the only one that showed selectivity toward cathepsin X. It is an irreversible inhibitor that also inhibits cathepsin B in prolonged incubation times. For treatment of various pathologic conditions associated with excessive proteolytic cleavage, reversible small molecular inhibitors are usually the preferred option.

In our study 579 compounds from the in-house library were tested for the relative inhibition of cathepsin X. K_i value was determined for a group of several compounds exhibiting the highest relative inhibition. Binding type, determined by the washout experiment, showed reversible inhibition of cathepsin X for all new inhibitors. Inhibitors with the lowest K_i values were further tested for the cathepsin specificity (cathepsins L, H, S and B exo- and endo-peptidase activity). A reversible and cathepsin X specific inhibitor Z9 with K_i 2.45 ± 0.05 mM was validated on PC-3 prostate cancer cells and PC-12 pheochromocytoma cells. It showed significant inhibition of PC-3 migration and PC-12 neurite outgrowth, two processes that are under control of cathepsin X carboxypeptidase activity.

PII-90

The effects of cystatin F on granule-mediated cytotoxicity of NK cells

Milica Perišić Nanut[1], Jerica Sabotič[1], Mateja Prunk[1], Janko Kos[2]

[1]*Institute Jožef Stefan, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Pharmacy, Slovenia*

Natural killer cells (NK) cells are a type of cytotoxic lymphocyte critical for cancer immunosurveillence and control of tumour progression. Yet, their cytotoxic function is frequently suppressed in tumour microenvironment. Cystatin F is a unique member of the cystatin type II family of cysteine protease inhibitors that is synthesized as inactive disulphide-linked dimer and converted, within endo/lysosomes to an active monomer by a cleavage of 15 N-terminal amino acids. In NK cells cystatin F is suggested to inhibit major granzyme convertases cathepsins C and H thus regulating their cytotoxic function. Our aim in this study was to analyse cystatin F dimerization, intracellular trafficking, peptidase inhibition and its effects on granule-mediated cytotoxicity of NK cells. To that end, we produced N-terminally cleaved form of cystatin F, mutants lacking specific oligosaccharide chains and C13 family protease-legumain binding site cystatin F mutants. Our results show that the extent of N-glycosylation affects the secretion, uptake and subcellular sorting of cystatin F. Modification of legumain binding site has no effect on these processes. Beside the dimeric, active N-terminally cleaved form of cystatin F is taken up by recipient cells and translocated to endo/lysosomes, where it inhibits cathepsins C and H. In addition, incubation with full length wild type cystatin F as well as with activated N-terminally cleaved cystatin F leads to decrease in the granzyme A and B activity in NK-92 cells and suppression of granule-mediated cytotoxicity in IL-2 stimulated both NK-92 and primary NK cells. By providing insights into the consequences of acquisition of cystatin F by NK cells our results highlight cystatin F protease inhibitor as a possible target for improvement of NK-cell based cancer treatment.

PII-91

Role of cathepsin X in neuroinflammation-induced neurodegeneration

Anja Pišlar[1], Biljana Božić[2], Nace Zidar[6], Larisa Tratnjek[3], Gordana Glavan[4], Marko Živin[3], Janko Kos[1,5]

[1]*University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Biology, Slovenia*
[2]*University of Belgrade, Faculty of Biology, Institute for Physiology and Biochemistry, Serbia*
[3]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[4]*University of Ljubljana, Biotechnical Faculty, Department of Biology, Slovenia*
[5]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[6]*University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Slovenia*

Neuroinflammation is closely implicated in the pathogenesis of neurodegenerative disorders, such as Parkinson's disease (PD), where the hallmark of neuroinflammation is activated microglia. Microglia-derived lysosomal cathepsins, including cathepsin X, are increasingly recognized as important mediators of the inflammation-induced neruodegeneration. Recent study revealed that up-regulated expression and activity of microglial cathepsin X as wells as increased release of cathepsin X after lipopolysaccharide (LPS) stimulation leads to microglia activation-mediated neurodegeneration. Cathepsin X inhibitor caused neuroprotection via its suppression of microglia activation. Moreover, the immunomodulatory role of cathepsin X has been also shown in microglial co-activation, where cathepsin X inhibition proved to diminish increased neuroinflammation by LPS and poly(IC) co-stimulation. Our recent study revealed that LPS also induced the expression and upregulated enzymatic activity of cathepsin X in brain regions observed in *in vivo* models of PD, with a preference cathepsin X upregulation in microglia cells and astrocytes in lesioned striatum. Taken together, these findings propose the potential function of microglial cathepsin X in inflammation-induced neurodegeneration. Knowing the involvement of cathepsin X in the neurodegenerative processes represent a step towards the development of new molecules for the treatment of neurodegenerative diseases.

PII-92

Localization of dipeptide repeat proteins and their interaction with lipid droplets in human cells

Anja Pucer Janež[1], Sonja Prpar Mihevc[1], Anja Kovanda[1], Mirjana Malnar[1], Toni Petan[2], Boris Rogelj[1,3,4]

[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Slovenia*
[3]*Biomedical Research Institute BRIS, Slovenia*
[4]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are devastating neurodegenerative diseases that represent two ends of a complex disease spectrum. They are characterized by selective loss of motor neurons. Aggregation of RNA binding proteins is one of the hallmark pathological features of ALS/FTLD, defining them as proteinopathies. Mutations in more than 50 different genes were linked to familial ALS/FTLD, including a hexanucleotide repeat expansion in the gene *C9orf72*. One of the major current hypotheses proposed for the pathogenicity of the expanded *C9orf72* repeat mutation is the accumulation of aggregates of pathogenic dipeptide repeat (DPR) proteins: poly(GA), poly(GR), poly(PR), poly(PA), poly(GP) and several studies show that DPR proteins are neurotoxic *in vitro*. Lipid droplets (LDs) are dynamic structures found nearly ubiquitously in cells. Recently it has become apparent that LDs play even broader cellular roles than it was previously described. In our study we wanted to contribute to the understanding of the potential molecular interactions between ALS/FTLD-associated aggregating DPR proteins and cellular LDs. To this end we first prepared GFP-conjugated constructs for mammalian expression of proteins containing 125 repeats of the dipeptides GA, GP, PA, PR and analyzed their subcellular localization after transient transfection in SH-SY5Y, NSC-34 and NTERA-2 model cell lines by means of confocal microscopy. The hydrophobic protein poly(GA) forms distinct cytosolic aggregates in all of the model cell lines, while poly(GP) and poly(PA) appear to be evenly distributed in the cytoplasm. Poly(PR) accumulates in nuclear aggregates. We also induced the accumulation of LDs by the addition of fatty acids in order to study the potential colocalization of DPR proteins with LDs and their potential effect on the neurotoxicity of DPR proteins.

PII-93

Genetic variability in *DRD2* and *DRD3* does not contribute to Parkinson's disease susceptibility

Sara Redenšek[1], Katja Goričar[1], Maja Trošt[2], Vita Dolžan[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Pharmacogenetics Laboratory, Slovenia*
[2]*University Medical Centre Ljubljana, Department of Neurology, Slovenia*

Introduction: Parkinson's disease (PD) is a sporadic progressive brain disorder with a relatively strong genetic background. Various genes have already been associated with PD susceptibility using genome wide approach. These genes participate in different pathways, but none of them take part in dopamine signalling, although a dopaminergic deficit is believed to be the most important pathogenic mechanism of PD. We used a candidate gene approach to evaluate the effect of polymorphisms in dopamine receptor genes (*DRD*), more specifically *DRD2* and *DRD3*, on PD susceptibility.
Methods: A retrospective pilot study included 82 PD patients, and 165 healthy blood donors older than 50 years of age. Three polymorphisms, *DRD2* rs1801028, *DRD2* rs1799732, and *DRD3* rs6280, were genotyped using real time PCR based method. Statistical analysis using logistic regression was performed.
Results: The patient group included 46 (56%) males and 36 (44%) females, median age 69.9 (63.9–77.2) years, while the control group included 128 (80%) males and 37 (20%) females, median age 55.3 (52.3–59.2) years. The observed minor allele frequencies were 0.008 for rs1801028, 0.003 for rs1799732, and 0.30 for rs6280. Genotype distributions in controls were in Hardy-Weinberg equilibrium. We found no association between an individual polymorphism and PD susceptibility. There were no differences between cases and controls after adjusting for differences in gender and age (rs1801028: $P = 0.549$, OR = 0.472, 95% CI = 0.040–5.499; rs1799732: $P = 0.437$, OR = 0.615, 95% CI = 0.181–2.094; rs6280: $P = 0.610$, OR = 1.233, 95% CI = 0.552–2.752).
Conclusions: The results of our pilot study do not suggest significant involvement of *DRD2* and *DRD3* receptor polymorphisms in PD susceptibility. As rs1801028 and rs1799732 polymorphic alleles were rare in our population further research is needed to determine the effect of *DRD2* and *DRD3* polymorphisms on PD susceptibility.

PII-94

Human *CYP51A1* polymorphisms and their effect on enzymatic activity

Tadeja Režen[1], Iza Ogris[2], Marko Sever[2], Franci Merzel[2], Simona Golič Grdadolnik[2], Damjana Rozman[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Centre for Functional Genomics and Bio-chips, Slovenia*
[2]*National Institute of Chemistry, Slovenia*

CYP51A1, lanosterol 14α-demethylase, is involved in cholesterol biosynthesis and its knockout is embryonically lethal. Heterozygous mice *Cyp51a1*$^{+/-}$ are healthy and fertile, however; feeding with high lipid diet revealed a hidden susceptibility. According to mouse model, we would expect to find heterozygous individuals with potentially detrimental variants existing in human population. The aim of this study is to evaluate the effect of natural missense mutations of the CYP51A1 protein on enzymatic activity. Search of different SNP databases revealed less than 200 SNPs casing a missense mutation in *CYP51A1.* Majority of them were labelled as tolerated by SIFT and PolyPhen-2. Potentially damaging *CYP51A1* polymorphisms are very rare with unknown effect on phenotype. We selected three missense variants for further analysis. Variants R277L and R431H lie in the region of the protein not previously connected to enzymatic activity, however; arginine on these two positions is highly conserved among biological kingdoms. Variant D152G lies within substrate recognition site 1, which was shown by previous studies to be essential for enzymatic activity. Two missense variants, R277L and R431H, were expressed to test the enzymatic activity *in vitro* but failed to produce a P450 spectrum. All three variants were used to perform molecular modelling in order to evaluate the effect of the amino acid change on interaction with the substrate lanosterol and the obligatory redox partner POR. Lower binding potential towards obligatory redox partner POR was shown for variant R277L and especially D152G. While, lower affinity towards the substrate was predicted for D152G and R431H. In conclusion, we propose to include selected damaging *CYP51A1* variants into personalized diagnostics of certain rare disease phenotypes.

PII-95

Altered expression of membrane progesterone receptors in endometrial cancer

Maša Sinreih[1], Snježana Frković Grazio[2], Peter Thomas[3], Tea Lanišnik Rižner[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia*
[2]*University Medical Centre, Department of Pathology, Division of Obstetrics and Gynecology, Slovenia*
[3]*The University of Texas at Austin, Marine Science Institute, USA*

Endometrial cancer (EC) is one of the most common gynecological malignancies worldwide and is associated with chronic exposure to endogenous or exogenous estrogens, together with insufficient protection by progesterone or progestins. Progesterone can act via the classical PGRs and the nonclassical mPRs. The mPRs are transmembrane proteins that are coupled to stimulatory or inhibitory G proteins. To date, five mPR isoforms have been identified in human: mPRα (PAQR7), mPRβ (PAQR8), mPRγ (PAQR5), mPRλ (PAQR6) and mPRε (PAQR9). The expression and role of mPRs in EC have not been studied yet, although higher mPRα mRNA levels have been seen in breast cancer, where progesterone has been shown to inhibit apoptosis through the mPRs [1, 2]. Although the pathogenesis of breast and endometrial cancer differ, we hypothesised that expression of mPRs is also changed in EC.

Our results showed that the genes that encode the mPRs are expressed in all 42 paired samples of control and cancer endometrium, where the mRNA levels of *PAQR7* and *PAQR8* were significantly decreased, by 1.6-fold and 1.4-fold, respectively, and the expression of *PAQR5* was not altered. Immunohistochemical staining of 26 paired samples supported the gene expression data, whereby the expression of mPRα and mPRβ in cancer tissue was lower, compared to control adjacent tissue. While mPRα was localized predominantly on luminal membrane in control endometrium, the protein was detected on either luminal membrane or in cytoplasm of tumour tissue samples. mPRβ was restricted to luminal membrane of both tumour and control tissue. mPRγ showed both cytoplasmic and nuclear localization in control tissue and only cytoplasmic in tumour tissue.

Whether reduced levels of mPRα and mPRβ can lead to increased cell apoptosis and might thus oppose progression of cancer, or whether reduced levels contribute to lower protective actions against endometrial cancer, needs to be further investigated.

References:

1. Dressing G. E. and Thomas P. (2007) Steroids 72, pp. 111–6.
2. Dressing G. E., *et. al.* (2012) Horm Cancer 3, pp. 101–12.

PII-96

Altered expression of genes that encode the enzymes of local progesterone synthesis and progesterone receptors in ovarian endometriosis tissue

Maša Sinreih, Tea Lanišnik Rižner

University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Slovenia

Endometriosis is a complex gynecological disorder, associated with excessive estrogen action together with reduced protective effects of progesterone, which is due to lowered progesterone formation, accelerated progesterone metabolism, or decreased expression of the nuclear progesterone receptors. Membrane progesterone receptors could also be implicated in pathogenesis of endometriosis similarly as in breast cancer, where they have been shown to be involved in the inhibition of cell apoptosis [1, 2]. We have previously shown that genes encoding progesterone metabolizing enzymes (*AKR1C1-C3, SRD5A1*) are upregulated in ovarian endometriosis tissue [3]. In this study, we examined the expression of genes that encode the enzymes of local progesterone synthesis (*STAR*, *CYP11A1*, *HSD3B1*, *HSD3B2*) and the progesterone receptors (*PGR*, *PAQR7*, *PAQR8* and *PAQR5*) in 31 tissue samples from patients with ovarian endometriosis and in 29 control endometrium samples from healthy women.

The genes involved in local progesterone synthesis were all statistically significantly up-regulated in the ovarian endometriosis tissue, compared to the control normal endometrial tissue. Likewise, the genes that encode the progesterone receptors were differentially expressed. We detected statistically significant down-regulation of *PGR*, while the expression of *PAQR7* and *PAQR8* was increased. The expression of *PAQR5* was borderline statistically significantly upregulated. The phase of the menstrual cycle had no statistically significant effects on the expression of these genes.

Our gene expression data suggest that in endometriosis, despite higher expression of the genes of local progesterone biosynthesis, due to the decreased levels of *PGR* and elevated levels of genes encoding progesterone metabolizing enzymes the protective actions of progesterone are likely to be reduced. As in breast cancer, increased expression of the *PAQRs* might contribute to lower apoptosis and thus to the pathogenesis of the disease.

References:

1. Dressing G. E. and Thomas P. (2007) Steroids 72, pp. 111–6.
2. Dressing G. E., *et. al.* (2012) Horm Cancer 3, pp. 101–12.
3. Hevir N., *et. al.* (2011) Chem Biol Interact 191, pp. 217–26.

PII-97

Can low intensity exercise prevent the damaging effects of a fructose-rich diet on cardiac insulin signalling in ovariectomized rats?

Jelena Stanišić, Goran Korićanac, Mojca Stojiljković, Tijana Ćulafić, Milan Kostić, Snježana Romić, Marija Pantelić, Snežana Tepavčević

University of Belgrade, Vinča Institute of Nuclear Sciences, Laboratory for Molecular Biology and Endocrinology, Serbia

There are indications that estrogen deficiency in postmenopausal women, in association with abdominal obesity and physical inactivity, increases risk for insulin resistance, which leads to development of metabolic and cardiovascular diseases. On the other hand, exercise training has been point out as a nonpharmacological treatment for these illnesses, but its effect on cardiac insulin resistance in menopause has been questioned. According to this, we used fructose rich diet model of insulin resistance in ovariectomized (OVX) female rats to test hypothesis that low intensity exercise can prevent disturbances in cardiac insulin signalling pathway. OVX female Wistar rats were divided into three groups: sedentary control and sedentary and exercise groups submitted to fructose diet (received 10% fructose for 9 weeks). We analyzed biochemical parameters relevant for insulin action. Expression and/or phosphorylation of cardiac insulin receptor (IR), insulin receptor substrate 1 (IRS1), tyrosine-specific protein phosphatase 1B (PTP1B), protein kinase B (Akt) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) in basal and insulin-stimulated conditions were evaluated. Fructose diet did not change blood glucose level but it increased plasma insulin level as well as homeostasis model assessment index, indicating decrease in insulin sensitivity. Exercise reversed these parameters to the control level. Fructose diet do not have effects on cardiac IR and PTP1B level as well as ERK1/2, Akt (Thr308) and IRS1 (Tyr632) phosphorylation but it reduced Akt (Ser473) and increased inhibitory IRS1 (Ser307) phosphorylation. Exercise returned Akt phosphorylation at Ser473 and increased at Thr308, without effect on IRS1 (Ser307). The obtained results showed that exercise can partially prevents disturbances in cardiac insulin action in OVX rats suggesting a moderate physical activity as an important treatment for prevention of development of cardiac insulin resistance.

PII-98

Insulin secretion is affected by *SLC6A4* 5HTTLPR polymorphism in patients with polycystic ovary syndrome

Barbara Šenk[1], Katja Goričar[1], Nika Aleksandra Kravos[2], Andrej Janež[2], Mojca Jensterle Sever[2], Vita Dolžan[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Pharmacogenetics Laboratory, Slovenia*
[2]*University Medical Center Ljubljana, Department of Endocrinology, Diabetes and Metabolic Diseases, Slovenia*

The association between the polymorphisms in serotoninergic system and insulin secretion has not been investigated in human, however studies in cell cultures and animal models point at that association. We tested the hypothesis that polymorphisms in genes coding for serotonin receptors 5HTR1A and 5HTR1B and serotonin reuptake transporter SLC6A4 affect basal and glucose-stimulated insulin secretion in women with polycystic ovary syndrome (PCOS).

The study included 65 female patients with PCOS who had oral glucose tolerance test (OGTT) performed before treatment. Blood glucose and insulin levels were measured before and after OGTT. DNA samples of 94 young healthy female blood donors were used as controls. *5HTR1A* rs6295 (–1019C>G) and *5HTR1B* rs13212041 (3' UTR-1997T>C) polymorphisms were genotyped by real-time PCR (KASPar). For the determination of *SLC6A4* 5HTTLPR polymorphism we amplified the promoter region by PCR and checked the amplicon length using agarose gel electrophoresis.

Genotype distributions were in accordance with HWE, except for *5HTR1A* rs6295 in healthy controls and *5HTR1B* rs13212041 in PCOS patients. Carriers of at least one polymorphic *5HTR1A* rs6295 G allele had almost three times lower risk for PCOS as homozygotes for the normal C allele (OR = 0.34; 95% CI = 0.16–0.75; *P* = 0.008). The investigated polymorphisms were significantly associated with neither basal nor glucose-stimulated blood glucose levels at any point of time during OGTT, nor with the basal insulin concentration. *SLC6A4* 5HTTLPR polymorphism was significantly associated with insulin secretion (*P* = 0.030) and with the area under the curve of insulin blood levels during OGTT (0.021). *5HTR1A* rs6295 and *5HTR1B* rs13212041polymorphisms were not associated with insulin secretion.

Our results suggest that serotoninergic system may play a role in insulin secretion in human. Pharmacological modulation of serotoninergic system could be used to modulate insulin secretion in women with PCOS.

PII-99

Pharmacological effects of metformin and dichloroacetate depend on intrinsic metabolic characteristics of cancer cells

Nives Škorja[1,2,3], Urška Matkovič[1], Klemen Dolinar[1,3], Matej Podbregar[1,4], Tomaž Marš[1], Erika Cvetko[2], Marija Meznarič[2], Mojca Pavlin[3,5], Sergej Pirkmajer[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Institute of Anatomy, Slovenia*
[3]*University of Ljubljana, Faculty of Electrical Engineering, Group for nano and biotechnological applications, Slovenia*
[4]*University Medical Centre Ljubljana, Clinical Department for Anesthesiology and Surgical Intensive Care, Slovenia*
[5]*University of Ljubljana, Faculty of Medicine, Institute of Biophysics, Slovenia*

Metformin, the most commonly used oral antidiabetic drug, and dichloroacetate (DCA), an experimental pharmacological compound, exert pronounced effects on metabolism of cancer cells. Metformin inhibits complex I of the respiratory chain, thus increasing glycolysis. Conversely, DCA stimulates pyruvate dehydrogenase and shifts glucose metabolism from glycolysis towards oxidation. Cancer cells are frequently highly glycolytic; however, different types of cancer cells display distinct metabolic characteristics, which may modulate their responsiveness to metformin and DCA.

We examined effects of metformin and DCA in MDA-MB-231 breast cancer cells and PC3 prostate cancer cells. Metformin increased lactate production in both cell types, demonstrating a shift from oxidative glucose metabolism to glycolysis. Conversely, DCA repressed lactate production, indicating stimulation of glucose oxidation. This was paralleled by reduced phosphorylation of E1α sub-unit of pyruvate dehydrogenase (Ser^{293}). As assessed by phosphorylation of the AMP-activated protein kinase (AMPK, Thr^{172}) and its target acetyl-CoA carboxylase (Ser^{79}) metformin induced energy stress and activated AMPK in both cell lines. In contrast, DCA activated AMPK only in MDA-MB-231 cells. Oxamate, which suppresses glycolysis by inhibiting lactate dehydrogenase, also activated AMPK only in MDA-MB-231 cells. Taken together with markedly lower basal fumarase activity in MDA-MB-231 cells, these results suggest that energy production in breast cancer cells is more dependent on glycolysis than in PC3 cells.

In sum, our results show that metformin and DCA induce similar metabolic responses in MDA-MB-231 and PC3 cells. However, due to intrinsic differences in their metabolic characteristics these metabolic responses produce divergent effects on energy balance and downstream signalling events. Collectively, this study suggests that cell type-specific metabolic characteristics impact pharmacological effects of metformin and DCA.

PII-100

Expansion of Slovenian newborn screening programme

Andraž Šmon[1], Barbka Repič Lampret[1], Urh Grošelj[1], Mojca Žerjav Tanšek[1], Jernej Kovač[1], Daša Perko[1], Sara Bertok[1], Katarina Trebušak Podkrajšek[1,2], Tadej Battelino[1,2]

[1]*University Medical Centre Ljubljana, University Children's Hospital, Slovenia*
[2]*University of Ljubljana, Faculty of Medicine, Slovenia*

Inborn errors of metabolism (IEM) are autosomal recessive disorders that can pose a major health problem, their consequences range from minor disabilities to sudden death. Many European countries have an expanded newborn screening (NBS) programme using tandem mass spectrometry, while the south-eastern European countries have no expanded screening programme. We conducted a pilot study of expanded NBS in Slovenia with 10048 included participants. 85 participants were later-on analysed at a metabolic follow-up. Of them, 75 participants were analysed also with next generation sequencing (NGS) for selected IEM. Five participants were found positive for screened IEM. Glutaric academia type 1 was confirmed in one patient who was a compound heterozygote for two known causative *GCDH* variants. Two participants were positive for 3-methylcrotoyl-CoA carboxylase deficiency, both had known causative homozygous variant in *MCCC1.* Seven other participants had elevated metabolites characteristic for 3-methylcrotonyl-CoA carboxylase deficiency, but were heterozygous, among them 2 had a novel genetic variant. A patient with mild very long-chain acyl-CoA dehydrogenase deficiency had negative metabolic follow-up tests, but had two heterozygous *ACADVL* variants; one known disease causing variant and one novel variant, predicted to be causative. The study also found one participants with classical phenylketonuria and one with hyperphenylalaninemia, which were confirmed through existing NBS in Slovenia. Estimated cumulative incidences of IEM in Slovenia are similar to the incidences in developed countries, where expanded NBS has been implemented. NGS as a confirmatory testing proved to be a valuable tool in explaining the abnormal metabolites in DBS. It enabled confirmation of affected patients and improved the turnaround time of genetic analysis.

PII-101

Good glycemic control of pediatric type 1 diabetic patients associates with decreased telomere shortening

Tine Tesovnik[1], Jernej Kovač[2], Tinka Hovnik[2], Klemen Dovč[1], Nataša Bratina[1,3], Tadej Battelino[1,3], Katarina Trebušak Podkrajšek[2,3]

[1]*University Medical Centre Ljubljana, University Children's Hospital, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Slovenia*
[2]*University Medical Centre Ljubljana, University Children's Hospital, Unit of Special Laboratory Diagnostics, Slovenia*
[3]*University of Ljubljana, Faculty of Medicine, Slovenia*

Type 1 diabetes (T1D) is a chronic disease with a characteristic state of hyperglycemia caused by the autoimmune destruction of insulin producing pancreatic β-cells. Good glycemic control and prevention of long-period hyperglycemia are preventive against increased cell stress and inflammation involved in development of diabetic complications. The aim of our study was to evaluate telomere length (TL) and telomere dynamics as glycemic control related biomarker in juvenile patients with T1D.
We included 53 juvenile T1D patients, 20 with poor (HbA1c > 9%) and 33 matched patients with good glycemic control (HbA1c < 7.5%). We assessed 264 chronological DNA samples (at least 4 DNA samples per patient) for chronological analysis of relative TL, telomere dynamics and its comparison with the matched controls. For glycemic control related nitrosative stress, 20 blood plasma samples of T1D patients were analyzed with 8-Nitroguanine DNA/RNA Damage ELISA kit.
Results indicate no statistical difference of telomere dynamics between matched patients with good and poor glycemic control. Nevertheless, the comparison analysis of both groups indicates that TL of T1D patients with poor glycemic control is shortened faster compared to patients with good glycemic control (P = 0.0375). Additionally, patients with poor glycemic control have significantly increased 8-Nitroguanine blood plasma levels (P = 0.0020).
Telomere lengths in whole blood DNA samples are dynamically changing and this process is complex due to different genetic, environmental and immune factors. Good glycemic control is associated with decelerated telomere shortening and lower nitrosative stress compared with poor glycemic control. These results indicate the importance of good glycemic control in T1D in reduction of cell stress and inflammations and related development of disease complications.

This work was supported by Slovenian National Research Agency grant J3-6800.

PII-102

Genetic basis of familial erythrocytosis in Slovenia

Lucija Vermiglio[1,2], Anja Kopitar[1,2], Anja Solarovič[1,2], Nina Stemac[2], Martina Fink[2], Tadej Pajič[2], Saša Anžej Doma[2], Tanja Belčič[2], Matjaž Sever[2], Peter Černelč[2], Nataša Debeljak[1], Irena Preložnik Zupan[2]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*
[2]*University Medical Centre Ljubljana, Clinical Department of Haematology, Slovenia*

Erythrocytosis is blood disorder defined by an increased hematocrit (Ht). It is caused by an inherited or acquired mutations or as a compensatory mechanism in some chronical diseases. Most common acquired erythrocytosis Polycythemia vera (PV) caused by mutations in Janus kinase 2 (*JAK2*) is routinely tested at University Medical Centre (UMC) Ljubljana and Maribor. Other genetic forms or familial erythrocytosis (FE) are indicated as *JAK2* negative. Primary inherited FE is linked with mutations in erythropoietin receptor (*EPOR*), secondary can be caused by mutations in O_2 sensing pathway [1]. Erythropoietin (*EPO*) polymorphisms were previously correlated with increased hematocrit [2].
The aims of our study are to determine frequencies of erythrocytosis in Slovenian population and genetic variations in this group of patients with emphasis on familial erythrocytosis. Lifestyle and comorbidities, contributing to disease development, will also be assessed to find out some correlations with clinical pictures.
From April 2011 till September 2016 ~3900 Slovenian samples were analyzed for *JAK2* mutation. Among them 1100 were *JAK2* positive and 2800 *JAK2* negative. From 1225 *JAK2* negative samples tested at Clinical Department of Hematology UMC Ljubljana, 171 samples were excluded from the study due to other mutations (*BCR-ABL1, CALR, MPL* and *c-KIT*). Remaining 1054 patients were reviewed for increased Ht in two separate testing at least 2 months apart. We found 34 women with Ht > 0.46 and 47 men with Ht > 0.50. All 81 patents are invited to collaborate in extensive questionnaire and additional genetic testing. The frequency of two *EPO* single nucleotide polymorphisms (SNP) were analyzed in *JAK2* positive and negative patients.
From literature review we conclude the prevalence of FE is less than 1 per 100.000, the incidence is unknown. We are expecting to find two families in Slovenian population.

References:

1. McMullin (2016) Int J Lab Hematol.
2. Khabour (2012) Transfus Clin Biol.

PII-103

Bioinformatic identification and experimental validation of new glioblastoma stem cell marker candidates

Marko Vidak[1], Mirjana Liović[1], Ivana Jovčevska[1], Damjana Rozman[1], Sašo Džeroski[2], Radovan Komel[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*
[2]*Jožef Stefan Institute, Slovenia*

The aim of our study was to find genes for cell surface proteins (CSP) that would fulfill criteria for glioblastoma stem cell (GSC) markers and could thus be proposed as new marker candidates.
We analyzed microarray-based messenger ribonucleic acid (mRNA) expression data from two unrelated datasets, GDS3885 and GDS1962, from the Gene Expression Omnibus (GEO) database. A series of four one-tailed t-tests was devised: glioblastoma stem-like cell lines (GSCL) vs conventional glioblastoma (GBM) cell lines (A), GSCLs vs GBM tumour samples (B), GBM tumours vs low-grade gliomas (C), and GBM tumours vs non-malignant brain tissue (D). Genes that scored $P < 0.001$ in all the four tests — i.e. genes with elevated expression ($P < 0.001$) in GSCLs at the tests A and B, and also over-expressed in GBM tumours at the tests C and D — were checked for their cellular localization according to the COMPARTMENTS database, and CSP genes were selected for further screening.
There were 13 CSP genes that had passed all the four statistical tests. These genes were ranked according to composite criteria based on their signal ratios from the tests A and B. The top-two ranked genes (candidates 1 and 2) were further validated by comparing their expression values from the both datasets with the values of established cell-surface GSC marker candidates *CD133* and *CD90*. Candidates 1 and 2 were found to be more consistently over-expressed at the transcriptome level in GSCLs (vs conventional GBM cell lines and GBM tumours) and in GBM tumours (vs low-grade gliomas and non-malignant brain tissue) than the established candidates *CD133* and *CD90*.
Genes 1 and 2 are thus proposed as novel GSC marker candidates, and are currently being experimentally validated with analysis of their expression at the protein level in GSCLs, conventional GBM cell lines, GBM tumour samples, and non-malignant astrocytes. The results of the experimental validation will be presented at the conference.

PII-104

Proteomic insights into cancer-related extracellular proteolysis with cathepsin K

Matej Vizovišek[1,2], Robert Vidmar[1], Barbara Sobotič[1], Veronika Stoka[1], Boris Turk[1,3,4], Marko Fonović[1,4]

[1]*Jožef Stefan Institute, Department for Biochemistry and Molecular and Structural Biology, Slovenia*
[2]*Jožef Stefan International Postgraduate School, Slovenia*
[3]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[4]*Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Slovenia*

Cysteine cathepsins are lysosomal proteases from clan CA that gained much attention over the recent years for their role in cancer development and progression. Among these cathepsin K has strong collagenolytic activity and participates in several physiological and pathological processes. Besides osteoclasts different cancer cells express significant amounts of cathepsin K and the use of selective inhibitors such as odanacatib can significantly reduce the cancer-related osteolysis and bone metastasis burden. Cathepsin K could thus have an important role in the processes behind bone metastasis, but the molecular functions of extracellular proteolysis by cathepsin K remain poorly understood.

Our lab established a mass spectrometry-based proteomic approach that provided the first evidence for sheddase activity of cathepsins L and S with potential role in cancer development and progression representing a good platform to extend our search for extracellular substrates to cathepsin K. For our study we selected different cancer cell lines inlcuding MDA-MB-231 (breast cancer), HT-144 (melanoma), PC3 (prostate cancer) and Saos-2 (osteosarcoma), known to metastasize or develop in bones and performed an identification of extracellular substrates of cathepsin K. A detailed bioinformatics analysis showed that the majority of them is well-conserved over selected cancer cells. Among them antigen CD166 and CD44, glycoprotein MUC18, ephrin receptor 2 type A, plexin B2 and transferrin receptor 1, all of them having important roles in cancer development and progression. Our results suggest that cathepsin K has important roles in cancer-related extracellular proteolysis and is potentially important in cancer progression and development of bone metastasis opening new avenues for development of new cathepsin K-based therapeutics.

PII-105

Evaluation of nanobodies against potential glioblastoma biomarkers

Alja Zottel[1], Neja Zupanec[1], Ivana Jovčevska[1], Jernej Šribar[2], Igor Križaj[2], Lorna Zadravec Zaletel[3], Radovan Komel[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*
[2]*Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Slovenia*
[3]*Institute of Oncology, Slovenia*

Glioblastoma multiforme is the most common primary brain cancer and also one of the deadliest cancers. Most of patients do not survive for more than two years after diagnosis, in spite of advanced surgery and post-operative chemo- and radio-therapy. The main reasons of failure are: late diagnosis, difficulty to remove tumour completely, presence of radio- and chemo-resistant glioblastoma stem cells and highly infiltrative nature of the tumour. Therefore, better diagnostic tools and therapy are essentially required. One of the promising way to improve diagnosis and therapy is the use of so called nanobodies. These are small antigen recognizing part of heavy-chain only antibodies that are naturally occurring in very few animals, such as llamas, camels and sharks. The advantages of nanobody regarding classical antibodies are among others easy and high yield production, high stability, larger repertoire of epitopes, faster penetration into tumour, and low immunogenicity.
The aim of our work was to evaluate the cytotoxic effect of nine nanobodies identified previously, on glioblastoma cells lines U251MG, U87MG, glioblastoma stem cells and astrocytes as a reference. We also compared the efficacy of nanobodies combined with temozolomide against temozolomide or nanobodies alone. Furthermore, we have determined the location of selected specific antigens using nanobodies and secondary anti-his antibody and have verified our results with commercial antibodies. We also performed scratch assay to determine which nanobodies inhibit or decrease migration of glioblastoma cells.

PII-106

Towards the structural solutions of proteins associated with ALS

Vera Župunski[1], Jakob Rupert[1], Bernarda Majc[1], Urša Hribar[1], Gregor Gunčar[1], Boris Rogelj[1,2,3]

[1]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Chair of Biochemistry, Slovenia*
[2]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[3]*Biomedical Research Institute BRIS, Slovenia*

Amyotrophic lateral sclerosis is a fatal progressive neurodegenerative disease. A key characteristic of the disease is a cytoplasmic mislocalization of otherwise predominantly nuclear TDP-43, forming cytoplasmic inclusions in neuronal and glial cells. As of today, an increasing number of genetic factors have been linked to the progression, age-of-onset and susceptibility of the disease, with RNA metabolism and RNA-protein substructure being a particularly fast evolving focus of research. On the opposite, the structural analysis of proteins of various ALS-associated genes, such as *TARDBP*, *FUS*, *C9ORF72* and the recently associated *ANXA11*, has yet to achieve a major breakthrough.
Our work is focused into expression and isolation of soluble proteins involved in ALS pathogenesis with aim to solve their crystal structure. We have expressed GST- or His-tagged proteins in bacterial and mammalian expression systems using affinity and size-exclusion chromatography for isolation. Proteins predominantly sequestered in inclusion bodies. We increased the protein solubility adding a reducing agent, glycerol and/or an increased concentration of EDTA. In order to obtain higher yield of a soluble TDP-43, the protein was co-expressed with interacting partners. Structures of proteins involved in ALS should give us an insight into interaction with known protein and RNA partners and explain physiological modifications of ALS proteins.

PII-107

In vitro systems toxicology assessment of a potential reduced risk product compared to a combustible cigarette

Ignacio Gonzalez Suarez, Florian Martin, Diego Marescotti, Emmanuel Guedj, Stefano Acali, Didier Goedertier, Manuel C. Peitsch, Julia Hoeng

Philip Morris Products SA, Philip Morris International R&D, Switzerland

Cigarette smoking causes respiratory and other diseases. Cessation is the most effective approach to minimize disease risk. However, for those who would otherwise continue to smoke, switching to reduced-risk products (RRP) may be a way to lower the risk of smoking related disease compared with smoking. The tobacco heating system (THS2.2) is a RRP where tobacco is heated rather than burned by means of an electronically-controlled heating blade. Tobacco heating generates an aerosol mainly composed of water and glycerol that contains significantly lower levels of harmful/potentially harmful constituents (HPHC) than cigarette smoke. Primary human bronchial epithelial cells were used to investigate the biological impact of THS2.2 compared to the 3R4F cigarette. Multiple toxicity endpoints were measured via real-time cellular analysis and high-content screening. Study was complemented by gene expression analysis, followed by a computational approach to identify and quantify perturbed molecular pathways. Chemical characterization of THS2.2 aerosol showed similar nicotine levels and lower levels of 54 HPHCs compared to 3R4F smoke. Cells were exposed to 3 different smoke/aerosol fractions: mainstream smoke/aerosol bubbled PBS, total particulate matter (TPM) and gas-vapor phase (the substance that passes through the filter pad during TPM collection). 3R4F smoke fractions caused a dose-dependent response in most toxicity endpoints and a significant level of perturbation in most biological pathways. By contrast, exposure to THS2.2 aerosol fractions had an overall lower biological impact. No cellular toxicity was observed at similar nicotine doses and up to 15-fold increased THS2.2 concentrations were necessary to elicit a level of response similar to 3R4F fractions. Moreover, the level of biological network perturbation was also significantly reduced compared to 3R4F. In summary, our systems toxicology study demonstrated reduced effects of THS 2.2 aerosol compared to 3R4F smoke.

PII-108

FAIR data in plant systems biology

Živa Ramšak, Anna Coll Rius, Andrej Blejec, Kristina Gruden

National Institute of Biology, Slovenia

Method developments in the post genomics era of biological research now allow us to capture snapshots of systems, both of the dynamic and spatial resolution. At the same time, computational capabilities have increased in terms of the ability to handle and analyse these huge amounts of data. However the challenge now lies in the scattered nature of data resources and replicability of experimental results. To facilitate knowledge discovery, we adopted a set of guiding principles, to make data FAIR — *Findable, Accessible, Interoperable and Re-usable*.

The field of plant systems biology is encountering such challenges as well, where the genotypic and phenotypic data of plant species can be found in multiple locations, with a large variation of their representations. Here we present the work done on the topic of data management system at our institute, starting on the bottom level of storage of high-throughput data, implementation of standard directory trees to support the researchers, to their analyses and metadata annotation.

We will also touch on the topics of standardisation in relation to the European distributed infrastructure for life-science information (ELIXIR), covering the expansion and evolution of standards relevant for plant systems biology. Lastly, a model covering the capture of metainformation about biological samples and publically exposing it jointly with the measured variables will be presented via a common infrastructure.

PII-109

QuantGenius: implementation of a decision support system for qPCR-based gene quantification

Špela Baebler, Miha Svalina, Marko Petek, Katja Stare, Ana Rotter, Maruša Pompe Novak, Kristina Gruden

National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia

Quantitative molecular biology remains a challenge for researchers due to inconsistent approaches for control of errors in the final results. Due to several factors that can influence the final result, quantitative analysis and interpretation of qPCR data are still not trivial. Together with the development of high-throughput qPCR platforms, there is a need for a tool allowing for robust, reliable and fast nucleic acid quantification.
We have developed "quantGenius" (http://quantgenius.nib.si), an open-access web application for a reliable qPCR-based quantification of nucleic acids. The quantGenius workflow interactively guides the user through data import, quality control (QC) and calculation steps. The input is machine- and chemistry-independent. Quantification is performed using the standard curve approach, with normalization to one or several reference genes. The special feature of the application is the implementation of user-guided QC-based decision support system, based on qPCR standards, that takes into account pipetting errors, assay amplification efficiencies, limits of detection and quantification of the assays as well as the control of PCR inhibition in individual samples. The intermediate calculations and final results are exportable in a data matrix suitable for further statistical analysis or visualization. We additionally compare the most important features of quantGenius with similar advanced software tools and illustrate the importance of proper QC system in the analysis of qPCR data in two use cases.
To our knowledge, quantGenius is the only qPCR data analysis tool that integrates QC-based decision support and will help scientists to obtain reliable results which are the basis for biologically meaningful data interpretation.

PII-110

Exploring potato-PVY interaction using systems biology approach identifies SAPK8 as novel regulator of plant immunity

Tjaša Stare[1], Anna Coll Rius[1], Katja Stare[1], Stefanie Wienkoop[2], Živa Ramšak[1], Kristina Gruden[1]

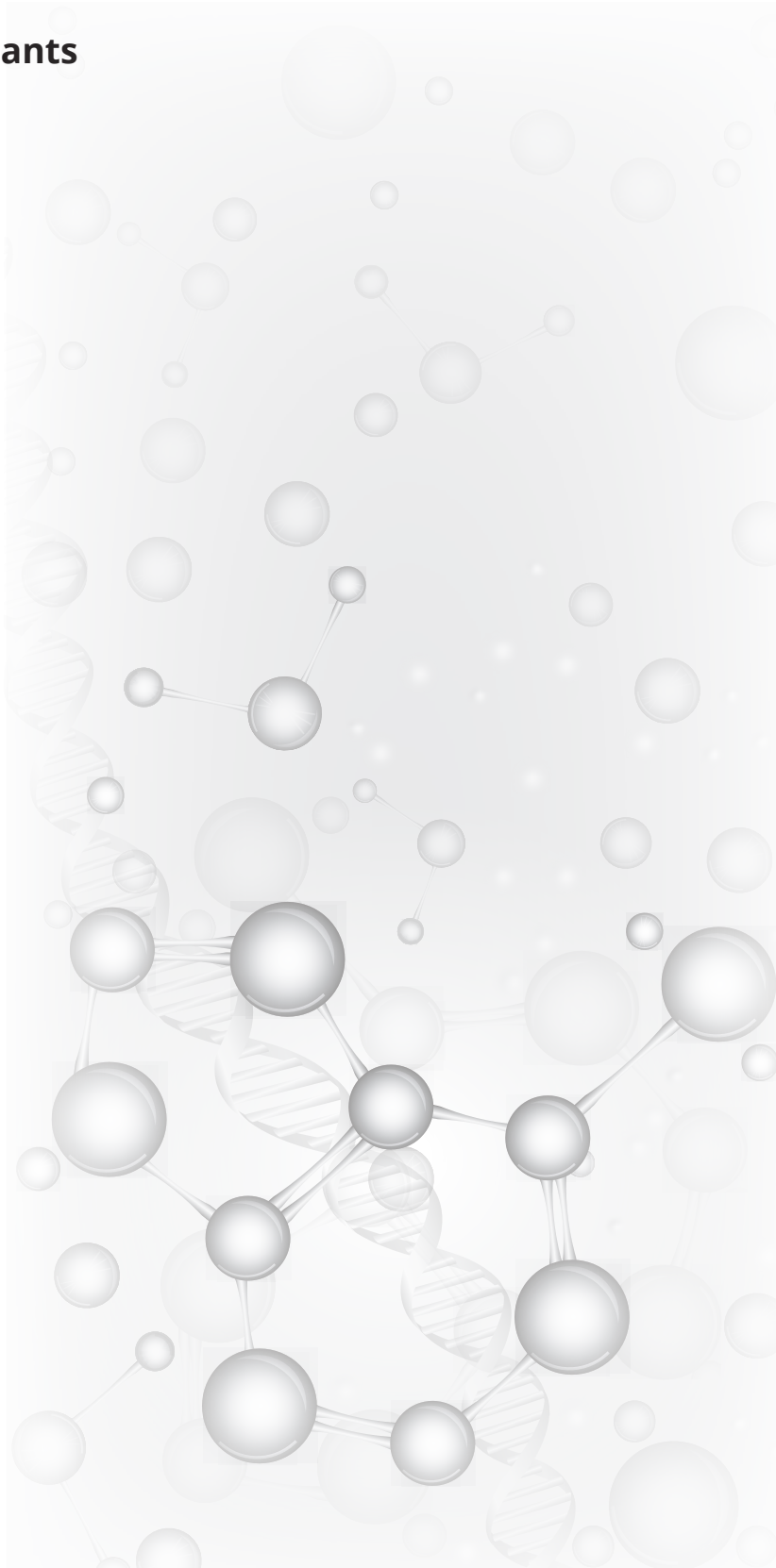
[1]*National Institute of Biology, Department of Biotechnology and Systems Biology, Slovenia*
[2]*University of Vienna, Faculty of Life Sciences, Department of Ecogenomics and Systems Biology, Austria*

The interaction between plant and its pathogen initiates a complex signalling network, resulting in massive changes of the gene activity and extensive reprogramming of the cell metabolism. In this study systems biology approach was used to model complex biological processes, understand mechanisms and dynamic involved in plant defence and identify novel regulators of plant immunity.

We constructed signalling network topology describing the biosynthesis, hormone recognition and signal transduction leading to activation of effector molecules of crucial phytohormones involved in plant defence: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). The primary model has been expanded with model with Arabidopsis (*Arabidopsis thaliana*) protein interactors and translated to potato. In parallel with model construction, analysis of dynamic potato response to PVY was evaluated. Dynamics of whole transcriptome changes of cultivar Désirée and NahG-Désirée were analysed in inoculated and systemically infected leaves following 0, 1, 3, 4, 5, 7, 8, 9, and 11 days after infection (dpi). Potato proteome changes on set of viral multiplication were analysed and compared to transcriptional dynamics. Dynamics of physiological changes were evaluated on the level of symptoms development, measuring virus accumulation and spread to uninfected tissue and callose accumulation.

Integration of both, modelling with novel biological data enabled us to identify novel regulators of plant defence against viral pathogens. The role of two kinases and two phosphatases has been evaluated with functional genomics. The results imply that novel players of virus induced-potato response gap the bridge between regulation of abiotic and biotic plant response signalling.

List of participants
Author Index



List of participants

Gregor Anderluh National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: gregor.anderluh@ki.si	Aleš Berlec Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: ales.berlec@ijs.si	Barbara Breznik National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: barbara.breznik@nib.si
Tina Arič Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection Dunajska 22, 1000 Ljubljana, Slovenia Email: tina.aric@gov.si	Rebeka Bernik Email: bernik.rebeka@gmail.com	Juan J. Calvete CSIC, Instituto de Biomedicina de Valencia Carrer de Jaume Roig 11, 46010 Valencia, Spain Email: jcalvete@ibv.csic.es
Špela Baebler National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: spela.baebler@nib.si	Monika Biasizzo Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: monika.biasizzo@ijs.si	Blaž Cigić University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: blaz.cigic@bf.uni-lj.si
Miha Bahun University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: miha.bahun@bf.uni-lj.si	Duško Blagojević University of Belgrade, Institute for Biological Research "Siniša Stanković" Bulevar despota Stefana 142, Belgrade, Serbia Email: dblagoje@ibiss.bg.ac.rs	Mateja Cigoj University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: mateja.cigoj@gmail.com
Ana Bajc Česnik Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: ana.bajc.cesnik@gmail.com	Kaja Blagotinšek University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: kaja.blagotinsek@mf.uni-lj.si	Anna Coll National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: anna.coll@nib.si
Aljoša Bavec University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: aljosa.bavec@mf.uni-lj.si	Tanja Blagus University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: tanja.blagus@mf.uni-lj.si	Andrej Cör University of Primorska, Faculty of Health Sciences Polje 42, 6310 Izola, Slovenia Email: andrej.coer@fvz.upr.si
Apolonija Bedina Zavec National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: polona.bedina@ki.si	Alexandra Bogožalec Košir National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: alexandra.bogozalec@nib.si	Tomaž Curk University of Ljubljana, Faculty of Computer and Information Science Večna pot 113, 1000 Ljubljana, Slovenia Email: tomaz.curk@fri.uni-lj.si
Mojca Benčina National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: mojca.bencina@ki.si	Anita Bosak Institute for Medical Research and Occupational Health Ksaverska cesta 2, 10 000 Zagreb, Croatia Email: abosak@imi.hr	Tanja Cvitanović University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: tanja.cvitanovic@mf.uni-lj.si

Katja Čop University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: katja.cop.07@gmail.com	Marinka Drobnič Košorok Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia Email: marinka.drobnic-kosorok@vf.uni-lj.si	Marko Goličnik University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: marko.golicnik@mf.uni-lj.si
Tanja Čufer University Clinic Golnik Golnik 36, 4204 Golnik, Slovenia Email: tanja.cufer@klinika-golnik.si	Jure Fabjan University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: jure.fabjan93@gmail.com	Ignacio Gonzalez Suarez Philip Morris Products SA Quai Jeanrenaud 5, 2000 Neuchâtel, Switzerland Email: ignacio.gonzalezsuarez@pmi.com
Vanja Čuk University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: vanja8cuk@gmail.com	Jana Ferdin University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: jana.ferdin@mf.uni-lj.si	Tjaša Goričan University of Ljubljana, Faculty of Chemistry and Chemical Technology Večna pot 113, 1000 Ljubljana, Slovenia Email: tjasa.gorican@fkkt.uni-lj.si
Nataša Debeljak University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: natasa.debeljak@mf.uni-lj.si	Klementina Fon Tacer St. Jude Children's Research Hospital Memphis, TN, USA Email: klementina.fontacer@stjude.org	Katja Goričar University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: katja.goricar@mf.uni-lj.si
David Dobnik National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: david.dobnik@nib.si	Vida Forstnerič National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: vida.forstneric@ki.si	Helena Gradišar National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: helena.gradisar@ki.si
Klemen Dolinar University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: klemen.dolinar@mf.uni-lj.si	Aljaž Gaber University of Ljubljana, Faculty of Chemistry and Chemical Technology Večna pot 113, 1000 Ljubljana, Slovenia Email: aljaz.gaber@fkkt.uni-lj.si	Urh Grošelj University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: urh.groselj@gmail.com
Bojan Doljak University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: b.doljak@ffa.uni-lj.si	Stanislav Gobec University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: stanislav.gobec@ffa.uni-lj.si	Kristina Gruden National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: kristina.gruden@nib.si
Vita Dolžan University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: vita.dolzan@mf.uni-lj.si	Oliver Goldenberg Sr Marketing Technical Specialist, Central Europe Illumina Email: ogoldenberg@illumina.com	San Hadži University of Ljubljana, Faculty of Chemistry and Chemical Technology, Večna pot 113, 1000 Ljubljana, Slovenia Email: sanahadzi@gmail.com

Iva Hafner Bratkovič National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: iva.hafner@ki.si	Tinka Hovnik University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: tinka.hovnik@kclj.si	Blaž Jug University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: blazjug9@gmail.com
Simona Hanželič University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: simona.hanzelic@gmail.com	Petra Hudler University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: petra.hudler@mf.uni-lj.si	Theodora Katsila University of Patras, Department of Pharmacy University Campus, Patras, Greece Email: thkatsila@upatras.gr
Henning Hermjakob European Molecular Biology Laboratory-European Bioinformatics Institute Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK Email: hhe@ebi.ac.uk	Adrijan Ivanušec Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: imajd7@gmail.com	Nadja Kern Prezelj VWR International GmbH Graumanngasse 7, 1150 Vienna, Austria Email: nadja.prezelj@vwr.com
Anžej Hladnik University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: anzej.hladnik@gmail.com	Polona Jamnik University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: polona.jamnik@bf.uni-lj.si	Anja Kerš University of Ljubljana, Faculty of Chemistry and Chemical Technology Večna pot 113, 1000 Ljubljana, Slovenia Email: anja.kers@fkkt.uni-lj.si
Vesna Hodnik University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: vesna.hodnik@bf.uni-lj.si	Vid Jan University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: vid.jan@mf.uni-lj.si	Mohammed Khurshed University of Amsterdam, Academic Medical Center Meibergdreef 15, Amsterdam, The Netherlands Email: m.khurshed@amc.uva.nl
Marko Hojnik University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: marko.hojnik@hotmail.com	Eva Jarc Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: eva.jarc@ijs.si	Nejc Kikelj University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: kikelj.nejc@gmail.com
Marjeta Horvat University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: marjetah3@gmail.com	Roman Jerala National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: roman.jerala@ki.si	Matic Kisovec National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: matic.kisovec@ki.si
Simon Horvat University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: simon.horvat@bf.uni-lj.si	Ivana Jovčevska University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: ivana.jovcevska@mf.uni-lj.si	Tina Kljun Lek Pharmaceuticals d.d. Verovškova ulica 57, 1000 Ljubljana, Slovenia Email: tina.kljun@novartis.com

Tajda Klobučar University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: tajda.klobucar@gmail.com	Janko Kos University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: janko.kos@ffa.uni-lj.si	Mirijam Kozorog National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: Mirijam.Kozorog@ki.si
Tamara Knific University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: tamara.knific@mf.uni-lj.si	Milan Kostić Vinča Institute of Nuclear Sciences PO Box 522, Mike Petrovića Alasa 12-14, 11001 Belgrade, Serbia Email: milankostic@vin.bg.ac.rs	Aleša Kristan University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: alesa.kristan@gmail.com
Eva Kočar University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: evi.kocar@gmail.com	Tjaša Košir University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: kosir.tjasa@gmail.com	Igor Križaj Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: Igor.Krizaj@ijs.si
Deja Kokot MIKRO+POLO d.o.o. Zagrebška 22, 2000 Maribor, Slovenia Email: deja.kokot@mikro-polo.si	Mihály Kovács Eötvös Loránd University, Department of Biochemistry Pázmány P. s. 1/c, 1117 Budapest, Hungary Email: mihaly.kovacs@ttk.elte.hu	Maja Križnik National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: maja.kriznik@nib.si
Radovan Komel University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: radovan.komel@mf.uni-lj.si	Jernej Kovač University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: jernej.kovac@kclj.si	Teja Krpan University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: krpan.teja@gmail.com
Rebeka Komel University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: rebeka.komel@gmail.com	Urša Kovač University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: ursa.kovac@mf.uni-lj.si	Nika Kruljec University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: nika.kruljec@ffa.uni-lj.si
Tilen Konte University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: tilen.konte@mf.uni-lj.si	Anja Kovanda Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: anja.kovanda@ijs.si	Tamara Lah Turnšek National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: Tamara.lah@nib.si
Nataša Kopitar Jerala Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: natasa.kopitar@ijs.si	Zrinka Kovarik Institute for Medical Research and Occupational Health Ksaverska cesta 2, 10 000 Zagreb, Croatia Email: zkovarik@imi.hr	Duško Lainšček National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: dusko.lainscek@ki.si

Tea Lanišnik Rižner University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: tea.lanisnik-rizner@mf.uni-lj.si	Adrijana Leonardi Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: adrijana.leonardi@ijs.si	Andreja Majerle National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: andreja.majerle@ki.si
Buday Laszlo Institute of Enzymology, RCNS, HAS 2. Magyar tudosok krt., 1117 Budapest, Hungary Email: buday.laszlo@ttk.mta.hu	Nataša Lindič Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: natasa.lindic@ijs.si	Tomaž Makovec University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: tomaz.makovec@mf.uni-lj.si
Zorica Latinović Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: zorica.latinovic@ijs.si	Ajasja Ljubetič National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: ajasja.ljubetic@ki.si	Mirjana Malnar Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: mirjana.malnar@ijs.si
Blaž Lebar University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: blazlebar1995@gmail.com	Uroš Logonder Roche farmacevtska družba d.o.o. Vodovodna cesta 109, 1000 Ljubljana, Slovenia Email: uros.logonder@roche.com	Mateja Manček Keber National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: mateja.mancek@ki.si
Tina Lebar National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: tina.lebar@ki.si	Remy Loris Vrije Universiteit Brussel Pleinlaan 2, 1050 Brussel, Belgium Email: Remy.Loris@vub.be	Nikola Maraković Institute for Medical Research and Occupational Health Ksaverska cesta 2, 10 000 Zagreb, Croatia Email: nmarakovic@imi.hr
Brigita Lenarčič University of Ljubljana, Faculty of Chemistry and Chemical Technology Večna pot 113, 1000 Ljubljana, Slovenia Email: brigita.lenarcic@fkkt.uni-lj.si	Tjaša Lukan National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: tjasa.lukan@nib.si	Tomaž Marš University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: tomaz.mars@mf.uni-lj.si
Tea Lenarčič National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: tea.lenarcic@ki.si	Denisa Maderova Biomedical Research Center SAS Dubravska cesta 9, 845 05 Bratislava, Slovakia Email: d.maderova@gmail.com	Urška Matkovič University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: urska.matkovic@mf.uni-lj.si
Metka Lenassi University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: metka.lenassi@mf.uni-lj.si	Gregor Majdič University of Ljubljana, Veterinary Faculty Gerbičeva 60, 1000 Ljubljana, Slovenia Email: gregor.majdic@vf.uni-lj.si	Mojca Mattiazzi Ušaj University of Toronto, The Donnelly Centre 160 College St., Toronto ON, Canada Email: m.mattiazziusaj@utoronto.ca

Julija Mazej Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: mazej.julija@gmail.com	Marek Mutwil Max Planck Institute of Molecular Plant Physiology Am Mühlenberg 1, 14476 Golm, Germany Email: mutwil@mpimp-golm.mpg.de	Miha Pavšič University of Ljubljana, Faculty of Chemistry and Chemical Technology Večna pot 113, 1000 Ljubljana, Slovenia Email: miha.pavsic@fkkt.uni-lj.si
Anja Medved Biomedis M.B. d.o.o. Slokanova 12, 2000 Maribor, Slovenia Email: anja.medved@bmgrp.si	Nejc Nadižar University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: nejc.nadizar@mf.uni-lj.si	Katarina Pavšič Vrtač Veterinary Faculty, University of Ljubljana Gerbičeva 60, 1000 Ljubljana, Slovenia Email: katarina.vrtac@vf.uni-lj.si
Katarina Miš University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: katarina.mis@mf.uni-lj.si	Marko Novinec University of Ljubljana, Faculty of Chemistry and Chemical Technology Večna pot 113, 1000 Ljubljana, Slovenia Email: marko.novinec@fkkt.uni-lj.si	Urša Pečar Fonović University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: ursa.pecarfonovic@ffa.uni-lj.si
Ana Mitrović Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: ana.mitrovic@ffa.uni-lj.si	Jakub Nowak NanoTemper Technologies sp. z o.o. Krakow, Poland Email: jakub.nowak@nanotemper.de	Milica Perišić Nanut Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: milica.perisic@ijs.si
Matej Mlinarič University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: matej.mlinarich@gmail.com	Tamara Obreza University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: tamara.obreza@kclj.si	Toni Petan Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: toni.petan@ijs.si
Helena Motaln Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: Helena.Motaln@gmail.com	Václav Pačes, FEBS Secretary General Academy of Sciences, Institute of Molecular Genetics, Vídenska 1083, 14220 Prague 4, Czech Republic Email: vpaces@img.cas.cz	Marko Petek National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: marko.petek@nib.si
Rolf Müller HZI, Helmholtz-Institute for Pharmaceutical Research Saarland University Campus E8.1, 66123 Saarbrücken, Germany Email: rolf.mueller@helmholtz-hzi.de	Anastasija Panevska University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: anastasija.panevska@bf.uni-lj.si	Špela Petelin University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: spela.petelin@mf.uni-lj.si
Werner Müller University of Manchester AV Hill Building, Oxford Road, Manchester M13 9PT, UK Email: werner.muller@manchester.ac.uk	Anja Pavlin University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, Ljubljana, Slovenia Email: anja.pavlin1@gmail.com	Uroš Petrovič University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: uros.petrovic@bf.uni-lj.si

Sara Pintar Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: sara.pintar@ijs.si	Nataša Poklar Ulrih University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: natasa.poklar@bf.uni-lj.si	Matjaž Rejec LABENA trgovina, svetovanje in proizvodnja laboratorijske opreme d.o.o. Verovškova 64, 1000 Ljubljana, Slovenia Email: matjaz.rejec@labena.si
Katja Pirc National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: katja.pirc@ki.si	Olivera Politeo University of Split, Faculty of Chemistry and Technology Ruđera Boškovića 35, 21000 Split, Croatia Email: olivera@ktf-split.hr	Barbka Repič Lampret University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: barbka.repic@kclj.si
Sergej Pirkmajer University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: sergej.pirkmajer@mf.uni-lj.si	Maruša Pompe Novak National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: marusa.pompe.novak@nib.si	Nathalie Reuter University of Bergen Thormøhlensgt 55, 5008 Bergen, Norway Email: nathalie.reuter@uib.no
Anja Pišlar University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: anja.pislar@ffa.uni-lj.si	Anja Pucer Janež Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: anja.pucer@ijs.si	Saša Rezelj National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: sasa.rezelj@ki.si
Janez Plavec National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: janez.plavec@ki.si	Pia Pužar Dominkuš University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: pia.puzar-dominkus@mf.uni-lj.si	Tadeja Režen University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: tadeja.rezen@mf.uni-lj.si
Tina Vida Plavec University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: plavec.tina@gmail.com	Živa Ramšak National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: ziva.ramsak@nib.si	Samuel Ristovski University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: samuel.ristovski@gmail.com
Ana Plemenitaš University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: ana.plemenitas@mf.uni-lj.si	Sara Redenšek University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: sara.redensek@mf.uni-lj.si	Boris Rogelj Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: boris.rogelj@ijs.si
Marjetka Podobnik National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: marjetka.podobnik@ki.si	Živa Rejc University of Ljubljana, Faculty of Computer and Information Science Večna pot 113, 1000 Ljubljana, Slovenia Email: ziva.rejc@gmail.com	Damjana Rozman University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: damjana.rozman@mf.uni-lj.si

Mateja Salobir Lek Pharmaceuticals d.d. Verovškova ulica 57, 1000 Ljubljana, Slovenia Email: mateja.salobir@novartis.com	Jelena Stanišić Vinča Institute of Nuclear Sciences PO Box 522, Mike Petrovića Alasa 12–14, 11001 Belgrade, Serbia Email: sjelena@vin.bg.ac.rs	Andreja Šink National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: andreja.sink@ki.si
Rok Sekirnik Lek Pharmaceuticals d.d. Verovškova ulica 57, 1000 Ljubljana, Slovenia Email: rok.sekirnik@novartis.com	Tjaša Stare National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: tjasa.stare@nib.si	Goran Šinko Institute for Medical Research and Occupational Health Ksaverska cesta 2, 10 000 Zagreb, Croatia Email: gsinko@imi.hr
Kristina Sepčić University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: kristina.sepcic@bf.uni-lj.si	Katja Stare National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: katja.stare@nib.si	Nives Škorja University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology Zaloška 4, 1000 Ljubljana, Slovenia Email: nives.skorja@mf.uni-lj.si
Tilen Sever University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: severtilen959@gmail.com	Jure Stojan University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: stojan@mf.uni-lj.si	Katja Škrlec Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: katja.skrlec@ijs.si
Maša Sinreih University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: masa.sinreih@mf.uni-lj.si	Martin Stražar University of Ljubljana, Faculty of Computer and Information Science Večna pot 113, 1000 Ljubljana, Slovenia Email: martin.strazar@fri.uni-lj.si	Andraz Šmon University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: andraz.smon@kclj.si
Mihaela Skrt University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: mihaela.skrt@bf.uni-lj.si	Aleksandra Šakanović National Institute of Chemistry Hajdrihova 19, 1000 Ljubljana, Slovenia Email: aleksandra.sakanovic@ki.si	Marko Šnajder University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: marko.snajder@bf.uni-lj.si
Nuša Slabajna Lek Pharmaceuticals d.d. Verovškova ulica 57, 1000 Ljubljana, Slovenia Email: nusa.slabajna@novartis.com	Amadej Šenk Juh Gimnazija Bežigrad Peričeva 4, 1000 Ljubljana, Slovenia Email: barbara.senk@amis.net	Igor Kristian Štern Lek Pharmaceuticals d.d. Verovškova ulica 57, 1000 Ljubljana, Slovenia Email: igor_kristian.stern@novartis.com
Jaka Snoj University of Ljubljana, Biotechnical Faculty Jamnikarjeva 101, 1000 Ljubljana, Slovenia Email: nalepka_@hotmail.com	Barbara Šenk University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: barbara.senk@amis.net	Borut Štrukelj University of Ljubljana, Faculty of Pharmacy Aškerčeva 7, 1000 Ljubljana, Slovenia Email: borut.strukelj@ffa.uni-lj.si

Ajda Taler-Verčič Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: ajda.taler@ijs.si	Katarina Uršič University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: katarina.ursic@mf.uni-lj.si	Igor Weber Ruđer Bošković Institute, Division of Molecular Biology Bijenička 54, Zagreb, Croatia Email: iweber@irb.hr
Tine Tesovnik University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: ti.tesovnik@gmail.com	Aleksandra Usenik Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: aleksandra.usenik@ijs.si	Antonio Zandona Institute for Medical Research and Occupational Health Ksaverska cesta 2, 10 000 Zagreb, Croatia Email: azandona@imi.hr
Špela Tomaž National Institute of Biology Večna pot 111, 1000 Ljubljana, Slovenia Email: tomazspela@gmail.com	Aleksandra Uzar University of Ljubljana, Faculty of Chemistry and Chemical Technology Večna pot 113, 1000 Ljubljana, Slovenia Email: aleks.uzar@gmail.com	Ulrich Zanger Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology Auerbachstr. 112, 70376 Stuttgart, Germany Email: uli.zanger@ikp-stuttgart.de
Nataša Toplak Omega d.o.o. Dolinškova 8, 1000 Ljubljana, Slovenia Email: natasa.toplak@omega.si	Cornelis van Noorden University of Amsterdam, Academic Medical Center Meibergdreef 15, Amsterdam, The Netherlands Email: c.j.vannoorden@amc.uva.nl	Tina Zavašnik Bergant Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: tina.zavasnik@ijs.si
Katarina Trebušak Podkrajšek University Medical Centre Ljubljana, University Children's Hospital Bohoričeva 20, 1000 Ljubljana, Slovenia Email: katarina.trebusak@kclj.si	Lucija Vermiglio University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: lucia.vermiglio@gmail.com	Alja Zottel University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: alja.zottel@mf.uni-lj.si
Boris Turk Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: boris.turk@ijs.si	Marko Vidak University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Vrazov trg 2, 1000 Ljubljana, Slovenia Email: marko.vidak@mf.uni-lj.si	Petra Zrimšek Veterinary Faculty, University of Ljubljana Gerbičeva 60, 1000 Ljubljana, Slovenia Email: petra.zrimsek@vf.uni-lj.si
Dušan Turk Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: Dusan.Turk@ijs.si	Monika Vidmar Stražišar MERCK d.o.o. Letališka ulica 29c, 1000 Ljubljana, Slovenia Email: monika.vidmar.strazisar@merckgroup.com	Vida Žgajnar LABENA trgovina, svetovanje in proizvodnja laboratorijske opreme d.o.o. Verovškova 64, 1000 Ljubljana, Slovenia Email: vida.zgajnar@labena.si
Uroš Urleb/Dominik Gaser Lek Pharmaceuticals d.d. Verovškova ulica 57, 1000 Ljubljana, Slovenia	Matej Vizovišek Jožef Stefan Institute Jamova cesta 39, 1000 Ljubljana, Slovenia Email: matej.vizovisek@ijs.si	Vera Župunski University of Ljubljana, Faculty of Chemistry and Chemical technology Večna pot 113, 1000 Ljubljana, Slovenia Email: vera.zupunski@fkkt.uni-lj.si

Author index

Corrigenda

1. Corrigendum: SL4; Bio-Rad's state of the art solutions to help you find answers: from DNA translation to single cell sequencing
Matjaž Rejec, Rok Košir
Labena d.o.o., Slovenia
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 45.

The authors regret that some authors were missing from the authorship. The correct citation is as follows:
SL4; Bio-Rad's state of the art solutions to help you find answers: from DNA translation to single cell sequencing
Uršula Prosenc Zmrzljak, Matjaž Rejec, Rok Košir
Labena d.o.o., Slovenia
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 45.

2. Corrigendum: PI-28; The conserved D0 domain of flagellin is involved in the activation of Toll-like receptor 5 and the intracellular Naip-NLRC4 inflammasome
Vida Forstnerič[1,2]
[1]*National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia*
[2]*EN-FIST Centre of Excellence, Slovenia*
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 102.

The authors regret that some authors were missing from the authorship. The correct citation is as follows:
PI-28; The conserved D0 domain of flagellin is involved in the activation of Toll-like receptor 5 and the intracellular Naip-NLRC4 inflammasome
Vida Forstnerič[1], Karolina Ivičak-Kocjan[1], Tjaša Plaper[1], Roman Jerala[1,2], Mojca Benčina[1]
[1]*National Institute of Chemistry, Department of Synthetic Biology and Immunology, Slovenia*
[2]*EN-FIST Centre of Excellence, Slovenia*
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 102.

3. Corrigendum: P-31; Characterisation of L1 ORF1p in mammalian cells
Mirjana Malnar[1], Boris Rogelj[1,2,3]
[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*Biomedical Research Institute BRIS, Slovenia*
[3]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 105.

The authors regret that some authors were missing from the authorship. The correct citation is as follows:
PI-31; Characterisation of L1 ORF1p in mammalian cells
Mirjana Malnar[1], Vera Župunski[2], Tomaž Bratkovič[3], Miha Modic[4], Micha Drukker[4], Jernej Ule[5,6], Boris Rogelj[1,2,7]
[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*

[4]*Helmoltz Zentrum München, Institute of Stem Cell Research, Germany*
[5]*University College London, Institute of Neurology, UK*
[6]*The Francis Crick Institute, UK*
[7]*Biomedical Research Institute BRIS, Slovenia*
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 105.

4. Corrigendum: P-55; HNRNPH and its localisation to nuclear G4C2 foci of C9ORF72 amyotrophic lateral sclerosis
Boris Rogelj[1,2,3]
[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*Biomedical Research Institute BRIS, Slovenia*
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 129.

The authors regret that some authors were missing from the authorship. The correct citation is as follows:
PI-55; HNRNPH and its localisation to nuclear G4C2 foci of C9ORF72 amyotrophic lateral sclerosis
Sonja Prpar Mihevc[1], Valter Bergant[1,2], Julija Mazej[1,2], Boris Rogelj[1,2,3]
[1]*Jožef Stefan Institute, Department of Biotechnology, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*Biomedical Research Institute BRIS, Slovenia*
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 129.

5. Corrigendum: PII-105; Evaluation of nanobodies against potential glioblastoma biomarkers
Alja Zottel, Neja Zupanec, Ivana Jovčevska, Radovan Komel
University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 181.

The authors regret that some authors were missing from the authorship. The correct citation is as follows:
PII-105; Evaluation of nanobodies against potential glioblastoma biomarkers
Alja Zottel[1], Neja Zupanec[1], Ivana Jovčevska[1], Jernej Šribar[2], Igor Križaj[2], Lorna Zadravec Zaletel[3], Radovan Komel[1]
[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Medical Centre for Molecular Biology, Slovenia*
[2]*Jožef Stefan Institute, Department of Molecular and Biomedical Sciences, Slovenia*
[3]*Institute of Oncology, Slovenia*
V: Goričar, K (ur.), et al. Book of Abstracts, 12th Meeting of the Slovenian Biochemical Society with International Participation, Bled, Slovenia, 20-23, 2017, p. 181.

Sponsors

The organisers wish to thank all the generous sponsors and donators for supporting the 12th Meeting of the Slovenian Biochemical Society.

General Sponsors

KEMOMED BRINGING SOLUTIONS

illumina AUTHORIZED CHANNEL PARTNER

nano TEMPER

Associate Sponsors

instrumentalia

mikro+polo vaš partner za laboratorij

Labena

Roche

Sponsors

bia TRUSTED LAB PARTNER

BIOMEDICA BIOMEDIS

CHEMASS MERILNI SISTEMI

eppendorf

NATIONAL INSTITUTE OF CHEMISTRY

mediline

MERCK

METTLER TOLEDO

Omega d.o.o.

Sanolabor

VWR We Enable Science

ZEISS

Donators

BEVOG BRAUHAUS • BREWERY

Mladinska knjiga Knjigarna Konzorcij www.konzorcij.si

LISJAK VIPAVSKA DOLINA

KEMOMED
BRINGING SOLUTIONS

Looking for Innovative Solutions in the Field of Genomics, Biomedicine and Biotechnology?

We Bring Them To You!

- Instruments and Laboratory Equipment
- Reagents for Research and Diagnostic Biomedicine
- Consumables
- Application Support
- Service and Technical Support
- Calibration

Kemomed
Svetovanje, trgovina in trženje d.o.o.

Kališka 9
PE: Stritarjeva 5
4000 Kranj
Slovenia

T +386 4 2015 050
F +386 4 2015 055
E info@kemomed.si
W www.kemomed.si

Affinity. Stability. Conformation.

nano TEMPER technologies

More than 1000 peer-reviewed publications with MST and nanoDSF.
Your project deserves the best data!

Discover our technologies:

- Easy to use
- Fast measurements
- Highest precision
- Fun

Visit our website to learn more!
www.nanotemper-technologies.com

NanoTemper Technologies

We care about your research

instrumentalia

lab im laboratorijska oprema

Združeni v en team.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Chromatography Systems

Acquity UPLC H-Class Bio coupled with QDa

ACQUITY UPLC H-Class Bio coupled with QDa Single Quad MS detector

Ready for your biomolecular analysis

A system purpose-built for the **analysis of proteins, peptides, nucleic acids and glycans,** adding MS detection capabilities to bring analytical confidence with additional mass data.

TA Instruments

TA Instruments Thermal Analysis Systems

Isothermal Titration Nano ITC

Isothermal Titration Calorimetry (ITC), Differential Scanning Calorimetry (DSC) and Isothermal Calorimetry Systems

Systems are powerful analytical techniques for in-depth characterization of molecular binding events and structural stability, for the most demanding applications in **drug discovery, protein-protein interactions, structure-function characterization and more.**

instrumentalia
T +386 1 524 0196
F +386 1 524 0198
A Leskoškova cesta 9E
1000 LJUBLJANA
E events@instrumentalia.si

lab im laboratorijska oprema
T +386 1 428 3684
F +386 1 427 3811
A Ziherlova ulica 6
1000 LJUBLJANA
E info@labtim.si

Labena

Complete Solutions in Laboratory and Process Analytics

- PCR, qPCR, Droplet Digital PCR
- BioSpectrometers
- Light and AFM microscopy
- Cell Imagers
- Cell Counters/Sorters
- Washers/Readers
- Electrophoresis
- Western blotting
- Antibodies
- Laboratory Grade Water Systems (Type 1, 2, 3)
- Centrifuges
- PH Meters
- Analytical Balances
- Pipettes
- Biological Safety Cabinets
- Laboratory Refrigerators & Freezers
- Other Laboratory Equipment

Labena

Ljubljana SLO / Zagreb HR / Sarajevo BIH / Skopje MAK / Beograd SRB

Contact:
All our Original Offices and www.labena.si or info@labena.si.

BIO-RAD ELGA VEOLIA JPK Instruments eppendorf ESCO WORLD CLASS. WORLDWIDE. OLYMPUS Your Vision, Our Future NEPTUNE Thermo SCIENTIFIC

NEW ENGLAND BioLabs Inc.

be INSPIRED
drive DISCOVERY
stay GENUINE

mikro+polo®
vaš partner za laboratorij

REQUEST A FREE SAMPLE AT THE MIKRO+POLO BOOTH

Lighting the way.

Luna® Universal qPCR & RT-qPCR Products.

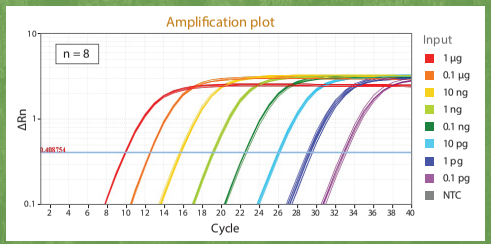
New England Biolabs is pleased to introduce a bright, new choice for your qPCR and RT-qPCR.

Luna products have been optimized for robust performance on diverse sample sources and target types.

Available for dye-based or probe-based detection, Luna products can be used across a wide variety of instrument platforms.

With so many qPCR and RT-qPCR options available, why not make a simpler, more cost-effective choice that delivers the sensitivity and precision you expect for your qPCR and RT-qPCR.

Request your free sample at the Mikro+Polo booth or at:www.LUNAqPCR.eu



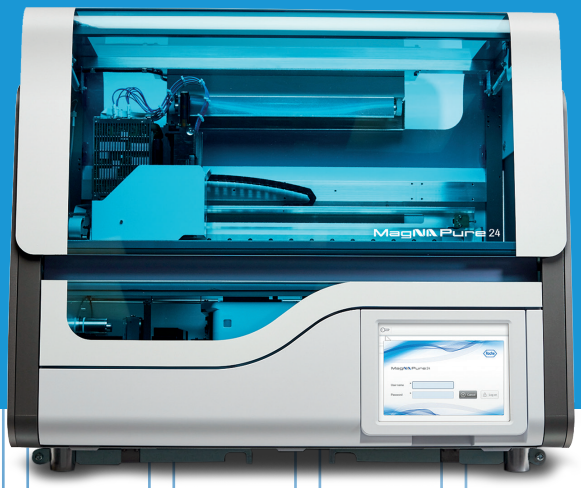
NEB's Luna Universal One-Step RT-qPCR Kit offers exceptional sensitivity, reproducibility and RT-qPCR performance.

RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 8 replicates at each concentration. Reaction setup and cycling conditions followed recommended protocols, including a 10-minute RT step at 55°C for the thermostable Luna WarmStart® Reverse Transcriptase. NTC = non-template control

Introducing the MagNA Pure 24 System

Enter a new world of productivity

Enhance your molecular testing workflow with the new MagNA Pure 24 System, a fully automated nucleic acid extraction system that provides more flexibility, reliable efficiency, and increased traceability.



Every result matters.
Life-changing decisions.
Making a difference.

These aren't dreams – they're destinations.
Getting here is a long journey,
and how you get there is key.

Whatever the workflow.
Whatever the goal.
Let's unlock the doors together.

Start here. Go Anywhere.

magnapure24.com

MagNA Pure

MagNA Pure 24 System is for *in vitro* diagnostic use.
MAGNA PURE is a registered trademark of Roche.

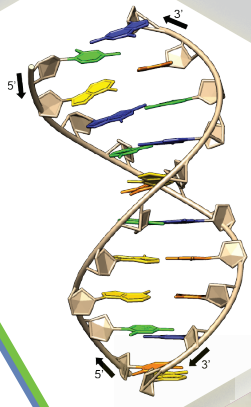
Roche farmacevtska družba d.o.o., Divizija za diagnostiko
Vodovodna cesta 109, 1000 Ljubljana, T: 01/56 80 260, F: 01/56 80 273
www.roche.si

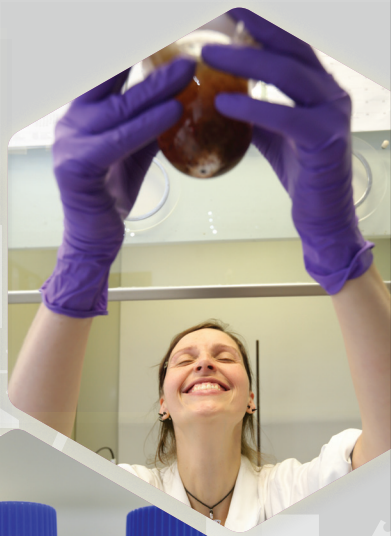
Dx-0052-2017

research EXCELLENCE

NATIONAL INSTITUTE OF CHEMISTRY

Hajdrihova 19
1000 Ljubljana
Slovenia
www.ki.si





Basic and applied research on materials, life sciences, biotechnology, chemical engineering, structural and theoretical chemistry, analytical chemistry and environmental protection.

In line with the priority areas of the EU Research and Innovation: nanotechnology, genomics and biotechnology for health, sustainable development, climate change, energy efficiency and quality and safety of food.

We expand knowledge and technology transfer to the domestic and foreign pharmaceutical, chemical, automotive and nanobiotechnologycal industries.

We are aware of the power of youth, so we transfer our knowledge on younger generations with providing many means of collaboration.

contact: www.mladi@ki.si

Vrhunska znanost ...

Vse, kar delamo, delamo za dobro ljudi.

Kakovost je temelj naše predanosti bolnikom in našega odnosa do zdravja. Naše delovanje temelji na dolgoletnem znanju in izkušnjah, medsebojnem zaupanju, vključevanju in spoštovanju različnosti ter na najvišjih etičnih vrednotah.

Stalna vlaganja v raziskave, inovacije in napredek proizvodnje omogočajo, da doma in po svetu ponujamo visokokakovostna, varna ter cenovno dostopna zdravila.

Z dolgoročno načrtovanim razvojem zagotavljamo pogoje za nova delovna mesta in izobraževanje ter napredovanje strokovnjakov v vrhunske znanstvenike.

Kot odgovoren delodajalec skrbimo za razvoj zaposlenih, odgovoren odnos z lokalnimi skupnostmi ter trajnostni razvoj okolja.

Lek je cenjen član Novartisa, vodilne svetovne družbe v farmacevtski industriji.

... za zdravje.

lek

član skupine Sandoz

UGLEDNI DELODAJALEC - 2016 - MojeDelo.com

POLNI CERTIFIKAT Družini prijazno podjetje

AMBASADOR KORPORATIVNE INTEGRITETE

Lek farmacevtska družba d. d., Verovškova 57, 1526 Ljubljana, Slovenija • www.lek.si • SI1706661950

Notes