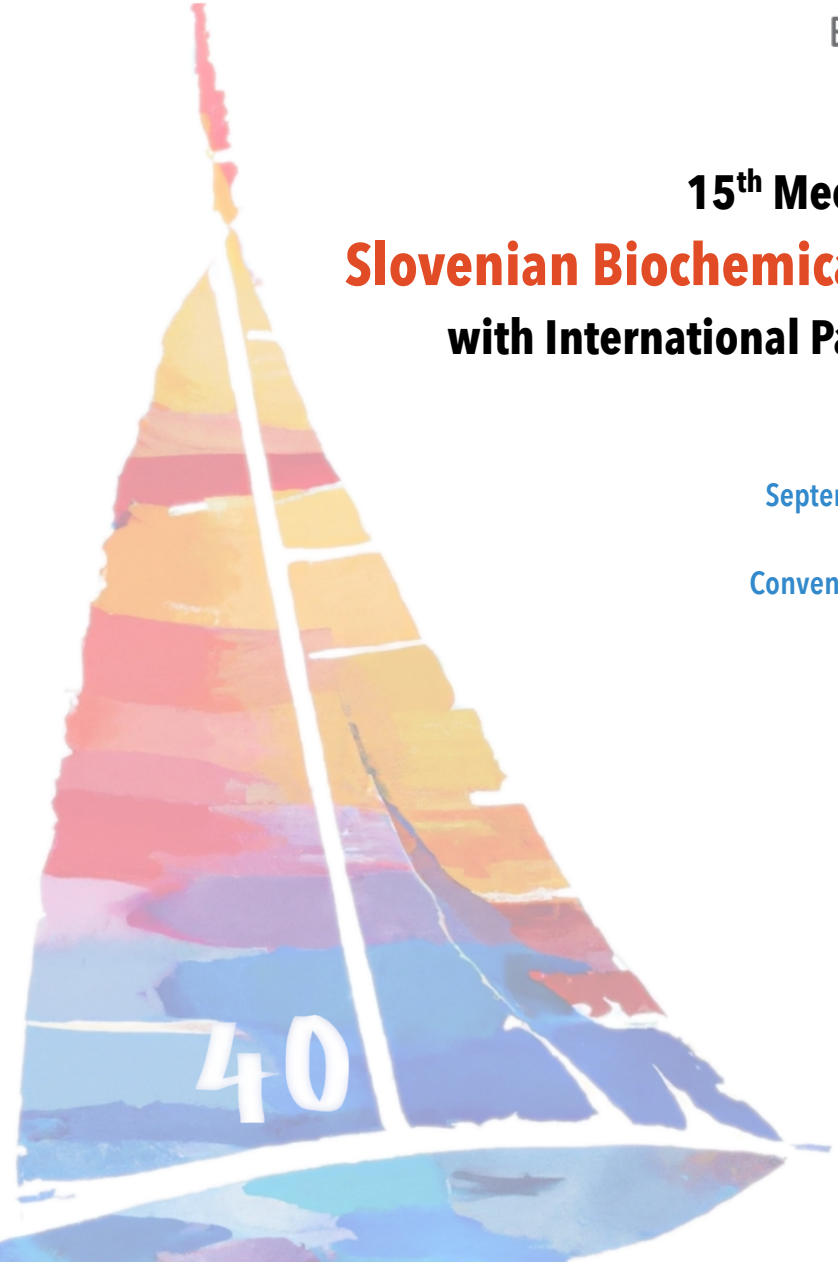
SLOVENIAN BIOCHEMICAL SOCIETY

15th Meeting of the Slovenian Biochemical Society with International Participation



September 20–23, 2023
Portorož, Slovenia
Convention Centre Portus

Book of Abstracts

15th Meeting of the Slovenian Biochemical Society
with International Participation

15. srečanje Slovenskega biokemijskega društva
z mednarodno udeležbo

Book of Abstracts

Zbornik povzetkov

Portorož, 20–23 September 2023

Organizers of 15th Meeting of the Slovenian Biochemical Society with International Participation:

- **Slovenian Biochemical Society** (https://sbd.si)
- **University of Ljubljana, Faculty of Chemistry and Chemical Technology** (https://www.fkkt.uni-lj.si)
- **University of Ljubljana, Biotechnical Faculty** (https://www.bf.uni-lj.si)

15th Meeting of the Slovenian Biochemical Society with International Participation, September 20–23, 2023, Portorož, Slovenia / Book of Abstracts

15. srečanje Slovenskega biokemijskega društva z mednarodno udeležbo, 20.–23. september 2023, Portorož, Slovenija / Zbornik povzetkov

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Foreword

It is with great pleasure and enthusiasm that the organizing and scientific committee welcome you to the 15th Meeting of the Slovenian Biochemical Society with International Participation. This event is a testament to the enduring spirit of scientific inquiry and the power of collaboration both within Slovenia as well as internationally.

The Slovenian Biochemical Society, an advocate for advancing biochemical research in Slovenia, has created the series of these meetings as a platform for researchers and students from diverse backgrounds to come together and share their discoveries. This year's meeting is particularly special as it not only celebrates the achievements of renowned and established scientists but also the 40th anniversary of the Society.

At this meeting you will have an opportunity to listen to presentations from diverse areas of biochemical research, from elucidating the fundamental mechanisms of cellular processes to advances in biotechnology and drug development. These presentations promise to not only deepen our collective knowledge but also inspire new ideas and directions for future research. Equally important are the contributions of the students participating at this meeting. Their presence reflects the society's commitment to education and mentorship of future generations of scientists and educators. In addition to the scientific program, this meeting also offers a unique opportunity for networking, collaboration, and the exchange of ideas.

Last but not least, we extend our gratitude to the members of organizing and scientific committees, the speakers, the students, sponsors and all participants who have made this meeting possible. We also extend our warmest welcome to our international guests, whose presence enriches the diversity of perspectives and strengthens the bonds of collaboration.

Sincerely,
Organizing and Scientific committee

Committees

Commitee members are listed in alphabetical order and with their main affiliation.

Organizing committee

Miha Pavšič (Chair), *University of Ljubljana, Faculty of Chemistry and Chemical Technology*
Vera Župunski (Co-chair), *University of Ljubljana, Faculty of Chemistry and Chemical Technology*
Miha Bahun, *University of Ljubljana, Biotechnical Faculty*
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Kristina Gruden, *National Institute of Biology*
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Janko Kos, *University of Ljubljana, Faculty of Pharmacy*
Igor Križaj, *Jožef Stefan Institute*
Brigita Lenarčič, *University of Ljubljana, Faculty of Chemistry and Chemical Technology*
Metka Lenassi, *University of Ljubljana, Faculty of Medicine*
Mateja Manček Keber, *National Institute of Chemistry*
Marjetka Podobnik, *National Institute of Chemistry*
Nataša Poklar Ulrih, *University of Ljubljana, Biotechnical Faculty*
Uroš Potočnik, *University of Maribor, Faculty of Medicine*
Tadeja Režen, *University of Ljubljana, Faculty of Medicine*
Boris Rogelj, *Jožef Stefan Institute*
Ferhan Sağin, *Ege University, Faculty of Medicine, Turkey*
Boris Turk, *Jožef Stefan Institute*
Tom Turk, *University of Ljubljana, Biotechnical Faculty*

General information

Conference venue
Convention Centre Portus (LifeClass Hotels), Obala 33, SI-6320 Portorož, Slovenia. Entrance to the convention centre is though the main entrance of Hotel Slovenija.

Registration
The registration desk is located in the lobby of the Convention Centre Portus. It is mandatory that all participants, both active and passive, members of the meeting committees, and sponsors' representatives **wear the meeting badge (given at the registration desk) in a visible manner during all conference events**.

Presentations
Speakers are kindly asked to upload their presentations one day before their talk either via a special link provided via e-mail, or through members of the organizing committee. Also, please obey the time limits (25 min + 5 min discussion for invited lectures, and 15 min + 5 min discussion for short presentations).
Sponsors' presentations are limited to 15 min.

Posters
Posters are expected to be on display from Wednesday (20 September) to Friday (22 September). There are two poster sessions, in each half of the posters will be presented. Materials for mouting the posters will be available on-site. Poster presenters are kindly asked to consult the list of posters and attach their poster to the correspondingly labeled stand. Posters must be dismounted on Friday, September 22, before lunch.

Social programme
Social programme includes:

- **Welcome dinner** on Wednesday, September 20, 20:00–22:00 at Meduza Beach Bar
- **Get together** during Poster session II on Thursday, September 21, 19:30–21:30
- **Conference dinner** on Friday, September 22, at 19:45

Coffee breaks and lunch
Coffee breaks will be held in the Pacific piano bar & Foyer of the Convention Centre Portus, at the exhibitors' stands. Lunch will be organized in Restaurant Adria of the Grand Hotel Portorož (neighbouring hotel).

Sponsors

Co-organizer and financial supporter of the meeting
University of Ljubljana, Faculty of Chemistry and Chemical Technology

General sponsor / exhibitor
Mikro+Polo

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Donators
- Krka
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Sponsors of the opening and closing lecture
- EMBO – European Molecular Biology Organization
- FEBS – Federation of European Biochemical Societies

Meeting programme outline

	Wednesday, 20th Sep	Thursday, 21th Sep	Friday, 22th Sep	Saturday, 23th Sep
8.30		**Molecular basis of disease I** *Cristoforo Colombo hall*	**Cell signaling and membranes** *Cristoforo Colombo hall*	
9.00				
9.30				**Protein structure and function** *Cristoforo Colombo hall*
10.00				
10.30		**Coffee break**	**Coffee break**	
11.00	**Registration** *entrance hall / Pacific piano bar & Foyer*	**Functional genomics and systems biology** *Cristoforo Colombo hall*	**Molecular basis of disease II** *Cristoforo Colombo hall*	**Coffee break**
11.30				**The FEBS National Lecture**: A. Becker
12.00				
12.30				
13.00		**Lunch** *Restaurant Adria (GH Portorož)*	**Lunch** *Restaurant Adria (GH Portorož)*	**Closing remarks**
13.30	**Opening ceremony**			
14.00	**The EMBO Keynote Lecture**: I. Tolić	**Biochemical education** *Cristoforo Colombo hall*	**Molecular interactions and networking** *Cristoforo Colombo hall*	
14.30				
15.00	**Coffee break**			
15.30	**Synthetic biology** *Cristoforo Colombo hall*			
16.00		**Coffee break**		
16.30		**Biotechnology and bionanotechnology** *Cristoforo Colombo hall*	**Assembly of the Slovenian Biochemical Society & Presentation of the Instruct.SI consortium** *(Galea hall)*	
17.00				
17.30	**Sponsors' lectures** *Christoforo Colombo hall*			
18.00				
18.30	**Poster session I** *Roald Amundsen & Robert Scott halls*	**Sponsors' lectures** *Christoforo Colombo hall*		
19.00				
19.30		**Poster session II** *Roald Amundsen & Robert Scott halls*	**Conference dinner** *Cristoforo Colombo hall*	
20.00	**Welcome dinner** *Meduza Beach Club*			
20.30				
21.00				
21.30				
22.00				

Opening lecture
The EMBO Keynote Lecture
Prof. Iva Tolić
Ruđer Bošković Institute, Zagreb, Croatia

Closing lecture
The FEBS National Lecture
Prof. Anke Becker
Philips-Universität Marburg, Marburg, Germany

Meeting programme

Wednesday, September 20, 2023

11:00 – 13:30		**Registration**	*entrance hall / Pacific piano bar & Foyer (Convention Centre Portus)*
13:30 – 14:00		**Opening ceramony**	*Cristoforo Colombo hall*
The EMBO Keynote Lecture			*Cristoforo Colombo hall*
14:00	PL1	**Mitotic Magic: How Cells Achieve Accurate Chromosome Seggregation** Iva Tolić (Ruđer Bošković Institute, Croatia)	
15:00 – 15:30		**Coffee break**	*Pacific piano bar & Foyer*
Synthetic Biology Chairs: Marko Dolinar, Iva Hafner Bratkovič			*Cristoforo Colombo hall*
15:30	L1	**Genetically controlled interfaces with mammalian cells** Gil Westmeyer (Technical University of Munich, Germany)	
16:00	L2	**First steps of a completely de-novo designed random protein walker** Ajasja Ljubetič (National Institute of Chemistry)	
16:30	S1	**Regulation of CD19 CAR-T cell activation based on an engineered downstream transcription factor** Duško Lainšček (National Institute of Chemistry)	
16:50	S2	**Hierarchical DNA assembly in yeast cells** Uroš Petrovič (University of Ljubljana, Biotechnical Faculty)	
17:10	S3	**A method for targeting a specified segment of DNA inside the bacterial microcompartment** Maja Hostnik (University of Ljubljana, Biotechnical Faculty)	
Sponsors' lectures			*Cristoforo Colombo hall*
17:30		**MGI Tech. co. / Mikro+Polo**: Innovative sequencing with DNA nanoball	
17:50		**NanoTemper**: Measure interactions of peptides and other biomolecules with Spectral Shift to minimize assay development time	
18:10		**TelesisBio / Kemomed**: From sequence design to automated assembly, cloning, NGS library and *in vitro* transcription	
18:30 – 20:00		**Poster session I**	*Roald Amundsen & Robert Scott halls*
20:00 – 22:00		**Welcome dinner**	Meduza Beach Bar

Thursday, September 21, 2023

Molecular Basis of Disease I *Cristoforo Colombo hall*

Chairs: Barbara Breznik, Boris Turk

8:30	L3	**New molecular probes for profiling active MMPs in complex proteomes** Laurent Devel (Université Paris-Saclay, CEA, INRAE, France)
9:00	L4	**Cysteine cathepsins in inflammation-associated diseases: the good the bad and the ugly** Boris Turk (Jožef Stefan Institute)
9:30	S4	**Stefin B increases autophagic flux and AMPK activation in macrophages** Nataša Kopitar Jerala (Jožef Stefan Institute)
9:50	S5	**Melanoma antigens in pancreatic cancer and glioblastoma are markers and drivers of therapy resistance** Klementina Fon Tacer (School of Veterinary Medicine, Texas Tech University, USA)
10:10	S6	**Phenylalanine-tRNA aminoacylation is compromised by ALS/FTD-associated C9orf72 antisense repeat RNA** Urša Čerček (Jozef Stefan Institute)

10:30 – 11:00 **Coffee break** *Pacific piano bar & Foyer*

Functional Genomics and Systems Biology *Cristoforo Colombo hall*

Chairs: Kristina Gruden, Uroš Potočnik, Tadeja Režen

11:00	L5	**Crises Abound: Health, Climate, Energy, Food, Pandemics. How Large-Scale Systems Biology Can Help Address the Major Challenges We Are Facing** Daniel A. Jacobson (Oak Ridge National Laboratory, USA)
11:30	L6	**A Modeler's Perspective on the Design and Analysis of Biological Systems: From Synthetic Biology to Systems Medicine Applications** Miha Moškon (University of Ljubljana, Faculty of Computer and Information Science)
12:00	S7	**Exploration of pharmacogenomic biomarkers of adalimumab (non)response in Crohn's disease patients using single-cell RNA sequencing** Mario Gorenjak (University of Maribor, Faculty of Medicine)
12:20	S8	**The Role of Sterol Intermediates in Gene Regulation: Insights from Targeted Knockouts of Cholesterol Synthesis Enzymes** Cene Skubic (University of Ljubljana, Faculty of Medicine)
12:40	S9	**3'UTR ribonucleoprotein reassembly drives selective mRNA decay to implement developmental signalling** Miha Modic (National Institute of Chemistry)

13:00 – 14:00 **Lunch** *Hotel restaurant Adria*

Biochemical Education *Cristoforo Colombo hall*

Chairs: Aljoša Bavec, Ferhan Sağin

14:00	L7	**Why and how should you develop and improve your educator skills?: Tips for both junior & senior scientists** Ferhan Sağin (Ege University Medical Faculty, Department of Medical Biochemistry, Turkey)
14:30	L8	**COVID - lessons for education** Blaž Cigić (University of Ljubljana, Biotechnical Faculty)
15:00	S10	**Current challenges and opportunities for AI in education** David Smith (Sheffield Hallam University, United Kingdom)

15:30	S11	**Ai and VR assisted teaching** Damjana Kastelic (Center for Genomic Regulation, Barcelona, Spain)	
16:00 – 16:30		**Coffee break**	*Pacific piano bar & Foyer*
Biotechnology and Bionanotechnology			*Cristoforo Colombo hall*
Chairs: Gregor Gunčar, Nataša Poklar Ulrih			
16:30	L9	**Functionalization of parametrically designed helix bundles by deviating from ideal geometries** Gustav Oberdorfer (Graz University of Technology, Department of Biochemistry, Austria)	
17:00	L10	**Hidden antibiotics in actinobacteria** Hrvoje Petković (University of Ljubljana, Biotechnical Faculty)	
17:30	S12	**Optimization of the extraction of extracellular polymeric substances from lactic acid bacteria and their characterization** Jerica Sabotič (Jožef Stefan Institute)	
17:50	S13	**Unravelling the biosynthesis of irregular polyprenyl backbone of the *Planococcus citri* (*Insecta*) sex pheromone** Mojca Juteršek (National Institute of Biology)	
18:10	S14	**Upgrading nanopore biosensing with unnatural amino acids** Ana Crnković (National Institute of Chemistry)	
Sponsors' lectures			*Cristoforo Colombo hall*
18:30		**Accela / Medipro**: Analyze and sort more than 40 markers in one tube with Cytek Spectral Technology	
18:50		**Promega / Kobis**: Lumit™ Immunoassays	
19:10		**Quantabio/ Avantor VWR**: Speed with precision: From cloning to sequencing, improve your PCR workflows	
19:30 – 21:30		**Poster session II**	*Roald Amundsen & Robert Scott halls*

Friday, September 22, 2023

Cell Signalling and Membranes *Cristoforo Colombo hall*

Chairs: Metka Lenassi, Tom Turk

8:30	L11	**Extracellular Vesicles: Diversity of Form and Function** Kenneth Witwer (Johns Hopkins University School of Medicine, Molecular and Comparative Pathobiology, USA)
9:00	L12	**Cystatin F potential in the immunotherapy of cancer** Milica Perišić Nanut (Jožef Stefan Institute)
9:30	S15	**Characterization of archaeosomes from polar lipids of Aeropyrum pernix K1 and stability in biological systems** Jan Kejžar (University of Ljubljana, Biotechnical Faculty)
9:50	S16	**New upstreaming signaling pathways of NIX regulation in development-induced mitophagy** Mija Marinković (School of Medicine, University of Split)
10:10	S17	**ASO-Mediated Inhibition of MyD88L265P-dependent signaling as a Therapeutic Approach for Waldenström's Macroglobulinemia** Peter Pečan (National institute of Chemistry)

10:30 – 11:00 **Coffee break** *Pacific piano bar & Foyer*

Molecular Basis of Disease II *Cristoforo Colombo hall*

Chairs: Vita Dolžan, Janko Kos, Boris Rogelj

11:00	L13	**Maintaining ER-mitochondria contacts is neuroprotective in C9orf72 ALS** Smita Saxena (University of Bern, Switzerland)
11:30	L14	**Understanding Fabry nephropathy: genetic and biochemical approach** Katarina Trebušak Podkrajšek (University of Ljubljana, Faculty of Medicine)
12:00	S18	**Inhibition of cathepsins B and X promote differentiation of cancer stem cells** Ana Mitrović (University of Ljubljana, Faculty of Pharmacy)
12:20	S19	**11-hydroxylated androgen metabolites as risk factors and source of androgen receptor-activating hormones in endometrial cancer** Marija Gjorgoska (University of Ljubljana, Faculty of Medicine)
12:40	S20	**Circular RNAs as potential peripheral blood biomarkers for amyotrophic lateral sclerosis** Metka Ravnik-Glavač (University of Ljubljana, Faculty of Medicine)

13:00 – 14:00 **Lunch** *Hotel restaurant Adria*

Molecular Interactions and Networking *Cristoforo Colombo hall*

Chairs: Mateja Manček Keber, Igor Križaj

14:00	L15	**Careful and systematic observations in basic and applied research** Manjunatha R. Kini (Faculty of Science, National University of Singapore, Singapore)
14:30	L16	**Intra and interspecies social behaviors by beneficial bacterium *Bacillus subtilis*** Ines Mandić Mulec (University of Ljubljana, Biotechnical Faculty)
15:00	S21	**Désirée in distress - a system biology approach: from statistics to networks** Maja Zagorščak (National Institute of Biology)
15:20	S22	**Impact of mislocalization on the TDP-43 protein network** Jerneja Nimac (Jožef Stefan Institute)

15:30	S23	**The role of AMP-activated protein kinase in regulation of Na+,K+-ATPase: the gauge does not always plug the sink** Sergej Pirkmajer (University of Ljubljana, Faculty of Medicine)	
16:00 – 16:30		**Reception for participants of the assembly**	*Galea hall (GH Portorož)*
16:30 – 19:00		**Assembly of the Slovenian Biochemical Society** **Presentation of the Instruct.SI consortium**	*Galea hall (GH Portorož)*
19:45 – 22:30		**Conference dinner** (with live music)	*Cristoforo Colombo hall*

Saturday, September 23, 2023

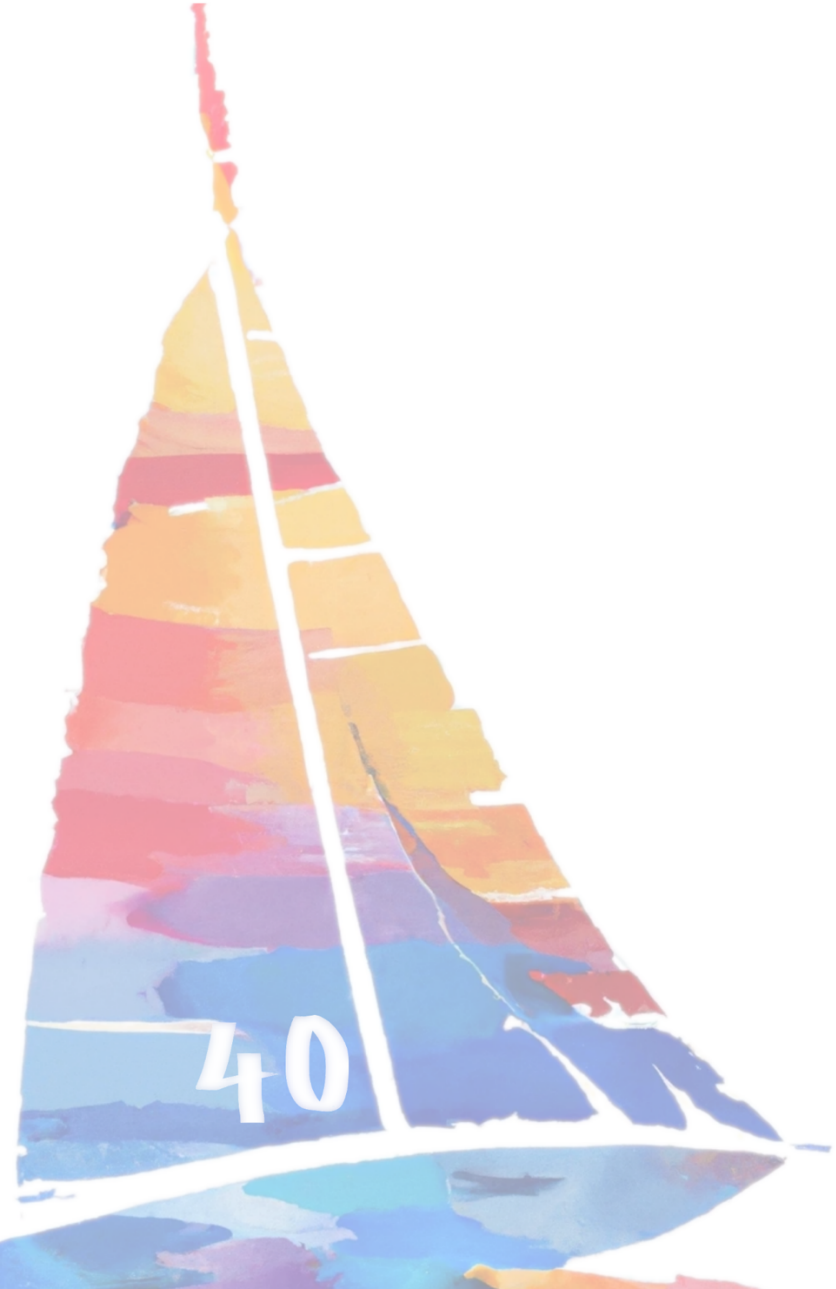
Protein Structure and Function			*Cristoforo Colombo hall*
Chairs: Brigita Lenarčič, Marjetka Podobnik			
9:30	L17	**Order from disorder: towards molecular architecture of the muscle Z-disk assembly by integrative structural biology** Kristina Djinović-Carugo (European Molecular Biology Laboratory (EMBL) Grenoble, France)	
10:00	L18	**Structure of protein nanopore reveals organization of membrane lipids and provides basis for designing nanopores for sensing applications** Gregor Anderluh (National Institute of Chemistry)	
10:30	S24	**Calpeptins: dual inhibitors of human cathepsins and SARS-CoV-2 main protease** Jure Loboda (Jožef Stefan Institute)	
10:50	S25	**Broad range phospholipase C of *Listeria monocytogenes* facilitates LLO-mediated destruction of lipid membranes** Nejc Petrišič (National Institute of Chemistry)	
11:10	S26	**Glycoengineering as a tool to design regression models for prediction of monoclonal antibody function from its structure** Tamara Cvijić (Novartis)	
11:30 – 12:00		**Coffee break**	*Pacific piano bar & Foyer*
The FEBS National Lecture			*Cristoforo Colombo hall*
12:00	PL2	**The pABC world: an open modular construction environment for synthetic multipartite bacterial genomes** Anke Becker (Philipps-Universität Marburg, Centre for Synthetic Microbiology, Germany)	
13:00 – 13:30		**Closing remarks**	*Cristoforo Colombo hall*

List of Posters

Poster session I (Wednesday, 18:30 – 20:00)

Poster session II (Thursday, 19:30 – 21:30)

Abstracts of Plenary Lectures (PL)



PL1
Mitotic Magic: How Cells Achieve Accurate Chromosome Segregation

Iva Tolić
Ruđer Bošković Institute, Division of Molecular Biology, Croatia

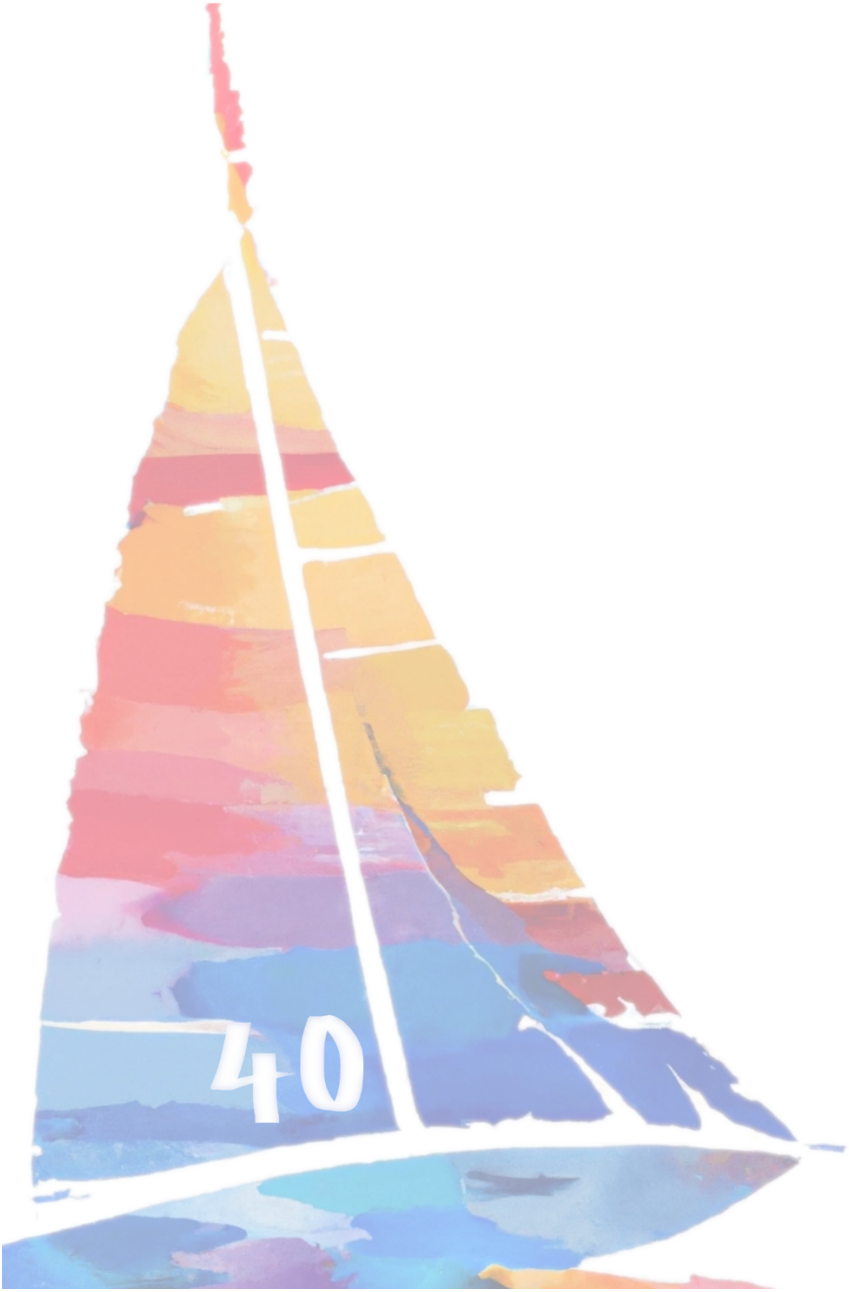
Segregation of the genome from a mother cell into two daughter cells during cell division is one of the fundamental processes of life. Physical separation of chromosomes is carried out by the spindle, a fascinating and complex molecular assembly composed of microtubules and numerous other proteins. The main task of the spindle is to accurately segregate the chromosomes, but sometimes errors occur that result in mis-segregation of one or more chromosomes, especially in chromosomally unstable cancer cells. Are all chromosomes at equal risk of mis-segregation? How do chromosomes reach the spindle? What brings them to the spindle midplane? I will discuss our efforts to answer these questions and understand spindle mechanobiology, focusing on microtubule forces that control chromosome movements and their role in ensuring chromosome segregation fidelity.

PL2
The pABC world: an open modular construction environment for synthetic multipartite bacterial genomes

Anke Becker
Philipps-Universität Marburg, Centre for Synthetic Microbiology, Germany

Plasmids as autonomously replicating extrachromosomal elements are well-established tools for engineering of bacteria. They are ideal DNA-carrying vectors due to easy construction, modularization, standardization, and intercellular transfer, and are used in a wide range of diverse applications. However, plasmid properties (e.g., copy number, cell-to-cell variation in copy number, plasmid incompatibility) can be advantageous or disadvantageous depending on the type of application. Low plasmid propagation stability is counteracted mostly by selection for plasmid-mediated antibiotic resistance. Moreover, their cloning capacity is usually limited to a few tens of kbp. This limits the scope of applications for standard plasmids, especially for implementing new functions requiring many additional genes, and for extensive remodeling of the chassis cell, as is increasingly envisioned in synthetic biology. We have developed a strategy for re-structuring and re-engineering of bacterial genomes based on the concept of segmented genomes. Genome segmentation is the rule in eukaryotes, but occurs only in a small fraction of bacteria. This, e.g., includes several α-proteobacteria with genomes composed of a main chromosome and additional single or low copy number replicons, stably integrated into the cell cycle and classified as secondary chromosomes, chromids, or megaplasmids, some even larger than 3 Mbp. Several α-proteobacteria carry multiple different secondary replicons, showing their potential to support a modular genome architecture. We exploited this rich resource of diverse secondary replicons to create an open modular construction environment for synthetic multipartite bacterial genomes. This “pABC world” and various examples of its applications will be introduced.

Abstracts of Lectures (L)



L1
Genetically controlled interfaces with mammalian cells

Gil Westmeyer[1,2]
[1]*Technical University of Munich, Department of Bioscience, Germany*
[2]*Helmholtz Munich, Institute for Synthetic Biomedicine, Germany*

In this presentation, I will overview our recent efforts to generate genetically controlled interfaces with mammalian cells to monitor key cellular processes and enable on-demand genetic modifications of cellular networks.

Firstly, I will introduce our exon-specific isoform expression reporter system (EXSISERS) [1] and the intron-encoded extranuclear cistronic transcripts (INSPECT) [2], and show how these reporters systems enable high-throughput screening, allowing us to identify modulators of isoform expression and regulation of non-coding RNAs.

Next, I will delve into our work on developing multiplexable gene reporters for correlative light and electron microscopy (CLEM), known as EMcapsulins [3]. These reporters can augment morphometric data at the nanoscale with genetically defined molecular information.

Lastly, I will present our work on applying these genetically encoded tools to hard-to-transfect cell systems, such as hIPSC-derived organoids, via transient RNA transduction or permanent gene editing.

We may then discuss how genetic interfaces with mammalian cells help to disentangle the intricate logistics of cell circuits and advance our capacity to monitor and control future cell therapies.

References
[1] EXSISERS | https://doi.org/10.1038/s41556-021-00678-x
[2] INSPECT | https://doi.org/10.1038/s41556-022-00998-6
[3] EMcapsulins | https://doi.org/10.1038/s41587-023-01713-y

L2
First steps of a completely de-novo designed random protein walker

Ajasja Ljubetič[1,2]
[1]*National Institue of Chemistry, Department of Synthetic Biology and Immunology, Slovenia*
[2]*University of Washington, Institute for Protein Design, USA*

Powered protein walkers such as kinesin, dynein or myosin are responsible for most movements within the cell and for the transport of crucial cargo. Design of static monomeric and oligomeric protein structures has advanced tremendously; however large dynamic protein mechanisms have not yet been designed. I will present the design and characterization of a random protein walker that can diffuse along micro-meter long fibers. This represents a scaffold for future powered molecular robots.

The requirements for such a system are threefold: a track, attachment points and a walker/roller scaffold. I will briefly present reversible heterodimers that I developed to serve as attachment points. These heterodimers behave well as monomers, have a range of affinities and fast binding/exchange kinetics in solution.

Next, I will show how I rigidly fused heterodimers and designed helical repeat (DHR) proteins to *de-novo* designed fibers to form a track for the walker. This turned out to be the hardest part of the project.

Finally, I will outline the different walker/roller scaffolds I used and show trajectories obtained from single molecule microscopy experiments.

L3
New molecular probes for profiling active MMPs in complex proteomes

Devel Laurent
Université Paris-Saclay, CEA, INRAE, France

Conventional proteomics approaches aiming at establishing the relation of enzymes/proteases to disease states are limited because they take the total protein amount into account, whereas the protease functional status is often the sole relevant parameter. Within the arsenal of chemical probes designed to tackle the proteases activation status in complex proteomes, activity-based probes (ABPs) have a prominent place. A typical activity-based probe (ABP) is composed of i) a targeting motif directed against the protease active-site, ii) an electrophilic "warhead" susceptible to react with a catalytic nucleophile (e.g., Serine or Cysteine residue for Serine and Cysteine proteases, respectively) in close proximity, and iii) an analytical handle for subsequent visualization and characterization of the covalent adduct between the probe and the protease. In the case of matrix metalloproteases (MMPs) that do not possess any catalytic nucleophile, the ABPs targeting those proteases are systematically composed of a reversible inhibitor to which a photolabile group has been attached. Such affinity-based probes (AfBPs) have been used for the detection of active metalloproteinases in fluids and tissue extracts but not in living animals where the photo-activation step is not feasible. By exploiting a ligand-guided chemistry, our team recently developed and validated a new generation of AfBPs capable of covalently modifying matrix metalloproteases (MMPs) active site without any external trigger. In the frame of this presentation, we will show how this approach has been expanded to a larger set of metalloproteases. We will also discuss the parameters that impact the probes selectivity as well as their labelling efficiency and will present our labeling results in complex proteomes including those obtained in animal models.

L4
Cysteine cathepsins in inflammation-associated diseases: the good the bad and the ugly

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Inflammation plays an important role in disease onset and progression in a vast number of diseases, called also inflammation-associated diseases including various cancers, psoriasis, inflammatory bowel diseases, various forms of arthritis, viral infections, and asthma. Proteases play a major role in a number of these diseases. However, understanding the precise role of an individual protease in a disease remains a major challenge for successful therapeutic applications. There are several ways how to address this issue, including the mass spectrometry-based proteomics and the chemical biology approaches involving small molecule inhibitors and activity-based probes, as well as by engineered macromolecules (e.g. DARPins). These approaches offer a major potential for identifying protease signaling pathways and biomerker identification as well as for noninvasive optical imaging by monitoring protease activities in situ, i.e. on disease site. Moreover, they enable also validation of proteases as drug targets, in vivo validation of drug candidates and evaluation of the diagnostic potential of the target proteases. Among the proteases found to be tightly linked with inflammation-associated diseases, including many types of cancer, are also cysteine cathepsins that can be found extracellularly at the sites of inflammation due to their secretion from primarily infiltrated immune cels, such as macrophages. Furthermore, since they are heavily upregulated in a number of inflammation-associated diseases, they are therefore perfect targets for such approaches. There is increasing evidence that monitoring cathepsin activity in vivo may be applicable to diagnostic imaging, such as demonstrated primarily for cancer. Moreover, cathepsins can be also used as targets for targeted drug delivery approaches combined with diagnostics, thereby offering a theranostic potential. Finally, the new role of cysteine cathepsins in modulating the complement systm will be also discussed.

L5
Crises Abound: Health, Climate, Energy, Food, Pandemics. How Large-Scale Systems Biology Can Help Address the Major Challenges We Are Facing

Daniel Jacobson
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The cost of generating biological data is dropping exponentially, resulting in an explosion in the amount of data available for the biological sciences. This flood of data has opened a new era of systems biology in which there are unprecedented opportunities to gain insights into complex biological systems. Integrated biological models need to capture the higher order complexity of the interactions among cellular components. Solving such complex combinatorial problems will give us extraordinary levels of understanding of biological systems. Paradoxically, understanding higher order sets of relationships among biological objects leads to a combinatorial explosion in the search space of biological data. These exponentially increasing volumes of data, combined with the desire to model more and more sophisticated sets of relationships within a cell, across an organism and up to ecosystems and, in fact, climatological scales, have led to a need for computational resources and sophisticated algorithms that can make use of such datasets. The disease, traits or phenotypes of an organism, including its adaptation to its surrounding environment and the interactions with its microbiome, are the result of orchestrated, hierarchical, heterogeneous collections of expressed genomic variants regulated by and related to biotic and abiotic signals. We have developed supercomputing and explainable-AI approaches to find complex mechanisms responsible for all measurable phenotypes as well as an organism's ability to detect and modulate its microbiome. The result is progress towards a comprehensive systems biology model of an organism and how it has adapted to and responds to its abiotic and biotic environment which has applications in bioenergy, precision agriculture, ecosystem studies, precision medicine, and pandemic prevention among other disciplines.

L6
A Modeler's Perspective on the Design and Analysis of Biological Systems: From Synthetic Biology to Systems Medicine Applications

Miha Moškon
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Computer modelling has become an indispensable tool in various scientific disciplines within modern science. One of its primary advantages is its ability to provide mechanistic insights into the systems under study, thus enhancing our understanding of complex phenomena. In this presentation, I will discuss our recent advancements in utilizing computer modelling for the design and analysis of biological systems. Firstly, I will present the proposed *in silico* design of a configurable biological processor, reconfigurable biological blocks, and the programmable evolution of engineered biological circuits. These innovative applications highlight the potential for using computer models to create sophisticated biological systems with customizable features. Furthermore, I will provide an overview of our efforts to enhance our comprehension of complex diseases using genome-scale metabolic modelling. In this case, our group has been focused on establishing a computational protocol for the reconstruction of context-specific models that exhibit high biological relevance. This protocol enables us to identify the most appropriate reconstruction or model extraction algorithm, along with its suitable parameterization, tailored to a specific dataset and its corresponding context. By employing models derived through this methodology, we can gain additional insights into experimental (omics) datasets. Our approach has been successfully applied in various studies, ranging from analysing metabolic signatures of COVID-19 infection to discovering novel biomarkers of HCC (Hepatocellular Carcinoma). Finally, I will delve into some of the tools we have developed, tailored for the analysis of specific experimental datasets, such as those reflecting rhythmic responses. The latter are designed to facilitate comprehensive analysis and interpretation of data derived from rhythmic biological processes.

L7
Why and how should you develop and improve your educator skills?: Tips for both junior & senior scientists

Ferhan Sağın
Ege University Medical Faculty, Department of Medical Biochemistry, Turkey

As an educator, you have the power to inspire and shape the minds of the next generation of scientists. But to be truly effective, you must continually develop and improve your skills. Effective teaching requires more than just knowledge of the subject matter; it requires the ability to communicate that knowledge to others in a way that is engaging, informative, and memorable.

For junior scientists, developing your educator skills can help you to effectively communicate your research findings to a broader audience, whether it be through classroom instruction, conference presentations, or public outreach. We will discuss ways to build confidence in your ability to teach, develop effective teaching experiences in different settings, and readjust mind-sets for shaping one's career also as an educator.

For senior scientists, improving your educator skills can help you stay current with the latest teaching techniques and technologies, and enable you to mentor the next generation of scientists. We will discuss strategies on how to stay updated in the fast-changing teaching and learning world, how to create a positive learning environment, promote diversity and inclusion, and how to use your experience and expertise to guide the next generation of scientists.

This talk is aimed to raise some awareness among young researchers and also in senior educators about the latest scientific research in teaching & learning with the hope that participants will continue to follow this interesting area besides their research field. Whether you're just starting out or have years of experience, investing in your educator skills is a powerful way to make a lasting impact on the future of science. So join us for this exciting and inspiring talk on how to develop and improve your educator skills!

L8
COVID - lessons for education

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The COVID-19 pandemic caused a large and involuntary shift to work from home (WFH) or teleworking, and widespread adoption of web-based platforms. In the higher education sector, there was a drastic shift to full WFH and online activities, for majority of students and educators. The pandemic can therefore be seen as an 'immersive laboratory'. In the time of lockdown, a web-based survey was performed among students and educators of the University of Ljubljana in order to uncover their attitude and perception of WFH and online education. When comparing the online educational process with the traditional one, the absence of traditional laboratory work, inadequate social interactions and limitations of online knowledge assessment were identified as major drawbacks by both, students and educators. A significant difference between students and educators was observed in their opinion on efficiency of online lectures compared to traditional ones, with the former being more favoured by students than educators. Commute time is the most important factor in relation to the adoption of WFH and online education. In the time of normalization, the proportion of online activities is reduced, but undoubtedly remains higher than before pandemic. We are entering the era of so-called blended learning. An additional post-COVID survey was conducted on the use of various online tools and information technologies by life science educators in teaching at the University of Ljubljana.

L9
Functionalization of parametrically designed helix bundles by deviating from ideal geometries

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Tremendous progress has been made in protein design in part due to advances in machine learning and growth of protein structure databases. However, the ability to reliably introduce function into genetically encodable *de novo* proteins is still a challenging task. Among the plethora of protein functions, the introduction of catalytic functionality into *de novo* proteins has proven to be particularly hard. One of the bottlenecks in this endeavor is the limited variability of starting backbones. Here we show that we can overcome this limitation by using parametric design. We used this approach to establish a general method for the *de novo* design of enzymes with metal cofactors of increasing complexity. Our show-case examples include copper, ruthenium half-sandwich complexes and lanthanides. This talk will highlight our challanges and findings during the development of the design pipeline.

L10
Hidden antibiotics in actinobacteria

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Multidrug resistant pathogens pose a serious problem to the treatment of infective diseases. Most of the recently developed antibiotics are based on semi-synthetic versions of old drug leads, thus allowing pathogens to develop resistance relatively quickly. Therefore, the search for truly new drug leads, preferentially with a new mode of action, is of primary importance. Actinobacteria are one of the richest sources of antibiotics. The genus *Streptomyces* is a particularly rich source of medically and industrially important antibiotics and of many other drugs. Over 90% of the antibiotics currently used in medicine are derived from natural products. Intensive *Streptomyces* genome sequencing projects, combined with advanced bioinformatic tools have identified a surprisingly large number of biosynthetic gene clusters (BGCs). A single linear *Streptomyces* species chromosome, of a size of approximately 10 Mb, typically contains between 15 and 60 biosynthetic gene clusters, thus representing a huge yet unexplored source of new antibiotics. Notably, most of the BGCs encoded in *Streptomyces* species have not yet been explored, since they are not considered to be active (often called »silent« gene clusters). Thus, when cultivating in laboratory or industrially optimised media, only a small fraction of metabolites encoded by any *Streptomyces* strain can be observed; this is considered one of the main obstacles to natural product drug discovery. Therefore, there is a critical need to develop technologies that may somehow activate these silent BGCs. *Streptomyce rimosus* is an industrialy important producer of the broad-spectrum antibiotic oxytetracycline (OTC). *S. rimosus* has been used for the industrial production of OTC for over 70 years; this strain represents a very attractive model system for the development of new antibiotics, while making use of the very advanced genome manipulation tools that have been developed in the recent years.

L11
Extracellular Vesicles: Diversity of Form and Function

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Extracellular vesicles (EVs) have received considerable attention in recent years because of their roles in cellular biology, including intercellular communication. Defined by their cell-derived lipid bilayer membrane and molecular content, EVs have great potential in biomarker and therapeutic settings. Here, through the lens of EV diversity, we will review progress in EV research and applications and examine several important but as-yet unanswered questions about EVs.

L12
Cystatin F potential in the immunotherapy of cancer

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Cancer immunotherapy, has revolutionized the cancer treatment. The most successful immunotherapies are focused on improving of the cytotoxic lymphocyte- based response to cancer, either through their release from inhibitory blockade- with immune checkpoint inhibitors (ICI), or trough adoptive therapy with improved cytotoxic cells. However, there is still an important stumbling block for this approach: cytotoxic cells' ability to kill can fade, a phenomenon often referred to as exhaustion. Therefore, understanding the mechanisms regulating the cytotoxic cell exhaustion and identification of mediators of immune suppression, which can be therapeutically targeted can lead to new, more efficient treatment modalities.

Cytotoxic cells such as NK cells and cytotoxic T lymphocytes (CTL) differ in many aspects of their activation and specificity, but they both use the perforin/granzyme cytotoxic pathway as the main targeted killing mechanism. Effector molecules, granzymes and perforin are stored in their inactive form and are activated from precursor forms by cathepsins C, H, and L. We have identified a protease inhibitor cystatin F as a potent down regulator of cytotoxic efficacy of NK cells and CTLs. Its expression was shown to be increased in anergized NK cells and hyporesponsive CTLs. Cystatin F is normally expressed by immune cells. We have shown that cystatin F expression is increased in tumor tissue by either tumor or immune cells. Additionally, cystatin F can be internalized to NK cells and CTLs which causes decrease in their cytotoxic potential. Therefore, cystatin F acts as a mediator of immunosuppression in the tumor microenvironment and we have developed several approaches to improve cancer immunotherapy by targeting cystatin F. Furthermore, since intracellular cystatin F acts as a regulator of functional states associated with reduced cytotoxic capacity in CTLs we have analyzed its expression as a possible predictive marker of clinical response to ICI therapy.

L13
Maintaining ER-mitochondria contacts is neuroprotective in C9orf72 ALS

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in adults with a prevalence of 6-7 per 100 000 people in Europe. Sporadic and familial cases of ALS share similar clinical-pathological hallmarks, including muscle atrophy, speech and swallowing disabilities and paralysis due to motoneuron degeneration in the spinal cord, brainstem and motor cortex. Mutations in the C9ORF72 gene characterized by a (G4C2) hexanucleotide repeat expansion is highly prevalent in familial ALS. Previous studies from our group have shown that endoplasmic reticulum (ER) stress and mitochondrial dysfunction are implicated in the pathogenesis of ALS. Here, we focused our attention on the intrinsic vulnerability of motoneurons to ER stress and the consequential adaptive response involving the mitochondrial-ER associated membranes (MAMs); these regions of contact between the two organelles are crucial for Ca^{2+} exchange and ER stress-associated UPR.

Using motoneurons differentiated from *C9ORF72* patients induced pluripotent stem cells (iPSCs), we have identified a linker molecule GRP75 that serves to not only physically connect the two cellular compartments, but whose transient enhanced expression has a neuroprotective effect during the progression of the pathology, enabling optimal ER-mitochondrial Ca^{2+} uptake and efficient ATP production. Moreover, taking advantage of the newly generated *C9orf72* pre-clinical model, we performed pharmacological and viral mediated modulation of ER stress and provide evidence that the ER compartment is critical for optimal mitochondrial function and inhibiting ER stress is sufficient to rescue mitochondrial dysfunction. Lastly, we validate the therapeutic potential of targeting mitochondrial function by using small molecules to restore mitochondrial Ca^{2+} uptake in ALS.

L14
Understanding Fabry nephropathy: genetic and biochemical approach

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Fabry disease is a genetic disorder characterized by accumulation of glycosphingolipids leading to damage of various organs. The disease progresses gradually, with the main late complications being renal failure, cardiomyopathy, neuropathy and others. The clinical picture of individual patients varies widely, even in patients from the same family with the same pathogenic *GLA* variant, and the factors affecting the clinical course are not fully known. The life expectancy of Fabry patients is shorter than that of the general population, and Fabry disease is therefore thought to be associated with premature aging. Progressive nephropathy is one of the main features of Fabry disease and contributes significantly to the overall morbidity and mortality of the disease. Due to the lack of specific biomarkers, heterogeneity of the disease, and nonspecific symptoms, the diagnosis is often delayed. Therefore, there is an undeniable need for noninvasive biomarkers that can be associated with early stages of kidney damage. Novel candidate biomarkers are being investigated using various state- of-the-art approaches. For example, we have investigated urinary extracellular vesicle-derived miRNAs, genomic telomere attrition and oxidative stress biomarkers to assess their potential suitability as biomarkers of development and progression of Fabry nephropathy. Such diagnostic and prognostic biomarkers would allow early diagnosis, which is crucial for effective treatment and prevention of severe irreversible and life-threatening complications.

L15
Careful and systematic observations in basic and applied research

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Toxins are thought as villains as they cause death and debilitation. In reality, they have contributed more to improving our lives than cause death. Toxins have played crucial roles in the discovery and development of therapeutic and diagnostic agents for human diseases. They have also contributed as important research tools and helped us to understand molecular mechanisms of normal physiological processes such as neurotransmission, blood coagulation and platelet aggregation. Our lab has been studying structure-function relationships and mechanism of actions of novel toxins from various sources. Our research has contributed to both basic and applied sciences. Based on the functional sites of the toxins, we have developed a number of therapeutic agents for various human diseases. I will describe the role of careful, keen and systematic observations in distinct lines of research straddling both basic and applied research.

L16
Intra and interspecies social behaviors by beneficial bacterium Bacillus subtilis

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Bacteria thrive in diverse communities, engaging in intricate social dialogs. In our research, we focus on the beneficial bacterium, *Bacillus subtilis*, to delve into the molecular mechanisms and implications of bacterial sociality at inter and intraspecific levels. Our investigations have revealed the correlation between phylogenetic kinship among *B. subtilis* isolates and their swarm's compatibility. This is visually detected through the merging of swarms among closely related isolates and a distinct boundary forming when two less related conspecific swarms come into contact. This kin discrimination (KD) like behavior, well studied in humans, animals, and plants, significantly impacts the distribution and fitness of interacting bacterial genotypes. Further examination revealed that KD's manifestation is contingent on the presence or absence of specific loci. It facilitates horizontal gene transfer between genotypes and serves as a policing mechanism, curtailing the spread of exploiters within species. Consequently, KD may play a pivotal role in shaping the evolutionary trajectory of *B. subtilis* species. In addition to bacterial KD, we also explore interspecies social behaviors by coculturing *B. subtilis* with the enteropathogenic *Salmonella Typhimurium*. In this context, our research highlights bacillaen as the main antibiotic responsible for inhibiting the growth of the pathogen. However, we also observed that environmental factors and cell-cell contact-dependent mechanisms influence the outcome of interspecies competition. As we uncover the secrets of bacterial social behaviors, we move closer to leveraging these insights for innovative applications in biotechnology and pathogen control.

L17
Order from disorder: towards molecular architecture of the muscle Z-disk assembly by integrative structural biology

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Sarcomeres are the smallest contractile units found in cardiac and skeletal muscle, where actin and myosin filaments move past each other to generate tension. This molecular machinery is supported by a subset of highly organised cytoskeletal proteins that perform architectural, mechanical, and signalling functions. The ultrastructure of a sarcomere is highly ordered and bordered by Z-disks, which play an important role in mechanical stability and force transmission.

In the Z-disks – the lateral boundaries of the sarcomere machinery – the protein α-actinin-2 cross-links antiparallel actin filaments from adjacent sarcomeres, and additionally serves as a binding platform for a number of other Z-disk proteins. In striated muscle cells, the Z-disk represents a highly organized three-dimensional assembly containing a large directory of proteins orchestrated in a multi-protein complex centred on its major component α-actinin, with still poorly three-dimensional interaction map. To investigate the structural architecture of the Z-disk, the assembly hierarchy, and structure-function relationships, we are employing an integrative structural biology strategy, combining molecular biophysics, structural, and biochemical approaches.

FATZ proteins interact with α-actinin and five other core Z-disk proteins, contributing to the assembly and maintenance of myofibrils as a hub for protein interactions. I will present our studies on the interaction of the major Z-disk protein α-actinin-2 with FATZ-1 and Z-portion of titin, forming dynamic fuzzy complexes, and discuss findings in view of asymmetric sorting of α-actinin and sarcomeric Z-disk architecture and assembly.

Furthermore, our recent finding that FATZ-1 phase-separates and forms biomolecular condensates with -actinin-2 and the other three Z-disk proteins raises the intriguing question of whether FATZ proteins can act as an interaction hub for Z-disk proteins during myofibrillogenesis via membrane-less compartmentalization.

L18
Structure of protein nanopore reveals organization of membrane lipids and provides basis for designing nanopores for sensing applications

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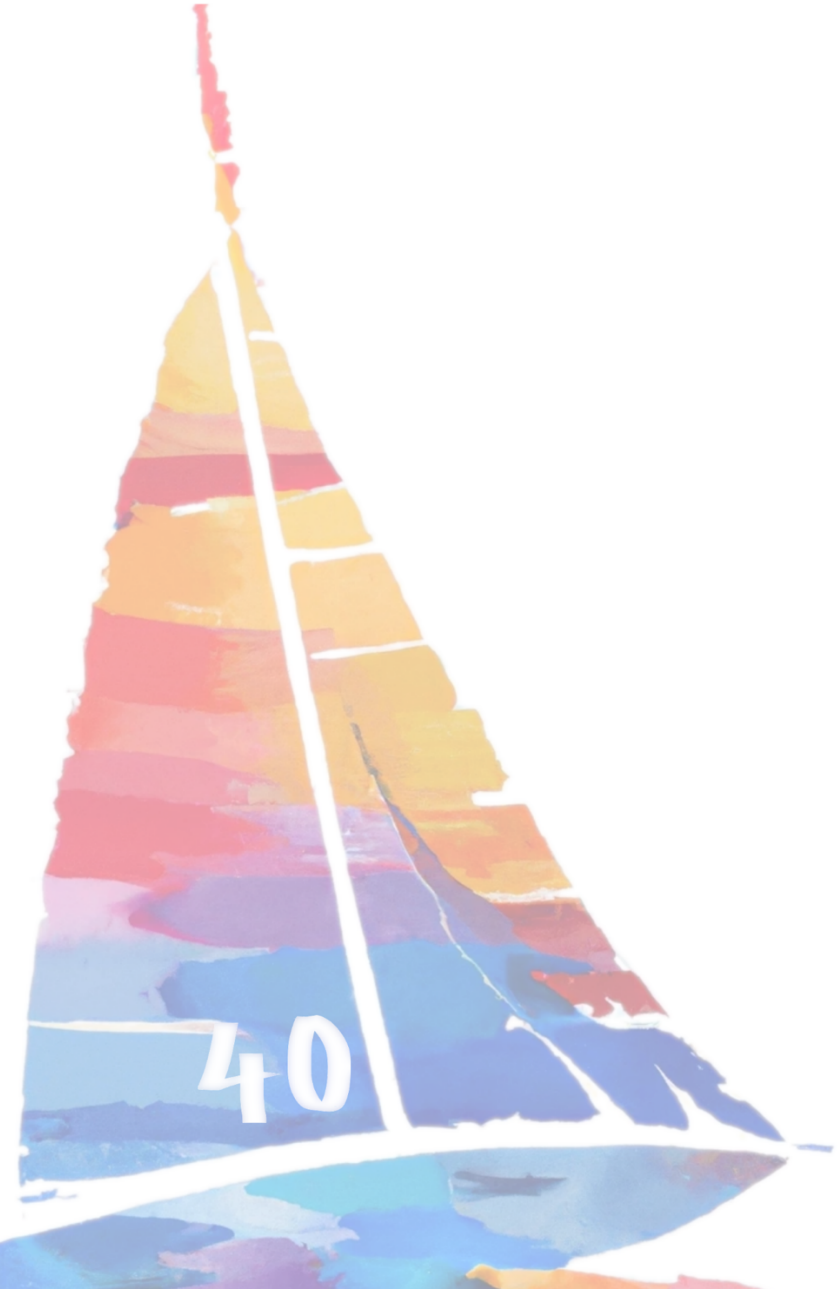
Nanopore sensing is becoming increasingly important as a direct, rapid, and cost-effective technology that can be used for high-throughput detection of various analytes. The use of protein nanopores has attracted considerable attention following the successful application of DNA sequencing. Despite significant progress in recent years, there are still a limited number of unique nanopores available. We are focusing on developing sensing approaches using natural protein-based nanopores and high-throughput biophysical methods. We used an actinoporin from mountainous star coral to produce stable pores by extracting them from model lipid membranes with detergents. The structure of the octameric pore, determined by cryo-electron microscopy, surprisingly revealed the presence of membrane lipids that formed an intricate network of protein-lipid and lipid-lipid interactions. The lipids were found to be indispensable for the assembly of the pore on the membrane surface and influenced the functional properties of the pore. The model of the pore was then used for protein engineering to improve pore incorporation into artificial membranes, stability, and reduction of electrical noise when the pore was characterized using planar lipid bilayers. We have also demonstrated the successful discrimination of different histone proteins using a high-throughput planar lipid bilayer approach. The combination of structural biology, protein engineering, and high-throughput biophysical methodology is therefore required for future advances in single-molecule and real-time sensing approaches.

Abstracts of Short Presentations (S)



S1
Regulation of CD19 CAR-T cell activation based on an engineered downstream transcription factor

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CD19 CAR T- cells present a therapeutic option for various malignant diseases based on their ability to recognize the selected tumour surface markers, triggering immune cell activation and cytokine production resulting in killing cancerous cells. Efficient tumour cell destruction by CAR-T may cause serious side effects so their activity needs to be carefully controlled. Several attempts were made to influence the CAR T cell proliferation and their activation by adding T cell growth factors, incorporating cytokine expressing cassettes in T cells etc., however this approach of regulating T cells activity with no external control can again lead to non-optimal therapeutic effects. Different improvements were made also by designing synthetic receptors or small molecule-inducible systems etc., which influence regulated expansion and survival of CAR T cells.

To regulate CD19 CAR-T cells, we developed a regulatory system for therapeutic effect of CD19 CAR-T cells, a unique mechanism to control T cell activation and proliferation based on the engineered NFAT2 artificial transcription factor. A key transcription factor in T cells, NFAT that result in IL2 production, was truncated by deletion of its own activation domain and joined with domains of different heterodimerization systems. The interaction counterparts were fused to a transcriptional activator or repressor domains, resulting in formation of an engineered artificial transcription factors with external control. Chemical regulators were used to either transiently trigger engineered T cell proliferation or suppress CAR-mediated activation when desired or to enhance activation of CAR T cells upon engagement of cancer cells, shown also *in vivo*. Additionally, an efficient sensor to monitor activated CD19 CAR-T cells *in vivo* was introduced. This implementation in CAR-T cell regulation offers an efficient way for on-demand external control of CAR-T cell activity to improve their safety.

S2
Hierarchical DNA assembly in yeast cells

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Construction of complex DNA assemblies is a technological challenge at the interface of genetic engineering and synthetic biology. Such DNA assemblies, with a range of applications in biotechnology and in basic research spanning from genetics to biomedicine, consist of many genetic elements with several variants of each element and sometimes without a predefined optimal consecutive order. To address this challenge, we have been developing a toolbox for hierarchical DNA assembly in *Saccharomyces cerevisiae* yeast cells. Hierarchical DNA assembly denotes a combination of *in vitro* and *in vivo* steps for genetic engineering whereby DNA fragments of various sizes – including at the whole chromosome or even whole genome level – are cloned and joined in a pre-defined or random order. Such DNA constructs are further modified using genome editing techniques including (multiplex) CRISPR-Cas and other scarless DNA cloning methods. Design and further modifications of a new series of yeast artificial chromosomes and of recombinant T7 bacteriophage genomes will be presented as proof-of-principle examples of hierarchical DNA assembly in yeast cells. The 'Design-Build-Test' iterative circuit will be discussed, with hierarchical DNA assembly in yeast cells as the building phase and high-throughput phenotyping as the testing phase. It is anticipated that further developments in human genetics and medicine will rely on synthetic biology tools for complex human DNA assembly, and it is within this context that the methodology in the presented work has been and will be developed further.

This work was supported by the ARRS research project L4-3181 *Hierarchical DNA assembly for advanced applications in biopharmaceuticals production and cell therapy*.

S3
A method for targeting a specified segment of DNA inside the bacterial microcompartment

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Microcompartments (MCPs) are proteinaceous organelles widely distributed across the bacterial kingdom. Selectively permeable shell of MCPs encapsulates a variety of enzymes involved in a specific metabolic pathway providing both enhanced flux and protection against toxic intermediates. A specific amino acid sequence located at the end of the enzymes represents a signal which directs the enzyme inside the MCPs. This gives MCPs high biotechnological potential since any protein of choice can be encapsulated within the lumen of MCPs.

In our study, we remodelled the *Citrobacter freundii* propanediol utilisation (Pdu) MCP to be used as a nucleic acid container. First, we constructed a plasmid carrying genes for shell proteins of the Pdu MCP and a transcription factor LacI labelled with the Pdu targeting peptide and the enhanced green fluorescent protein (EGFP). Since these genes were under pBAD promoter, we achieved self-assembly of the synthetic organelle containing fluorescently labelled LacI by adding arabinose to the transformed *Escherichia coli* cells. The fusion of the major Pdu subunit with the "Twin-Strep-tag" allowed us to isolate MCPs by affinity chromatography. The presence of LacI in the isolated MCPs was confirmed by fluorescence microscopy and mass spectrometry. Since LacI is a DNA-binding protein, we assumed that a specific DNA sequence could also be encapsulated within the MCP. Therefore, we introduced an additional plasmid with multiple binding sites for LacI into *E. coli* cells carrying the plasmid with genes for assembly of Pdu MCP containing fluorescently labelled LacI. The DNA was indeed purified from isolated MCPs, and the results of high-throughput sequencing show that the selected DNA segment with the LacI target sites was successfully encapsulated within the MCP.

The ability to pack the desired DNA into MCPs, holds potential across various applications ranging from the development of novel gene delivery platforms to improved nano bioreactors.

S4
Stefin B increases autophagic flux and AMPK activation in macrophages

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Stefin B (cystatin B) is an inhibitor of nuclear and lysosomal cysteine cathepsins. The gene encoding stefin B is find on human chromosome 21 and its expresson is increased in the brains of individuals with Down syndrome. Loss-of-function mutations in the gene encoding stefin B are associated with a neurodegenerative condition called Unverricht-Lundborg disease (EPM1), which is characterised by progressive myoclonus epilepsy and ataxia. We previously reported that stefin B-deficient mice are significantly more sensitive to lethal lipopolysaccharide (LPS)-induced sepsis and have increased NLRP3 inflammasome activation. Here, we found lower caspase-11 gene expression and lower interleukin 1-β processing in bone marrow-derived macrophages of stefin B trisomic mice —mice with an additional copy of stefin B gene. Stefin B trisomy prevented mitochondrial reactive oxygen species (ROS) formation and impaired the NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in macrophages. Recent studies of cellular metabolism in macrophages demonstrated alterations in metabolic profiles during macrophage activation. Increased AMP-activated kinase activation and suppressed mTOR activity were observed in stefin B trisomic macrophages and cells with increased stefin B expression. Our study demonstrated that increased stefin B expression induced autophagy and downregulated mitochondrial ROS generation. Our findings reveal the basis of the anti-inflammatory properties of stefin B and may facilitate the development of a novel therapeutic approach for preventing NLRP3 inflammasome overactivation.

S5
Melanoma antigens in pancreatic cancer and glioblastoma are markers and drivers of therapy resistance

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Introduction: Brain tumor glioblastoma (GBM) and pancreatic cancer (PC) are one of the deadliest tumors in adults due to their therapeutic resistance. Melanoma-associated antigens (MAGE) are the first tumor antigens discovered more than 30 years ago and correlate with the more aggressive disease progression and resistance to the therapy in many solid tumors, however, their role in glioblastoma and pancreatic cancer is mostly unknown. Our preliminary data suggest that the evolutionary role of MAGE proteins in the protection of germ cells is hijacked during oncogenesis, including MAGEA3/6-mediate resistance of pancreatic cancer cells to nutrient stress. Aim: The purpose of this study is to determine the expression and role of the MAGE protein family in GBM and PC upon therapy-induced stress. Methods: The Cancer Genome Atlas (TCGA) and Cancer Cell Line Encyclopedia (CCLE) were analyzed to determine the expression of MAGEs in human tumors and cell lines and their association with clinical parameters. Further, we have applied 2D and 3D cellular models coupled with gene silencing and overexpression to evaluate the role of MAGEs in GBM and PC progression and therapy response. We analyzed the response to the metabolic stress, induced by the glycolysis pathway inhibitor (2DG) or nutrient depletion, and to the standard chemotherapy, e.g. temozolomide. Results and Future directions: Our data show that MAGE genes are expressed in 10-20% of GBM and PC tumors and are associated with poorer patients' prognosis. Furthermore, in GBM and PC cells, MAGEC2 and MAGEA3/6 promote cancer growth and therapy resistance, respectively, however, the underlying mechanism are different. We are currently investigating the molecular underpinnings of their function, and what may provide novel therapeutic opportunities, in particular in therapy-resistant patients.

S6
Phenylalanine-tRNA aminoacylation is compromised by ALS/FTD-associated C9orf72 antisense repeat RNA

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The most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the C9orf72 gene mutation, which results in expanded hexanuleotide repeat – GGGGCC. It transcribes in sense (G4C2)n and antisense (C4G2)n direction and leads to the formation of nuclear RNA foci. We performed RNA-pull down assay from mouse and human brain lysates followed by mass spectrometry to determine protein interactors of antisense repeats and validated them using WB, FISH/ICC and developed a modified RNA-protein proximity ligation assay to observe cytoplasmic interactions of the repeats, since up until now FISH method only detected nuclear interactions. Interactors are involved in protein synthesis, cytoskeleton stability and mRNA processing. For the first time we observed the interaction with phenylalanine-tRNA synthetase (FARS). To evaluate the impact this interaction on tRNA aminoacylation, protein synthesis and cellular stress we used two aminoacylation assays, western blot analysis and Click-chemistry. Antisense RNA-FARS interaction resulted in significant decrease in aminoacylation rate in *in vitro* assay and lower charged $tRNA^{phe}$ levels in patient derived lymphoblasts. Additionally, we observed a decreased expression of phenylalanine-containing proteins at the whole protein level using modified click-chemistry experiment and of four unbiasedly selected proteins with high phenylalanine content in patient derived lymphoblasts, FARSA KD HEK293, differentiated FARSA KD NSC-34 cells and post-mortem patient tissue. In the presented study we investigated and confirmed protein interactions with the biologically relevant 32×C4G2 RNA repeats and how this affects cellular processes. Our discovery highlights the role of aminoacyl-tRNA synthetases in C9orf72 ALS/FTD where they may be important contributors to the development of these diseases. Other studies have previously linked irregularities in aminoacyl-tRNA synthetases to other neurodegenerative disorders.

S7
Exploration of pharmacogenomic biomarkers of adalimumab (non)response in Crohn's disease patients using single-cell RNA sequencing

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Anti-TNFα biologicals such as adalimumab have revolutionized management of the severe Crohn's disease (CD) cases refractory to the standard therapies. Alas, over 30% of the patients do not respond to the anti-TNFα therapy, another 30-40% loose response within a year. This calls for a personalized approach to treatment of these patients, based on reliable biomarkers.

Non-invasive approaches, such as use of the bulk peripheral blood immune cells, are favoured for novel biomarker discovery. Recent studies however suggest that cell subtype-specific biomarkers may be superior to those measured in the bulk peripheral blood. In this regard, the single-cell RNA sequencing (scRNA-seq) technology can prove highly valuable. By simultaneously capturing and profiling all the cells in a sample, scRNA-seq allows the analysis of cellular heterogeneity and gene expression in vast landscapes of the immune cell sub-populations, targeted or adversely affected by the treatment.

Here we utilized 10x Genomics scRNA-seq technology to analyze peripheral blood immune cells from CD patients responsive or non-responsive to the adalimumab. Confounding of disease conditions and therapy on marker genes was eliminated using Seurat package and computational integration anchors in order to identify conserved marker genes across all states.

Using an automated annotation (Seurat-Azimuth) combined with subsequent manual validation we reliably identified 20 sub-populations of immune cells, including classical monocytes, non-classical monocytes and several T-cell sub-populations (naïve, central and effector memory $CD4^+$T-cells, Tregs, MAIT, etc.). We further profiled differentially expressed genes between the responsive and non-responsive patients in the most interesting cell sub-populations, in particular monocyte and T-cell sub-populations, which were previously identified as relevant for the adalimumab/anti-TNFα therapy (non)response in CD.

S8

The Role of Sterol Intermediates in Gene Regulation: Insights from Targeted Knockouts of Cholesterol Synthesis Enzymes

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Sterols are essential components of cell membranes and play important roles in various cellular processes. The biosynthesis of cholesterol involves a series of enzymatic reactions that convert acetyl-CoA to cholesterol via several intermediates our focus on sterols. In this study, we investigated the interplay between sterol intermediates and gene regulation by conducting targeted knockouts of consecutive enzymes involved in cholesterol synthesis in human hepatoma cells. Our goal was to determine the specific roles of individual sterols.

By conducting targeted knockouts of consecutive enzymes (CYP51A1, DHCR24 and SC5D) from in cholesterol synthesis, and performing targeted metabolomics using LC-MS and global transcriptome analysis, we identified key roles of individual groups of sterols in influencing gene regulation. We identified many changed pathways due to sterol accumulation. Specific sterols proved to have diverse effects on gene regulatory pathways. Early sterols, such as 24,25-dihydrolanosterol, promote cell proliferation, cell cycle progression and activate transcription factors LEF1, NF-kB and the WNT pathway, while accumulation of desmosterol and lathosterol results in slower proliferation and promotion of apoptosis and tumor suppressor pathways like HNFA1.

Our study advances understanding of the interplay between the sterol part of cholesterol synthesis and gene regulation. These results highlight the crucial role of non-polar sterols in regulating gene expression and their potential importance in relation to processes such as LEF1 activation and cell cycle control. Our results suggest that the balance between different sterols (coupled cholesterol synthesis) is critical for maintaining normal cellular functions.

S9
3'UTR ribonucleoprotein reassembly drives selective mRNA decay to implement developmental signalling

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Changes in gene expression are key effectors of signalling pathways, but remodelling of protein-RNA networks by signalling remains elusive. Here we find that developmental signalling drives a major reassembly of protein-mRNA interactions centred on LIN28A, which coordinates pluripotency progression with embryonic morphogenesis. Upon embryo implantation, MEK phosphorylates the intrinsically disordered domain of LIN28A, which then reassembles onto terminal regions of 3'UTRs. In pluripotency mRNAs, these regions form multivalent AUU-rich hubs for convergent binding of pLIN28A and PABP, which drives selective MEK/ERK-induced mRNA decay. Upon blocking this LIN28A function, cells retain naïve expression programme, while morphogenesis proceeds to unforeseen multiple lumens. This demonstrates the potency of signal-transduced pLIN28A/PABP reassembly on 3' UTRs in selecting mRNAs for decay, thereby coordinating pluripotency progression with embryonic morphogenesis.

One-sentence summary: Multivalent RNA hubs for IDR-dependent RNP reassembly coordinate pluripotency progression with morphogenesis via selective mRNA decay

S10
Current challenges and opportunities for AI in education

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Assessment in education is changing AIs such as ChatGPT and other language models move rapidly into everyday life. Here we investigate how artificial intelligence (AI) has already been used in bioscience education to address UnGoogleable questions. In this talk we investigate how AIs can deal with existing assessment styles followed by grading the outputs against existing assessment criteria. Each assessment prompt returned viable answers but was vague, lacked depth and was limited in creative aspects. However, all would achieve a good grade close to the class average and seen to be comparable to first-year undergraduate essays and satisfactory online exam answers. Our response to AI entering the learning space will be to look at what we are assessing and why we are assessing it. Ai can do more than beat the system, when used well it can be a valuable learning tool for our students and a powerful teaching assistant for the tutor. This talk will conclude by presenting practical solutions to the integration of AIs into the skills development of our students and how these skills can be developed through the curriculum.

S11
Ai and VR assisted teaching

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Recently, the convergence of artificial intelligence (AI) and virtual reality (VR) has opened up exciting possibilities for transforming the field of education. This talk explores the ground-breaking potential of AI and VR assisted teaching, showcasing how these immersive technologies can revolutionize traditional educational practices and enhance the learning experience for students. The combination of AI and VR holds immense promise for creating dynamic, inclusive, and immersive learning environments that cater to diverse learning styles and enable students to develop critical thinking, problem-solving, and collaboration skills necessary for success in the digital age.

S12
Optimization of the extraction of extracellular polymeric substances from lactic acid bacteria and their characterization

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In nature, microorganisms organize themselves into biofilms in which they are surrounded by a self-produced matrix of extracellular polymeric substances (EPS) that enable long-term persistence. The EPS consists of polysaccharides, proteins, extracellular DNA, lipids, and other substances and can comprise 90% of the dry mass of the biofilm. EPS constituents are critical for the establishment of the mature biofilm and are responsible for most of the benefits of bacterial life in the biofilm. EPS compounds are responsible for adhesion to a surface, cohesion within the biofilm, aggregation of bacterial cells, retention of water (prevention of desiccation), protection from environmental conditions including antimicrobial agents (as a protective barrier), absorption of organic and inorganic compounds (as an energy reservoir), and enzymatic activity. However, little is known about which components of EPS contribute most to biofilm formation that can promote or inhibit the survival of foodborne pathogenic bacteria. Our objective was to analyse the involvement of EPS from five different lactic acid bacterial species in competitive or cooperative interactions within the microbial biofilm community with the pathogen *Campylobacter jejuni*. We used five different methods to extract EPS to compare their content of polysaccharides, proteins and eDNA. Then, the effect of EPS on *C. jejuni* biofilm formation was analysed. Selected EPS were further investigated using solid-state magnetic resonance (NMR) to highlight the compounds that modulate *C. jejuni* biofilm. Understanding the effects of lactic acid bacteria EPS and their composition on *Campylobacter* biofilm development will highlight EPS components that are important for biofilm formation and therefore can potentially be used to limit *Campylobacter* survival and persistence in biofilms. This could provide an alternative strategy to prevent pathogen contamination and animal infection, as well as safer food production.

S13

Unravelling the biosynthesis of irregular polyprenyl backbone of the Planococcus citri (Insecta) sex pheromone

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Synthetic mealybug (Insecta, Coccoidea) sex pheromones have become important for sustainable population control in agriculture, but further commercialization would benefit significantly from development of their biotechnological production. However, little is known about the biosynthesis of the prenyl backbones of mealybug sex pheromones, presumably resulting from irregular coupling activity of unidentified isopentenyl diphosphate synthases (IDS). We have set to establish a basis for a bioproduction platform for the sex pheromone of *Planococcus citri*, a widespread agricultural pest, by conducting a comprehensive search for sequences homologous to IDSs in the *P. citri* genome and *de novo* transcriptome. We identified a set of sequences with homology to short- and long-chain IDSs and proposed their putative functions based on sequence analysis and expression profile. For some IDS sequences, we found multiple *P. citri* paralogs, indicating possible gene family expansion and functional diversification. Testing the activity of eleven IDS candidates for the production of mono- and sesquiterpene regular and irregular backbones confirmed *in vitro* regular activity for five IDS candidates, one of which also produced lower amounts of irregular prenyl diphosphates maconelliyl and lavandulyl diphosphate, but not planococcyl diphosphate, the terpenoid moiety of the *P. citri* sex pheromone. The identified IDS sequences provide an important basis for deciphering terpenoid biosynthesis in *P. citri* and other mealybugs and are a potential source for biotechnological exploitation.

S14
Upgrading nanopore biosensing with unnatural amino acids

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Many molecules found in the environment are present only in trace amounts, but have an enormous impact on individual living organisms and ecosystems. Detection of these molecules using ensemble techniques remains difficult due to the complex pre-processing of samples and the need for large equipment. Single-molecule techniques based on portable devices such as nanopore biosensing offer excellent alternatives, but detection of trace-level analytes in complex mixtures can be thwarted by a large number of interactions between the nanopore and more abundant analytes. To enable simple and highly specific detection of sodium azide, a common environmental pollutant, we have developed biological nanopores decorated with an unnatural amino acid (UAA) whose side-chain chemistry is tailored to react with the analyte. Using genetic code expansion, we incorporated 4-propargyloxy-L-phenylalanine (pPpa), an UAA with an alkyne handle, into actinoporin monomers in *E. coli*. To use this pPpa-bearing actinoporin for sodium azide biosensing, we selected positions in the transmembrane region of the actinoporin whose side chains extend into the lumen of the pore. All five selected residues facing the lumen could be reassigned to pPpa, and three of them also allowed the formation of functional pores carrying pPpa. Importantly, pPpa-containing nanopores exhibit low noise and stable open pore currents in nanopore experiments. Structural characterization of pPpa-containing nanopores by cryo-EM shows that the orientation of alkyne-containing side chains is appropriate for biosensing. The use of pPpa-containing nanopores in single channel experiments shows that the pPpa side chains undergo a click chemistry reaction with free azide analytes in the presence of copper(I) ions, which can be monitored as a decrease in open pore currents. The chemoselectivity of UAAs makes nanopores decorated with UAAs ideal for the detection of analytes in complex mixtures such as environmental samples.

S15
Characterization of archaeosomes from polar lipids of Aeropyrum pernix K1 and stability in biological systems

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Liposomes are spherical structures made from one or more phospholipid bilayers enclosing an aqueous phase. They are promising drug delivery system as they enable capsulation of both polar and non-polar bioactive compounds, along with increasing their efficacy, stability, and lowering toxicity. However, due to their relatively low stability and difficult production, their presence on the market is very limited. One of the solutions is the formation of vesicles from archaeal lipids – archaeosomes, which are characterized by their thermostability, long-term storage stability, resistance to enzymatic degradation, and immunomodulatory properties. Archaea are unique group of organisms known for their wide distribution in extreme environments, which is partly enabled by the unique lipid structure of their cell membrane. One of such organisms is archaeon *Aeropyrum pernix* K1 that thrives in solfataric vents at an optimum growth temperature of 90 – 95 °C. The isolated total polar lipids from *Aeropyrum pernix* K1 consist exclusively of glycerol ether lipids with isoprenoid groups attached to glycerol *via* ether linkages. More specifically, the two major polar lipids extracted from the membranes are $C_{25,25}$-archaetidyl(glucosyl)inositol and $C_{25,25}$-archaetidylinositol. An overview of the results of the effects of temperature and pH on the stability, structural organization, fluidity, and permeability of archaeosomes composed of pure $C_{25,25}$ was examined by a combination of techniques, including fluorescence emission spectroscopy, electron paramagnetic resonance, differential scanning calorimetry, and confocal microscopy. We also compared the physicochemical properties of pure vesicles composed of either archaeal lipids or conventional lipids (e.g. DPPC) with mixed vesicles composed of both lipid types. Archaeal lipids are discussed in terms of their potential use as a targeted drug delivery system based on the results of *in vivo* and cytotoxicity studies.

S16
New upstreaming signaling pathways of NIX regulation in development-induced mitophagy

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Mitophagy, a process that selectively clears damaged or dysfunctional mitochondria through autophagic machinery, maintains metabolic and energy balance and contributes to mitochondrial homeostasis. As a cellular quality control mechanism, mitophagy primary targets dysfunctional mitochondria. However, mitophagy is also activated during remodeling of mitochondrial networks in a number of cell types. Mitochondria and mitochondria-mediated signaling pathways are known to control the development of fully functional erythrocytes through the elimination of mitochondria at terminal differentiation of erythroblasts. NIX, mitophagy receptor, has a specific role in targeting the mitochondria for clearance by mitophagy during erythroid maturation since Nix deficiency prevents fully functional mitochondria to enter the autophagosome. Recently, we have described completely novel mechanism underlying NIX-mediated mitophagy. This mechanism involves the interplay between C-terminal NIX dephosphorylation and receptor dimerization, consequently. Analysis of C-terminal intermembrane part of NIX has revealed that receptor dimerization is achieved by specific Ser212 dephosphorylation and has the positive effect on mitophagy initiation and progression as well as phosphorylation of the LC3-interacting region (LIR), essential for the recruitment of autophagy machinery, described earlier. This interplay between NIX phosphorylation and dimerization, together with LIR phosphorylation, is crucial for proper NIX-dependent mitophagy. Currently, the focus of our research is in detailed analysis of interactions between NIX and identified kinases/phosphatases to unveil upstream signaling pathways that trigger and regulate mitophagy especially in erythroid cell lines. This knowledge is crucial for better understanding the mechanisms of particular cell`s differentiation and the development of pathological conditions that underlie the disturbed mitophagy process.

S17
ASO-Mediated Inhibition of MyD88L265P-dependent signaling as a Therapeutic Approach for Waldenström's Macroglobulinemia

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MyD88 is an adaptor protein involved in the signaling pathways of Toll-like receptors (TLRs) and the Interleukin-1 receptor. Upon ligand binding, the TIR domains of TLR or IL-1R dimerize and associate with the TIR domain of MyD88. This interaction leads to the formation of a complex called the Myddosome, resulting in NF-κB activation and an inflammatory response. The $MyD88^{L265P}$ mutation triggers its spontaneous oligomerization, causing constitutive signaling and serving as a driving mutation in lymphoid malignancies such as Waldenström's macroglobulinemia (WM). A naturally occurring variant of MyD88 lacks exon 2, resulting in an isoform that acts as a dominant negative variant. Previously, antisense oligonucleotides (ASOs) have been used to direct pre-mRNA processing, skipping exon 2 and inhibiting the signaling of the $MyD88^{WT}$. Our aim is to improve the technology of exon skipping using ASOs and apply it to inhibit the L265P variant of the protein, which could be therapeutically beneficial for WM patients. We designed ASOs targeting sites involved in pre-mRNA processing, including the branch point, splicing enhancers and the splice sites. Following transfection of the ASOs into cells, we showed the expression of short MyD88 isoform resulting in decreased cytokine expression. We demonstrated the applicability of this inhibition strategy to the constitutively active $MyD88^{L265P}$. ASOs that were effective in HEK293-T cells were also tested in the MWCL-1 (WM) cell line, which harbors the L265P mutation. ASOs proved highly effective in reducing MyD88-dependent secretion of IL-6, IL-8, IL-10, and TNFα. Moreover, combining ASOs with approved WM drugs like ibrutinib further suppressed cytokine secretion and increased apoptosis in MWCL cells. Considering the potential of this approach for therapeutic use in WM, we are also developing a delivery method utilizing antibody-conjugated lipid nanoparticles (LNPs) to specifically target B lymphocytes in the spleen and bone marrow.

S18
Inhibition of cathepsins B and X promote differentiation of cancer stem cells

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Tumor relapse emerges mainly due to the presence of small population of cancer stem cells (CSCs) that are resistant to the most conventional antitumor therapies. CSCs are capable of self-renewal, differentiation and generation of new tumors and are thus the main driving force for tumor initiation and progression. The major challenge in the development of new therapeutics for cancer treatment is to effectively target also CSCs. Recognizing molecular targets activated in CSC responsible for induction and maintenance of stemness is thus a crucial step in the development of new therapeutic strategies. Lysosomal cysteine cathepsins are important enzymes that participate in multiple stages of tumor progression and have been recognized as promising targets in anticancer therapy. Among them important contribution is attributed to cathepsins B and X that are unique due to their carboxypeptidase activity. Here we showed increased protein levels and activity of cathepsins B and X in CSCs, isolated from different breast cancer cell lines based on their ability of tumorsphere formation, compared to single adherent differentiated tumor cells. Cathepsins B and X are thus promising molecular targets to impair CSCs. Therefore, selective, reversible small molecular inhibitors of cathepsins B and X were used to evaluate the effect of cathepsin B and X inhibition on CSCs. We showed that cathepsin B and X inhibitors effected CSC phenotype by decreasing the expression of stemness markers and markers of mesenchymal cell phenotype. Next, effect of cathepsin B and X inhibition on CSCs was demonstrated also in functional assays mimicking processes of tumor progression. Additionally, our results show that cathepsin inhibition effects signaling pathways important for tumor progression. Taken together, we demonstrate that cathepsin inhibition is a promising new approach to reduce a pool of CSCs and to improve existing antitumor therapy resulting in increased effectiveness of cancer treatment.

S19
11-hydroxylated androgen metabolites as risk factors and source of androgen receptor-activating hormones in endometrial cancer

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Background: Endometrial cancer (EC) is a gynaecological pathology for which a growing incidence rate is observed worldwide, as the population grows and ages. Epidemiological studies associate classic androgens with EC risk, whereas 11-oxygenated androgen metabolites have not been investigated yet in the context of EC.

Methods: Serum 11-oxyandrogen levels were determined by a validated LC-MS/MS method in 70 tumour-free women and 62 EC patients, from which 56 had endometroid, 6 had serous EC. Logistic regression model adjusted for body mass index (BMI), age and parity was used to calculate odds ratio (OR) per unit increase in 11β-hydroxy-androstenedione (11βOHA4), 11β-OH-testosterone (11βOHT), 11-keto-A4 (11KA4) and 11-keto-testosterone (11KT). To investigate whether endometrial tumours contribute to the 11-oxyandrogen pool, we incubated cell lines of various EC grades with classic androgen and 11-oxyandrogen precursors. The profile of formed metabolites was investigated by LC-MS/MS. Analysis was performed in R studio 4.3.0.

Results: EC patients have higher 11βOHA4 and 11βOHT levels and comparable 11KA4 and 11KT levels comparing to tumour-free women. The OR per unit increase is: 11βOHA4 (OR, 1.06; 95% CI, 1.00-1.13, p=0.04), 11βOHT (OR, 2.13; 95% CI, 1.10-4.61, p=0.04), 11KA4 (OR, 0.94; 95% CI, 0.79-1.12, p=0.52), 11KT (OR, 1.21; 95% CI, 0.78-1.90, p=0.39). In EC cell lines, 11-oxymetabolites form from 11-oxyandrogen precursors, and not from classic androgen precursors. Cell lines of lower grade form higher levels of bioactive 11KT, comparing to poorly differentiated cells.

Conclusions: EC patients have higher levels of 11-hydroxylated androgens comparing to tumour-free women. 11βOHA4 and 11βOHT levels are associated with higher EC risk. EC cell lines of lower grade have enriched microenvironment with androgen receptor-activating 11-oxyandrogens.

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S20
Circular RNAs as potential peripheral blood biomarkers for amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive fatal adult-onset neurodegenerative disease in which both upper and lower motor neurons are affected. The diagnosis is often delayed due to initial non-specific clinical symptoms. Identifying and validating diagnostic biomarkers is therefore essential. In this study, we investigated the usefulness of circRNAs as potential biomarkers for ALS. We first performed a microarray analysis of circRNAs on peripheral blood mononuclear cells of a subset of ALS patients and controls. For further analyses, we selected two approaches. In one approach we collected only circRNA with a host gene that harbors any evidence of genetic constraints, which could hypothetically have a major role in determining a trait or disease. We used only the set of genes labeled as a “conserved set” for regression analyses. With a False Discovery Rate threshold of 0.1, only six circRNAs passed the filtering. In another approach we used different clustering algorithm including kmeans and Mclust to find the smallest dataset of circRNA which have the best True positive rate and True negative rate in identifying cases and controls. 13 circRNA had the best power to discriminate between case and controls. In both approaches, hsa_circ_0060762 and its host gene CSE1L were detected. Further analysis in larger sets of patients and controls revealed significant differences in expression levels for both hsa_circ_0060762 and CSE1L and receiver operating characteristics curve analysis showed diagnostic potential for CSE1L and hsa_circ_0060762. CSE1L is a member of the importin β family and mediates inhibition of TDP-43 aggregation, the central pathogenicity in ALS, and hsa_circ_0060762 has binding sites for several miRNAs that have been already proposed as biomarkers for ALS. In conclusion, Hsa_circ_0060762 and CSE1L thus represent novel potential peripheral blood biomarkers and therapeutic targets for ALS.

S21
Désirée in distress - a system biology approach: from statistics to networks

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Potato is a dominant staple food across the globe. It requires relatively minimal amounts of fertilisers and crop management, thus being a perfect candidate for economic, environmental, and social sustainability. On the contrary, keeping up with major factors negatively affecting agricultural yield, such as abiotic and biotic stressors, is becoming more and more difficult in the face of intensifying climatic changes.

The Horizon 2020 EU project Accelerated Development of multiple-stress tolerAnt PoTato (ADAPT) is focused on understanding potato responses to a combination of abiotic stresses and thus identify targets for breeding of new potato varieties. High-throughput phenotyping and panomics analysis of potato under single- and combined abiotic stresses was conducted on the well-known cultivar *Désirée*.

The main questions addressed were: i) what are the markers (phenotypical, physiological, molecular) for individual vs. combined stress treatment, ii) which response mechanisms are triggered in each stress/multi-stress exposure, and iii) what are the common stress mechanisms (shared between all stresses). To address beforementioned, deep systems biology approach was used, including common and more complex statistical analyses, machine learning, and graph theory. The resulting output was supplemented with a expert knowledge network built upon hormone synthesis, regulation and signalling pathways and pathways involved in plant responses to stress. We believe that integrated results will facilitate domain knowledge experts to answer the addressed questions in a holistic way.

The protocol for species-agnostic panomics data integration is publicly available from institutional GitHub (https://github.com/NIB-SI/multiOmics-integration).

Acknowledgement:
We would like to thank the ADAPT consortium for generating Phenomics, Metabolomics, Proteomics, and Hormonomics data (https://adapt.univie.ac.at/).

S22
Impact of mislocalization on the TDP-43 protein network

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TDP-43 is a DNA/RNA-binding protein that is mainly located in the nucleus. In amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), it mislocalizes and becomes the main component of protein aggregates in the cytoplasm. In the present study, we investigated the interactome of wild-type TDP-43 and TDP-43 lacking nuclear localization signal (dNLS), mimicking a pathological condition in ALS. We established inducible mammalian cell lines stably expressing the recombinant fusion protein TDP-43wt or TDP-43dNLS with the biotin ligase BioID2 and control cell line expressing only BioID2. We performed the biotin identification (BioID) method and isolated biotinylated proteins from the cell lysates by pull-down assay. The isolated proteins were detected by mass spectrometry (MS), resulting in a list of unique TDP-43wt and TDP-43dNLS interactors that were further validated. Cellular localization and function of the interactors were investigated using DAVID bioinformatics analysis. It revealed that TDP-43wt interacts mainly with proteins of the ribonucleoprotein and spliceosome complexes and with paraspeckles, whereas the mutant TDP-43dNLS interactors are components of cytoplasmic stress granules (SG) and processing bodies (P-bodies). Validation of selected interacting proteins (NONO, SFPQ, FUS, MAML1, PUM1, and ATXN2L) revealed that MAML1 is unique TDP-43wt interactor, whereas NONO, SFPQ, and FUS are common interactors of TDP-43wt and TDP-43dNLS and are more abundant in the TDP-43wt fraction. ATXN2L and PUM1 are unique interactors of mutant TDP-43. Our results suggest that the development of ALS may involve impaired regulatory functions related to transcription/paraspeckle function and a potential link to stress granules (SG) and processing bodies (P-bodies) due to their increased association with mutant TDP-43. In addition, the newly identified interactors of TDP-43 in this study may contribute to the understanding of the aggregation process.

S23

The role of AMP-activated protein kinase in regulation of Na+,K+-ATPase: the gauge does not always plug the sink

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AMP-activated protein kinase (AMPK) is a cellular energy gauge and a regulator of energy metabolism. Under the energy-deprived conditions AMPK stimulates the ATP production and suppresses the ATP consumption. Na^+,K^+-ATPase (NKA), which contributes 20-25% to whole-body ATP consumption, is a major sink of energy and might therefore seem like an ideal target for the energy-saving measures. Consistent with its role as a suppressor of the ATP consumption, AMPK stimulates the endocytosis of NKA in alveolar cells, thus suppressing ion transport in the lungs. However, skeletal muscle contractions activate NKA, which opposes rundown of ion gradients, as well as AMPK, which plays an important role in adaptations to exercise. While inhibition of NKA under these conditions would promote loss of excitability and accelerate fatigue, evidence suggests that AMPK does not inhibit or even stimulates NKA in skeletal muscle, which appears to contradict the idea that AMPK always suppresses the ATP consumption. The dephosphorylation of the catalytic NKA α1-subunit at Ser18 was previously shown to be important for the AMPK-mediated regulation of NKA in myotubes. We now show that AMPK promotes the dephosphorylation of NKA α1-subunit at Tyr10, indicating existence of a new regulatory pathway between AMPK and NKA. Moreover, we show that glucose deprivation not only induces energy stress, but also alters the ratio between the different isoforms of NKA subunits in myotubes. Notably, in rat L6 myotubes, energy stress increases the abundance of the catalytic NKA α2-subunit, while gene silencing of AMPK decreases it, again demonstrating that energy deficiency and AMPK activation do not necessarily suppress NKA in skeletal muscle cells. Taken together, our results highlight a close link between the regulation of NKA and energy metabolism in skeletal muscle. Furthermore, they are consistent with the notion that skeletal muscle possesses specific pathways of NKA regulation by AMPK and energy stress.

S24
Calpeptins: dual inhibitors of human cathepsins and SARS-CoV-2 main protease

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A great need for specific antiviral drugs targeting various stages of viral cell entry and replication has led to our recent X-ray crystallographic sreen of two repurposing drug libraries of more than 10.000 compounds that identified calpeptin, a cysteine cahepsin inhibitor, as a covalent active site inhibitor of SARS-CoV-2 main protease, M^{pro}, an essential protease for viral replication and a key drug target of SARS-CoV-2 infection (Günther *et al.*, 2021). Dual targeting of viral M^{pro} and human host cathepsins seems an attractive approach for SARS-CoV-2 treatment. In particular as it was found that also host cysteine cathepsins mediate cleavage/activation of the viral Spike-protein (S-protein) upon endosomal cell entry.
To test this hypothesis, we have determined the inhibition constants of various human cathepsins L, V, K, and B by calpeptin and calpeptin-derived compounds including S-calp, the sulfonated “prodrug”, N-calp, the cyano derivative, and GC-376 which is structurally related to calpeptins (Reinke *et al.*, 2023). Cathepsins K, V, and L were inhibited in the picomolar range, which imply that in the cellular context several cysteine cathepsins, all endopeptidases, are inhibited much more effectively than M^{pro}. The crystal structures of calpeptin and S-calp in complex with these cathepsins revealed binding at the non-prime sites from S3 – S1 and formation of a covalent bond. Treatment of SARS-CoV-2 infected hamsters with the S-Calp led to a significant reduction of the viral load in trachea on day 5, further highlighting their therapeutic potential. Following X-ray crystallographic screen against cathepsin L, performed next, we focused on 11 compounds that bound in the active site of cathepsin L. Their inhibitory properties were further assessed by nanoDSF and enzyme kinetic assays, their anti-viral properties on a Vero E6 cell line, while the experiment on SARS-CoV-2 infected mice is undergoing (Falke *et al.*, in preparation).

S25
Broad range phospholipase C of Listeria monocytogenes facilitates LLO-mediated destruction of lipid membranes

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Listeriosis is one of the deadliest foodborne diseases caused by the intracellular *bacterium Listeria monocytogenes* (*Lm*), killing thousands of patients worldwide each year. Broad-range phospholipase C (PC-PLC) is a zinc metalloenzyme and an important virulence factor of *Lm*. A crucial step in the pathophysiology of *Lm* is its escape from the lipid-enclosed phagosome after internalization. PC-PLC together with the cholesterol-dependent pore-forming toxin listeriolysin O (LLO) and other bacterial and host proteins, disintegrates the lipid membrane of the phagosome and releases *Lm* into its replicative niche, the cytosol. We determined the crystal structure of PC-PLC and complemented it with the functional analysis of this enzyme. This revealed that PC-PLC has evolved several structural features to regulate its activity, from the structurally plastic active site and the Zn^{2+}-dependent activity to the tendency to form enzymatically inactive oligomers. Next, we investigated the interaction of PC-PLC and LLO on the model lipid membranes. PC-PLC caused a significant increase in LLO binding to liposomes and LLO induced vesicle leakage, whereas PC-PLC alone did not cause permeabilization. Preincubation with less active PC-PLC mutants resulted in lower LLO binding and vesicle permeabilization by LLO. These results suggest that the activity of PC-PLC may increase the availability of the cholesterol in the membrane. Using cryoEM, we observed enhanced binding and changes in the morphology of LLO oligomers on lipid membranes in the presence of PC-PLC. Furthermore, we demonstrated specific inhibition of the mature PC-PLC by its propeptide *in trans*, providing a new platform for the development of alternative solutions to combat listeriosis. Our results contribute to better understanding of the molecular mechanisms of listerial virulence factors, which is crucial for understanding the interaction of the pathogen with the host.

S26
Glycoengineering as a tool to design regression models for prediction of monoclonal antibody function from its structure

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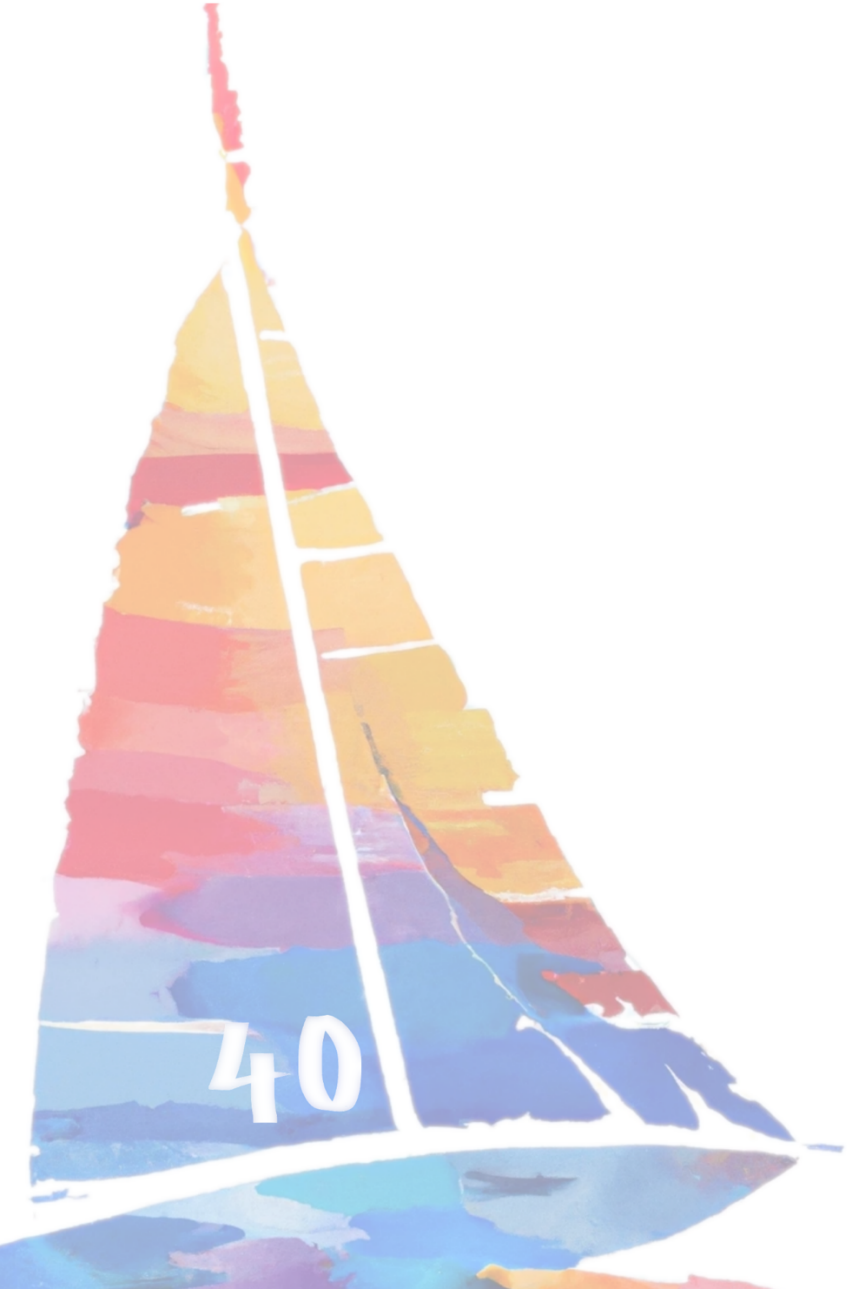
Development of novel therapeutic proteins and biosimilars requires a thorough understanding of the relationship between the structure and function of the therapeutic proteins. The importance of Fc-glycosylation of therapeutic proteins regarding their effector functions is extensively discussed and has been reported in various studies. As an important example, to ensure proper safety and efficacy profiles of therapeutic proteins, it is crucial to understand the effect of glycosylation on glycoprotein effector function. It is widely acknowledged, for example, that even a 1% change in Fc fucosylation of an immunoglobulin G1 (IgG1) antibody can significantly influence its efficacy. However, the effects of other glycan structures remain a matter of debate in the literature. The glycosylation variability observed during development and production is always observed to be intercorrelated for multiple glycan species. Therefore, studying the effect of individual glycan structure is challenging due to the lack of well-defined differences in glycan patterns of samples used. We systematically addressed this structure-function relationship by methodical exploration of the IgG glycosylation traits using samples with high variability of individual glycan structures, generated with in-vitro glycoengineering (IVGE). A variety of analytical assays, including Bio Layer Interferometry (BLI) and cell-based antibody dependent cellular cytotoxicity (ADCC), antibody dependent cell phagocytosis (ADCP), and complement dependent cytotoxicity (CDC) bioassays, were applied to investigate the effect of galactosylation, mannosylation, fucosylation, sialylation and agalactosylation on effector functions of the IgG1 molecule. The regression models developed for Fc gamma R binding (FcyR), ADCC, CDC and ADCP provide a quantitative explanation and prediction of the impact of individual glycan features on the binding and bioactivity of the therapeutic protein.

Abstracts of Posters (P)



P1
Engineering and characterization of lactic acid bacteria expressing cellulases

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The circular economy and sustainable society are important goals of the European Union. To meet these goals, it is crucial to reduce dependence on fossil fuels, and this can be achieved by making better use of plant biowaste as a cost-effective feedstock for bacteria. Lactic acid bacteria (LAB) are the industrially important producers of lactic acid; however, their growth on cellulosic substrates is limited by the absence of cellulases in their genome.

We have prepared a series of plasmid constructs for inducible and constitutive expression of three heterologous cellulases from different cellulolytic bacteria in LAB *Lactococcus lactis*. A secretion signal sequence for cellulase secretion, two anchor sequences for cellulase surface display, and a peptide tag for detection of cellulases were inserted. Inducible and four constitutive promoters were used to drive transcription. Expression and secretion of cellulases was compared in concentrated conditioned culture media using SDS PAGE and western blot analyses, and surface display was confirmed by dot-blot analysis of *L. lactis* cells. Cellulolytic ability was confirmed on carboxymethylcellulose (CMC) agar plates with Congo red staining, and the ability of heterologous cellulases to bind crystalline cellulose was demonstrated by incubating conditioned medium with cellulose, followed by SDS-PAGE and western blot analysis.

We have successfully engineered *L. lactis* strains that express, secrete and display all three heterologous cellulases. The ability to degrade CMC varied among the three cellulases, and two of them were able to bind strongly to crystalline cellulose. This work provides a starting point for the development of genetically engineered *L. lactis* strains that can utilise plant waste for lactic acid production.

P2

Rewiring innate immune pathways by synthetic biology tools for the development of novel biosensors of microbial activity

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Inflammasomes are intracellular multiprotein complexes that form in response to various pathogenic and endogenous danger signals and execute pyroptosis, an inflammatory form of programmed cell death. CARD8 is a human sensor that upon activation with HIV protease or DPP8/9 inhibitors assembles an inflammasome through direct recruitment and activation of pro-caspase-1. Active caspase-1 activates proinflammatory cytokines and gasdermin D, which assembles pores in cellular membranes, leading to pyroptosis. CARD8 is composed of a disordered N-terminal tail, ZU5-UPA and CARD domains. Upon cleavage of the N-terminus by HIV protease, the N-terminus becomes destabilized and undergoes proteasome degradation, while the UPA-CARD fragment remains intact and forms filaments that facilitate the recruitment and activation of pro-caspase-1. We aimed to develop a viral detection biosensor by reformatting CARD8 to act as a biosensor of microbial activity independently of its function as a component of the inflammasome. We directly fused the CARD8 molecule with an enzymatic component (i.e. split luciferase system) that becomes functional only upon UPA-CARD filament formation, which leads to a restoration of enzyme activity that can be used as a readout. We first demonstrated the functionality of the system using HIV protease and through the incorporation of other viral protease cleavage sites illustrated the high adaptability of CARD8-based biosensor for the detection of other viral proteases. Unlike methods that rely on detecting a specific nucleic acid sequence and can thus fail to detect mutated viruses, our biosensor detects the activity of pathogen proteases, which are crucial for virus propagation. Such biosensors have the potential to be used for diagnostics and also for the development of antiviral drugs targeting viral proteases.

P3
Binding Gas Vesicles to the Surface of Mammalian Cells

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Gas vesicles are hollow protein structures found in bacterial and archaeal cells. They play a crucial role in providing buoyancy, enabling microorganisms to position themselves near the water's surface where nutrients and sunlight are abundant. These cylindrical structures have lengths ranging from 100 to 4000 nm and diameters ranging from 45 to 200 nm. They feature closed ends with two cone-shaped caps, creating an internal volume that is filled with gaseous phases from the surrounding cytosol. This unique property makes gas vesicles valuable in combination with MRI and ultrasound, as they provide contrast to the surrounding tissue.

Gas vesicles primarily consist of a single protein, either GvpA or GvpB. However, a cluster of genes encodes several other proteins that contribute to the formation of functional gas vesicles. In our research, we investigated the binding of various proteins from the *Bacillus megaterium* cluster to isolated gas vesicles using flow cytometry. Our findings revealed that GvpJ exhibits strong binding affinity to the vesicles, even in the presence of 6M urea.

To further expand the capabilities of gas vesicles, we introduced the accessory protein GvpC from the *Anabaena flos aquae* gas vesicle cluster to isolated vesicles originating in *Bacillus megaterium*. By attaching GvpC, we were able to confer integrin binding capability to the gas vesicles. Integrins are cell-specific molecules that allow us to target particular cells using specially designed RGD sequences. Consequently, we successfully bound gas vesicles to HEK293 cells, enhancing the effects of ultrasound stimulation on the cell membrane.

Overall, our research highlights the multifunctional nature of gas vesicles and their potential applications in biomedical and biotechnological fields. By understanding the binding properties and incorporating accessory proteins, we can harness the unique characteristics of gas vesicles for targeted cell manipulation and enhanced therapeutic approaches.

P4
Designed multi-chain polypeptide nanostructures stabilized by intein cyclization

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Non-natural self-assembling bio-nanostructures based on modular coiled-coils (CC) units can be built from scratch and tuned extensively, as the rules underlying the specificity of the CC dimer-forming peptides are well-understood. However, achieving a predicted fold from multiple polypeptide chains presents challenges. Here, we present a new strategy for assembling oligomeric CC-based cages by incorporation of cyclized polypeptide subunits achieved by the spontaneous self-splicing of split intein gp41. Cyclized chains in the complexes provide additional structural constraints for assembling the structure in the desired conformation.

To demonstrate the advantages of this strategy a 12 CC-segment coiled-coil protein origami (CCPO) cage that adopts a tetrahedral shape was decomposed into a heterodimeric complex. The two subunits were symmetric 6-segment long chains containing complementary peptides able to drive the assembly of chains through the formation of dimeric CC. However, the assembly with correct tetrahedral conformation (SAXS analysis) was achieved only when at least one of the subunits was cyclized. Next part of the study tackled the design of larger CCPO structures such as a 109 kDa trimeric cage, comprising 24 CC-forming segments. In this case, intein cyclization was required to facilitate the assembly of a *de novo* irregular octahedral protein.

In this work, we pushed the boundaries of CCPO-based multimeric assembly design and showcased the application of protein stabilization via split intein cyclization, which is an easy-to-use yet very powerful biochemical tool.

P5
MicroRNAs as biomarkers in spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a severe neurodegenerative disorder characterized by the degeneration of anterior motor neurons, leading to progressive muscle weakness and paralysis. In addition to the homozygous deletions in survival of motor neuron gene (*SMN1*), loss-of-function mutations or rarer point mutations leading to a deficiency of the survival motor neuron (SMN) protein are responsible for SMA.

Three medications (nusinersen, risdiplam, and onasemnogene abeparvovec) have been approved by the FDA and EMA to enhance SMN production in SMA patients. However, evaluating the effectiveness of therapies for SMA presents challenges in finding reliable measures. Circulating miRNAs are emerging as biomarkers that reflect disease progression as well as predict and monitor clinical response to treatments in various conditions, including SMA. Moreover, the SMN protein, which plays an important part in RNA processing, is most likely implicated in miRNA synthesis.

We performed a thorough literature search to gather information on miRNAs associated with the pathogenesis of SMA and their potential use as circulating biomarkers. Four muscle-specific miRNAs (myomiRs), namely hsa-miR-1-3p, hsa-miR-133a-3p, hsa-miR-133b, and hsa-miR-206, were underlined as potential biomarkers, as they were most frequently found elevated in SMA patients compared with controls and/or showed a decrease in their levels after nusinersen treatment. In addition, target genes of the identified myomiRs were found to play a role in neurological inflammatory processes. Further research is needed to validate myomiRs in a series of healthy controls and SMA patients before and after their treatment to confirm their suitability as potential SMA biomarkers.

P6
Genetic basis of erythrocytosis in Slovenia

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Erythrocytosis is a haematological disorder with a heterogeneous background. Patients who are negative for acquired causes and *JAK2* variants indicative for Polycythaemia Vera are additionally screened for variants involved in the hereditary form of the disease, Familial Erythrocytosis (ECYT). The low diagnosis rate suggests that additional genes contribute to the development of the disease.

As part of the Genetic Basis of Erythrocytosis in Slovenia (GenEry) project, we 1) updated the national diagnostic algorithm, 2) explored additional disease-causing molecular mechanisms, and 3) applied targeted next-generation sequencing (NGS) to 4) erythrocytosis patients identified in a retrospective study.

A three-stage diagnostic algorithm for erythrocytosis was established and 116 patients with non-clonal erythrocytosis were identified in the 8-year retrospective study, including 25 patients with no family history (Idiopathic Erythrocytosis, IE) and five families (1). A comprehensive review of the literature and various in silico tools were used to introduce a targeted NGS approach covering 39 genes associated with erythrocytosis and iron metabolism (2). The pathogenic variant *EPAS1* c.1609G > A (p.Gly537Arg), responsible for the development of congenital erythrocytosis type 4 (ECYT4), was discovered in one IE patient. In other patients, several variants of unknown significance (VUS) were identified, including in *EPAS1, EGLN1, JAK2, SH2B3* and *SEC23B*. A high proportion of patients with heterozygous *HFE* variants suggests a link between iron metabolism and erythrocytosis (3).

The effect of VUS variants on increased red blood cell production is currently being investigated. In families where the disease-causing variants have not been identified, a broader approach using WES or WGS is being used.

References:

1. Anžej Doma S et al, Ann Hematol. 2021; 100(8):1965-1973.
2. Kristan A et al, J Clin Lab Anal. 2021; 35(4):e23715.
3. Kristan A et al, Front Genet. 2021; 12:689868.

P7
Monitoring for phytopathogenic fungus Phyllosticta citricarpa in air and rain collected in European and Tunisian citrus orchards

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Phyllosticta citricarpa is a pathogenic quarantine fungus that causes citrus black spot disease, which destroys the fruits and shoots of citrus trees, so its spread in Europe is strictly controlled. It is most common in humid geographical areas, and all epidemiological information was obtained there. More than 600 samples from at-risk orchards were analyzed as part of the SMART-Surveillance project, and the suspected presence of the fungus in Europe was disproved. In 2019, its presence was confirmed in arid regions of Africa (Tunisia), and further studies are currently being conducted there. As part of the CBS Epidemiology project, we are collecting information on the impact of weather conditions and agricultural practices on spore dispersal and disease progression in Mediterranean climates. This will contribute to a better understanding of the epidemic and better preparedness in case of a possible outbreak in Europe. Airborne ascospores were assumed to be the main source of infection spread, while waterborne conidia were expected to play only a minor role. Sampling in Tunisia includes both collecting airborne particles on tapes as well as rain under citrus canopies. To identify spores in air and rain samples, we optimized the procedure for DNA isolation from fungal spores. We used a kit designed for isolation of difficult-to-lyse material with a high content of polysaccharides. The procedure was automated using a KingFisher™ Apex nucleic acid isolation instrument. The isolated DNA is tested with qPCR assays specific for *P. citricarpa*. To date, analysis of 113 samples has shown that the relative number of spores in the air varies over time. This demonstrated the importance of conidial dispersal in the airborne spread of infection, which was not previously the case and will have an important impact on understanding the course of the epidemic. With our colleagues from Spain (IVIA), we will try to find correlations between weather data and spore counts in samples.

P8

Adalimumab response negatively correlates with IL1B expression in macrophages differentiated from Crohn's disease patients' monocytes

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Research on colon biopsies and bulk peripheral blood immune cells has yielded promising personalized therapy biomarkers for Crohn's disease (CD) treated with anti-TNF therapeutics. However, recent studies indicate that investigating particular cell sub-types is required to develop better biomarkers that might be translated to clinical settings. Prior research has suggested that peripheral blood monocytes and macrophages are crucial for both CD pathogenesis and anti-TNF treatment response. In this study, we examine the relationship between inflammatory cytokine expression and monocyte-to-macrophage differentiation in CD patients, as well as (non)-response to adalimumab.

We extracted peripheral monocytes from CD patients responsive/non-responsive to adalimumab, differentiated them into macrophages, and then incubated those cells under induced inflammation and subsequent adalimumab therapy. We compared *in vitro* outcomes with the donor patients' clinical outcomes.

Our study shows a relationship between the expression of the inflammatory cytokines *TNF*, *IL1B*, *IL6* and *CXCL8* and the expression of two macrophage differentiation-related genes, *CD68* and *MMD*. Furthermore, adalimumab non-responders had higher levels of inflammatory cytokines in monocytes and *in vitro* differentiated macrophages than do responders, with *IL1B* expression having highest variance. Furthermore, we demonstrate a negative correlation between *IL1B* expression in CD patients' *in vitro* differentiated macrophages and their clinical response to adalimumab.

Our results suggest that *IL1B* macrophage expression could serve as a potential biomarker for adalimumab response in CD patients.

P9
IELs, their metabolic reprogramming and dependence on metabolites during during activation

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The metabolic properties of immune cells are tightly coordinated and can adapt to the demands imposed by the environmental compartment and pathogenic challenges. The intestinal epithelial barrier is home to one of the largest T cell populations in the body, recognized as intestinal intraepithelial T lymphocytes (IELs). Because of their location in an environment that is colonized by gut microbiota under the constant challenge by pathogens, dietary antigens, and toxins, IELs must have specific metabolic and functional adaptations that enable them to mount an appropriate immune response. One of the functional adaptations of IELs is that they contain high levels of granzymes and express activation markers, indicating their increased activation state already in poised state. We have shown that IELs are capable of a unique metabolic response to challenge; IEL activation is metabolically faster than activation of circulating CD8+ T cells. Glycolysis and oxidative phosphorylation (OXPHOS) of IELs are regulated in a mutually dependent manner, and depend on rapid access to metabolites from the environment. This metabolic characteristic is not observed with circulating CD8 T cells. Additionally we identified nutritional metabolites that sustain IELs metabolic transformation and enhance their ability to clear an intestinal pathogenic infection through secretion of interferon-gamma. Importantly all the metabolic properties of IELs that we identified, enable them to preform tightly regulated immune response within the fragile and diverse environment of the intestinal epithelial barrier.

P10

A dynamic interplay of cytosolic and lysosomal lipid droplet breakdown pathways supports the adaptation of cancer cells to nutrient stress

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Lipid droplets (LDs) are cytosolic fat storage organelles involved in various cellular stress responses. Starving cells typically employ cytosolic neutral lipases for LD breakdown, but nutrient deprivation also upregulates various forms of autophagy, including the selective breakdown of LDs via lipophagy. Here, we aimed to investigate the interplay between autophagy/lipophagy and the regulation of LD turnover in starving cancer cells. Our findings reveal that LDs are continuously formed and broken down during both acute, severe amino acid starvation and during chronic, but milder serum starvation. Autophagy was required for LD biogenesis mediated by diacylglycerol acyltransferase (DGAT) in amino acid-deprived cells. However, DGAT-driven LD accumulation occurred also after several days of serum starvation, but autophagy did not contribute to LD biogenesis under these conditions. Instead, autophagy/lipophagy facilitated LD breakdown and was active alongside lipolysis during prolonged serum starvation. Depletion of adipose triglyceride lipase (ATGL), which is responsible for the initiation of intracellular lipolysis, reduced LD breakdown, prevented fatty acid transfer from LDs to mitochondria, and impaired lipophagy. Suppression of autophagy/lipophagy by silencing the essential autophagy genes ULK1 or ATG5 during prolonged starvation resulted in a compensatory activation of ATGL-mediated lipolysis leading to LD depletion. These findings suggest a dominant role for ATGL in the regulation of LD turnover via lipolysis and lipophagy. However, even when both ATGL and autophagy/lipophagy were inhibited, cancer cells could still break down LDs through an autophagy-independent lysosomal mechanism mediated by lysosomal acid lipase. Overall, our findings demonstrate that a dynamic interplay between cytosolic lipolysis and both autophagy-dependent and autophagy-independent lysosomal LD breakdown mechanisms participate in the protection of cancer cells against nutrient stress.

P11
Macular Telangiectasia Type 2 (MacTel 2): retrospective analysis of Slovenian patients with overview of genetic background

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Macular telangiectasia type 2 (MacTel 2) is a slowly progressive bilateral retinal disease. Symptoms typically start in the fifth or sixth decade of life. It was originally thought to be a vascular disorder. However, novel imaging techniques have provided new insight into the pathogenesis of this condition, which appears to be primarily neurodegenerative with secondary vascular involvement. The prevalence of MacTel 2 is estimated to be 0.0045–0.1% [1,2]. Typical clinical findings include vascular abnormalities, loss of retinal transparency, redistribution of macular pigment, and thinning of the central macula. Vision loss is related to photoreceptor atrophy. A genetic predisposition to MacTel 2 has been identified in studies of the disease in monozygotic twins, siblings, and families. The cause of the disease remains unknown. The aim of our study was to retrospectively analyse the clinical features of Slovenian patients with MacTel 2 that have been managed at the Department of Ophthalmology, UMC Ljubljana in the past 5 years. Furthermore, previously published literature and data from databases were reviewed. Currently, the disease has been diagnosed in 49 patients. To date, two genome-wide association studies were conducted on 1067 patients [3]. These studies identified 22 single nucleotide variants (SNVs) overlapping 19 genes, 151 transcripts, and 8 regulatory features. The SNVs are associated with changes in retinal vascular diameter, implicated in the glycine/serine metabolic pathway, and lipid metabolism. Consistent with these findings, patients have significant deficiencies in blood serine and glycine, elevated alanine levels, and elevated deoxysphingolipids. In this study, we will elucidate the genetic background of patients with MacTel 2 to provide the basis for early diagnosis and personalised treatment.

References:
[1]Aung, K.Z. et al. *Retina*, 2010. 30:473–478
[2]Klein, R. et al. *Am J Ophthalmol*, 2010. 150:55–62
[3]Bonelli, R. et al. *Commun Biol*, 2021. 4:274

P12
Leukocyte telomere length dynamics in patients with Fabry disease

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Fabry disease is a rare lysosomal disorder caused by variants in the *GLA* gene. Due to the shorter life expectancy of Fabry patients, Fabry disease is thought to be associated with premature ageing. Telomeres are short, repeating TTAGGG sequences that cap the end of chromosomes. They play an important role in protecting chromosomes from end-to-end fusions and chromosomal instability. Shortening of telomere length (TL) is a well-known feature of cellular senescence and organismal aging. The aim of our study was to investigate the dynamics of TL and the impact of late complications (nephropathy, hypertrophic cardiomyopathy, and stroke) on TL shortening in Fabry patients.

We included 33 patients with genetically confirmed Fabry disease managed at the Centre for Fabry Disease in Slovenj Gradec. A total of 147 chronological samples collected over a 10-year period were included. gDNA was isolated from EDTA samples using a commercial kit. Monochrome multiplex quantitative PCR was used to determine the relative TL in leukocytes given as a ratio of the amount of DNA between the telomere and albumin as the housekeeping gene.

TL shortened significantly over time and shortening was significantly faster in patients with a longer baseline TL (both $p < 0.001$). On the other hand, TL shortening was not significantly altered by the presence of nephropathy, hypertrophic cardiomyopathy, or stroke. The number of late complications was also not significantly associated with TL shortening.

In the present longitudinal study, TL dynamics in leukocytes from Fabry patients was not influenced by the presence and number of late complications characteristic of Fabry disease.

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P13
Structural insight into the enzymes involved in the synthesis of the S-layer-anchoring polysaccharide ligand of *Clostridioides difficile*

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In the past, the extensive use of broad-spectrum antibiotics led to emergence of pathogenic strains resistant to multiple antibiotics. One example of such "superbug" is *Clostridioides difficile*, a spore-forming nosocomial pathogen. Its outermost surface layer (S-layer) is a 2D array of proteins, attached to the cell through the peptidoglycan-protruding secondary polysaccharides (PSII). Impairment of enzymes involved in *C. difficile* PSII synthesis is either lethal or leads to growth defects, diffused cell wall, changes in PSII anchoring, shedding and deposition, defects in S-layer morphology and assembly, and finally, changes in biofilm formation and virulence. Thus, we aimed to validate the proteins involved in the synthesis of PSII as potential novel targets for *C. difficile* specific infection treatment.

Using X-ray crystallography we determined the structure of one representative of each group of enzymes involved in the PSII synthesis: glycosyltransferases (Mg^{2+}-dependent 27760), mannose converting enzymes (alpha-D-phosphohexamutase Pgm2) and enzymes anchoring the PSII to the peptidoglycan (phosphotransferase LcpB). All three structures revealed domain organization similarities to their homologues and conserved cation/ligand binding regions, but the flexible loops-rich of the active sites require further structural elucidation. We complemented our studies via molecular simulations to assist in ligand design for possible inhibition and get insight in substrate interactions to elucidate the mechanisms of their enzymatic activity. Our results illuminate possible ways of specifically treating *C. difficile* infections, and are applicable to other Gram-positive bacteria in possession of homologous proteins.

P14
Protective and adverse effects of aripiprazole on the liver cells

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Aripiprazole is a second-generation antipsychotic with one of the fewest side effects. Despite this, severe aripiprazole hepatotoxicity can develop in persons with a history of cocaine and alcohol abuse, as it is metabolized in the liver. Therapeutically relevant aripiprazole concentrations, equal to plasma levels in patients' serum (laboratory alert levels), reduce the rate of liver cells' division [1]. Aripiprazole is moderately toxic when added to the liver cells in vitro. However, it upregulates genes and proteins associated with oxidative stress response and antioxidative enzyme activity thus having a protective role under oxidative stress conditions [2]. This may be one of the underlying mechanisms of aripiprazole's successful use in the clinic, as schizophrenia and the patients' lifestyle choices promote oxidative stress. Aripiprazole's liver toxicity can be an underlying mechanism of reported severe liver injury development in patients with a history of alcohol and cocaine abuse, as these hepatotoxic agents require the increased ability of liver self-regeneration. Monitoring liver functions is therefore important in patients when aripiprazole is co-prescribed or used with drugs with potential hepatotoxic effects.

1. Pirc Marolt T, Kramar B, Bulc Rozman K, Šuput D, Milisav I. Aripiprazole reduces liver cell division. PLoS One. 2020 Oct 26;15(10):e0240754. doi: 10.1371/journal.pone.0240754. PMID: 33104743
2. Kramar B, Pirc Marolt T, Monsalve M, Šuput D, Milisav I. Antipsychotic Drug Aripiprazole Protects Liver Cells from Oxidative Stress. Int J Mol Sci. 2022 Jul 27;23(15):8292. doi: 10.3390/ijms23158292. PMID: 35955425

P15
Abl-kinase mediated phosphorylation of FUS in FTLD

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Nuclear to cytoplasmic mislocalization and aggregation of multiple RNA-binding proteins (RBPs), including Fused in sarcoma (FUS), are the main neuropathological features of the majority of cases of amyotrophic lateral sclerosis (ALS) and frontotemporal lobular degeneration (FTLD). In ALS-FUS these aggregates arise from disease-associated mutations in FUS, whereas in FTLD-FUS the cytoplasmic inclusions do not contain mutant FUS, suggesting different molecular mechanisms of FUS pathogenesis. We have previously shown that phosphorylation of the C-terminal Tyr526 of FUS results in increased cytoplasmic retention of FUS due to impaired binding to Transportin 1 (TNPO1). In this study we developed a novel antibody against the C-terminally phosphorylated Tyr526 FUS ($FUS^{p\text{-}Y526}$) that is specifically capable of recognizing phosphorylated cytoplasmic FUS, which is poorly recognized by other commercially available FUS antibodies. Using this $FUS^{p\text{-}Y526}$ antibody, we demonstrated a FUS phosphorylation-specific effect on the cytoplasmic distribution of soluble and insoluble $FUS^{p\text{-}Y526}$ in various cells. In addition, we found that $FUS^{p\text{-}Y526}$ expression pattern corelates with active pSrc/pAbl kinases in specific brain regions of mice, indicating preferential involvement of cAbl in the cytoplasmic mislocalization of $FUS^{p\text{-}Y526}$ in cortical neurons. Finally, altered cytoplasmic distribution of $FUS^{p\text{-}Y526}$ was observed in cortical neurons of post-mortem frontal cortex tissue from FTLD patients compared with controls. Given the overlapping patterns of cAbl activity and $FUS^{p\text{-}Y526}$ distribution in cortical neurons, and cAbl induced sequestration of $FUS^{p\text{-}Y526}$ into G3BP1 positive granules in stressed cells, we propose that cAbl kinase is actively involved in mediating cytoplasmic mislocalization and promoting toxic aggregation of wild-type FUS in the brains of FTLD patients, as a novel putative underlying mechanism of FTLD-FUS pathophysiology.

P16
Interactome of FUS and FUSdNLS

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Neuronal degeneration has been recognized as a predominant driver of disability and disease progression in central nervous system diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Aggregation of RNA-binding proteins (RBPs) has been recognized as a hallmark pathological feature in these disorders, defining them as proteinopathies. Fused in sarcoma (FUS), normally a nucleus residing RBP, is known to aggregate into physiological granules and pathological inclusions, which can impair cell homeostasis leading to neuronal cell death. Mutations in FUS that alter its C-terminal nuclear localization signal (NLS) proved autosomal dominant in ALS and showed to disrupt its nucleo-cytoplasmic shuttling and increase its cytoplasmic localization. Since protein interactors of FUS and the exact signalling pathways involved in cytoplasmic toxicity of FUS remain unknown, we aimed to identify the interactomes of FUS and FUSdNLS (without NLS) proteins overexpressed in a model cell line using BioID2 proximity labelling. We prepared constructs of the FUS and FUSdNLS conjugated to the BioID2 enzyme by a flexible linker and transiently expressed them in HEK293T cells. The biotinylated proteins were analysed by mass spectrometry. Bioinformatic analyses of the proteomic data identified interaction candidates involved in RNA processing and degradation, protein translation and various signal transduction pathways. Selected interactions were validated by pull-down assays and cell co-localization analyses *in vitro.* These analyses showed that FUS interacts with NUDT21 and decreases its nuclear expression, whereas NUDT21 interaction with FUSdNLS is abolished, which may have downstream effects on 3‘RNA cleavage and polyadenylation processing. The interactome differences between FUS and FUSdNLS provide a detailed insight into the function of FUS that could be used for therapeutic interventions.

P17
Decoding the enigma: sex-specific differences in IC/BPS

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Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic urinary bladder inflammation characterized by increased urinary frequency and persistent pelvic pain. It predominately affects women, but the underlying mechanisms for this sex-specific occurrence remain unclear. To investigate these differences, a mouse model of IC/BPS was created using repetitive i.p. injections of cyclophosphamide (CYP). Twelve adult C57BL6/J mice of both sexes were divided into CYP-treated and Ctrl (NaCl-treated) groups. Urinary bladders were collected post-euthanasia and processed for total RNA isolation. mRNA sequencing was performed on the Illumina Novaseq platform and enrichment analysis of differentially expressed genes (DEGs) was conducted using clusterProfiler. Sex-specific differences in the enrichment of KEGG pathways pointed to cell-cycle processes in males and innate immunity processes in females, suggesting attenuated bladder tissue regeneration in females. GlycoMaple analysis of relevant DEGs uncovered sex-specific differences in mucin-type O-glycans biosynthesis, specifically in the expression of *Gcnt3*, which may contribute to the compromised urothelial barrier in females. Analysis of miRNAs revealed significant difference in the expression of miR-301a-3p between treated males and females. Among predicted targets of this miRNA, 51 genes were significantly deregulated either in males or in females. GO analysis showed significant enrichment of these genes in processes of nerve activity and signal transduction, which could potentially explain the disrupted signaling observed in the IC/BPS bladders of females. To validate the results of molecular-genetic analyses, various cell-biological and histological methods were employed. Our findings demonstrate significant sex-dependent disparities in the pathophysiology of the IC/BPS mouse model, which could potentially be translated to patients and aid in the development of more effective treatments in the future.

P18
Serum proteome profiles in children with severe asthma

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Patients with severe asthma and other allergic disease may be offered treatment with targeted biologics: anti-immunoglobulin E (omalizumab), anti-interleukin 5 (mepolizumab) or anti-IL-4 receptor α (dupilumab) antibodies. In children, there is little evidence that guides the choice of biologics treatment.

The PERMEABLE study is a multi-centre multi-omic collaboration recruiting children and adolescents with severe asthma that are currently receiving biological therapy. It aims to understand individual patient response to currently available biologic treatments in allergic diseases.

We analysed of the blood serum proteome in a cohort of 14 paediatric patients (age 9-17) treated with omalizumab, dupilumab or mepolizumab. Biosamples including blood serum and extensive clinical data were collected at the time of enrolment (T=0) and at two follow-up visits (T=3 months, 6 months). After abundant protein depletions, serum proteins were digested with trypsin, mass spectra for desalted peptides were acquired using data-dependent acquisition on Q Exactive Plus in duplicate, and proteins identified with Proteome Discoverer 2.4.

We quantified >500 proteins present in >75% of samples across 40 samples and compared protein abundances between patients receiving omalizumab, dupilumab or mepolizumab, correcting for patient age and sex. We investigated differences between patients on different therapies and changes in the proteome profile over time. Characterisation of the serum proteome could give new insight in patient response to biologic therapy.

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P19
Amyloid beta peptide interaction with profilin 1

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Profilin 1, a 15 kDa protein has a role in actin polymerisation, a mechanism involved in a large number of cell processes. As the central protein in cell cytoskeleton organisation and consecutive molecular/organelle transport, cell adhesion, cell migration and other process it is of vital importance in brain development and function. It is also important for correct myelination of peripheral nervous system. Thus, irregular expression and action of profilin 1 can lead to various neuronal pathologies. Amyloid b peptides, soluble and insoluble, are important players in the pathology of Alzheimer's and Parkinson's disease. Numerous mechanisms of their action have already been studied that show that amyloid b peptides have important physiological functions and that they become toxic when there is an imbalance between their production and degradation.

Neuroblastoma SH-SY5Y cells were treated with subtoxic concentrations of amyloid b peptide 1-42 in non-aggregated and aggregated forms. Amyloid b monomers and formation of fibrils were confirmed by transmission electron microscopy. qPCR showed changes in *PFN1* expression after cell treatment with fibrils and monomers. There were little or no changes in profilin 1 protein level in cells, however profilin 1 secretion from the cells was altered. The role of secreted profilin 1 is only now becoming a popular research topic; profilin 1, for example, was found down-regulated in a secretome of several types of cancer and its importance for cell migration was demonstrated. Thus, function of extracellular profilin 1 in brain tissue needs to be further investigated.

P20
Analysis of the inflammatory potential of alumina matrix ceramic composite wear particles on osteoarthritic patients immune cells

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The biological reactivity of metal implants has been extensively studied after patients undergoing total hip arthroplasty (THA) experienced adverse reactions to metal debris. Conversely, ceramic implants are known for their high wear resistance and excellent biocompatibility. This study aimed to investigate whether BIOLOX®delta particles trigger the local and systemic host defense mechanisms of osteoarthritic patients. CoCr and BIOLOX®delta alumina matrix composite (AMC) particles were generated by cryo-pulverization with a D(50) of 574nm and 238nm, respectively. Human primary were exposured to wear particles (CoCr, AMC) at different concentrations and for different time intervals (24, 48, 96h). The immune response was measured by ELISA and quantitative PCR. The control group was exposed to pure alumina powder (APA 0.5, Sasol). Our results indicated that AMC particles did not induce cytotoxic reactions in human macrophages or monocytic cell line even after 96h of incubation, confirming the biological safety of BIOLOX®delta ceramic. In the control group, the exposure to alumina powder did not cause any changes in cell viability as measured by MTS cell proliferation assay. Treatment of THP-1 macrophages with CoCr (50 µm3/cell) particles for 48h resulted in a 3-fold ($p=0.02$) increase in TNF-α level, whereas treatment of THP-1 macrophages with AMC particles did not cause significant changes in the level of excreted cytokines TNF-α and IL-6. When human primary macrophages were exposed to alumina and AMC particles for 24, 48 and 96h, no increase in TNF-α and IL-8 level was detected. On the contrary, CoCr particles caused elevated levels of endogenous IL-8. In conclusion, our study confirms that BIOLOX®delta particles, in clinically relevant concentrations, are not cytotoxic. Moreover, we demonstrate the low inflammatory potential of BIOLOX®delta particles in vitro.

P21
Low-activity MTHFR elicits higher susceptibility to methotrexate, trimethoprim and sulfasalazine

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Adequate folate levels are vital for proper cellular growth and proliferation. Many commonly prescribed medicines act or exhibit side effects due to their effect on the folate cycle. The increased use of medicines reported in pregnant women and in those of child-bearing age could lead to more frequent disturbances in folate homeostasis and have detrimental consequences for the developing fetus. We investigated the effect of methotrexate, trimethoprim and sulfasalazine as antifolates on proliferation mechanisms using *in vitro* cell models. We measured metabolic activity and analysed cell cycle characteristics in the neuroblastoma cell line SH-SY5Y and lymphoblastoid cell lines (LCLs) of 20 different individuals. We found that the investigated antifolates lower relative metabolic activity on LCL ($MA_{25\ nM\ MTX}$ = 30.9% ± 3,9%; $MA_{500\ \mu M\ SSZ}$ = 36.5% ± 5,7%) and SH-SY5Y ($IC50_{MTX}$ = 87.4 nM) cells. Methotrexate also arrested cells in the S phase of the cell cycle up to 9.9% ± 2.6%. Lowered metabolic activity was accompanied by induced apoptosis of cells. Furthermore, antifolate activity was even more pronounced when the cells were cultured in folate-deprived media, especially in the case of trimethoprim. Upon folate depletion, metabolic activity in LCLs and SH-SY5Y cells treated with 50 µM TMP decreased by 59.8% and 22.2%, respectively. Using TaqMan genotyping we also determined the most common genetic polymorphisms of tested LCLs in genes encoding for key enzymes of the folate cycle. In our panel of LCLs, we also observed a significant impact of genetic polymorphisms in *MTHFR*, namely *MTHFR* 677C>T and *MTHFR* 1298 A>C eliciting higher cytotoxicity of methotrexate (p=0.049) and trimethoprim (p=0.0098). Our results demonstrate a general mode of action by which antifolates affect cellular growth and proliferation, indicating the significance of dietary folate supplementation during and before pregnancy.

P22
HMEC-1 cell line as a model to study microvascular endothelial cell abnormalities associated with hemophilia

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Clinical evidence suggests possible alteration or functional differences of microvascular endothelium (ME) in patients with hemophilia, presumably due to ME interactions with factors of the coagulation cascade. As a model system for this issue, we use the HMEC-1 microvascular cell line cultivated in perfusion. MCDB culture medium is enriched with human plasma isolated from whole heparinized blood of healthy donors, to be compared with plasma from hemophilia patients in the next steps. At the starting point, we focused on studying the basic characteristics of HMEC-1 microvascular cells in response to various shear stress levels. HMEC-1 cells differ in many aspects from the more frequently used macrovascular cell lines or macrovascular primary cells.

Confocal microscopy and microimpedance measurement were used to monitor the cell behavior, morphological changes and actin cytoskeleton rearrangement under static or flow conditions. In our experiments, we show visible changes in the organization of F-actin filaments in response to unidirectional flow. However, in contrast to many macrovascular cells, HMEC-1 cells do not show any sign of orientation in the flow direction. We also observe significant changes in intercellular interactions following cell treatment with plasma.

P23
Impairment of cystatin F activation can modulate the cytotoxicity of NK cells

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Natural Killer (NK) cells are innate immune effectors that use the granzyme perforin pathway as the primary mechanism to target and kill cancer cells. This process relies on the activation of granzymes and perforin by cathepsins C, H, and L. However, the effectiveness of NK cells can be compromised by immunosuppressive factors present in the tumor microenvironment, such as cystatin F, which inhibits the activity of cathepsins C, H, and L. Cystatin F activity is regulated by various factors, including expression levels, N-glycosylation, and proteolytic activation. In lysosomes, cathepsin V cleaves 15 N-terminal amino acids from cystatin F, thereby activating it from an inactive dimeric form to an active monomer. Cystatin F was found expressed in glioblastoma tumor tissue. The aim of this study was to assess NK cell function in glioblastoma patients and to enhance NK cell cytotoxicity by inhibiting cystatin F activation. Cytotoxicity of healthy donor NK cells was higher than that of patient-derived NK cells. However, even healthy NK cells were susceptible to the effects of cystatin F. Recombinant cystatin F reduced cytotoxicity, increased IFN-γ secretion, and decreased cathepsin C and granzyme B activity. To counteract the effects of cystatin F on NK cell cytotoxicity, a new small molecular inhibitor of cathepsin V was developed. After molecular docking of small molecular compounds from commercial libraries with cathepsin V, selected compounds were evaluated by enzyme kinetics for enzyme inhibition, selectivity, and reversibility of binding. The effect of the most potent, selective, and reversible-acting cathepsin V inhibitor on cystatin F activation was tested by western blot. Cathepsin V inhibition decreased the conversion of cystatin F from dimer to active monomer form in NK cells and increased cytotoxicity against glioblastoma stem cells. In conclusion, by targeting the activating peptidase of cystatin F, we can increase the cytotoxic potential of NK cells.

P24

Brain-Derived Neurotrophic Factor DNA Methylation and Expression in Alzheimer's Disease and Mild Cognitive Impairment

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Due to the increasing number of progressive dementias in the population, numerous studies are being conducted that seek to determine risk factors, biomarkers and pathological mechanisms that could help to differentiate between normal symptoms of aging, mild cognitive impairment (MCI) and dementia. Among dementias, Alzheimer's disease (AD) is the most common form. This progressive neurodegenerative disease is characterized by memory loss and cognitive dysfunction. Of all genetic biomarkers, only apolipoprotein E is being used for predicting the possibility of the development of AD. However, based on its function, BDNF could be an interesting candidate gene. It belongs to the family of proteins that promote neuronal survival, development and function. It is involved in neurogenesis, neurotransmission, proliferation, regeneration, the promotion of synaptic growth and the modulation of synaptic plasticity, and it is essential in the survival and function of hippocampal, cortical, cholinergic and dopaminergic neurons.

The aim of this study was to investigate the possible association of levels of *BDNF* and *COMT* gene expression and methylation in peripheral blood cells with the development of Alzheimer's disease (AD). Our results revealed higher expression levels of *BDNF* ($p < 0.001$) in MCI subjects compared to individuals diagnosed with AD. However, no difference in *COMT* gene expression ($p = 0.366$) was detected. DNA methylation of the CpG islands and other sequences with potential effects on gene expression regulation revealed just one region (BDNF_9) in the *BDNF* gene ($p = 0.078$) with marginally lower levels of methylation in the AD compared to MCI subjects. Here, we show that the level of *BDNF* expression in the periphery is decreased in subjects with AD compared to individuals with MCI. The combined results from the gene expression analysis and DNA methylation analysis point to the potential of *BDNF* as a marker that could help distinguish between MCI and AD patients.

P25
Evaluating the Role of Cathepsin X and Its Target γ-Enolase in Animal Model of Autoimmune Inflammatory Diseases

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Cathepsin X, a lysosomal peptidase, is primarily expressed in immune cells but is also found in brain cells and plays a role in neuroinflammatory processes that are closely implicated in the pathogenesis of several neurodegenerative and autoimmune disorders. Cathepsin X proteolytic activity in neuronal cells was found to cleave the C-terminal amino acids of γ-enolase, leading to the loss of its neurotrophic activity. However, the expression patterns and functional roles of cathepsin X and its target γ-enolase in inflammatory brain pathology have not yet been fully elucidated. In this study, we aimed to evaluate the role of cathepsin X and its regulation of γ-enolase in inflammatory processes by studying their changes in the expression and activity in the rat model of experimental autoimmune encephalomyelitis (EAE). We used immunofluorescent staining to assess the presence of inflammatory processes in the injured peripheral nerve with specific markers of inflammatory processes. Cathepsin X upregulated expression was observed in the peripheral nerve, localized in the inflammatory cell type (M1 macrophages) and co-localized with γ-enolase. At the peak of the disease, a significant increase in the expression and activity of cathepsin X was observed in the spinal cord. There was also a higher expression of γ-enolase compared to the control. Moreover, it was shown that the administration of B-complex vitamins, known for their anti-inflammatory properties, resulted in a reduction of cathepsin X activity at the peak and the end phase of the disease. Additionally, this treatment also altered the expression levels of cathepsin X and γ-enolase. Overall, our findings suggest that cathepsin X is involved in inflammation-related degenerative processes by regulating γ-enolase. Therefore, investigating their role is essential in developing new treatments for inflammatory-related disorders.

P26
Sequestration of hnRNPH in nuclear G4C2 foci and cytoplasmic stress granules of C9orf72 amyotrophic lateral sclerosis

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The most common genetic cause of ALS is hexanucleotide G4C2 repeat expansion in the first intron of C9orf72. One of the hallmarks is the formation of RNA foci in the nucleus, G_4C_2 foci, which contain aberrant repeat transcripts and sequester a variety of RNA-binding proteins (RBP). hnRNPH is a member of a large protein family of RBPs involved in the regulation of alternative splicing, mRNA stabilization, transcription, and translation. In many neurodegenerative diseases, including ALS, increased oxidative stress characterised by the formation of stress granules (SG) is a concomitant pathological factor. In ALS brain tissue, hnRNPH colocalizes with nuclear RNA G_4C_2 foci, whereas under cellular stress conditions, it is localized in cytoplasmic stress granules. Sequestration of hnRNPH in insoluble RNA aggregates correlates with dysregulation of splicing and may contribute to neurodegeneration. Our goal was to reveal the domains of hnRNPH that determine its localization in G_4C_2 foci and stress granules. Nuclear foci share a group of interacting proteins with stress granules and their simultaneous presence in ALS neurons could have further pathological implications. We designed a series of hnRNPH1 protein constructs based on its domain structure and introduced mutations into individual qRRM domains to disable their RNA-binding activity. Quasi (q)RRM2 and qRRM3, but not qRRM1, were sufficient for localization of hnRNPH in stress granules. Localization of hnRNPH in G_4C_2 foci was independent of the RNA-binding activity of any individual qRRM domain. Using RBDmap, we demonstrated that the putative ZnF domain of hnRNPH may have RNA-binding activity. Surprisingly, hnRNPH protein localized to G_4C_2 foci even after the removal of the RNA-binding activity of the qRRM and ZnF domains. This result suggest that RNA-binding activity may not be the only driving force for the sequestration of hnRNPH into the G_4C_2 foci associated with C9orf72 ALS.

P27
Exploring the Influence of Genetic Variability in Inflammatory Pathways on the Severity and Short-Term Consequences of COVID-19

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Background: COVID-19 patients often experience an excessive immune response known as a "cytokine storm," characterized by the overproduction of pro-inflammatory cytokines (IL-6, IL-12, IFN-γ) and chemokines (CXCL10, CCL2). This hyperactive immune reaction is associated with a more severe disease progression and an increased risk of fatal outcomes. Identifying genetic markers that can rapidly predict which COVID-19 patients are at higher risk of developing a severe course and experiencing adverse outcomes is crucial. Therefore, our aim was to investigate the potential associations between common functional *IL1B* and *IL6* polymorphisms and disease severity, hospitalization duration, the need for oxygen therapy, and requirement for intensive care unit (ICU) treatment.

Methods: We enrolled 175 hospitalized COVID-19 patients from whom we obtained peripheral blood samples for DNA extraction. All patients were genotyped for *IL1B* (rs1143623, rs16944, rs1071676) and *IL6* (rs1800795) polymorphisms. Statistical analyses included χ2/Fisher's test, Mann-Whitney test, and logistic regression.

Results: The study group comprised 66.3% males and 33.7% females, with a median age of 56.8 years (range: 41–67). The majority of patients (66.1%) presented with severe disease, while 19.9% had critical, 11.7% had moderate, and 2.3% had mild COVID-19. None of the investigated polymorphisms showed significant associations with disease severity, hospitalization duration, type and duration of oxygen therapy, or the need for ICU treatment.

Conclusion: This preliminary study does not indicate an association between *IL1B* and *IL-6* polymorphisms and COVID-19 severity or short-term outcomes. Future investigations should encompass a broader range of genes involved in inflammatory pathways to gain a more comprehensive understanding.

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P28
Vitamin A-enriched diet affects gene expression of inflammatory and retinoic acid signalling pathways in a mouse model of cystitis

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Introduction: Vitamin A and retinoids affect the expression of more than 500 genes *via* the retinoic acid signalling pathway and also have anti-inflammatory properties. However, their effects on urinary bladder inflammation (cystitis) have not yet been studied. One of the most commonly used animal models of cystitis is a mouse model of acute cyclophosphamide (CP) - induced cystitis, in which the urothelium is damaged and the urinary bladder wall is inflamed. The aim of our study was to investigate the effects of a vitamin A-enriched diet on the expression of inflammation-related genes (*CCL8, IL33, IL13ra2* and *VEGFD*) and genes that are part of the retinoic acid signalling pathway (*LRAT, RXRα* and *ALDH1a1*) in a mouse model of cystitis.

Methods: Thirty female 8 weeks old BALB/c mice were used (permit number U34401-4/2022/10). All mice had *ad libitum* access to drinking water and food. One week prior to the single intraperitoneal injection of CP (150 mg/kg) or saline, the diet of 15 animals was changed from normal diet to vitamin A-enriched diet. Animals were euthanised 1 day and 3 days after administration of CP and 1 day after administration of saline solution. Bladder samples were collected and prepared for qPCR.

Results: Our results showed that genes associated with inflammation (*CCL8, IL33, IL13ra2, VEGFD*) and *ALDH1a1* were upregulated by administration of CP, whereas vitamin A-enriched diet 3 days after administration of CP resulted in a minor downregulation of these genes. Administration of CP also led to downregulation of *LRAT*, which was slightly reduced by the vitamin A-enriched diet. Due to the large standard deviations within the groups, we were not able to detect statistically significant differences.

Conclusions: Our results show that administration of CP affects the expression of selected genes of inflammatory and retinoic acid signalling pathway, whereas a vitamin A-enriched diet has no significant effect on the acute phase of cystitis.

P29
Endophytic bacteria isolated from sterile potato plants: A genomic approach to characterization

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Endophytes encompass microbes, which for all or part of their lifetime colonize the internal of plant tissues. They can be commensals with no apparent effect on their host, beneficial plant growth-promoting microbes or also latent pathogens. Beneficial endophytes can directly promote plant growth through nitrogen fixation, synthesis of enzymes or peptides that provide nutrients, or by the production of phytohormones. On the other hand, they can protect the plant either directly, by antibiosis or competition for nutrients with the pathogens, or indirectly, by priming the plant's immune response.

We isolated two bacteria, from tissue culture cultivated potato plants propagated in a sterile environment for several years without any visible signs of infection. Thus, it is reasonable to assume that they are endophytes tightly associated with potato plants. To precisely determine their phylogeny and get insight into their potential functions, we sequenced their genomes by a combination of Nanopore and Illumina sequencing. Most contiguous genomes were obtained using *trycycler*, by combining the output of several long-read assemblers followed by Illumina reads polishing. Taxonomic analysis suggests that one of the isolates belongs to a novel species. We will present its gene repertoire, comparison to phylogenetically most similar bacteria and biochemical determination of some metabolic functions. Our assembled genomes facilitate hypothesis-driven functional characterisation of the isolated endophytes and the understanding of their interactions with the host plant.

P30
RSAD2 is a potential predictor of response to omalizumab in paediatric asthma

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Biologics are increasingly being considered for the treatment of children with corticosteroid-resistant asthma. However, a multitude of available therapies and lack of guidelines to prescribe them to the children call for reliable biomarkers to help select the optimal therapy.

Here we used a functional *ex vivo* approach to identify potential biomarkers associated with efficacy of omalizumab, an anti-IgE biological, in paediatric asthma patients. Peripheral blood immune cells from children with moderate to severe asthma, naïve to omalizumab therapy, were isolated and cultured in the presence of patient's own serum for 24 hours with/without omalizumab. Then, an asthma attack was simulated *in vitro* using patient-specific allergens and Basophil activation test performed. Based on the test results, the patients were classified as good (Re) or poor/non- responders (P/NR) to omalizumab. In parallel, the *in vitro* cultured basophils from the same patients were sorted and used for RNA analysis. A whole transcriptome analysis (RNA-seq) was performed on samples from 3 Re and 3 P/NR, identifying *OAS3, HLA-C, EPSTI1, HERC5, CMPK2, RSAD2, HBB, HBA1, and HBA2* as potential biomarkers of (non)response to omalizumab. Gene ontology (GO) analysis revealed Response to viruses, Regulation of the extrinsic apoptotic pathway, Cellular response to type I interferon, Regulation of cytokine-mediated signalling as signalling pathways involved in the (non)response to omalizumab. Based on the RNaseq data and GO analysis, *OAS3, HERC5*, and *RSAD2*, all decreased in P/NR patients, were selected for qPCR confirmation on 21 additional samples. Only *RSAD2* expression showed a trend towards a decrease in the basophils of the P/NR patients and negatively correlated to the *in vitro* response to omalizumab, confirming the RNA-seq results.

By combining the *in vitro* cell model and cell-specific transcriptomics we highlight *RSAD2* expression as a potential biomarker of omalizumab (non)response in paediatric asthma.

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P31
The role of Hnrnph1 decoy splice site in regulating gene expression

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Regulatory role of deep intronic space is still largely unknown. One of such regulatory elements are decoy splice sites. They are described as cryptic splice sites that compete with the canonical splice sites for spliceosomal recruitment and interfere with catalysis of splicing. Outcome of this interference can be exon skipping, exon inclusion or intron retention. Unpublished data from the Ule lab shows that they are present in a much greater number, suggesting a wider role in regulation of gene expression.

In the Ule lab they used iCLIP of spliceosomal component proteins SmB and PRPF8 to identify decoy splice sites in high throughput manner. Making spliceosomal iCLIP the method for valid identification of decoy splice sites. It also provides us with additional information of the stage of spliceosomal assembly.

Analysis of spliceosomal iCLIP results has identified *Hnrnph1* to have a strong binding of both spliceosomal components. Complementary RNA-seq data has shown retention of that specific intron. The hnRNPH1 protein is a splicing factor and it has a major role in splicing regulation. Making it a good case study example to better understand the workings of decoy splice site and its involvement in the potential regulatory network of splicing factors via intron retention.
Using mESC and CRISPR/Cas9 genome editing system we generated cell line where the identified *Hnrnph1* decoy splice site is deleted from the genome. Were decoy site was deleted out we detected a big reduction in intron retention isoform, and an increase in correctly spliced isoform which resulted in a higher level of protein expression. We also saw that the decoy splice site deletion in *Hnrnph1* appears to affect the splicing and expression of other splicing factors. This hints at a possibility that a network of splicing factors could be co-regulated via mutual cross-regulation of intronic decoys that control their intron retention.

P32

PeptideVisualizer: A novel software solution for PROTOMAP-based determination of protease substrates

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Background: Proteolysis is an irreversible process where proteins undergo proteolytic cleavage to break them into smaller fragments. One of the most important protein-centric approaches for the identification of protease substrates is PROTOMAP, developed by the Cravatt group in 2008. This proteomic technique detects proteolysis through differential migration rates of proteins due to changes in molecular weight on a gel electrophoresis.

Aim: In this work, we aimed to provide a new software solution and several improvements to the original PROTOMAP approach. The tool should enable data input from free and broadly used MaxQuant software. additionally, the PROTOMAP analysis should be upgraded to give quantitative instead of semi-quantitative information, visualization of protein features, and provide a mismatch factor to quickly detect proteolysis.

Results: We present the PeptideVisualizer – an open-source cross-platform offline software solution for PROTOMAP-based proteomics. The PeptideVisualizer is a user-friendly Python 3 script with a graphical user interface and backward compatibility for command-line use. The script takes output from MaxQuant, the user is then required to group detected experiments, set the number of replicates and optionally adjust other parameters. The LFQ intensities visualized by the script, instead of semi-quantitative MS counts, provide information on whether a protein is up- or down-regulated. Furthermore, if more replicates are provided, the missing data is imputed and volcano plots are drawn to detect statistically significant changes in protein abundance. Using protein features obtained from UniProt database, the script visualizes known molecular processing, regions and secondary structure information. Finally, we present a reliable mismatch factor to quickly detect proteolysis substrates between two conditions making it a valuable tool for researchers working with PROTOMAP data.

P33
Assessing the potential of cholesterol-related sterols in predicting the progression of COVID-19 disease

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As COVID-19 becomes endemic, the search for biomarkers to characterize the disease and aid in patient stratification remains important. Herein, we examined the role of lipid metabolism in disease progression and investigated, whether cholesterol-related sterols could improve prediction of COVID-19 disease severity.

We collected data from 165 patients admitted to the Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, between July 2020 and July 2021. We assessed the concentrations of 10 sterol intermediates at three different time points during hospitalization: at admission, at disease worsening or in the middle of the disease, and at discharge. Changes in 10 sterol intermediates from the Bloch and Kandutsch-Russell (K-R) pathways were measured during the course of the disease. In addition, we combined the sterol measurements with clinical parameters collected at patient admission to the hospital to develop a machine learning based prediction of disease severity.

Our study revealed statistically significant differences in sterol concentrations during the course of the disease for most representatives of Bloch pathway, including T-MAS, zymosterol, 24-dehydrolathosterol, and desmosterol, as well as for zymostenol from the K-R pathway. Our machine learning models showed that eight clinical features were sufficient to predict disease severity with high accuracy (AUC = 0.96), which is an improvement over currently used clinical risk scores. In addition, the performance of the model remained excellent even when sterol measurements were included, with AUC = 0.95.

To our knowledge, this is the first study to examine changes in sterol intermediates over the course of COVID-19. Additionally, our computational models demonstrate a promising prognostic tool for stratifying patients based on only a handful of readily accessible clinical features, with cholesterol-related sterols showing moderate performance, compared with other clinical risk scores.

P34
Variant in the EGLN1 gene has a potential impact on the development of erythrocytosis

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Erythrocytosis is a blood condition with increased number of erythrocytes, which could lead to severe thromboembolic events. In rare cases, erythrocytosis appear due to variants in genes involved in erythropoiesis and oxygen homeostasis regulation, but majority of the patients remain undiagnosed. Identifying the specific cause of erythrocytosis is the only way to enable proper treatment. Next-generation sequencing (NGS) allows high diagnostic yield, but also increases the number of identified variants with unknown significance (VUS). VUSs are usually problematic for clinical interpretation, however functional studies can elucidate their role in the development of disease. In our previous study we applied targeted NGS on a national cohort of patients with symptoms of erythrocytosis, to identify variants with potential clinical impact (1, 2).

The aim of the present study is to functionally assessed selected variants with unknown significance by localization on 3D protein model, protein expression analysis and luciferase reporter assay.

With targeted NGS, we detected four variants in the *EGLN1*, *EPAS1*, *JAK2* and *SH2B3* genes, that were classified as VUS (3). Variant in the *EGLN1* gene was identified with strong co-segregation with the disease in multiple family members. Protein expression analysis showed that *EGLN1* variant have an effect on decreased protein accumulation. Variant was positioned in an active site of the EGLN1 hydroxylase and amino acid substitution could have an effect on enzyme activity. However, luciferase reporter assay did not confirm the effect of variant on impaired EGLN1 activity.

Further functional assessment of VUS in the *EGLN1* gene is necessary to resolve the role of variant in the development of erythrocytosis.

References:
1. Anžej Doma S et. al., Ann Hematol. 2021; 100(8):1965-1973.
2. Kristan A et. al., J Clin Lab Anal. 2021; 35(4): e23715.
3. Kristan A et. al., Front Genet. 2021; 12:689868.

P35
Transcriptomics of plaque lesions at different anatomical locations obtained by non-invasive skin tape-stripping in psoriatic patients

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Plaque psoriasis is skin disease, defined by sharply demarcated plaques, which occur in different anatomical locations. Although, biological agents are efficient and effective, some lesions are more resistant to treatment. Evidence have shown differences in treatment period between lesions, where scalp and palmoplantar are more resistant compared to lesions on limbs and trunk. Therefore, we hypothesize that different molecular mechanisms between anatomical lesions are present.

Invasive skin biopsy is the gold standard for obtaining RNA samples. Therefore, non-invasive approaches, such as tape-stripping, are in demand. Herein, we aimed to optimize the number of tape-strips needed to obtain sufficient amount of RNA. Afterward, we performed RNA-seq analysis on three different lesions sites of a well-characterized psoriatic patient.

Our results will serve as a framework for future RNA-seq experiments for multiple locations of plaque psoriasis. A major advantage of our protocol is its non-invasive nature, enabled by the use of tape strips. Different transcriptome profiles of each sampled plaque location could provide new insight in the biological pathways of psoriasis and biomarkers for personalized treatment.

P36
Link Prediction on Comprehensive Knowledge Graph

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Network analysis can give insights about complex biological systems such as the mechanisms underlying the interaction between plants and their pathogens. This can be helpful for developing novel crop breeding strategies for sensitive plants like potato (Solanum tuberosum). This work presents a machine learning method on graphs for inferring continuous numeric values that can validate interactions between entities (genes, transcripts, proteins, small RNAs) in an Arabidopsis thaliana comprehensive knowledge graph (CKN; [1]), which represents a set of potential binary interactions between the network constituents, such as binding or transcription factor regulation.

The graph structured data (CKN) was obtained through a collaboration with the National Institute of Biology (NIB) and was the basis for the construction of a directional graph with multiple edge types with a total of 26350 nodes and 909857 edges. Highly reliable interactions are based on targeted experimentally confirmed connections while a large proportion of connections were experimentally shown using high-throughput experimental techniques. The goal was to calculate reliability estimations for the interactions and represent them as weights on the edges of the CKN.

The machine learning (ML) task has been framed as link prediction on a graph, a supervised learning problem that uses node representations (embeddings) that were computed with unsupervised representation learning using the node2vec algorithm [2]. Binary classifiers have then been trained to predict whether there is an edge between any two nodes in the graph and the best performing one selected (logistic regression with binary operator_l2) and evaluated on unseen data to see how it generalizes (ROC AUC 0.97). Its prediction probabilities have been added to the graph as edge weights. The calculated node and edge embeddings of the CKN open up various options for further exploratory analyses, e.g. calculating similar nodes and edges in the CKN.

P37
Sleep diary outperforms actimetry and gene expression analysis from blood plasma in evaluating obstructive sleep apnea

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Introduction. Obstructive sleep apnea (OSA) is a breathing disorder where the airway is partially or completely obstructed during sleep. OSA incidence is increasing and is associated with cardiovascular and metabolic disorders. Recent research suggests circadian dysregulation's role in OSA and its comorbidities development. Current diagnostic methods include polysomnography or respiratory polygraphy, with additional actimetry and sleep diary as supportive methods. Nevertheless, stratification of patients based on biochemical markers is necessary. We aimed to assess circadian gene expression in OSA patients' blood plasma and compare their sleep and activity rhythms with healthy controls.

Methods. The study included 25 adult participants with suspected OSA at the Clinical Institute of Clinical Neurophysiology, University Medical Centre in Ljubljana. OSA risk was assessed using STOP-Bang and Epworth questionnaires, and chronotype with Morningness-Eveningness Questionnaire. For the assessment of sleep and activity rhythms, participants underwent 14-day actimetry monitoring coupled with a sleep diary. Based on the results of overnight polygraphy 20 participants were assigned to the patient group and 5 to the control group. Peripheral venous blood was collected at 13.00, 19.00, 1.00 and 7.00. The core circadian clock genes expression (*PER1,2*, *BMAL1, CRY2*) was measured by q-RT-PCR from RNAs isolated from blood plasma.

Results. We discovered that sleep diary was more effective than actimetry in OSA diagnosis. The blood plasma was tested as a source of RNA for monitoring circadian gene expression for the first time. However, the method requires further optimization as no statistically significant circadian RNA markers were identified in the plasma of OSA patients. Circadian gene expression analysis was suboptimal due to size of samples, therefore new samples are being collected to increase the statistical power.

P38
Nanopore sequencing enables phasing of a tetraploid potato locus with tandemly repeated TGA genes

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TGA transcription factors, belonging to the basic region leucine zipper (bZIP) protein family, have been extensively studied in Arabidopsis. In this model plant, ten TGAs (AtTGAs) are categorized into five clades. Notably, Clade II AtTGAs play a crucial role as regulators in the salicylic acid-mediated defence response following pathogen infection. These transcription factors co-regulate the expression of key defence-related genes involved in salicylic acid synthesis and signalling by interaction with NPR (NON-EXPRESSOR OF PR) cofactors, including the major regulator NPR1, and are also involved in jasmonic acid and ethylene-mediated signalling pathways.

In potato, we identified a clade II TGA transcription factor, StTGA2.1, which possesses unique characteristics compared to the AtTGAs. Specifically, StTGA2.1 lacks most of the bZIP DNA-binding domain and has a shorter N-terminus [1]. This gene is located on chromosome 10 within a genomic region containing three tandemly repeated TGAs exhibiting high sequence similarity. In the DM potato genome, this region spans approximately 35 kbp. To confirm the consistency of this genomic structure in the tetraploid cultivar Rywal, we initially employed Illumina targeted locus amplification sequencing (TLA-Seq). However, due to low coverage and ambiguous short read mappings, we could not resolve the structure of the region using this strategy. Consequently, we employed targeted enrichment of the genomic region using the Xdrop workflow in conjunction with Nanopore sequencing. By mapping Nanopore reads to the reference genome and performing polyploid allele phasing, we successfully resolved the structure of the region. Through this approach, we unveiled the allelic variability of this genomic locus.

References:
1. Tomaž Š., *et al.* (2023) Plant physiology 191(3), pp. 1934-52.

P39
Importance of PVY coat protein in plant virus movement

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Potato virus Y (PVY) is a devastating plant virus affecting potato production worldwide. Understanding the interaction between the plant and the virus is critical for developing sustainable and efficient crop protection strategies. We are studying the movement of the virus from cell to cell and aim at identifying sites in the viral coat protein (CP) that are essential for this process.

Our current work is based on results published by Kežar et al. (2019). There we showed that the C-terminal region of CP is essential for the establishment of efficient viral replication. In contrast, deletion of 50 N-terminal amino acids from CP seems to hinder the ability of the virus to move from cell to cell.

We introduced short deletions at the CP N-terminus of a PVY clone labelled with GFP (Lukan et al. 2023). Deletions dN50, dN40, dN30, dN20 and dN10 with different cleavage sites for proteases and +/- one to three amino acids were prepared using PCR site-directed mutagenesis. We infected *Nicotiana clevelandii* with the prepared PVY clones by biolistic bombardment. For each construct, we observed symptom development, virus assembly by negative-stain transmission electron microscope (TEM) and cell-to-cell movement ability by confocal imaging of bombarded leaves. We are also imaging fluorescence signal of the entire plant to follow systemic spread of the virus.

So far, we have confirmed that N50 and N40 deletions of CP still allow the virus to assemble, but they affect its ability to move from cell to cell in the bombarded leaves, as the mutated virus was only observed in single cells.

References

Kežar A, Kavčič L, Polák M, Nováček J, Gutiérrez-Aguirre I, Žnidarič MT, Coll A, Stare K, Gruden K, Ravnikar M, et al. 2019. Sci Adv 5: eaaw3808.
Lukan T, Županič A, Mahkovec Povalej T, Brunkard JO, Kmetič M, Juteršek M, Baebler Š, Gruden K. Chloroplast redox state changes mark cell-to-cell signalling in the hypersensitive response. New Phytol. 2023 Jan;237(2):548-562

P40
Uncovering the circRNA Transcriptome in Hepatocellular Carcinoma using Long-read Nanopore Sequencing

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The incidence of liver cancer has experienced a significant surge in recent decades. Globally, liver cancer ranks third among all cancer-related mortalities, following lung and colorectal cancers. Hepatocellular carcinoma (HCC) is a prevalent type of liver cancer in individuals with chronic liver disease, accounting for more than 75%. Even though circular RNAs (circRNAs) have gained great attention in cancer research in recent years, their role in HCC is still unclear and transcriptomic data is very limited. The latter is based solely on patient samples from medical institutions in China and Singapore, where, unlike in the Western world, HBV is still the main risk factor for HCC. Furthermore, microarray and Illumina technologies used in these studies do not allow for precise circRNA length and sequence determination.

Our study aims to address these limitations by using long-read Nanopore sequencing to obtain circRNA transcriptome in a European population of HCC patients. We will sequence RNA from samples of tumor and adjacent non-tumor liver tissue from 20 HCC patients, on which poly(A) transcriptome has already been obtained. To overcome challenges, such as a small share of circRNAs in the total RNA pool and the absence of their poly(A) tail, required for library preparation, we will use a protocol that enriches for circRNAs and adds poly(A) tails after their linearization. Out of three tested protocols, the one with the following sequence (1) ribosomal depletion, (2) poly(A) tailing, and (3) RNase R treatment, turned out the most efficient for circRNA enrichment.

Given the widespread prevalence of HCC, it is crucial to gain a comprehensive understanding of its pathogenesis. By analyzing the resulting data from the European population of HCC patients without HBV or HCV etiology, we hope to contribute significantly to the understanding of circRNA expression in this pathology, particularly with regard to its etiology and molecular subtypes.

P41
Mechanisms of chemical toxicity inferred by functional genomics approaches in Chlamydomonas reinhardtii

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Herbicides are extensively studied chemicals with a strong focus on their environmental impact. Unlike many other chemicals, herbicides have been thoroughly researched, providing information not only on their direct molecular targets but also on the subsequent events that lead to weed death.

In this particular study, our aim was to investigate whether functional genomics, utilizing a comprehensive mutant library, could offer fresh insights into the susceptibility of the unicellular green alga Chlamydomonas reinhardtii to three herbicides: diuron, atrazine, and paraquat. Diuron and atrazine are herbicides and algicides that bind to different sites on the D1 protein of the PSII reaction center, hindering ATP synthesis. Paraquat disrupts the electron flow in PSI and damages plant tissue by generating free radicals that attack lipid membranes. Our hypothesis was that the screening of mutants would support the known mechanisms of action for these herbicides while uncovering novel details regarding their precise modes of action. Regarding diuron and atrazine, we observed some overlap among the genetic markers identified. Most of these markers were associated with photosynthesis: Mutants of Cgl54 (PSII D1 precursor processing protein) displayed greater sensitivity to both herbicides, whereas the homolog of Lpa2 (PSII accumulation protein) exhibited increased sensitivity to diuron alone. Concerning paraquat, several mutants displayed greater tolerance to the herbicide. This included a potential paraquat transporter and genes involved in the synthesis of fatty acids. Furthermore, certain mutants associated with PSI, such as the recently discovered PSI-associated kinase Cpl3, exhibited heightened sensitivity.

Drawing on functional genomics and existing knowledge, we have formulated a new hypothetical adverse outcome pathway specific to green algae. This pathway commences with the production of reactive oxygen species (ROS) in the chloroplast and culminates in cell death.

P42
Increasing yield of IVT reaction with at-line HPLC monitoring for continuous production of mRNA

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The COVID-19 pandemic triggered an unprecedented surge in development of mRNA-based vaccines. Despite the need to increase process productivity and thus decrease the cost of mRNA vaccines, limited scientific literature is available on strategies to increase the yield of in vitro transcription (IVT) reaction, the unit operation with highest cost of goods, which has traditionally been performed as a batch reaction.

We developed an IVT optimization workflow based on rapid at-line high pressure liquid chromatography (HPLC) monitoring of consumption of nucleoside triphosphates (NTPs) with concomitant production of mRNA, with a sub-3 min read- out, allowing for adjustment of IVT reaction parameters with minimal time lag. IVT was converted to fed-batch resulting in doubling the reaction yield compared to batch IVT protocol, reaching 10 mg/ml for multiple constructs. Fed-batch approach was then applied to produce 2 g mRNA in a single-use bioreactor with a starting volume of 100 mL, reaching a maximum mRNA concentration of 12 g/L, thus demonstrating the feasibility of continuous fed-batch production, and paving the way towards continuous manufacturing of mRNA.

Analytical method used for monitoring IVT was also coupled with exonuclease digestion to monitor capping efficiency of mRNA. HPLC monitoring was applied to production of an anti-reverse cap analog (ARCA)-capped mRNA construct, which requires an approximate 4:1 ARCA:guanidine triphosphate ratio, the optimized fed-batch approach achieved productivity of 9 mg/ml with 79 % capping.

P43
Innovative affinity-based filter for isolation of the CD90-expressing human mesenchymal stem cells from cell suspensions

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Efficient and reliable isolation of specific cells is a prerequisite for many analytical and preparative methods and tests. Stem cell isolation is of particular interest for medical applications. Systems currently providing high purity and reliability of cell isolation rely on active pre-labelling of the target cells with specific antibodies. This can be problematic for downstream applications. Labelling-independent (passive) techniques isolate unlabelled target cells, but do not allow the separation of cells of similar size and are limited to a few cell types.
Here we present an innovative, patented, affinity-based filter for isolation of the human mesenchymal stem cells. The basic structure of the filter is a biocompatible, 3D printed hydrogel mesh made from a mixture of alginate, carboxymethyl cellulose and nanofibrillated cellulose. Specific antibodies against CD90, a surface antigen present on human mesenchymal stem cells, are fixed on the surface of the filter to capture the target cells. This allows for specificity of cell separation, while at the same time keeps the target cells unlabelled and thus useful for a full range of downstream applications in both research and medical applications.
We have optimised the method of isolation of target cells using the affinity filter. The biological sample from which the target cells are to be isolated must be prepared as a single-cell suspension, without large cell aggregates. This input cell suspension is applied to a system of three identical affinity filters in series. After removal of the unbound, non-target cells, the target cells are washed out of the system using a special buffer and under elevated pressure.
We have used the filter to successfully isolate mesenchymal stem cells from stromal vascular fractions of lipoaspirates. The isolated target cells ($CD90^+$ stem cells) were well viable and fully functional - capable of tri-lineage differentiation.

P44
Following the rate of cell death initiation in potato hypersensitive response by digital macroscope

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Potato virus Y (PVY), which is considered the most important viral pathogen of potatoes, in potato plants of cv. Rywal triggers a hypersentive response (HR), an effective defense mechanism, which is mediated by temperature dependent *Ny-1* resistance gene. In HR, restriction of pathogens to the infection site is associated with a form of localized programmed cell death (PCD), which is manifested as the formation of necrotic lesions on inoculated leaves 3 days after inoculation (dpi). However, higher temperature leads to abortion of resistance manifested as lack of cell death zone formation and systemic virus spread. When the plants are transferred to a lower temperature, cell death is initiated. This feature was exploited to follow the rate of cell death initiation by imaging inoculated leaves in the time between the cell death initiation and the cell death appearance. Digital macroscope enables continuous imaging in desired intervals, which allows to determine an accurate cell death initiation rate. In addition, if this feature is studied in transgenic plants with altered component of interest, the protocol enables us to determine if the decreased level of a studied component affects the rate of cell death initiation. By that, components involved in cell death initiation can be identified using this protocol. A protocol for studying cell death initiation will be demonstrated.

P45
Environmentally friendly protection of potatoes against Colorado potato beetle

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Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera: Chrysomelidae; CPB) is one of the most devastating insect pests of potato causing important yield losses worldwide. To combat this pest various crop protection strategies have been employed, including chemical insecticides, entomopathogens, and biotechnological products, such as the proteinaceous endotoxins from *Bacillus thuringiensis* (Bt) and double stranded RNAs. However, CPB has already demonstrated rapid resistance development against chemical pesticides and biopesticides, highlighting the importance of integrating multiple modes of action for its effective management in the future.

Recently, lipid-binding aegerolysin proteins derived from edible oyster mushrooms (*Pleurotus sp*.) have gained interest as new biopesticides and a solution with possibly less chances for CPB resistance development. These aegerolysin proteins, in combination with their protein partner pleurotolysin B, interact specifically with sphingolipid-enriched insect membranes, resulting in pore formation in the CPB midgut. This unique mechanism of action exhibits potent and selective toxicity towards CPB, making it a potential breakthrough in pest control strategies. We will present the strategy used to obtain potato plants expressing a bioinsecticidal aegerolysin-based protein complex and show the results of feeding trials with the transgenic plants that demonstrate its effective protection against CPB infestation. In addition, we will showcase the genome-wide expression response of CPB larvae exposed to the protein complex, providing insights into its physiological impact on the pest.

P46
Establishing protocol for inoculation of potato plants with Fusarium oxysporum to study complex plant microbial interactions

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Understanding complex interactions between plants and microbes is essential for effective agricultural practices and ecosystem management. Studies focusing on two-way systems can overlook the intertwined nature of interactions seen in natural habitats. Hence it is crucial to establish systems for studying interactions between multiple associated organisms. We will present the initial progress made towards implementing such a system.

We isolated a strain of *Fusarium oxysporum* (*F. oxysporum*) from our potato plants grown in sterile conditions of tissue culture. Our study started with sequencing of *F. oxysporum* genomic DNA, utilizing Illumina short read and Oxford Nanopore Technologies (ONT) long-read sequencing. The WENGAN hybrid assembler yielded the most favorable genome assembly results. Moreover, the extraction of high molecular weight DNA using the CTAB method, combined with preliminary sorbitol buffer washing and size selection of long DNA fragments using PEG 8000, enhanced genome assembly contiguity and resolution of repeat regions. Functional annotation of the genome assembly was performed using the funannotate pipeline, providing insights into the genome's functional aspects.

Furthermore, a protocol for efficient dip inoculation of Desiree potato plants with adjusted concentration of *F. oxysporum* spores (microconidia) was established and two different inoculation methods were tested. Efficiency of inoculation and spread of the hyphae in the plant was evaluated using real-time polymerase chain reaction (qPCR), revealing higher concentration of *F. oxysporum* in lower plant tissues (roots) compared to upper ones. Additionally, *F. oxysporum* inoculation efficiency was verified by culturing surface-sterilized nodules and chopped plant material on MS-30 media.

Using the set tools we will study the biology of this plant-microbe interactions. Our study paves the way for more holistic approaches in terms of transition to sustainable agriculture.

P47
Following chloroplast redox state and stromules frequency around the cell death zone in potato hypersensitive response

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A defense mechanism in potato plants, called hypersentive response (HR), is activated by *Ny-1* gene after being infected with potato virus Y (PVY) and results in formation of lesions – programmed cell death zones – on inoculated leaves. HR is accompanied by salicylic acid (SA) synthesis and accumulation of reactive oxygen species (ROS). After infection, PVY multiplication and cell death also induce stromule formation. However, the exact connection between apoplastic ROS (apoROS), chloroplastic ROS (chlROS), SA and stromule formation remains unclear. To investigate this, we conducted detailed spatiotemporal analysis of chloroplast redox state and stromule count in the cells of upper epidermis and palisade mesophyll in PVY-inoculated transgenic potato plants with altered accumulation of multiple components, including reduced SA accumulation, reduced apoROS levels and consequently altered SA levels and reduced chlROS levels in different combinations. By confocal microscopy and customized script for image analysis, we visualized stromules and measured chloroplast redox state in cell death proximity in all transgenic lines. Results showed that spatiotemporal patterns of stromules induction and redox state differ between transgenic lines.

P48
The influence of codon usage on translation dynamics and protein folding

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Translation and protein folding are interconnected processes that mutually regulate each other by impacting the speed at which a ribosome operates. Codon usage significantly influences translation speed by accessing synonymous codons with different translation rates governed by the tRNA concentration. Minor modifications to codon sequences, such as two synonymous substitutions, can increase misfolding. Through years of evolution, organisms adapted their codon usage to align with protein folding dynamics to ensure efficient production. By simulating the ribosome movement on the mRNA, we estimated the translation speed for all *E. coli* protein-coding genes up to codon resolution. We paired the estimated speed with protein structures to observe common patterns. Due to the ribosome tunnel-induced latency between translation and folding, we cannot directly compare codon usage influence. Thus, we derive an approach to account for the tunnel latency when comparing the translational velocity and protein structure. We observe that the main drivers of ribosome stalling are not closely positioned rare codons but islands of approximately seven codons with slow translation. Furthermore, stalling does not happen randomly but at specific positions, arguably to facilitate the regulation of folding. It is only present in larger proteins, especially those with multiple domains and multiple hydrophobic cores. Although the codon sequence carries additional information about the translation velocity, it seems to only modulate the inherent bias of the amino-acid translation velocity.

P49
Diverse structural landscape of potyviral coat protein assemblies

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Potato virus Y (PVY) is a flexible filamentous single-stranded RNA virus that belongs to the *Potyvirus* genus, and is the most important viral pathogen of potato worldwide. Its coat protein (CP), which has a high degree of intrinsic disorder, is the only structural component of infectious virions. It plays many other roles in the viral life cycle, but the structural ensemble of forms that enables such multitasking has never been previously explored in detail.
Using combined structural and biophysical approaches, we show that CP self-assembles into three distinct filament types via a distinct organization of intrinsically disordered regions (IDRs), one of which encapsidates RNA similarly to the native virion. Based on their high-resolution cryo-EM structures and additional protein engineering, we can redirect the assembly of protomers towards desired filament morphology as well as increase their thermal stability by disulfide bond stapling. Furthermore, by partially truncating IDRs in combination with single amino acid substitutions, we can unleash entirely novel modes of CP self-assembly, leading to single or double octameric rings, highly ordered cubes, spherical particles, and more. By fusing CP to other proteins, we can additionally control the self-assembly process spatially and temporally. In summary, we show that by making simple and controlled changes to CP and thus its self-assembly pathway, one can utilize the enormous potential of PVY CP intrinsic plasticity to generate diverse structural assemblies with desired architectural and chemical properties not previously observed for filamentous CPs.

P50
Localisation of potato virus Y VPg protein during the infective cycle using confocal microscopy

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Potato virus Y (PVY) is the most devastating potato pathogen worldwide (1). It belongs to the genus *Potyvirus* and forms flexous filamentous particles containing a single-stranded RNA. Its genome encodes for a polyprotein that is cut into 10 mature proteins. In addition, another protein is produced from an overlapping coding sequence (2).

Most of the viral proteins are multifunctional. They are involved in different stages of the viral cycle, establishing a complex and dynamic interaction network between viral and plant proteins (3). In order to develop sustainable and efficient plant protection strategies it is important to understand the role of different viral proteins in the PVY infection cycle.

To that end, here we focus on localisation of the VPg protein using a PVY clone. First, we prepared a clone with fluorescently tagged native VPg and followed its localisation over time in bombarded epidermal leaf cells of *Nicotiana clevelandii* using confocal microscopy. We observed single cells with a signal in the nucleus, cytoplasm and as granules and filaments within cytoplasm. The localisation seemed to change over time. To follow the localisation of infective virus, we prepared another clone with an ectopically expressed VPg-GFP protein. We transiently transformed *Nicotiana bethamiana* with different organelle markers and infect it with the prepared infectious clone in order to study the subcellular distribution of VPg at early stages of the infective cycle. These preliminary results will help, to not only decipher the role of VPg in the viral cycle, but also to better understand the complex mechanism of the PVY infection cycle.

References

1. Kreuze JF et al. 2020. Viral Diseases in Potato. The *Potato Crop. Springer, Cham*
2. Chung BYW et al. 2008 An overlapping essential gene in the *Potyviridae*. *PNAS* 105 (15): 5897–5902
3. Rodrigo G et al. 2017. Virus-host interactome: Putting the accent on how it changes. *J Proteomics* 156: 1–4

P51
Chimeric vaccine for hypercholesterolemia treatment

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Atherosclerosis, a leading cause of global morbidity, poses multiple challenges due to disease's complex etiology. Impaired cholesterol clearance and subsequent accumulation of serum cholesterol, resulting from PCSK9-mediated inhibition of LDL-receptor, significantly contribute to the development of atherosclerotic lesions. Since PCSK9 deficiency does not cause any adverse pathologies, it represents an ideal target for preventive therapy of atherosclerosis. Our primary objective is to develop an innovative vaccine against PCSK9 by designing an "autologous" chimeric protein. Thus, chPCSK9 consists of key human PCSK9's solvent-accessible amino acids, which act as antibody formation-inducing B-cell epitopes and are transferred to a distant homologue. Retaining PCSK9's conformation, we want to induce formation of efficient antibodies, neutralizing PCSK9-LDLR interaction. Simultaneously, we aim to prevent systemic autoimmune attacks on healthy tissue. To avert T-cell cytotoxicity we strategically mutated predicted $CD8^+$ T-cell epitopes (MHCI binding peptides) of PCSK9. Our *in vivo* study demonstrates that vaccination with chPCSK9 DNA constructs generates a humoral immune response in $ApoE^{-/-}$ transgenic mice (model for murine atherosclerosis). It reduces cholesterol and PCSK9 serum levels, increases liver LDLR amount and furthermore manifests similar effects over a long-term period. Additionally, we assess the efficacy of neutralizing antibodies by inhibiting PCSK9-LDLR interaction *in vitro* and establish safety of the vaccine by stimulation of isolated splenocytes. This work provides proof-of-concept results for a therapeutic approach to hypercholesterolemia prevention and opens a discussion on directed vaccine design, which could be extended to other therapeutic targets and diseases.

P52

Recombinant Probiotic Lactococcus lactis Secreting Endolysins Against Pathogenic Bacteria Clostridioides difficile

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Clostridioides difficile is an enteric pathogen bacterium that causes life-threatening intestinal infections and diarrhoea, while *Lactococcus lactis* is a probiotic lactic acid bacterium that is part of the normal gut microbiota and prevents the growth of pathogenic bacteria. Moreover, bioengineered *L. lactis* has been demonstrated as a promising oral delivery system expressing heterologous proteins for the treatment of various human diseases.

In the present study, we aim to combine the benefits of intrinsic probiotic properties of *L. lactis* with antimicrobial activity of recombinant *L. lactis* against *C. difficile* infection, developed to lyse *C. difficile* cells by secreting specific endolysins, bacteriophage enzymes that degrade bacterial cell wall.

Endolysins are a promising class of highly efficient antibacterial agents as they are bacteria-specific and hence do not disrupt the normal microbiota, as well as are not susceptible to bacterial resistance. They generally consist of two domains – enzymatically active domain (EAD), and cell wall-binding domain (CBD). Endolysins are also encoded by prophages, phage genomes integrated in bacterial chromosomes.

In order to find new candidate *C. difficile*-specific endolysins, a bioinformatic study was conducted to investigate putative prophage endolysins from 151 *C. difficile* genomes. Several bioinformatic tools were used, including PHASTER for prophage identification, InterPro for EAD and CBD domain prediction, EMBOSS package for automated pairwise sequence alignment and comparison using the Needleman–Wunsch algorithm, as well as Orange for cluster analysis and visualisation.

Thus, 79 novel *C. difficile*-specific endolysins have been identified, composed of EAD and CBD domains grouped into several clusters. The newly selected and previously reported (CD27L in PlyCD) *C. difficile*-specific endolysins were cloned and expressed in *L. lactis*, and their lytic activity against *C. difficile* cell wall has been demonstrated.

P53
New insights in biology of fungal lipid-biding aegerolysins

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Aegerolysins are relatively small proteins (15 kDa) with unique biotechnological potential. The main biochemical characteristic of aegerolysins is their high affinity for specific membrane lipids or lipid mixtures. In addition, they are non-toxic, and protocols for their isolation in recombinant form have already been developed. In some production organisms, aegerolysins co-occur with MACPF (Membrane Attack Complex / Perforin) protein partners.

The so-far known aegerolysins are characterized by their high affinity for ceramide phosphoethanolamine (CPE), which is an insect-specific membrane sphingolipid. In addition, some of these aegerolysins less specifically bind to the mixture of sphingomyelin (SM) and cholesterol (Chol), which is a characteristic lipid mixture in vertebrate membranes. Fluorescently labeled aegerolysins can be used to study these lipids, in the similar manner as antibodies are used to study the localization or presence of proteins in the cells. Although aegerolysins are non-toxic, they may, in the presence of a protein with the MACPF domain, become part of a cytolytic complex that permeabilizes the cell membrane containing the lipid receptor recognized by aegerolysin. Aegerolysins are therefore bicomponent cytolysins - component A (aegerolysin) recognizes the lipid target, and component B (protein partner with the MACPF domain) further allows the formation of pores in the target membrane.

We are currently studying four novel fungal aegerolysins and their MACPF protein partners from basidiomycetes *Heterobasidion irregulare*, *Trametes versicolor*, *Mucidula mucida* and *Lepista nuda*. Here we present lipid binding affinities of these four aegerolysins and their pore forming activity in concert with MACPF protein partners.

P54
Human histone fingerprinting based on the novel actinoporin homolog pores

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Nanopore sensing is a method in which biological pores inserted into an electrically non-conductive membrane allow charged particles to translocate through the membrane. The pore constriction acts as a sensing region responsible for the occurrence of analyte-specific electrical blockages. Nanopores have already been adapted to allow precise, long-read DNA sequencing, while the detection and identification of proteins based on nanopore sensors is under development. To facilitate the analysis of diverse protein analytes that differ in size, charge and hydrophobicity, new pores with new properties are required. We recombinantly expressed the actinoporin homologue from *Orbicella faveolata*, which forms stable octameric pores on large unilamellar vesicles in the presence of sphingomyelin. The solubilised pores were stably inserted into the robust artificial MinION membranes and showed improved open pore signal quality compared to the wild type pores. Examination of the signal traces of hundreds of inserted pores enabled us to distinguish between different human histone variants. Histones are highly charged proteins about 20 kDa in size that are susceptible to post-translational modifications. Although they are predominantly found in the nucleus, they can also be found in human serum in certain diseases and therefore have the potential to serve as proteinogenic biomarkers. Different blockage specifics such as dwell time, amplitude, and noise indicate that the main driving force for protein capture and possible translocation is the electrostatic interactions between the negatively charged pore lumen and positively charged histone proteins.

P55

Engineering of vaginal lactobacilli, their incorporation into electrospun nanofibers and interaction with epithelial cells.

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Lactobacilli are the dominant species in the vagina. They are crucial for maintaining a normal microbial balance by producing antimicrobial substances. Depletion of the normally present lactobacilli leads to overgrowth of opportunistic pathogens and the occurrence of vaginal infections. This can be countered by restoring the normal vaginal microbiota through application of lactobacilli as probiotics. However, vaginal probiotics are hampered by the lack of a suitable delivery system for local administration.

In our research, we used three lactobacilli that are dominant in the vagina: *L. crispatus*, *L. gasseri* and *L. jensenii*, and as a control *L. plantarum*. We genetically engineered them to produce fluorescent proteins with different spectral properties: IRFP, mCherry, GFP and mTagBFP2, which allow easy and effective imaging and their distinction within a mixture. The genes of the fluorescent proteins were cloned into the pNZ8148 plasmid under the control of the *ldh* promoter. The newly engineered plasmids were electroporated into the four species using customized electroporation protocols. The expression of fluorescent proteins differed between the lactobacilli, with *L. plantarum* showing the highest fluorescence, followed by *L. gasseri, L. jensenii* and *L. crispatus*. We also developed a new delivery system for local administration using electrospinning and incorporating lactobacilli into nanofibers composed of polyethylene oxide, or its combination with alginate and sucrose. The lactobacilli remained viable for 8 weeks after incorporation, retained their fluorescence and adhered to the surface of Caco-2 cells after release from the nanofibers. Nanofibers and lactobacilli did not impair the viability of Caco-2 cells, showing their potential for further *in vivo* studies.

In this study, we successfully incorporated vaginal lactobacilli into a promising nanofiber-based delivery system and demonstrated their viability, imaging, safety and adhesion to human epithelial cells.

P56
Regenerating embryos from single cells of grapevine cv. Zweiglet

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Advances in plant genetic engineering and genome editing have made it possible to investigate gene functions in a prolific manner and produce various improved crops by both genetic engineering and more informed selection. These processes are very well established in small leafy plants, such as the model plant *Arabidopsis thaliana*, or small crop plants but remain challenging in woody plants. Transforming single cells to obtain non-chimeric plants has become of more interest in grapevine. To succeed in such endeavors, it is necessary to improve the regeneration of single cells into embryos and subsequently whole plants, the former step being especially difficult for grapevine. Only a couple of cultivars have been effectively regenerated from single cells up to now. We report here the first successful regeneration of single cells from grapevine cv. Zweigelt into embryos.

P57
Monitoring and optimization of Listeria innocua biofilm eradication by antibacterial nanoparticles

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Biofilms are clusters of microbes bound together by a self-produced matrix of extracellular polymeric substances (EPS) composed of polysaccharides, proteins, and DNA. It hydrates the microorganisms and protects them from mechanical injury and antimicrobial agents, making biofilms extremely difficult to remove from surfaces. We propose a method for removal of biofilms using antibacterial nanoparticles. Biofilms of the model strain *Listeria innocua* were grown on polystyrene and treated with antibacterial nanoparticles either intact or washed, removing loosely bound bacteria. The treatment time, the use of three different types of antibacterial nanoparticles, and the effect of the magnetic field were adjusted to achieve optimal reduction of viable biofilm bacteria. Eradication efficiency was determined by monitoring fluorescence of expressed reporter protein or biofilm staining and viability reduction in logs. Intact biofilms of the model strain could not be reproducibly assessed by the viability assay, so treatments were applied to washed biofilms in which only persistent bacteria were present. The applied magnetic field itself had limited effect on the biofilms. The three types of antibacterial nanoparticles showed varying degrees of biofilm eradication. The results are promising, but require further optimization of conditions consistent with large-scale applicability for further development of this innovative approach to removal of biofilms by antibacterial nanoparticles.

P58
Removal of Escherichia coli biofilm by magnetic nanoparticles

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Microorganisms frequently form biofilms on living and inanimate surfaces as an alternative lifestyle to ensure their survival in various environmental niches. Biofilms represent a significant clinical and public health problem, as up to 80% of bacterial infections in humans are associated with biofilms. One of the main problems with biofilms is their lower sensitivity to antimicrobial agents, which creates a need for new effective approaches to combat biofilms.

In this study, we propose the use of magnetic nanoparticles to enable removal of bacterial biofilms by exposure to a magnetic field. We established biofilms of *Escherichia coli* transformed with a pSEUDO-CP-25-GFP plasmid in microtiter plates and measured the effects of magnetic nanoparticles by monitoring green fluorescent protein (GFP) fluorescence and counting colony forming units (CFU). Preliminary experiments showed that nanoparticles were able to remove 50% of the biofilm after 30 minutes of exposure to the magnetic field.

The use of magnetic nanoparticles to remove biofilms is a promising approach to address the problem of biofilm-associated infections. This technology has the potential to reduce the need for high doses of antibiotics and minimize toxicity to humans. Further studies are needed to optimize this technology and explore its potential applications in the clinical setting.

P59
An innovative process for the production of bioethanol: Optimization and kinetic assessment

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Given the growing concern about the depletion of fossil fuels, global warming, and the loss of natural resources, bioethanol made from sugar cane, molasses, and corn continues to garner interest globally and is regarded as the safest and cleanest alternative to oil. Starch is a widely available renewable carbohydrate from which bioethanol is conventionally obtained through energy demanding liquefaction and saccharification processes. A significant simplification of the process and a reduction of starch processing costs would be possible by applying raw starch hydrolysis using enzymes capable of degrading starch below the gelatinization temperature. A novel strategy for highly concentrated raw corn starch (30 % w/v) hydrolysis based on a modified simultaneous saccharification and fermentation process is optimized for the production of bioethanol. Different ratios of *Bacillus paralicheniformis* ATCC 9945a (*Bli*Amy) and glucoamylase (Dextrozyme® GA), glucoamylase addition time, incubation time, and pH were investigated using a Box–Behnken experimental design to ensure high process efficiency. A two-step synergistic hydrolysis and fermentation with *Saccharomyces cerevisiae* at 30 °C was carried out in a single bioreactor vessel at the same pH (4.5). The obtained bioethanol concentration at 129.2 g/L, with a productivity of 2.94 g/L/h and ethanol yield ($Y_{P/S}$) at 0.50 g EtOH/g total sugar, equivalent to 87.8 % theoretical yield indicates the viability of the proposed innovative process.

P60
Newly developed PVY-N605(123)-mTurquoise2 infectious clone for functional analyses in plant-PVY interaction

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Due to its global distribution and economic impact on crop production, potato virus Y (PVY) is one of the most studied plant viruses today. A powerful research tool for virologists are infectious clones, where the entire viral genome is inserted into one or more plasmids suitable for multiplication outside the host plant. Such clones can be tagged with fluorescent and nonfluorescent proteins. We have previously constructed a PVY infectious clone, tagged with the green fluorescent protein (GFP), i.e., PVY-N605(123)-GFP. Unfortunately, GFP-tagged PVY cannot be imaged in combination with proteins tagged with GFP or other fluorescent proteins from this part of the spectrum. Therefore, here we present a protocol for the construction and imaging of a newly developed PVY infectious clone, tagged with mTurquoise2 (mTq2). PVY-N605(123)-mTq2 was constructed by inserting the mTq2 coding sequence between the coding sequences for viral proteins NiB and CP and introduced in *Nicotiana clevelandii* plants by bombardment. We confirmed PVY-mTq2 abundance in bombarded and systemic leaves by imaging the plants with optimized imaging systems that allow observation of PVY-mTq2 in whole plants and thus allow high spatial and temporal resolution. To confirm stability of the inserted tag in the viral progeny, we followed virus infectivity and systemic spread in several passages in *N. clevelandii* plants. To confirm its functionality in potato, in the last passage, we infected PVY resistant potato plants of cv. Rywal, which develop hypersensitive response after infection and susceptible NahG-Rywal transgenic plants. Necrotic lesions developed in both genotypes, while the virus could spread systemically only in NahG-Rywal. We conclude that this construct is biologically comparable to a non-tagged and GFP-tagged PVY, allows *in vivo* tracking of the virus in inoculated and systemic leaves, and successfully infects *N. clevelandii* and potato plants, while its systemic spread is not impaired.

P61
Effect of differentiation of primary human skeletal muscle cells on expression of receptors for the IL-6 family cytokines

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One of key functions of skeletal muscle, which is being actively researched, is its function as an endocrine organ. Skeletal muscle produces and releases cytokines, which were named myokines to highlight their tissue origin. The first identified and most researched myokine is interleukin-6 (IL-6). The IL-6 family of cytokines is defined by its common signaling through the membrane bound β-receptor glycoprotein 130 (gp130). IL-6 cytokines have an important role in regulation of metabolism in skeletal muscle and in regulation of various biological processes in other tissues and organs. They are also implicated in the pathophysiology of various metabolic, liver and gastrointestinal disorders, which makes them interesting pharmaceutical targets and thus a focus of attention in recent research. The systemic effects of IL-6 cytokines are well known, but their mechanisms of action and their production in skeletal muscle are still not very well understood, which will be crucial for the development of treatments of pathophysiological states in which myokines are thought to play a prominent role. All IL-6 cytokines, except for IL-31, signal through at least one gp130 β-receptor subunit and induce common intracellular signaling pathways. Gp130 receptors function either as a homodimer or a heterodimer, comprised of one gp130 β-receptor subunit in combination with one of the other three β-receptors. Despite their importance it is still not very well known whether and how gp130 signaling pathways are activated during skeletal muscle cell differentiation. To shed further light on this topic, we aimed to determine the regulation of expression of selected receptors of IL-6 family cytokines at different stages of differentiation. For the experimental model we used cultured primary human skeletal muscle cells. Cells were differentiated into myotubes for seven days and expression of target genes was analyzed using quantitative real-time PCR at three stages of differentiation.

P62
Expression of secretory pathway kinase FAM20C and its regulator FAM20A depends on differentation stage of cultured skeletal muscle cells

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FAM20C, a kinase of the secretory pathway, is involved in phosphorylation of the majority of extracellular proteins. We explored whether differentiation of cultured skeletal muscle cells affects expression of FAM20C and FAM20A, a pseudokinase and an allosteric activator of FAM20C. We also determined whether FAM20C affects expression and action of IL-6, a phosphorylated myokine and FAM20C substrate, which plays an important role in myogenesis, muscle regeneration, inflammation and metabolism.

Real-time PCR and immunoblotting were used to characterize the expression of FAM20C and FAM20A in rat L6 muscle cell line and primary human skeletal muscle cells. FAM20C and FAM20A were expressed throughout the muscle cell differentiation. Notably, we observed a significant upregulation of FAM20A expression in differentiated myotubes compared to undifferentiated myoblasts. Conversely, the expression of FAM20C kinase exhibited a decline as the muscle cells underwent differentiation. Using gene silencing we investigated the functional significance of FAM20A and FAM20C in muscle cells. The FAM20A and FAM20C mRNA expression was reduced by ~50 %, which was paralleled by minor changes in IL-6 mRNA expression. Silencing of FAM20A resulted in moderate elevation in phosphorylation of STAT3 and expression of IL-6 and IL-6Rα and gp130, whereas silencing of FAM20C caused no alterations in expression of IL-6 and IL-6 receptors.

Collectively, our results show that expression of FAM20C and FAM20A depends on the differentiation stage of cultured skeletal muscle cells, indirectly suggesting that extracellular phosphoproteome is altered during myogenesis. In addition, FAM20C and FAM20A may also modulate expression of IL-6 and its receptor. Understanding the interplay between FAM20C and IL-6 in skeletal muscle cells is likely to provide valuable insights into the complex regulation of muscle physiology and pathology.

P63
Characteristics of plasma EVs in healthy adults and the influence of recent mild COVID-19

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Extracellular vesicles (EVs) are phospholipid bilayer-enclosed particles, released into body fluids, reflecting their cell of origin. They are used in disease biomarker research, yet little is known about EVs in healthy population. Here we analyzed plasma EVs (pEVs) in healthy adults, assessing interindividual variability and impact of recent mild COVID-19.

Blood and clinical (height, weight, blood pressure, menopause), demographic (sex, age), lifestyle (exercise, smoking) data were collected from 208 pre-COVID-19 donors (HDC19-) and 60 post-recent COVID-19 (HDC19+) donors (ethically approved, informed consent). HDC19- samples underwent complete blood count (CBC), CRP and insulin analysis. HDC19+ samples were analyzed for CBC and anti-SARS-CoV-2 antibodies. pEVs were enriched from all plasma samples (ultracentrifugation on a 20% sucrose cushion, sUC), then analyzed for concentration (NTA) and 37 surface proteins (MACSPlex). The variability in pEV characteristics of the HDC19- group was assessed, correlating with individuals' parameters. The effect of recent mild COVID-19 on pEV characteristics was analyzed.

HDC19- subjects, evenly distributed by sex and age, were healthy with normal CBC, insulin, and CRP levels. Enriched HDC19- pEVs (conc: $5.7*10^9$ part./mL; mode size: 151.0 nm) displayed blood-cell and endothelial markers, predominantly platelet-derived EVs. These markers did not correlate with CBC or other parameters, except for smoking, which positively correlated with increased pEVs from platelet, endothelial, and leukocyte origins. HDC19+ pEVs showed no significant changes in surface protein expression or correlation with anti-SARS-CoV-2 levels or time after recovery (1-12 weeks). However, HDC19+ had significantly fewer pEVs (conc: $3.6*10^8$ part./mL) that were smaller (mode size: 121.8 nm) compared to HDC19-.

pEV characteristics in healthy adults were not associated with analyzed variables, except for smoking. Mild COVID-19 altered pEV size and concentration.

P64
Unveiling MLKL Protein: Exploring Nanobody Interaction, Membrane Disruption, and Oligomerization

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Mixed lineage kinase domain-like (MLKL) protein plays a central role in necroptosis, a regulated form of cell death characterised by membrane disruption. Understanding the molecular mechanisms underlying MLKL effector function is essential for elucidating the intricate processes of necroptosis.

We investigated the interaction between MLKL and a newly developed nanobody and explored the formation of MLKL oligomers and their involvement in membrane disruption. To study the interaction between MLKL and the nanobody, we performed *in vitro* experiments using recombinant variants of MLKL and the nanobody. Our results demonstrated a specific interaction between MLKL and the nanobody, suggesting its potential as a valuable tool for modulating MLKL activity in various biological contexts.

To further investigate the functional implications of MLKL in membrane disruption, we generated stable inducible FlpIn HEK293 cell lines expressing different MLKL constructs. This allowed us to examine the properties of MLKL variants and their effect on cell death. Using confocal fluorescence imaging of fixed cells and functional assays, we observed that MLKL expression led to membrane disruption, consistent with its role in necroptosis.

To gain insight into the oligomeric state of MLKL, we employed cross-linking experiments. Our data revealed the presence of MLKL dimers, trimers, tetramers, and higher-order oligomers, supporting the hypothesis that oligomerization is a crucial step in MLKL-mediated membrane disruption. Further investigations into the structural properties and dynamics of MLKL oligomers will contribute to a comprehensive understanding of the MLKL effector function and may offer potential therapeutic targets for diseases involving dysregulated cell death pathways.

P65
Cell Bank of Primary Human Skeletal Muscle Cells: An In Vitro System for Investigating Idiopathic Inflammatory Myopathies

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Idiopathic inflammatory myopathies (IIMs) are a group of diverse autoimmune diseases characterised by progressive muscle weakness, atrophy, and immunohistological alterations in skeletal muscle tissue. Although they primarily affect skeletal muscle, IIMs are systemic diseases that often lead to cancer and other secondary complications in the heart, lungs, and gastrointestinal tract. In Slovenia, the estimated annual incidence of IIMs is 11.5 cases per million adults, and the 5-year survival rate is 75%.

We aim to establish and expand a cell bank of skeletal muscle cells from patients with IIM and healthy controls without neuromuscular disease. We isolate CD56+ myogenic cells from skeletal muscle tissue by proteolytic release followed by magnetic-activated cell sorting. Healthy muscle cells are obtained from residuals of semitendinosus muscle tissue discarded during orthopaedic surgery, while IIM muscle cells are isolated from biopsy samples of deltoid muscle taken as a part of the standard diagnostic procedure for suspected IIM. During culturing, satellite cells differentiate into myoblasts and subsequently fuse to form myotubes. Cultured skeletal muscle cells serve as a suitable *in vitro* system for investigating physiological and pathological properties at the molecular level, as their (patho)physiological characteristics resemble those of the donor's myofibers *in vivo*.

To date, our continually expanding bank consists of skeletal muscle cells isolated from samples obtained from more than 50 healthy individuals and 20 patients diagnosed with all subtypes of IIM, i.e. dermatomyositis, polymyositis, immune-mediated necrotizing myopathy, inclusion body myositis, antisynthetase syndrome, and overlap myositis. Additionally, we are collecting clinical data from the IIM patients to establish correlations between molecular and clinical findings and hopefully contribute to the understanding of IIMs.

P66
Glucose-mediated regulation of isoform-specific expression of Na^+,K^+-ATPase in cultured skeletal muscle cells

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Skeletal muscle is an important site for insulin action and transport of Na^+ and K^+. Dysregulation of Na^+,K^+-ATPase (NKA), a heterodimeric (α/β) Na^+-K^+ pump, occurs in diabetes mellitus, but it remains unknown whether the altered hormonal *milieu* or hyperglycemia *per se* affects NKA in skeletal muscle. The aim of our study was to investigate the effects of glucose concentration on the expression of NKA subunits and the NKA regulator FXYD1. We also determined whether AMP-activated protein kinase (AMPK), an important sensor of cellular energy, acts as a link between glucose availability and NKA expression.

Glucose deprivation decreased NKAα1 mRNA expression in cultured human myotubes and, on the other hand, increased protein abundance of NKAα1 and NKAα2 subunits in rat L6 myotubes. In human myotubes, glucose deprivation led to upregulation of ChREBP mRNA, while it had no effect on the transcription factors Sp1, Zeb1, and Atf1, which are known to be involved in the regulation of NKA expression. In contrast, glucose deprivation in L6 myotubes resulted in downregulation of Sp1 and Zeb1 expression and upregulation of Atf1 expression. Expression of Fxyd1, a negative regulator of NKA, was suppressed by glucose deprivation and the AMPK activators AICAR and A-769662, indirectly suggesting an increase in NKA activity. Finally, siRNA-mediated gene silencing of AMPKα1/α2 catalytic subunits in L6 myotubes decreased protein abundance of NKAα2, while increasing protein abundance of NKAα1 and mRNA levels of NKAβ1.

In summary, our findings demonstrate that glucose and AMPK modulate NKA α/β-subunits in an isoform-specific manner. Furthermore, they suggest that suppression of NKA expression by AMPK is not a primary energy-saving measure in cultured myotubes.

P67
Evaluation of the functional reserve of the IL-6 receptors IL-6Rα and gp130 in cultured human skeletal muscle cells

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Interleukin-6 (IL-6), a key myokine, mediates its effects by forming a complex with either a transmembrane or soluble receptor IL-6Rα, which binds IL-6, and gp130, which facilitates signal transduction. Typically, target cells possess an abundance of receptors, surpassing the required number for achieving the maximal response. This excess creates a functional reserve within the signalling system. The functional reserve serves as a safety mechanism, enabling cells to respond effectively even when receptors are partially depleted. However, when evaluating receptor function through gene silencing, a mere partial silencing of the receptors may prove insufficient to suppress the response in cells possessing a substantial functional reserve.

We aimed to assess the effectiveness of gene silencing in disrupting the downstream signalling of the IL-6Rα/gp130 receptor complex in cultured primary human myoblasts (n = 8 donors). Using siRNA the expression of IL-6Rα mRNA and gp130 mRNA was reduced by 65 % ($P < 0.05$) and 70 % ($P < 0.05$), respectively. Our objective was to determine whether this partial knock-down would diminish the responsiveness of myoblasts to IL-6. To evaluate the impact, myoblasts were treated with 50 ng/mL of recombinant human IL-6 for 30 minutes. We monitored the phosphorylation of STAT3 (Tyr705), a crucial transcription factor downstream of IL-6Rα/gp130 receptor complex, as an indicator of IL-6 activity. Comparing the IL-6-induced phosphorylation of STAT3 in IL-6Rα and gp130 knock-down myoblasts with that of control myoblasts, we observed reductions of 78 % ($P < 0.05$) and 44 % ($P < 0.05$), respectively. These results suggest that the level of gene silencing achieved was sufficient to suppress the response to IL-6.

In summary, gene silencing effectively unveils the role of the IL-6Rα/gp130 receptor complex in cultured human myoblasts, indicating a limited functional reserve for these receptors.

P68
Contribution of APOBEC proteins 3A and 3B to the oncogenicity of HPV viruses

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Human papillomaviruses (HPV) cause nearly 5% of all human malignancies, including cervical cancer and head and neck cancer. In persistent HPV infection, a group of cytosine deaminases, APOBEC3 (A3), is involved in the accumulation of certain mutational signatures in the host cell genome, leading to high levels of DNA damage and oncogenesis. Two members of this family, A3A and A3B, have been associated with a high mutational burden in HPV-related cancers, while the editing-independent role of A3A and A3B in human tumorigenesis remains largely unknown. We first analysed the expression profile of head and neck cancer patients from The Cancer Genome Atlas (TCGA) and identified genes correlated with either A3A or A3B. Genes whose expression correlated with A3A appeared to be generally downregulated in HPV-positive patients. GO Analysis of these genes revealed enrichment of genes associated with epidermal differentiation and innate immunity. Genes correlated with A3B were associated with the cell cycle, chromosome organisation, DNA replication, and DNA repair and were overexpressed in HPV-positive cancer patients. This suggests a distinct role for the two enzymes. A3A being more involved in the antiviral response and therefore mostly present in HPV-negative cancers, whereas A3B affects the expression of genes involved in cell transformation and is more abundant in HPV cancer patients. HPV host cells HFK (human foreskin keratinocytes) deficient in A3A or A3B protein showed altered expression of selected genes correlating with the expression of A3A or A3B proteins and HPV oncoproteins. These cell lines also exhibited altered patterns of cell proliferation, migration, and invasion, confirming the role of editing-independent activity of A3 proteins in cell transformation, particularly in the context of HPV.

P69
CTLA4 polymorphisms play a role in asbestos-related diseases

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Background: Exposure to asbestos is a known risk for the development of asbestos-related diseases, including pleural plaques (PP), asbestosis and malignant mesothelioma (MM). In addition, inflammatory processes and the immune response also contribute to the development and the progression of these diseases. Peripheral T lymphocytes, as an important part of immune response, are controlled by multiple checkpoints, one of them being cytotoxic T-lymphocyte-associated protein 4 (CTLA4; CD125). CD125 binds to antigens expressed on tumor cells and consequently repress the immune response. Within this study, we aimed to investigate the potential associations between *CTLA4* polymorphisms and asbestos-related diseases.

Methods: We included 824 Slovenian patients with either PP (N=376), asbestosis (N=151) or MM (N=297), and 78 healthy occupationally asbestos exposed controls from our previous retrospective association studies. All subjects were genotyped for common *CTLA4* polymorphisms rs4553808, rs5742909, and rs231775. Logistic regression was used in statistical analysis.

Results: Lower risk for developing asbestos-related diseases was observed in carriers of two polymorphic rs4553808 G-alleles (OR=0.32, 95% CI=0.15-0.72, P=0.006) when compared to carriers of two normal A-alleles. Specifically, these subjects had a lower risk for developing PP in the dominant and additive genetic models (OR=0.61, 95% CI=0.37-0.995, P=0.048 and OR=0.28, 95% CI=0.11-0.67, P=0.005, respectively) and for MM in the dominant genetic model (OR=0.56, 95% CI=0.32-0.98, P=0.044). Furthermore, higher risk for developing MM was observed in carriers of two polymorphic rs231775 G-alleles (OR=1.96, 95% CI=1.21-3.15, P=0.006) when compared to all other subjects. *CTLA4* rs5742909 polymorphism was not associated with the risk for any of the investigated asbestos-related diseases.

Conclusion: *CTLA4* polymorphisms may play a role in the development of asbestos-related diseases.

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P70
Chemotherapy affects the metabolism of glioblastoma stem cells and differentiated cells differently

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Background: Common feature of cancer cell metabolism is the Warburg effect, which states that tumour cells rely mainly on glycolysis to produce ATP even when oxygen is present because mitochondria do not function properly. Recent research has shown that OXPHOS is not defective in most types of cancer cells and remains the main source of ATP despite the increased level of glycolysis. OXPHOS plays an important role in meeting the energy needs of cancer stem cells, including glioblastoma stem cells (GSCs). Chemotherapeutic genotoxic drugs cause a shift in cancer cell metabolism towards upregulated OXPHOS and mitochondrial biogenesis. The metabolic state of GSC is different from that of a differentiated glioblastoma (GB) cell. The ability of GB cells to use multiple metabolic pathways for energy production makes them resistant to therapies targeting single metabolic pathways.

Aim: To understand the metabolic activity and ultrastructure of mitochondria of GB cells upon chemotherapeutic agent as well as explore the differences between GSCs and more differentiated GB cells.

Results: GSCs have large, elongated mitochondria that occupy a larger portion of the cytoplasm. Their cristae are aligned along the long axis of the mitochondria. Treatment with chemotherapeutic temozolomide for 48h caused dilatation of the cristae but did not affect the GSC viability. In contrast, GB cells have smaller, rounder mitochondria with cristae oriented in different directions. Treatment with temozolomide caused swelling of the mitochondria, but the viability of the cells was not significantly altered.

Conclusion: The GSC and GB function of mitochondria and their OXPHOS capacity are closely related to their ultrastructure. The elongated mitochondria with highly organized cristae of GSCs indicate enhanced biogenesis and fusion of mitochondria supported by OXPHOS, whereas the differentiated GB cells with fragmented mitochondria which exhibit less organized cristae are more glycolytic.

P71
New inhibitors of AKR1C enzymes and their effect on ovarian cancer cell lines

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High-grade serous ovarian cancer (HGSOC) is the most common type of ovarian cancer and accounts for approximately 80% of all ovarian cancer deaths. Although HGSOC treatment has advanced significantly over the last decade, the five-year survival rate for OC remains below 30%. Despite high initial response to first line chemotherapy, most patients relapse in 18 months after the initial treatment. AKR1C enzymes are involved in chemoresistance, either by metabolising chemoterapeutics or indirectly by eradicating the cellular stress created by chemoterapeutics. The aim of our study was to investigate 11 compounds as inhibitors of AKR1C enzymes and to evaluate their effects on proliferation of control and HGSOC cell lines. Six compounds can be classified as core-modified estrane derivates (DTP-153, DTP-154, DTP-155, DTP-158, AD-13), the other five are tetrahydronaphtalen-1-one derivates (AD-4, AD-5, DTP-036, DTP-026, DTP-150, DTP-150-KK). 13α-Estrone derivatives were more effective inhibitors than tetrahydronaphtalen-1-one derivates. The two most active inhibitors of the AKR1C2 isoenzyme, DTP-036 and AD-4, had an IC_{50} value of 3.3 μM and 14.2 μM, respectively. At 100 μM concentration two of tetrahydronaphtalen-1-one derivates (DTP-154 in DTP-158) also showed more than 50% inhibition of AKR1C3. The control ovarian cell line (HIO-80) and HGSOC cell lines (COV-362 and OVSAHO) were next exposed to 10 μM and 50 μM inhibitors for 48 hours. Cell viability of the OVSAHO cells was significantly decreased when exposed to 50 μM DTP-153, DTP-155, AD -13, and DTP-150. There were no effects on control cell line HIO-80 and HGSOC cell line COV-362 . To conclude, we have evaluated a series of compounds for their inhibition of AKR1C isoenzymes and discovered some new, selective inhibitors of AKR1C2 isoform.

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Whole blood transcriptomics for biomarker discovery in endometriosis

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Endometriosis is a chronic gynaecological condition that affects up to 10% of all women in reproductive age. The symptoms of endometriosis are non-specific and include severe pain and infertility. Currently, definitive diagnosis relies on laparoscopy, an invasive surgical procedure. Therefore, there is a clear need for a non-invasive diagnostic test that allows earlier detection and treatment of endometriosis. The aim of this study was to identify novel biomarker candidates for the non-invasive diagnosis of endometriosis using whole-genome RNA sequencing. Patients enrolled in this study had symptoms suggestive of endometriosis and were divided into case and control groups based on laparoscopy and histology. Patients were also divided into proliferative and secretory groups according to the phase of their menstrual cycle. Blood samples from the patients were collected at the University Medical Centre Ljubljana (Slovenia) and at the Medical University of Vienna (Austria). RNA sequencing was performed using the Illumina platform (Novogene, UK). Raw data were analysed using CLC Genomic Workbench 21 software (Qiagen, DE) and differentially expressed genes (DEGs) and transcripts (DETs) were determined (logFC > 0.5/FDR < 0.05). The identified DEGs and TEGs were analysed using the Reactome database. In the proliferative group, we identified 7 DEGs and more than 400 DETs between cases and controls. In the secretory group, we found 379 DEGs and 457 DETs between the case and control groups. The detected DETs were significantly enriched in signalling pathways related to the immune system (interferon and cytokine signalling) and gene transcription in both the proliferative and secretory groups. To our knowledge, this is the first study in which whole genome RNA sequencing has been used to discover endometriosis biomarkers in blood samples. Validation of selected DEGs in a larger number of patients is currently underway.

P73
The role of far upstream element binding protein 3 in bone remodeling

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Genome-wide association studies (GWAS) are powerful approaches for identifying genetic loci associated with bone mineral density (BMD). GWAS have identified hundreds of associations with BMD, but few have been functionally evaluated, and functional characterisation remains a challenge. One of the loci significantly associated with femoral neck BMD on a genome-wide level (p=3.4×10-22) is SNP rs7851693 from the intron of the far upstream element binding protein 3 (FUBP3) gene. Here, we investigated the functional role of FUBP3 in bone biology across multiple in silico, in vitro and in vivo functional screens in human cells and model organisms. The expression of FUBP3 in 43 osteoporotic and osteoarthritic human bone tissue samples was compared with healthy controls. The expression of FUBP3 was significantly decreased in bone tissues from osteoporotic patients compared to controls (p=0.004). In addition, we examined the expression of FUBP3 in whole fish during zebrafish development and adulthood and during fin regeneration using in situ hybridisation and Q-PCR. The twofold increase in FUBP3 expression (p=0.003) in the newly formed zebrafish fins suggests that FUBP3 is involved in tissue regeneration and bone tissue formation. Furthermore, we investigated the expression of FUBP3 during osteogenic, adipogenic and myogenic differentiation of human mesenchymal stem cells. Indeed, silencing of FUBP3 inhibited osteogenic differentiation, confirming the involvement of FUBP3 in osteoblast formation. Transcriptome analysis of FUBP3 osteosarcoma knockout cells suggests multiple pathways by which FUBP3 impairs osteogenesis. All in all, our results suggest that FUBP3 plays an important role in bone biology.

P74
Role of estrogen metabolism in chemoresistance of high-grade serous ovarian cancer cell lines

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High-grade serous ovarian cancer (HGSOC) is the most aggressive and chemoresistant form of epithelial OC and is responsible for ~80% of OC-related deaths. OC is associated with disturbed estrogen action. In postmenopausal patients, estrogens are formed locally from steroid precursors. To date, the interplay between estrogen synthesis and HGSOC chemoresistance remains unclear. The aim of this study was to investigate the role of estrogen synthesis in the chemoresistance of HGSOC cell lines OVSAHO, OVCAR-3, Kuramochi, OVCAR-4, Caov-3, and COV362. We first determined the expression of genes involved in estrogen biosynthesis/metabolism (*STS*, *SULT1E1, HSD17B1*, *HSD17B2, HSD17B14*, *PAPSS1*, *PAPSS2*), steroid transport (*SLCO1A2*, *SLCO1B3*, *SLCO2B1*, *SLCO4A1*, *SLCO4C1*, *ABCC1*, *ABCC4*, *ABCC11*, *ABCG2*, *SLC51A*, *SLC51B*), estrogen action (*ESR1*, *ESR2*, *GPER*) and oxidative metabolism (*CYP1A1*, *CYP1A2*, *CYP1B1*, *SULT1A1*, *SULT2B1*, *SULT1E1*, *UGTB7*, *COMT*, *NOQ1*, *NOQ2*, *GSTP1*) by qPCR. Next, we evaluated the formation of active estrogens in HGSOC cells by their incubation with estrone-sulfate followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

Expression analysis revealed that estrogen receptor *ESR2* was highly expressed in the most resistant cell lines COV362 and Caov-3. The mRNA levels of estrogen biosynthesis and oxidative metabolism genes *PAPSS2*, *STS*, *HSD17B14, NOQ1* and *GSTP1* were increasing with carboplatin resistance of HGSOC cell lines and the efflux transporters *ABCG2* was highly expressed in the most resistant cell lines COV362 and Caov-3. These results indicate a potential of *ESR2*, *PAPSS2*, *STS, HSD17B14, NOQ1*, *GSTP1* and *ABCG2* as predictive markers of HGSOC chemoresistance. Furthermore, LC-MS/MS analysis showed that active estrogens are formed only in cell lines sensitive to carboplatin and not in the most resistant cell line COV362.

Further studies are ongoing to elucidate the mechanism of the interplay between local estrogen metabolism and HGSOC chemoresistance.

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Innovative Probes of Metalloproteases 2 and 9 for Targeting Brain Tumor Glioblastoma

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Increased expression and activity of matrix metalloproteases (MMPs) 2 and 9 are associated with invasiveness of the most malignant brain tumor glioblastoma. To date, the exact role of MMP-2 and -9 and their therapeutic potential for cancer treatment has not been fully elucidated due to lack of selective MMP probes and inhibitors. The aim of this study was to develop novel small molecule activity-based probes (ABPs) to monitor MMP-2 and -9 activity in glioblastoma. ABPs exclusively target active forms of MMPs, consist of a pseudopeptide scaffold as a recognition motif for the active site of MMPs, a cleavable linker as a smooth electrophile that can react with nucleophiles in proximity to the catalytic cleft, and an analytical handle that is covalently transferred to MMPs for subsequent detection. We have demonstrated the full synthesis and characterization of ABPs using an Fmoc strategy on a solid support, specifically designed for higher selectivity in MMP labeling experiments. We incorporated a PEG into the structure of the probes to limit hydrophobic interaction with biomatter and thus unselective binding of the probe, while aiming to limit most other unwanted MMPs by targeting the S3' region of the MMP for nucleophilic attack. Probes were fully characterized and tested by labeling experiments, followed by SDS-PAGE /in-gel fluorescence analysis against MMP-2, -9, and -12, which revealed positive binding to these MMPs. Primary and established glioblastoma cells, as well as glioblastoma organoids, were screened for expression and secretion of MMP-2 and -9 to establish cellular models for testing the selective MMP probes and inhibitors *in vitro*. Overall, we have synthesized ABPs selective for MMP-2, -9, and -12 that are now being tested in glioblastoma cellular models. Further experiments are needed to fully understand how the changes we have made to the structure of the probes compared to previous probes affect the binding properties and their selectivity.

P76
The impact of oxygen level on stress response triggering

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Oxygen is necessary for normal cellular function, as it strongly influences cellular physiology as an electron acceptor and through the formation of reactive oxygen species (ROS) with roles ranging from signalling to causing cellular damage. The oxygen pressure in arterial blood (partial pressure of oxygen, pO_2) differs among the tissues in an organism and is higher in the lungs than in other tissues. Mammalian cell cultures are routinely conducted under atmospheric oxygen levels, yet there are reports of different cell responses related to differences in oxygen supply, not just lower (hypoxia), but also higher than is tolerated by the tissue (hyperoxia).

Caspase-9 inactivation is central to the stress adaptation preapoptotic cell stress response (PACOS) that is triggered in liver cells (hepatocytes) upon the moderate stressor. ROS are crucial in the cell conversion from normal to stress response state, as increased levels of H_2O_2 are necessary for the stress response phenotype. Therefore, we investigated the role of 20 % and 3 % oxygen on PACOS activation and observed that these conditions had no influence on this stress response activation.

P77
Gastrointestinal hormone gastrin affects extracellular vesicle secretion and function

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Various molecular and genetic factors contribute to the development and progression of gastric cancer. Elevated plasma concentration of gastrointestinal hormone gastrin is associated with increased risk for gastric cancer. It could affect the concentration and content of extracellular vesicles secreted from cancer cells. These are small membrane-bound particles that transport oncogenic factors between cells and affect carcinogenesis. Our aim was to study the effect of gastrin on extracellular vesicle secretion and function in gastric cancer cell line.

We exposed gastric cancer cell line MKN45 to gastrin. We isolated large oncosomes, microvesicles and exosomes using differential centrifugation and characterized them by western blotting, TEM and confocal microscopy. Flow cytometry and NTA were used for concentration and size determination. Functional analysis of gastrin-induced extracellular vesicles on the naïve gastric cancer and normal epithelial cell lines was performed with cell proliferation and migration assays. We isolated total RNA and performed miRNASeq and pathway enrichment analysis in gastrin-exposed and control cells.

We isolated three distinct populations of extracellular vesicles with specific protein content and size. Exosomes were enriched in endosomal proteins Alix and CD9, whereas microvesicles and large oncosomes were enriched in Cytokeratin 18. Gastrin increased the secretion of microvesicles by 1.802-fold (P = 0.0466). Gastrin-induced exosomes increased the proliferation of naïve gastric cancer cells by 1.380-fold (P_{adj}=0.0001), but did not affect cell migration. Thirty-five cellular miRNA were up-regulated and eighteen were down-regulated in gastrin-exposed cells in comparison to control. Differentially expressed miRNAs regulate targeted genes that were predominantly involved in metabolic pathways.

Gastrointestinal hormone gastrin affects miRNA expression in gastric cancer cells, as well as extracellular vesicle secretion and function.

P78
The establishment and characterization of 3D models of high grade serous ovarian cancer

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High grade serous ovarian cancer (HGSOC) is the most lethal gynecologic malignancy. Studies of HGSOC usually involve 2D cell lines, which are only partially suitable models due to the lack of spatial tumor formation. 3D cellular models, such as spheroids, present promising alternatives. With a spatial structure more similar to *in vivo* tumors, spheroids better mimic biochemical changes leading to the loss of cellular polarity and the gaining of migratory and invasive cellular properties (epithelial-mesenchymal transition, EMT). Despite advantages, HGSOC spheroids are not yet commonly used due to higher costs and technical challenges and have not yet been morphologically and biochemically evaluated.

In our study, the aim was to establish 3D spheroids of well-characterized HGSOC cell lines OVCAR-4, Kuramochi, OVSAHO, and COV362 and compare their characteristics to 2D models. Our 3D models show reproducible morphological features, with spheroid diameters ranging from 400 µm (Kuramochi cells) to 1400 µm (OVCAR-4 cells), and the roundness for all cell lines exceeding 0.85. We next evaluated the expression of 22 genes of EMT, extracellular matrix proteases, angiogenesis-associated factors, mutation markers, and proliferation markers. As expected, we observed higher expression of EMT markers *VIM* and *SLUG*, indicating a more invasive phenotype of cells in spheroids. Furthermore, the increased *WNT11B* expression in spheroids suggested a higher impedance of these cells to attach to the extracellular matrix. Decreased *BRCA1* expression was observed in spheroids, suggesting a closer resemblance of spheroids to *in vivo* tumors. Interestingly, we also observed lower expression of the proliferation marker Ki67 in spheroids compared to 2D cells.

Our results confirm the higher suitability of HGSOC spheroids over 2D cell lines. Functional studies showing differences in cell proliferation, migration, invasiveness, and response to chemotherapeutics between 3D and 2D models are currently in progress.

P79
Investigating Response Factors to Thiopurines in Pediatric Acute Lymphoblastic Leukemia Patients

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As cytostatic and immunosuppressive drugs, thiopurines play indispensable role in treatment of acute lymphoblastic leukemia and autoimmune diseases. Due to their narrow therapeutic index, small changes in their branched metabolism can cause severe side effects or leave the patient, treated with thiopurines, unresponsive. In pursuit of personalized dosing, we investigated the factors influencing their response. We established a cohort of 37 pediatric patients with acute lymphoblastic leukemia. We gathered comprehensive demographic data and treatment protocol information, and collected blood samples at multiple time points during their consolidation treatment phase. Using TaqMan assays, Sanger sequencing or amplicon-based sequencing by synthesis, we genotyped the patients for most common genetic polymorphisms in thiopurine metabolism and folate cycle. We employed HPLC protocols to measure the level of thiopurine metabolites, specifically 6-thioguanines (6TGN) and 6-methylmercaptopurines (6MMP). Our findings revealed a significant correlation between the dosage of thiopurines and the 6MMP/6TGN ratio in hemolysates (r=0.5, p=0.003). Furthermore, activity of liver enzymes AST or ALT in patients significantly correlated with levels of 6MMP in hemolysates (r=0.4, p=0.001 and r=0.4, p=0.001, respectively), indicating hepatotoxic activity associated with this metabolite. Although we did not identify any clinically established genetic polymorphism in *TPMT* within this cohort, next-generation sequencing of *TPMT* in selected individuals (N=15) unveiled several different single nucleotide polymorphisms, some of them identified for the first time. Bioinformatic analysis of certain detected variants predicted potential effects on the enzymatic activity of TPMT. Future determination of consequences that these polymorphisms have on TPMT activity, may further elucidate the effect of identified factors in thiopurine treatment.

P80

Genetic variability in Alzheimer's disease risk loci associated with CSF biomarkers and cognitive test scores in Slovenian patients

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Background: Alzheimer's disease (AD) is a prevalent neurodegenerative disease with a complex genetic background. The familial form is linked to rare monogenic mutations, while the susceptibility for the more common sporadic form is influenced by a combination of multiple risk loci dispersed throughout the genome. Several genome-wide association studies (GWAS) already led to identification of numerous AD risk loci. Based on the disease associated changes in cerebrospinal fluid (CSF) biomarkers and advanced imaging techniques, AD-related brain dysfunction can be detected even before the onset of clinical symptoms. This initial, pre-dementia stage of the disease that can progress to AD is known as mild cognitive impairment (MCI). In this study we aimed to assess the associations between SNPs in well-established GWAS AD risk loci and CSF biomarker levels or cognitive test result in a Slovenian population with cognitive decline.

Methods: We included 82 AD patients, 28 MCI patients with pathological CSF biomarker levels, and 35 MCI patients with normal CSF biomarker levels. Patients were genotyped for 9 polymorphisms using competitive allele specific PCR.

Results: Carriers of at least one polymorphic *TOMM40* rs157581 C allele had lower $A\beta_{1-42}$ ($p = 0.033$) and higher total tau ($p = 0.032$) and p-tau_{181} levels ($p = 0.034$). Carriers of at least one polymorphic *SORCS1* rs1358030 T allele had lower total tau ($p = 0.019$), while *SORCS1* rs1416406 polymorphic C allele was associated with lower total tau ($p = 0.013$) and p-tau_{181} ($p = 0.036$). Additionally, carriers of at least one polymorphic *BCHE* rs1803274 T allele achieved lower MMSE scores ($p = 0.029$).

Conclusion: Our findings may contribute to the identification of genetic markers associated with AD and MCI and provide further insights into early diagnosis of the disease.

P81
CD44, TNFSF14 and HOXD13 are potential PD-L1 related glioblastoma biomarkers

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Background: Glioblastoma is the most common primary brain tumor with an exceptionally low survival rate, as most patients die within 2 years of diagnosis. The main factors contributing to treatment failure are lack of reliable biomarkers and efficient therapy. In our research, we analyzed gene expression of the PD-L1 network, one of the most de-regulated immune pathways in cancer, as potential glioblastoma biomarkers.

Methods: Network was constructed using Cytoscape, with STRING PubMed query and STRING protein query. Gene expression was analyzed using CGGA (mRNAseq_693 dataset), TCGA (TCGA GBMLGG dataset retrieved from GlioVis), and Rembrandt (retrieved from GlioVis). Survival analysis of GBM patients was determined based on optimal cutoff, calculated by surv_cutpoint function in R (version 4.1.1), using package survminer. The expression of selected genes was determined on glioblastoma, lower-grade glioma and normal brain tissue with qPCR.

Results: Enrichment data analysis shows that the genes are mainly involved in immune system processes. Next, we selected genes that were overexpressed in glioblastoma compared with tumors of lower grade glioma, overexpressed in glioblastoma compared with normal brain tissue and their higher expression was associated with poorer overall survival. Most promising genes were at last validated on tissue samples by qPCR. Results show that *CD44*, *TNFSF14* and *HOXD13* are overexpressed in glioblastoma (validated in silico and in vitro) and related to worse overall survival (validated in silico).

Conclusion: Our results suggest that *CD44*, *TNFSF14* and *HOXD13* genes may be novel biomarkers and therapeutic targets for glioblastoma.

P82

Epigenetic changes in Slovene male suicides: Histone tail posttranslational modifications and microRNAs

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Suicide is a significant public health issue, with more than 700,000 deaths occurring annually. Because of its complex and multifactorial phenomenon, a multidisciplinary approach is needed for a better understanding of its underlying mechanisms. Epigenetic studies offer a promising avenue to explore the links between environmental and biological factors contributing to suicide. Three of the most studied epigenetic modifications are DNA methylation, histone posttranslational modifications, and non-coding RNAs. DNA methylation and histone posttranslational modifications regulate transcription of DNA to messenger RNA, while micro RNAs (miRNAs) regulate mRNA translation into proteins. Furthermore, miRNAs have been identified as cargo of extracellular vesicles (EVs) used for intercellular communication, which can be found also in cerebrospinal fluid (CSF) vesicles.

To investigate epigenetic changes, we examined the differential expression of selected miRNAs in EVs from CSF and the acetylation of lysine 14 on histone 3 (H3K14ac) in the hippocampus of male suicide completers and controls. Chromatin immunoprecipitation and next-generation sequencing (ChIP-seq) were used to study H3K14ac modification.

Out of 20 analyzed miRNAs, 9 were present in EVs of the CSF. We found that miR-19a-3p and miR-4516 reached statistical significance. These miRNAs have been previously studied in suicide and have been found to target SLC6A4 and TNF-α expression. The study of H3K14ac showed an overall decrease in acetylation of lysine 14 in the hippocampus of suicides. From 293046 peaks, 1682 peaks reached statistical significance ($q \leq 10^{-5}$). Further research is needed to determine genes affected by this modification.

There is importance of epigenetic research in psychiatry for the identification of relevant biomarkers. However, the role of epigenetic modifications in the pathophysiology of suicide needs further investigation to develop a more comprehensive understanding of this complex phenomenon.

P83
Glioblastoma biomarkers identified with RNAseq in glioblastoma tissue for non-invasive liquid biopsy

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Glioblastoma, the most prevalent and lethal type of brain tumor, presents a major challenge in terms of early diagnosis due to the appearance of non-specific symptoms and the tumor's aggressiveness. As a result, there is a critical need for biomarkers that can be detected non-invasively via liquid biopsy for diagnostic and therapeutic purposes. Additionally, GBM is known for its intra-tumor heterogeneity, which is an outstanding characteristic of the disease. To capture overall tumor heterogeneity, we determined expression changes by RNAseq of brain samples from patients diagnosed with glioblastoma compared to tissue postmortem brain samples from healthy individuals. A cDNA library was prepared from isolated RNA from normal brain tissues of 13 patients and 10 glioblastoma tissues with NEBNext rRNA depletion kit. The quality of the library was checked with a Bioanalyzer. Sequncing of the library was performed on NovaSeq 6000 system (Illumina). The differential mRNA expression in glioblastoma samples was determined by bioinformatics analysis of NGS mRNA data based on the SnakePipes tool, which includes Cutadapt to remove adaptor sequences and sequences of lower quality. After the analysis, sequences where the coverage was less than 70 % and the percentage of dimers greater than 40 % were removed from further analysis. The Salmon Pseudo-alignment algorithm tool was utilized to quantify and count mRNA sequences and the R DEseq library was applied to display basic differential expression metrics. The genes with log2FC > 1 and log2FC < 1 with adj. p-value < 0.05 were considered as significant differentially expressed genes. We identified 5132 differently expressed genes and the most promising among the overexpressed genes for detection in liquid biopsy are *EMILIN, TUBB6, PLA2G2A, FMOD, CHRDL2.*

P84
Surface plasmon resonance approach to study drug interactions with SARS-CoV-2 RNA-dependent RNA polymerase

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The SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) is essential for virus replication, therefore it is a promising drug target. Here we present a surface plasmon resonance approach to study the interaction of RdRp with drugs in real-time. We monitored the effect of favipiravir, ribavirin, sofosbuvir triphosphate PSI-7409 and suramin on RdRp binding to RNA immobilized on the chip. Suramin precluded interaction of RdRp with RNA and even displaced RdRp from RNA.

P85
Interaction between molecules involved in DNA-protein crosslink repair

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DNA-protein crosslinks (DPCs) are DNA lesions which occur when a protein becomes irreversibly covalently linked to DNA. DPCs have adverse effects on the organism, including cancer, premature aging and neurodegenerative diseases. Due to their bulky nature, DPCs affect all DNA transactions such as replication, transcription and repair, making DPC repair an essential cellular pathway.

DPC repair includes diverse mechanisms, which are still largely unknown. We are studying two proteins of human origin involved in the DPC repair process: a DNA dependent metalloprotease called SPRTN and a hexameric AAA+ unfoldase called p97. The N-terminal protease domain of SPRTN is well structured, whereas the C-terminal part (roughly half of the protein) is intrinsically disordered. Within this unstructured region, there are many interaction motifs, one of them is the SHP motif, which is thought to be the main interaction motif between the SPRTN and p97 and is also used by many other proteins that bind to p97.

We have examined the molecular interactions between p97 and SPRTN, and SPRTN with DNA using various biochemical and biophysical approaches such as pull-down assays, size exclusion chromatography (SEC), microscale thermophoresis (MST), surface plasmon resonance (SPR), and isothermal titration calorimetry (ITC). The interactions studies were performed using both the full-length SPRTN and a truncated version that has a shorter unstructured part, but still contains the SHP interaction motif. Due to low sample consumption and rapid assay setup and optimization the majority of interaction experiments were performed using MST. We have shown that, in human cells, overexpression of SPRTN mutant which cannot bind to p97 leads to a significant increase in DPC levels in cells lacking SPRTN-WT.

In this presentation, we will show and compare the results obtained with different approaches and discuss their contribution in understanding the molecular and mechanistic background of the DPC repair.

P86
Insights into Transcriptional Regulation of Potato in Response to Viral Infection

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One of the most widely spread crop pathogen is potato virus Y (PVY), which causes vast crop loss all over the globe. Plants have developed a multi-layered immune response based on the recognition of pathogens by plant cell-surface and intracellular receptors encoded by *R*-genes and it comprises of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Whereas studies show great importance of the RNA silencing as the third layer of plant immunity. All those processes are thoroughly studied in model plants such as *Arabidopsis thaliana,* while we still lack a lot of information about mechanisms in crops. Hence, we decided to study transcriptional regulation of potato (*Solanum tuberosum*, L.) in response to viral infection.

The first part of our study focuses on the group of ARF transcription factors (TFs), known as auxin response factor. Among them, StARF10 was described as an interesting TF involved in potato response to PVY and previous transcriptomic studies identified the peroxidase gene *StPRX28* as its potential target. We tested here StARF10 involvement in the regulation of expression of *StPRX28* using transactivation assays in *Nicotiana benthamiana* plants. We showed that StARF10 can significantly increase the activity of *StPRX28* promoter.

In the second part we examined the interactions of TGA TFs, belonging to the basic region leucine zipper (bZIP) protein family. Previous studies recently identified a mini-TGA, StTGA2.1, lacking most of the bZIP domain and thus unable to directly bind DNA target. However, we expected that it can regulate promoter activity by forming heterodimers with other members of StTGAs family. We tested our hypothesis using transactivation assays in *N. benthamiana* transiently expressing StTGAs and their potential target, *StPRX07* promoter. We confirmed that StTGA2.1 by itself has only minor effect on the promoter activity, while heterodimer StTGA2.1–StTGA2.3 can significantly increase the activity of *StPRX07* promoter.

P87
The role of ubiquitination, proteasome and autophagy in degradation of pyruvate dehydrogenase kinase 1 in cultured skeletal muscle cells

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Pyruvate dehydrogenase complex (PDC), which converts pyruvate to acetyl-CoA, is a gatekeeper between glycolysis and the tricarboxylic acid cycle. One of the most important regulators of PDC is pyruvate dehydrogenase kinase 1 (PDK1), which inhibits its activity through phosphorylation, thus suppressing oxidation of glucose and increasing lactate production.

Despite its importance, the mechanism by which PDK1 is degraded has not been thoroughly researched. We previously established that inhibition of the proteasome or mitochondrial proteases does not increase or even decreases PDK1 protein levels in cultured skeletal muscle cells. Here we examined the role of ubiquitination and autophagy in degradation of PDK1 in cultured L6 and human myotubes.

Inhibition of protein ubiquitination with PYR-41 decreased the level of PDK1 protein. Conversely, the levels of HIF-1α, known to be degraded by the proteasome, were increased by PYR-41. Treatment with chloroquine, an inhibitor of autophagy, followed by PYR-41 treatment, did not increase PDK1 protein levels. Proteasomal inhibitor MG-132 decreased both PDK1 protein and mRNA levels. Inhibition of transcription and translation with actinomycin D and puromycin, respectively, did not significantly decrease PDK1 protein levels within the chosen timeframe.

These results demonstrate a paradoxical decrease in PDK1 protein levels following proteasomal inhibition, which seems to exclude proteasome as the site of PDK1 degradation in cultured myotubes. Moreover, autophagy is also unlikely to play a significant role in PDK1 degradation. Finally, the experiments involving transcription and translation inhibition suggest that PDK1 protein is stable, with a relatively long half-life. Reduction in PDK1 mRNA caused by MG-132 therefore cannot account for the decrease in PDK1 protein levels. Collectively, our results suggest that PDK1 is degraded via a proteolytic pathway that does not involve ubiquitination, proteasome, or autophagy.

P88

Newly discovered miR160-PRX28 module regulates programmed cell death in potato in response to Potato virus Y and Phytophthora

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MicroRNAs (miRNAs) are a class of small noncoding RNAs that modulate the abundance and spatiotemporal accumulation of target mRNAs at the post-transcriptional level, thereby playing an important role in various biological processes in plants. Using non-targeted spatial transcriptomics approach, we uncovered miR160 as important component in hypersensitive response (HR)-conferred resistance of potato (Solanum tuberosum L.) to potato virus Y (PVY) infection. To functionally characterize the role of miR160 in HR, we generated two sets of transgenic lines: one with miR160 overexpression and the other with silenced miR160. When we challenge the plants with PVY, we found that miR160 does not directly participate in restricting the virus. However, we revealed a previously unknown involvement of miR160 in programmed cell death (PCD), which is accompanied by HR, leading to lesion formation. We observed that overexpression of miR160 in the transgenic potato plants resulted in a reduction in both the size and number of lesions formed on the leaves upon PVY infection. Conversely, when we silenced miR160 expression in the transgenic lines, it induced larger lesion formation. We analyzed the processes in the cell death zone and surrounding tissue at the gene expression level to reveal the spatiotemporal regulation of the immune response. We discovered that the level of the peroxidase gene 28 (PRX28) was significantly reduced when miR160 was upregulated, thus showing a negative correlation with miR160 levels. Furthermore, we employed another pathosystem in which we challenged the potato plants with the cell death elicitor Pep-13, derived from Phytophthora. Again, overexpression of miR160 resulted in a decreased cell death zone and lower PRX28 levels. These findings suggest that the role of the miR160-PRX28 module extends beyond PCD during HR, highlighting its broader significance in regulating cell death processes in plants.

P89

Fungal lectins show antibiofilm activity against foodborne strains of *Listeria monocytogenes* and *Listeria innocua*

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Listeria monocytogenes is a highly pathogenic bacterium that can form biofilms in food processing facilities, allowing the bacterium to persist in the environment despite the control measures applied. Since the bacterial surface is covered with versatile polysaccharides, these have a major impact on the bacterium's interactions with any surface. Lectins are proteins that bind glycans with high specificity without altering them, and they are ubiquitous in all organisms. Therefore, due to their unique properties and high stability, fungal lectins are ideal candidates for bacterial control. In this study, we investigated the antibacterial and antibiofilm activity of fungal lectins against model strains of L. monocytogenes (serotype 4b) and L. innocua (serotype 6). The tested fungal lectins showed antibiofilm activity but no antibacterial activity. They act selectively on biofilms of the species tested and interfere with adhesion to the surface of L. innocua but not L. monocytogenes; several lectins were also found to disrupt mature biofilm on polystyrene. Fungal lectins also affected biofilm development on biological model surfaces, mucin and fibronectin, in both species, but had no effect on adhesion of L. monocytogenes to the Caco-2 cell line. We confirmed that glycans and proteins on the cell surface play an important role in the interactions between the surface and Listeria. Modulation of the interactions by lectins has therefore been shown to be a suitable strategy for developing new approaches to prevent surface contamination by bacteria.

P90
Phosphoproteomic analysis of legumain deficient mice

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Legumain, a member of the cysteine proteinase family, acts as an asparaginyl-specific protease. It is primarily located in the endolysosomal system but can also be present in the cytoplasm, cell nucleus, and extracellular space under specific physiological conditions, such as a tumour environment. The amino acid sequence of legumain is highly conserved across various species, suggesting its importance in normal physiological function. Remarkably, when legumain is knocked out in mice, they exhibit a mild phenotype. These mice remain viable, fertile, and show no behavioural abnormalities. However, they do exhibit reduced body mass, irregular kidney function, and hyperinflammation compared to their wild-type counterparts. Despite these observations, a comprehensive understanding of the molecular basis for this phenotype is largely unknown due to the lack of systemic studies in these animals. One notable finding is the significant increase in EGF receptor levels in legumain null mice, which may lead to global changes in cellular signalling. Along with EGFR, different peroxidases are also upregulated in legumain knock-out animals. Furthermore, legumain may also influence the function of other receptors and kinases. To elucidate potential molecular interactions that explain the observed phenotype in legumain knock-out mice, we employed phosphoproteomic techniques to study changes in levels of protein phosphorylation. Additionally, we utilized immunological methods to validate selected target proteins while measuring the expression levels of specific target proteins. The results obtained from these studies will provide insights into legumain's role in organism physiology and its possible involvement in the immune response.

P91
The NLP cytolysin pore-forming action finally unveiled: small, transient and unique

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Necrosis and ethylene-inducing peptide 1-like proteins (NLPs) are produced by a plethora of plant pathogens, imposing an alarming threat to worldwide food security. Many NLPs are cytotoxic, causing cell death and tissue necrosis by disrupting the plant plasma membrane. Glycosylinositol phosphorylceramides (GIPC), the most abundant class of plant sphingolipids, are receptors for NLP binding to membranes. Just recently, it was shown that this lipid recognition is eletrostatic-driven and leads to shallow membrane binding, protein aggregation and transient pore formation.

We are exploiting various model lipid systems, composed of plant-isolated GIPC, e.g. cell-sized vesicles, which are conveniently followed with confocal microscopy. Visual information after NLP_{Pya} – membrane interaction about localised toxin binding, changes in morphology of the vesicles and differential leakage of different-sized probes allows to make predictions about membrane-damaging mechanism. Furthermore, molecular dynamics simulations revealed that the C-terminal α-helix of NLP_{Pya} undergoes conformational rearrangements during membrane interactions. We are tackling this clue by designing mutants of NLP_{Pya}, among which cysteine ones are of special interest. Introduced cysteines are labelled with IANBD to monitor the insertion of this residues into the lipid bilayer by changes in its fluorescence. Double cysteine mutants have C-terminal α-helix locked to the core of the protein upon disulfide formation. Reduced cytolitic activity would be the sign that flexible C-terminal region is indeed a pre-requisite for proper membrane interaction.

Our study will reveal specific molecular insights into the toxic NLPs- plant membrane interaction which are crucial for the development of better strategies for crop protection.

P92
Generation of synthetic nanobodies for non-invasive detection of cathepsin B in cancer

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Aberrant cathepsin activity contributes to different pathological states such as cancer, arthritis, osteoporosis, and infectious, cardiovascular as well as neurodegenerative diseases. Increased cathepsin expression has been associated with poor prognosis in several cancers. Cathepsins function as acid hydrolases within the lysosome and are effectors of protein catabolism, thereby aiding increased metabolism of proliferating cancerous cells. Secreted cathepsins are involved in the modification of tumours through proteolytic processing and degradation of the extracellular matrix, cytokines and growth factors. Because of that and their extremely high overexpression in tumour microenvironment, cathepsins are promising targets for development of non-invasive diagnostic imaging platforms. In addition to small molecules, different types of binding proteins such as full-sized antibodies, alternative antibody formats and small protein scaffolds have been utilized as imaging agents. However, these molecules penetrate tumours poorly and require considerable manufacturing efforts to achieve sufficient expression levels. Single-domain antibody fragments or nanobodies are attractive alternatives because of their small size (12-14 kDa) which allows them to disperse through the bloodstream and reach targets with high affinity and specificity. In the present study, we generated synthetic nanobodies known as sybodies against human and mouse cathepsin B by employing an *in vitro* approach based on ribosome display and mRNAs encoding sybody library followed by a phage display selection. We identified several sybody candidates that neutralized recombinant cathepsin B cleavage of the fluorogenic subtrate zRR-AMC and bound to the target in a macrophage cell line and primary tumour cells. The sybody platform thus offers a potential to be used for future development of non-invasive assessments of cathepsin expression at cancer site or monitoring of disease progression.

P93
Synergistic effects of polyphenols from propolis

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Propolis is a lipophilic substance collected by bees from various plant sources. It is known for its biological activity, as more than 850 different molecules have been identified in different samples. The most important of them are polyphenols. It has long been suspected that the activity of propolis is due to the synergy between different molecules. The possible synergism of compounds in propolis was therefore studied on five polyphenols, namely chrysin (CHRY), quercetin (QUER), (-)-epigallocatechin-3-gallate (EGCG), trans-ferulic acid and caffeic acid (CAFF). All five were selected based on their demonstrated biological activity and occurrence in propolis samples. Their effects on the bilayer were studied on DPPC and phospholipon PL-90G liposomes mainly by DSC. To compare the effects of mixtures, both pure compounds and mixtures of two (CHRY /QUER; CAFF /QUER) or all five compounds at different molar ratios between compound(s) and lipid were studied. The results show that certain combinations exhibit strong (CHRY /QUER) or at least visible synergistic effects (mixture of all five polyphenols), while certain combinations show no effect at all (CAFF /QUER). Surprisingly, QUER showed better single effect than the mixture of all five compounds at twice higher molar ratio. On the other hand, the mixture of all five compounds showed a stronger effect than the other four molecules and inhibited UV-induced oxidation of lipids more successfully than EGCG. However, compared to the propolis extract, propolis showed a stronger effect on the bilayer than any mixture or single compound. This confirms not only that the potent effect of propolis is due to the unique synergy between its constituents, but also that not all molecules act synergistically. Certain compounds in a mixture may even exhibit antagonistic effects. Since the synergism of complex mixtures of substances is difficult to predict, their effects on lipids should be studied individually.

P94
Protein interactors of ORF1p from human retrotransposon L1

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Retrotransposon LINE1 is the only autonomous mobile genetic element still active in humans. It accounts for up to 17% of the human genome and also mobilizes non-autonomous elements. The LINE1 element contains open reading frames for three proteins, namely ORF0p, ORF2p and ORF1p which is an RNA-binding protein that acts as a nucleic acid chaperone. Elevated levels of LINE1 retrotransposition have been associated with several diseases, including certain cancers and neurodegenerative diseases. A comprehensive understanding of LINE1 retrotransposition is essential for the development of potential therapeutics. Therefore, it is important to discover novel proteins and RNA molecules that interact with LINE1 components - particularly ORF1p, and modulate retrotransposition. Using BioID we determined potential protein interactors of ORF1p from LINE1. Immunodetection of biotin identification mass spectrometry results confirmed 5 ORF1p interactors: IGF2BP1, TDP-43, ELAVL1, FUS, and hnRNPK. Gene ontology term enrichment analysis revealed that the potential ORF1p interaction partners are localized in stress granules, cytoplasm, nucleus, and nucleoli, just like ORF1p. Our results contribute to a better understanding of LINE1 and the factors regulating retrotransposition. We also have insight into the processes in which LINE1 may be involved.

P95
Maturation pathways of a subtilase from the hyperthermophilic archaeon Aeropyrum pernix

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Pernisine is an extracellular subtilisin-like proteinase produced by the hyperthermophilic archaeon *Aeropyrum pernix*, a colonizer of hydrothermal vents. This proteolytic enzyme is stable at temperatures around 100 °C and degrades resistant protein aggregates such as infectious prion proteins [1]. These properties make pernisine attractive for use in industrial applications. Pernisine is synthesized as an inactive zymogen (propernisine), consisting of a propeptide and a catalytic domain. Propernisine is autocatalytically matured into the active pernisine by degradation of the propeptide. The maturation of propernisine occurs only at high temperatures in the presence of Ca^{2+} ions, which thermally stabilize the propernisine structure [2]. However, we showed that the Ca^{2+}-bound catalytic domain of propernisine is proteolytically unstable but is further stabilized after cleavage of peptide linker between the propeptide and the catalytic domain. In the resulting noncovalent complex, the propeptide tightly interacts with and inhibits the catalytic domain, preventing rapid propernisine autoactivation even at high temperatures. The interaction between the catalytic domain of pernisine and its propeptide proved to be highly selective, as the pernisine propeptide is unable to bind to and inhibit mesophilic pernisine homologs. Instead, pernisine propeptide is rapidly degraded by these heterogeneous proteases, which in turn allows transactivation of propernisine. The transactivation of propernisine by mesophilic proteases occurs at a higher rate and at lower temperatures compared to the propernisine autoactivation. Therefore, this alternative propernisine maturation pathway might be applied to simplify the procedures for preparation of the active recombinant pernisine produced by mesophilic hosts.

1. Šnajder M. et al. 2012. *PLoS One* 7: e39548.
2. Bahun M. et al. 2020. *Applied Environ Microbiol* 86(17): e00971-20.

P96
Reversible and transient thrombocytopenia induced by snake C-type lectin-like proteins (snaclecs) from nose-horned viper venom

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In Slovenia, the nose-horned viper (*Vipera a. ammodytes, Vaa*) is the most medically important snake. Its venomous bite can result in severe thrombocytopenia. No agonists of platelet aggregation have yet been detected in *Vaa* venom. We hypothesized that snake C-type lectin-like proteins (snaclecs) could be responsible for this pathological effect. The aim of our work was therefore to isolate snaclecs from the venom and to investigate their ability to bind platelet receptors and trigger agglutination/aggregation. In the biochemical part of this study, we isolated snaclecs from a crude *Vaa* venom by a combination of different liquid chromatography techniques and then confirmed their purity by LC-ESI-MS/MS. We investigated them for their effect on platelet agglutination/aggregation by turbidometry and for their binding to platelet receptors and platelet activation by flow cytometry. *Ex vivo* studies showed that isolated *Vaa*-snaclec-3&2 binds to the platelet GPIb receptor and causes platelet agglutination inducing a drop of the platelet count. The process was reversible and it did not activate platelets. *Vaa*-snaclec-3&2 is an acidic, non-glycosylated 30 kDa protein. It is a heterodimer consisting of the snaclec subunits *Vaa*-snaclec-3 and *Vaa*-snaclec-2. By thromboelastometry, we evaluated platelet functionality in patients envenomed by the *Vaa* venom that suffer from thrombocytopenia, before and after antivenom therapy with $F(ab')_2$ fragments raised against the whole *Vaa* venom. Thrombocytopenia was reversed in all patients within one hour after the antivenom treatment. We are currently conducting an *in vivo* study in a mouse model of arterial thrombosis to validate the potential of *Vaa*-snaclec-3&2 to prevent arterial occlusion and to determine its potential for medical applications, for example in interventional angiology and cardiology.

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P97
SARS-CoV-2 spike protein protomer dynamics alters its protease cleavage efficiency and fusogenic activity

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After three years of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, the virus appears here to stay and has yet to settle into a predictable pattern. Spike protein, which plays an essential role in initiating SARS-CoV-2 infection, requires cleavage at the S1/S2 and the S2' cleavage sites. Subsequent major conformational changes enable the S2 subunit to mediate the fusion of viral and cell membranes. Studies on coronaviruses have shown the perplexing activation of spike proteins involving diverse host proteases.

The molecular mechanisms of viral infection remain insufficiently clarified; therefore, further understanding of the pathogen's structural modifications could help us against the current (or future) pandemic. We introduced several mutations into different domains of the spike protein to study pre- to post-fusion changes necessary for protease cleavage and membrane fusion. By utilizing sensitive split reporter cell assays, we demonstrated that trimer destabilizing mutations in the NTD or SD1 region of the S1 subunit did not significantly impact ACE2 receptor binding efficiency but led to reduced spike fusogenicity and protease cleavage. Additionally, the introduction of disulfide bonds within or between protomers as well as the insertion of different length linkers in flexible domains of the protein, such as the receptor binding domain, resulted in altered syncytia formation, in some cases even to its complete inhibition. Interestingly, titrations of mutant spike proteins with its wild-type version revealed some details on trimer formation, showing the potential of viral adaptability.

Taken together, protomer dynamics and spike assembly are very sensitive to relatively small changes in spike protein, thus affecting its furin cleavage efficiency, cell surface expression, and fusogenic activity.

P98
Effect of different ions on lactoperoxidase conformation stability and enzymatic activity

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Lactoperoxidase is a glycoprotein that belongs to the superfamily of mammalian haeme-containing peroxidases. Lactoperoxidase consists of a single polypeptide chain and contains a haeme-moiety, a calcium binding site, and conserved glycosylation sites. Lactoperoxidase is an important component of host defence due to its antimicrobial activity against a variety of Gramme-positive and Gramme-negative bacteria, viruses, fungi, and parasites. Lactoperoxidase has great potential as a natural food preservative. In addition to the food industry, the application of lactoperoxidase in human medicine has also been demonstrated. There are several factors that can positively or negatively affect the structure or activity of the enzyme during isolation, purification, storage and application. Therefore, for the application of lactoperoxidase as a biopreservative in the food industry and medicine, its structural integrity and enzymatic activity should remain intact. In our study, we investigated how different ions (Ca^{2+}, Mg^{2+}, and Na^{+}) affect the conformational stability and enzymatic activity of lactoperoxidase. UV-VIS Spectroscopic measurements of the conformational stability of lactoperoxidase in acetate buffer (pH 5.6) in the absence and presence of Ca^{2+}, Mg^{2+}, and Na^{+} ions showed that all ions used increased the conformational stability of lactoperoxidase. However, when the effects of these ions on the enzymatic activity of lactoperoxidase were examined, it was found that the presence Ca^{2+}, Mg^{2+}, and Na^{+} ions had no effect on the activity. To better understand the effects of different ions on the conformational stability and enzymatic activity of lactoperoxidase, we prepared lactoperoxidase that was partially depleted of iron and calcium ions (de-LPO). This de-LPO was thermally less stable compared to native lactoperoxidase, and the enzymatic activity also decreased. The addition of Ca^{2+}, Mg^{2+}, and Na^{+} ions to the de-LPO also had no effect on the enzymatic activity.

P99
Insight into non-muscle α-actinin heterodimerization using model half dimers

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Cell migration is an intricate cellular process that is involved in tissue formation and remodeling, immune response, and metastasis. The orchestration of cell migration requires precise spatial and temporal regulation of the underlying machinery, which is provided by the actin cytoskeleton, a sophisticated and dynamically regulated network of proteins. At its core, actin filaments serve as the principal components onto which various proteins can associate, thereby modulating their functionality. α-actinins play a prominent role as major F-actin bundling proteins. Non-muscle isoforms, α-actinin 1 and 4, are found in all types of cells. A common structural feature of α-actinins is their antiparallel dimerization. Recently, heterodimers of non-muscle α-actinins have been identified in cells, where they are the predominant form. It is unknown how these differ from homodimers in structure and F-actin bundling. To investigate heterodimerization, we designed recombinant proteins, which could be combined in different ways to form structural approximations of homo- and heterodimers. We determined the thermodynamic parameters of their formation using isothermal titration calorimetry, revealing similar nanomolar affinity and thermodynamic profiles among the various combinations. These results indicate that the ratio of hetero- to homodimers *in vivo* is likely influenced by other factors such as post-translational modifications, interactions with other proteins or expression levels of individual isoforms.

P100
Introducing NH-triazole into biomolecules

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Over the past two decades, there has been a rapid increase in the number of approved biopharmaceutical drugs based on peptides and proteins. Consequently, there is a need for new synthetic methods to selectively modify biomolecules.[1] Such methods hold the potential to enhance or modify their activity and stability. However, their modifications are challenging due to their highly complex structures and the required specific reaction conditions, i.e. aqueous media, required low reaction temperatures and precise pH values. *N*H-1,2,3-Triazole is a moiety closely related to imidazole structure. Although imidazole is commonly found in natural molecules and plays a crucial role in proteins and peptides as histidine residue, its relative, *N*H-triazole is not naturally occurring. Although closely related, *N*H-triazole possesses different properties such as different pKA value and hydrogen bonding abilities due to additional nitrogen atom in its structure. *N*H-Triazoles are synthesized by many approaches but most of them suffer from harsh conditions, specific reagents or multi-step procedures which are not applicable to sensitive substrates, such as biomolecules. We recently reported a new method for preparation of *N*H-triazoles which relies on copper catalyzed azide-alkyne cycloaddition (CuAAC) between terminal alkynes and *in situ* generated hydrazoic acid (HN_3).[2] The reaction employs mild reaction conditions and can be carried out in water.[2] Reaction was successfully applied on unprotected peptides with incorporated alkyne handles, resulting NH functionalized peptides in clean form. Using described protocol high yielding synthesis of non-canonical amino acid *aza*histidine, i.e. histidine analogue with *N*H-triazole instead of imidazole, was achieved. A reliable synthetic procedure opens up interesting possibilities for the incorporation of *N*H-triazole into various biomolecules, and the outlooks will be discussed.

[1] *Chem. Rev.* 2015, *115*, 2174–2195.
[2] *J. Org. Chem.* 2022, *87*, 4018–4028

P101
Cryo-EM structure determination of a complex between major histocompatibility complex class II molecules and invariant chain

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Major histocompatibility complex class II (MHCII) – invariant chain (Ii) complex plays a central role in adaptive immunity. During this process Ii is removed from the complex and pathogen antigenic peptides, generated by endosomal/lysosomal proteases, bind to the MHCII binding cleft for presentation at the surface of professional antigen presenting cells.

α- and β-chains of MHCII are synthesized in endoplasmic reticulum (ER), where they assemble into heterodimer and associate with Ii, forming presumably a nine-subunit complex consisting of three αβ dimers and Ii trimer. The stoichiometry of nonameric MHCII-Ii complex has been primarily reported, however, pentameric and heptameric assemblies of MHCII-Ii were also documented. From ER MHCII-Ii complex is transported into the late endosomal compartment, where it is further processed by the lysosomal cysteine proteases. Using cell biology studies the processing scheme of MHCII-Ii was established quite well, whereas the precise molecular mechanism of its processing and its synchronization with antigen processing is still not well understood. Structural insight of MHCII-Ii complex will provide a better understanding of MHCII maturation and loading, whereas biochemical insight in processing of Ii may specify the significance of cysteine proteases.

We established a baculovirus expression system for production of the MHCII-Ii complex. In order to obtain a soluble protein complex we designed the constructs so that the transmembrane regions responsible for formation of the MHCII dimers and Ii trimers were replaced by the leucine zipper motifs that self-associate into corresponding oligomers. Using cryo-electron microscopy we have determined a 3D structure of the MHCII-Ii complex, that confirms a nonameric assembly and reveals its geometry.

P102
Structural characterization of p97 in complex with the protease by cryo-EM

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DNA-protein crosslink (DPC) is a type of DNA lesion in which a protein becomes irreversibly covalently bound to DNA when exposed to endogenous or exogenous crosslink inducers. DPCs are common DNA lesions that represent a physical blockage to all DNA transactions: replication, transcription, recombination and repair. If not repaired, DPCs cause genomic instability and adverse phenotypes in humans including premature ageing, neurodegeneration and cancer. Mammalian protease SPRTN initiates the removal of DPCs through proteolytic digestion of cross-linked proteins. Molecular mechanisms and structural knowledge behind the protease-mediated DNA-protein crosslink repair (DPCR) is lacking. Data from yeast indicate that SPRTN might work in concert with the ATP-dependent AAA family segregase p97, another essential protein linked to DPCR.

Our research project aims to characterize the SPRTN:p97 complex *in vitro* and *in vivo* and solve three-dimensional structures of human SPRTN:DPC and SPRTN:p97 complexes. We have successfully produced p97 homo-hexamer in *E. coli*, purified it and, after extensive sequence and sample preparation optimization, determined the 3D structure of the wild-type p97 hexamer and its conformationally more rigid mutant using cryo-EM at the National Institute of Chemistry (NIC). For the purpose of testing whether the interaction between SPRTN and p97 is essential for the DPC repair *in vivo*, we have created a SPRTN mutant with impaired binding to p97 and showed that DPC levels significantly increase in human cells with impaired complex formation. To our knowledge, this is the first time that p97 structure has been determined using a 200 kV microscope. We will also describe our attempts to obtain structures of the p97:SPARTN complex, as it is very challenging to obtain a stable complex due to the relatively low affinity between the two components and the structural flexibility of SPARTN.

P103
A comprehensive structural study of filamentous potyviruses and their virus-like particles

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Potyviruses are plant pathogens that pose a significant threat to crops worldwide. Despite their economic importance, the molecular mechanisms underlying the infection process of potyviruses remain poorly understood. Potyviral virions are flexible, filamentous particles composed of genomic single-stranded RNA encapsidated by multiple copies of the potyviral coat protein (CP). In our study, we analyzed the three-dimensional structure of potato virus A (PVA) using cryo-EM. Similar to the structure of potato virus Y (PVY) and other known potyvirus structures, PVA exhibited tight, left-helical arrangement of CPs around the viral genome. In contrast, significant differences were observed in the cryo-EM structures of virus-like particles (VLPs) obtained upon bacterial expression of CPs from different potyviruses. The CP sequences originated from three potyviruses from different phylogenetic clades - PVY, PVA and Johnsongrass mosaic virus (JGMV). While PVY CP and JGMV CP were able to form VLPs with three different morphologies, namely helical with RNA (virion-like), RNA-free helical, and RNA-free stacked rings, PVA CP could form only two types of filaments, lacking the type with RNA-free stacked-ring morphology. Importantly, VLPs composed of CPs from different viruses formed VLPs with different helical parameters, resulting in various filament diameters. Furthermore, we were also able to obtain stacked-ring filaments for PVA VLPs by structure-based mutagenesis. These results can serve as a basis for further studies on the subtle differences between members of the genus *Potyvirus* and their biological consequences. Moreover, the structural information we provide may serve as a basis for the development of antiviral strategies aimed at preventing or mitigating potyvirus infections in crops or as platforms in nanobiotechnology.

P104
Recombinant expression of marine bacterial enzymes, involved in jellyfish and comb jellies detrital organic matter degradation

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Blooms of jellyfish (hereinafter scyphozoan jellyfish (Cnidaria) and comb jellyfish (Ctenophora)) occur seasonally every year in the northern Adriatic Sea. Often these blooms are followed by a rapid decline. Sinking jellyfish detrital organic matter serves as a great source of organic matter for marine microorganisms and is rapidly incorporated into biomass and respired by opportunistic bacteria. However, only few studies have investigated the link between detrital organic matter and marine microorganisms. Our research focused on enzymes that are secreted by marine bacteria and are involved in organic matter degradation, as our preliminary multi-omics analysis suggests. After identifying the nucleotide and amino acid sequences of key marine bacterial enzymes involved in the *Aurelia aurita* and *Mnemiopsis leiydi* organic matter degradation, we first performed bioinformatics analysis to predict the proteins' signals and conserved domains, active sites, and three-dimensional structures. We then optimized genetic constructs for expression of the enzymes in *E. coli*. Selected constructs were sequenced and the expression of the enzymes was carried out at different growth conditions. We successfully expressed several active enzymes and characterized their biochemical properties. From a biotechnological point of view, the leucine aminopeptidases and collagenases are the most interesting ones due to their wide range of potential applications.

P105
Proteomics of the haemolymph of the terrestrial crustacean Porcellio scaber, a model organism for environmental research

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Terrestrial crustaceans are commonly used test organisms in environmental science [1]. One of them, the isopod *Porcellio scaber* (*P. scaber*, common name: woodlouse), especially its haemolymph, has become an important model for studying the physiological state of the organism exposed to various stressors, e.g. microbial infections, pesticides, nanoparticles and microplastics [2-3]. Mass spectrometry is currently one of the most efficient tools for exploratory proteomic studies. Most proteomic studies have focused on identifying differences in haemolymph proteomes between untreated and stressor-treated organisms. Our study is the first to perform an analysis of the whole haemolymph proteome of *P. scaber* under baseline conditions when the organism is not exposed to an infection or any other stressor. Using the classical gel-based proteomics approach, available public sequence databases and our *P. scaber* transcriptome data, we have identified 76 proteins in the molecular mass range of 10 to 300 kDa. They are involved in many cellular processes such as cytoskeleton formation, protein degradation, vesicular transport, genetic information processing, detoxification, and carbohydrate and lipid metabolism. Of particular interest for the future ecotoxicity studies are those proteins associated with the immunity in *P. scaber*. According to data reported for other crustaceans, 28 of the identified proteins are potentially involved in *P. scaber* immunity, among them haemocyanin as the most abundant protein, phenoloxidase, superoxide dismutase and haemolymph clottable protein. Our results thus provide a solid basis for studying the innate immune response of *P. scaber* at the level of the haemolymph proteome to identify potential biomarkers of exposure to environmental stressors and to understand the underlying mechanisms of toxic action.

[1] Van Gestel et al., 2018, ZooKeys 2018
[2] Dolar et al., 2021, Sci. Total Environ. 772
[3] Dolar et al., 2022, Sci. Total Environ. 818

P106
A platform for protein production and crystal structure determination

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A platform for protein production and crystal structure determination involves several stages: molecular cloning, small-scale expression screening, large-scale protein production, protein purification, protein crystallization, and protein structure determination. We developed a high throughput system that involves parallel cloning into a range of *E. coli* expression vectors containing different tags and different *E. coli* strains. Small-scale expression screening is performed in 96 deep-well format with auto-induction medium. This approach enables the screening of multiple constructs simultaneously and the selection of the most promising ones for large-scale expression and protein production. We also have the capacity and know-how for yeast, protozoan, insect, and mammalian cell expression systems. For the expression of multi-protein complexes, we use MultiBac/MultiMam technology. Standard protein purification usually involves the ÄKTA chromatography systems, with Ni-affinity and size-exclusion chromatography as the first two steps. When necessary, additional purification steps such as ion-exchange and affinity chromatography are introduced. Protein tags are removed before crystallization or electron microscopy grid preparation. We use mass spectrometry to confirm the identity and molecular weight of expressed proteins. For protein characterization, nano-differential scanning fluorimetry (nanoDSF), DSF, and solubility tests are implemented. In order to establish initial crystallization conditions, commercial screens are applied. To ensure rapid set up of crystallization plates and their observation, an automated pipetting robot and imaging system, which records plates by predefined schedule, are used. Optimized crystals are tested on an in-house rotating anode (X-ray). We collect the final high-quality diffraction data for crystal structure determination at a synchrotron source and test the suitability of materials for cryo-EM at other facilities (KI or abroad).

P107
Gp1: A novel repressor of the temperate phage GIL01 lytic cycle

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GIL01 is a temperate phage that infects the insect pathogen *Bacillus thuringiensis*. As a temperate phage, it can choose between two life cycles after infection of its host: a dormant lysogenic cycle, in which transcription of phage genes is repressed, or an active lytic cycle, in which new virions are formed and released from the cell by host lysis. Transcription of phage GIL01 genes is controlled by three promoters. The weak and strong tandem promoters, *P1* and *P2*, respectively, control transcription of gene cluster required for replication of the phage genome and regulation of phage life cycles, while *P3* controls expression of phage structural genes and genes required for bacterial lysis. To establish the lysogenic cycle, GIL01 hijacks the bacterial protein LexA, the master regulator of the bacterial SOS response, which together with the small phage protein gp7, which increases the binding affinity of LexA, represses the transcription of phage genes. In addition to gp7, gp1, another small protein, has also been identified as critical for establishing and maintaining the lysogenic state, but with an unknown mechanism of action. In this work, we show that gp1 binds to the operator at the *P2* promoter of GIL01 and forms a filament that prevents transcription from *P2*. *P2* is the stronger promoter of the *P1-P2* tandem controlling genes required for replication and regulation of the phage genome. Gene for gp1 is the first phage gene to be transcribed from *P2* after infection, creating a negative feedback loop. While most temperate phages insert into the host genome and replicate along with the bacterial chromosome, GIL01 resides in the host cell as an extrachromosomal linear plasmid in 10-15 copies per cell. We therefore believe that gp1 is the safety net of phage GIL01, maintaining sufficient copy number to ensure its transmission to the daughter cell.

P108
Molecular dynamics simulations reveals divergent evolutionary paths of modern EpCAM orthologues

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EpCAM is a homodimeric type-I transmembrane protein that arose in vertebrates and has no known homologues in non-vertebrate species. It modulates cell adhesion and has proliferative signaling functions. It is necessary for proper epithelial development and is frequently highly expressed in carcinomas, which makes it a potential cancer therapy target.

We explored potentially function-impacting differences that arose during the evolution of EpCAM by comparing the structures and molecular dynamics of five different orthologuous EpCAM homodimers (human, house mouse, chicken, African clawed frog, zebrafish) from species belonging to four different vertebrate classes. Using the crystal structure of human EpCAM and four AlphaFold-predicted homodimeric EpCAM structures, we performed 5 independent 500 ns molecular dynamics simulations for each of the proteins. We calculated Cα RMSF, subunit orientation, dimer interface area, dimer binding energy and the volume of a hydrophobic binding pocket. In most of the comparisons, either zebrafish or frog EpCAMs exhibited a statistically significant difference from others, as would be expected due to the larger evolutionary distance. Simulations indicate that in the zebrafish EpCAM inter-subunit angle is substantially different than in other orhologues, frog EpCAM has a smaller dimer interface area, and the thyroglobulin loop of zebrafish EpCAM has a smaller interface area with the opposing subunit. Chicken EpCAM has the greatest free-energy change upon dimer formation, while zebrafish EpCAM has the lowest, which could impact proteolysis rates and thus regulated intramembrane proteolysis signaling. We also found differences between the hydrophobic pocket properties—zebrafish EpCAM has a smaller pocket volume, which could imply a different ligand or different affinity, while frog EpCAM pocket is more rigid, which could mean a smaller entropy loss upon binding. The observed differences reflect unique divergent evolutionary paths from common anchestors, and can be important when using these organisms as model systems for functional studies.

P109

An improved method for determining enzyme-kinetic parameters from time-concentration progress curves: A study on patients with dementia.

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Traditionally, an enzyme's reaction rate is reported in enzyme units (U/mL). Although easy to measure, the reaction rate is dependent both on the enzyme's concentration and its affinity for substrate. More information is acquired if one separately measures concentration and the kinetic parameters K_m (Michaelis constant), V_{max} (maximum velocity) and k_{cat} (turnover number); K_m and V_{max} can be determined by measuring initial velocities. A newer approach is to calculate K_m and V_{max} directly from individual time-concentration progress curves, e.g. with the integrated Michaelis-Menten (MM) equation; however, the equation doesn't perform well when the ratio $[S]_0/K_m$ is very high. To remedy this, we used a formula that calculates t_Q, the time interval during which the progress curve has maximum curvature and hence most information about kinetic parameters. We coupled this formula with the integrated MM equation to produce iFIT, a program that calculates K_m and V_{max} from the progress curve's area of maximum curvature.

For a reaction of the enzyme paraoxonase 1 (PON1) on the substrate dihydrocoumarin, we compared iFIT with established methods for determining kinetic parameters: we found that iFIT performs equally or better than these approaches. Consequently, we used iFIT for the analysis of PON1 enzymatic activity on two large cohorts of patients: 231 patients with Parkinson's disease (PD) and 161 patients with suspected Alzheimer's dementia (AD) or mild cognitive impairment (MCI). For the AD/MCI cohort, we found a strong correlation between K_m for DHC and K_m for phenylacetate (PA), between V_{max} for both substrates, and between V_{max} and protein concentration, as well as previously-known correlations between kinetic parameters and the genotype of different SNPs. We also found a strong correlation between kinetics and genotype for the PD cohort, where we only determined genotype and DHC activity. This shows iFIT is a reliable method for enzyme progress curve analysis.

P110

Advancement in the study of the anticoagulant serine protease homolog from the snake venom: toward rational peptide drug design

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Serine proteases (SPs) are major components of viperid venoms and their action is attributed to their enzymatic activity. However, their enzymatically inactive homologs also exhibit pharmacological activities by acting as ligands for various receptors. VaaSPH-1, the serine protease homolog from the venom of the nose-horned viper (*Vipera a. ammodytes*), has been recognized as a promising anticoagulant with specific action on the intrinsic pathway of the blood coagulation cascade, which plays a crucial role in venous thromboembolism (VTE). VaaSPH-1 has great potential as a lead structure for the development of drugs that would safely attenuate thrombus formation. With two mutations in its catalytic triad, VaaSPH-1 is a 34 kDa glycoprotein with no proteolytic activity. It acts as an antagonist of FIXa in binding to FVIIIa and in this way inhibits the formation of the intrinsic tenase complex. Our objective is to design peptides based on the structure of the VaaSPH-1-FVIIIa-interacting area that retain the high binding affinity for FVIIIa. To this end, we developed and optimized the production of recombinant VaaSPH-1 and the A2 domain of FVIII in the HEK293 FreeStyle expression system. We demonstrated that the recombinant VaaSPH-1 was correctly folded and functional, as it prolonged the activated thromboplastin time like the natural protein. By generating mutants, using surface plasmon resonance and bioinformatics tools, we will identify the key amino acids responsible for binding to the A2 domain of FVIIIa. These findings will enable the identification of essential pharmacophores and design of peptides that exclusively target the intrinsic pathway and can be used as drugs to treat VTE with reduced risk of excessive bleeding.

References:
Latinović, Z. *et al. Thromb Haemost* 118, 1713–1728 (2018).
Venkateswarlu, D. *Biochem Biophys Res Commun* 452, 408–414 (2014).

P111
pH responsive four helix bundle switches for use in coiled-coil protein origami

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We aim to engineer dynamic protein coiled coil (CC) bundles, which can act as molecular switches, that respond to changes in the environment, such as pH. Our objective is to integrate these molecular switches into larger CC origami assemblies, specifically a tetrahedral protein cage, to achieve precise control over the opening and closing of the cage structure.

We selected four helix bundles designed by Boyken et al. [1] which features a buried network of histidine residues that form hydrogen bonds under physiological pH. Under acidic conditions the histidines become protonated, which causes steric clashes in the core of the bundle. This should cause dissociation of the bundle, however, at low pH the bundle oligomerized or aggregated. Therefore, we used proteinMPNN (a deep learning method for side-chain design, [2]) to redesign the bundle. With AlphaFold2 predictions, we filtered out designs with high predicted homodimerization or propensity to form parallel heterodimers. We reasoned that with improved solubility of proteinMPNN and increased negative charge, we could achieve complete separation of the two halves of the protein under acidic conditions, without the formation of higher order oligomers.

To monitor oligomerization at low pH with FRET (Förster resonance energy transfer), we introduced a cysteine residue into each half of the protein to enable labeling with dyes Sulfo-Cy3 and Sulfo-Cy5. To further validate oligomerization state, we also used combination of SEC-MALS (size exclusion chromatography with multi angle light scattering), CD (circular dichroism), native page and acidic native page to monitor oligomerization in a spectrum of pH values from 3 to 8. This work has potential applications in a variety of fields, including drug delivery and nanotechnology.

[1] Scott E. Boyken et al. Science364,658-664(2019)
[2] J. Dauparas et al. Science378,49-56(2022)

P112
Multilevel regulation of Src kinase through phosphorylations

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The activity of Src kinase in cells is controlled by intramolecular inhibitory interactions mediated by SH3 and SH2 domains, which impose structural constraints on the kinase domain holding it in an inactive state. We identified and described in detail phosphorylation of tyrosine 90 (Y90) within the SH3 domain as a new regulatory mechanism of controlling kinase activity, structure, binding ability, and membrane mobility of Src. To analyze the effect of Y90 phosphorylation, we used classical approaches such as mutational analyses, cellular transformation and invasion assays, as well as advanced methodological approaches including quantitative targeted MS, FRET or FCS. We found that phosphorylation of Y90 reduces binding affinity of the SH3 domain to its interacting partners, opens the Src structure, and renders Src catalytically active. This is accompanied by an increased affinity to the plasma membrane, decreased membrane motility and slower diffusion from focal adhesions.

Our data allowed us to come up with a new, comprehensive model for the regulation of Src by tyrosine phosphorylations. In this model, the SH2 and the SH3 domain serve as cooperative but independent regulatory elements of the Src kinase. Their function and their engagement in the intramolecular inhibitory lock are controlled by phosphorylation on tyrosines 527 and 90. Both phosphorylations affect the opening of Src structure and its catalytic activity, but in opposite ways.

Taken together, the modular system of regulations through phosphorylation of these key tyrosines and intramolecular interactions enables the Src kinase to adopt several different conformations of varying kinase activities and interacting properties. That allows Src to operate not as a simple on/off switch but as a tunable regulator functioning as a signalling hub in a variety of cellular processes.

P113
Surfactant protective properties on recombinant paraoxonase 1 during catalytic substrate turnover

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Human paraoxonase-1 (PON1) is a well-studied plasma metalloenzyme belonging to the paraoxonase (PONs) family. It exhibits catalytic activity in hydrolyzing a wide range of compounds, including aryl (thio)esters (e.g., phenyl (thio)acetate), (thio)lactones (e.g., d-valerolactone, homocysteine thiolactone), estrogen esters, phosphorous esters (such as paraoxon, organophosphate pesticides, and nerve gases). Numerous clinical studies link PON1 to oxidative stress-related diseases such as cardiovascular disease, diabetes, HIV infection, autism, Parkinson's, and Alzheimer's, where the kinetic behavior of an enzyme is characterized by initial rates or by methods that obtain enzyme kinetic parameters by fitting the computed curves over the entire time-courses of product formation (progress curves). Lactonase and arylesterase activity of PON1 is frequently determined by enzymatic hydrolysis of artificial substrates, such as dihydrocoumarin (DHC) and phenylacetate (PA). These chemicals are not physiological substrates and can be unstable in buffer solutions. Moreover, in the analysis of progress curves, the behavior of PON1 during hydrolytically catalyzed turnover cycles is unknown. Therefore, progress curves for enzyme-catalyzed hydrolysis of the lactone substrate DHC by recombinant PON1 (rePON1) were studied to determine the influence of catalytic DHC turnover on rePON1 stability. rePON1 was significantly inactivated during the catalytic DHC turnover. The activity of rePON1 was not lost due to product inhibition or spontaneous inactivation in the sample buffers. The progress curves of DHC hydrolysis by rePON1 indicate self-inactivation during the catalytic turnover of DHC. Additionally, human serum albumin or surfactants such as Triton X-100, polysorbate 20, n-dodecyl-β-d-maltoside, or polyethylene glycol 4000 protected rePON1 from inactivation during this process. This is important as PON1 activity in clinical samples is measured in the presence of albumin.

P114
Exploring the influence of iodine on Arabidopsis thaliana proteins: insights into plant biochemistry

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Most of us associate iodine more with the proper functioning of the thyroid gland in the animal world. However, it is important to know that iodine also plays an important role in plant physiology. In plants, iodine has been shown to have a positive effect on biomass accumulation and can even promote early flowering when supplied in sufficient amounts.

Recent studies have shed light on the mechanisms by which iodine affects plant defence responses. It has been suggested that iodine treatment may enhance the plant's ability to defend itself against both biotic (related to living organisms) and abiotic (related to environmental factors) stresses. This highlights the potential role of iodine in protecting plants under challenging conditions.

To gain a deeper understanding of the effects of iodine on plants, our study focuses on proteins from *Arabidopsis thaliana*, a commonly studied model plant. We produce selected proteins and investigate how their biochemical properties are affected by iodination.

By studying the changes in protein structure, activity and interactions that result from iodination, we hope to gain valuable insights into the functional significance of iodinated proteins in plant biology. This research will contribute to our understanding of the broader role that iodine plays in plant physiology, defence mechanisms and general adaptation to various environmental stresses.

P115
Structure(s) of thyroglobulin:insight in hormone production

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Thyroglobulin is the protein precursor of thyroid hormones, which are essential for growth, development and control of metabolism in vertebrates. Hormone synthesis from thyroglobulin (TG) occurs in the thyroid gland via the iodination and coupling of pairs of tyrosines and is completed by TG proteolysis. The limitations of crystal growth of TG samples were surpassed by the progress in cryo-electron microscopy (cryo-EM), which enabled us to determine 3-dimensional atomic structure of human thyroglobulin at ~3.5 Å resolution (Coscia et al., 2020). Combining literature data, insight in positioning of proximate tyrosine residues, expression and site directed mutagenesis of thyroglobulin in HEK cells we were able to identify and confirm all hormonogenic tyrosine pairs. Structure analysis revealed that proximity, flexibility, and solvent exposure of the tyrosines are the key characteristics of hormonogenic sites. The coupled, iodinated tyrosine residues were however not present in our human TG structures, however, a later work by Kim et al. visualized two pairs of well resolved coupled tyrosine residues in the ~2.6 Å cryo-EM structure of natively iodinated bovine TG. How to use these structures to further research and improve healthcare knowing that 20 % of population, predimonantly women, suffers from thyroid disroders?

References:
Coscia, F., Taler-Verčič, A., Chang, V.T., Sinn, L., O'Reilly, F.J., Izoré, T., Renko, M., Berger, I., Rappsilber, J., Turk, D. & Löwe, J. (2020) The structure of human thyroglobulin. Nature, 578, 627–630.
Kim, K., Kopylov, M., Bobe, D., Kelley, K., Eng, E. T., Arvan, P. & Clarke, O. B. (2021). The structure of natively iodinated bovine thyroglobulin. Acta Cryst. D77, 1451–1459.

P116

Proteomic data and structural analysis reveal the interplay of structural rigidity and flexibility on the selectivity of cysteine cathepsins

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Addressing the elusive specificity of cysteine cathepsins, which in contrast to caspases and trypsin-like proteases lack strict specificity determining P1 pocket, calls for innovative approaches. To address this problem proteomic analysis of cell lysates incubated with human cathepsins K, V, B, L, S and F. It provided 30 000 protein cleavages, which analysis by software platform SAPS-ESI (Statistical Approach to Peptidyl Substrate-Enzyme Specific Interactions) revealed heterogeneous substrate positions with non-normal distribution of amino acid residues and homogeneous positions with normal distribution. The heterogeneous positions were used to generate clusters which enabled the selection of training datasets for the generation of support vector machine models. Cleavage site predictions on the SARS-CoV-2 S protein, confirmed experimentally, expose the most probable first cut under physiological conditions and suggested furin-like behavior of cathepsins. To get insight into the structural basis of cathepsin specificity, 30 peptidyl sequences of cathepsin V substrates were chosen. 20 crystal structures of their complexes with cathepsin V were determined at resolutions from 1.5 to 2.1 Å. Structural analysis showed that the heterogeneous positions are structurally restrained, whereas residues at homogeneous positions exploits structural variability of the protease. Comparison with variability observed in the crystal structures of cathepsins K and S is consistent with this interpretation. Taken together, specificity and promiscuity of substrate binding is explained by the restraining substrate binding interactions resembling the lock and key mechanism, which are complemented by the induced fit and conformational variability of the rest of the binding region.

Reference

L. Tušar *et al.*, Proteomic data and structure analysis combined reveal interplay of structural rigidity and flexibility on selectivity of cysteine cathepsins. *Communications biology* 6, 450 (2023).

P117
Structural and biochemical characterisation of the cell wall protein Cwp5 from Clostridioides difficile

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C. difficile is a Gram-positive, spore-forming, anaerobic bacterium and a leading cause of antibiotic-associated diarrhea that can lead to life-threatening complications. To gain understanding of the interactions of the cell surface proteins within the bacterial cell wall and with their environment we previously determined the crystal structures of multi-domain cell wall proteins (CWPs) Cwp8 and Cwp6. The revealed crystal structure of the trimeric cell wall binding type 2 (CWB2) module that is shared among 29 CWPs of *C. difficile* 630 represents one of the two evolutionary conserved surface layer (S-layer)-anchoring modules in Gram-positive bacteria. Although it served as a molecular replacement model for the crystal structure of the main CWP and S-layer protein SlpA, which led to electron microscopy model of the S-layer assembly, most of the CWPs remain structurally and functionally uncharacterised. The aim of our studies is to gain structural insight into the CWPs to understand the functions of these proteins acting either alone or as a part of the S-layer assembly.

The mature Cwp5 (i.e. without the signal sequence) from *C. difficile* 630 was overexpressed in *Escherichia coli* BL21(DE3) and purified by Ni-affinity and size exclusion chromatography. After spontaneous degradation of Cwp5 the resulting C-terminal fragment was identified by mass spectrometry and crystallized by sitting-drop vapor-diffusion technique using optimised commercial screens. Platinum derivatives of the crystals were prepared by soaking. Single-wavelength anomalous dispersion method revealed a two-faced right-handed β-helix crystal structure of the functional domain of Cwp5. Site-directed mutagenesis showed that Cwp5 undergoes intramolecular autoproteolysis, most likely similar to the maturation mechanism of CwpV, indicating a possible common autoprocessing mechanism of *C. difficile* CWPs and other CWB2 module-containing S-layer proteins of Gram-positive bacteria.

P118
Interaction of murine cathepsin B and DARPin and its prospects

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Cysteine cathepsins are lysosomal proteinases that influence many cellular processes. They express as preproenzymes, get processed into proenzymes and are activated in the acidic milieu of lysosomes through cleavage of the propeptide by other active cathepsins. In healthy tissue, they are important for homeostasis and are involved in processes, such as bone remodelling, antigen processing and its presentation, thyroglobulin processing and protein turnover in general, including extracellular and adhesion proteins. They are known to overexpress and to be involved in the progression, invasion and metastasis of solid tumours as well but it is arduous to ascribe exact individual roles to individual members owing to the heterogeneity of tumours. Cathepsin B (CtsB) has been most studied in the context of cancer which can be located at the surface of invadopodia of tumour cells (association with annexin A2) as a proenzyme and in membrane invaginations (caveolin-1) where it was linked with extracellular degradation. Designed ankyrin repeat proteins (DARPins) are genetically engineered antibody mimetic proteins based on natural ankyrin proteins used for binding and involved in numerous cell functions. DARPins can be used as diagnostic or therapeutic agents due to their high specificity and affinity for the selected target. DARPin 4m3 shows a high affinity and selectivity for murine CtsB. Until now, the structure of murine CtsB has remained unresolved due to a lack of success in crystallization efforts but DARPin 4m3 was successfully used for chaperone-assisted crystallization and the crystal structure of the complex has been successfully resolved. Due to similar biochemical and physiological properties between human and murine CtsB, the structure of the mouse CtsB can offer important insight for structure-based drug design, especially if we consider the role of mice in the development of drugs.

P119
Lipids play an important role in the pore structure of a novel actinoporin Fav

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Pore-forming toxins (PFTs) are a diverse group of proteins that form pores in lipid membranes. The final pore complex usually consists of circularly arranged protomers of one or more protein species that cross the membrane with their amphipathic regions. In some cases, lipids are not only the medium in which the pores are formed but also a building block of the pore that remains bound to the larger pore complex even after membrane solubilization. One such example is actinoporins, a family of α-PFTs from cnidarians that form pores in membranes containing sphingomyelin. Currently, only one crystal structure of actinoporin pore is known and that is that of an octameric pore of FraC, an actinoporin from strawberry anemone (*Actinia fragacea*) in which sphingomyelin has been shown to play some structural role. Here we present cryo-EM studies of the actinoporin Fav, an actinoporin from mountainous star coral (*Orbicella faveolata*), which revealed pores in different stoichiometries, including heptamers, octamers, and nonamers. The different stoichiometries were caused either by mutations driven by directed evolution or by changes in lipid membrane composition during pore preparation. The high-resolution cryo-EM maps also revealed several sphingomyelin molecules surrounding the pore, with some also exposed in the pore lumen. This suggests that the involvement of sphingomyelin in the final pore structure is greater than previously thought. We were able to observe cholesterol molecules in high-resolution cryo-EM maps, which allowed us to describe interactions between the Fav pore and the cholesterol and sphingomyelin present in the lipid membranes.

P120
Phytophthora parasitica NLP shares three-dimensional fold and pore-forming activity of model Nep1-like protein, NLPPya

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Necrosis- and ethylene-inducing 1-like proteins (NLPs) constitute a superfamily of proteins found in diverse phyla of plant-associated microorganisms. Numerous NLPs are cytotoxic and facilitate infections in a wide range of crops. The evidence of NLPs being toxic to both monocot and dicot plants is inconclusive. The target for interaction with the plant plasma membrane is a sphingolipid glycosyl inositol phosphoramide (GIPC). Studies of NLP_{Pya} from *Pythium aphanidermatum* highlight important residues for interaction with the terminal hexose unit of GIPC and a unique mode of membrane damage was observed. Despite 1,700 identified homologs, 3D structures of only 3 NLPs are solved. Considering their wide taxonomic distribution and crucial role in pathogenesis, structural information on NLPs is critically lacking.
Studies of NLPs from evolutionary distant organisms are essential to understand these proteins' evolution and mechanistic action. We study the structural and functional characteristics of NLP_{Pp} from *Phytophthora parasitica*, an important plant pathogen. Results of *Nicotiana tabacum* infiltration assays indicate NLP_{Pp} causes necrosis and ion leakage of plant tissue. The protein binds to vesicles consisting of both mono- and dicot-derived GIPC, and binds to the terminal hexose unit found on GIPC polar head. Despite NLP_{Pp} is exhibiting less profound binding and plant tissue damage in comparison to model NLP_{Pya}, modes of action seem to be similar. Surprisingly, NLP_{Pp} and NLP_{Pya} exhibit pore-forming on monocot GIPC-derived vesicles, which was unexplored until now. We have determined the crystal structure of NLP_{Pp} at 1.7 Å resolution, revealing this protein shares structural characteristics with other NLPs, with a central β-sandwich flanked by α-helices. In addition, NMR experiments were conducted to characterize NLP_{Pp} in solution.

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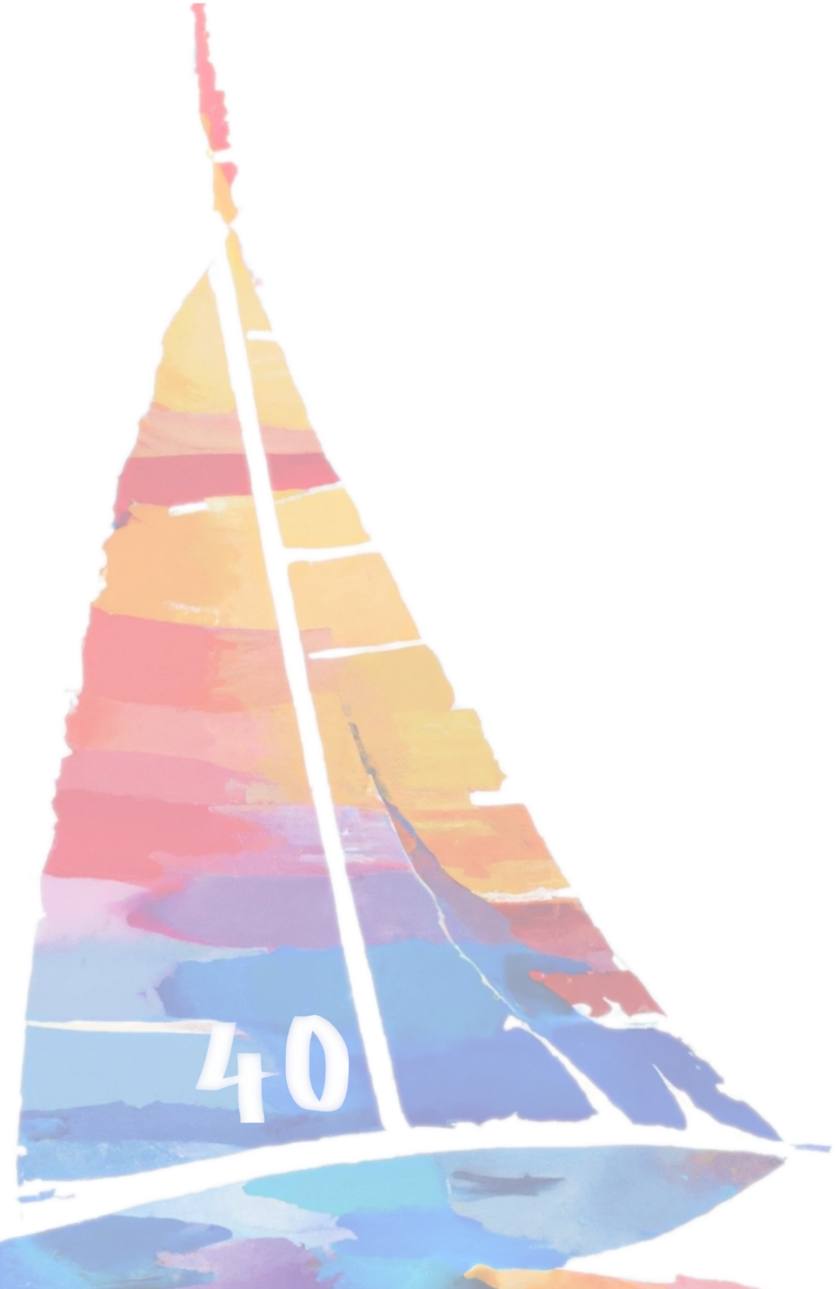

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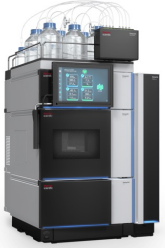


HPLC, UHPLC & nano-flow LC

IC, HPIC & IC-MS

Chromatography columns & consumables

- HR – ICP-MS
- Triple & Single Quadrupole ICP-MS

AAS

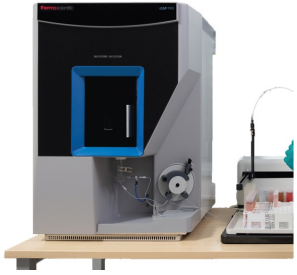
LC-MS:

- Triple quadrupole LC-MS/MS
- Orbitrap HRAM systems
- Tribrid HRAM systems with combied ion trap and orbitrap analyzers

GC-MS:

- Triple quadrupole GC-MS/MS
- Orbitrap HRAM systems

ICP-OES



LKB

Exellence in Routine and Science

Malvern Panalytical

Particle size analyzers

Zetasizer Advance

Mastersizer 3000

Microcalorimeters

MicroCal Peaq – ITC

MicroCal Peaq – DSC

(Automated)

cytiva

Chromatography systems

Akta Pure

Imaging systems for proteins

Amersham ImageQuant 800

SPR systems

Biacore T200

MERCK

IMPACTING

LIFE & HEALTH WITH SCIENCE

omega

Že skoraj 30 let ponujamo rešitve na področju kemije, biokemije, molekularne biologije in mikrobiologije, farmacije, imunologije, onkologije, forenzike in diagnostike

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