The 20th FEBS Young Scientists' Forum

# FEBS 2021


CROATIA • SLOVENIA

VIRTUAL EVENT

15-18 JUNE 2021

# PROGRAMME AND ABSTRACT BOOK

# The 20th FEBS Young Scientists' Forum


virtual event

15–18 June 2021

# PROGRAMME AND ABSTRACT BOOK

**Programme and Abstract Book** – presenting abstracts accepted to the 20th FEBS Young Scientist's Forum, 15–18 June 2021, Virtual event. Abstracts from YSF participants (poster and short oral presentation abstracts in this booklet) are also accepted to the virtual 45th FEBS Congress, 3–8 July 2021.


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## ORGANISERS

The Federation of European Biochemical Societies (www.febs.org) in collaboration with The Croatian Society of Biochemistry and Molecular Biology (www.hdbmb.hr) and the Slovenian Biochemical Society (www.sbd.si)

## CONGRESS SECRETARIAT

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# Contents

## ORGANISING COMMITTEE

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**Prof Irene Díaz-Moreno**
Chair of the FEBS Working Group on the Careers of Young Scientists
University of Seville – CSIC, Seville, Spain

## WELCOME NOTE

Dear YSF participants,

In these uncertain times of the COVID-19 pandemic, the 20th FEBS Young Scientists' Forum (YSF), originally planned for Lovran, Croatia, and already postponed from 2020, is being held for the first time ever in a virtual format.

Nonetheless, we are sure this will be an enjoyable and valuable, albeit different, experience. We will work our magic to make the most of various online meeting tools and give you the best of two worlds – science and social interactions – through four mornings from June 15th to June 18th, 2021. In such a way you will have some time to gather your thoughts and have a break before meeting again online at the virtual 45th FEBS Congress from July 3rd till July 8th, 2021.

This online event will bring together about 100 PhD students and postdocs from across the FEBS area, with FEBS continuing to support those selected by covering their registration fee for the ensuing virtual 45th FEBS Congress. The scientific programme will comprise four keynote lectures delivered by distinguished scientists as well as eight short talks given by the selected participants, all presenting new and interesting findings in different areas of biochemistry and molecular biology. Furthermore, participants will have the opportunity to learn and develop new skills, tutored by scientists and experts in the field of communication, writing and presentation of scientific results. So we hope this will be an excellent opportunity for a rewarding scientific and personal experience, including exchanging ideas with colleagues and establishing new acquaintances.

We are looking forward to welcoming you online!

Maja Katalinić

Maja Katalinić, 20th YSF Chair
On behalf of the Organising Committee

Prof Irene Díaz-Moreno, Chair, FEBS Working Group on the Careers of Young Scientists

# Information

## General info

The YSF will be run on a virtual platform provided for the YSF and FEBS Congress by the company C-IN. Within the platform you will find the **Session room**, **E-Poster room**, **Practical exercise room** (with four Zoom rooms) and **Chat room**:

- The keynote lectures, SOPs, "Careers – Fellowship Opportunities" and "Science From Another Point of View" sessions will be accessible through the Session room.
- For practical exercises marked in the programme as "CAREER SKILLS TIPS & TOOLS and POSTERS" participants will be divided into four groups named as Lipa/Marun/Zmaj/Lovor. Each group will have two practical exercises per day accessible through the Practical exercise room.
- E-Poster hall will contain all the posters from participants together with the 1-min presentation videos and abstracts, and will be accessible during all four days of the YSF.
- In the Chat room participants will be able to talk virtually to each other as well as to organisers and speakers.

## Online meeting conduct guidelines

Test your setup and please make sure your internet connection is working properly. Join early. For practical exercises, presence with both audio and video stream is a must. Be aware of your sourroundings and mute your microphone when you are not talking. Minimise distractions. If any problem occurs notify the organizers as soon as possible; there will be an informatics technician from C-IN present at all times to assist you (the help/info desk button is accessible via the YSF lobby).

## Language

The official language of the congress is English. There will be no simultaneous translation.

## Awards

Each day there will be a short Quiz to participate in. The winners (one each day) will each be awarded a special prize. Furthermore, based on participants' votes the best short oral presentation and the best 1-min poster presentation will receive prizes too.

## Certificate of attendance

The certificate of attendance will be downloadable from your participant account on the Congress website at https://2021.febscongress.org/ upon completing the survey at the end of the YSF meeting.


FEBS 2021
YSF20
CROATIA
SLOVENIA
INFO DESK
COFFEE &CHAT
PRACTICAL
EXERCISES
E-POSTERS
SESSIONS
The 20th FEBS
Young Scientists' Forum
YSF 2021

# Programme

## Tuesday, June 15, 2021

| | |
|---|---|
| *9:15- 9:30 Signing in* | |
| 09:30 | OPENING<br>*Opening remarks*<br>Maja Katalinić, Chair YSF2021<br>Irene Díaz-Moreno, Chair FEBS WGYS<br>Jerka Dumić, HDBMB<br>Janko Kos, President SBD<br>Václav Pačes, FEBS Secretary General |
| 9:50 | WELCOME ADDRESS BY FEBS<br>**Israel Pecht**, past FEBS Secretary General (Israel)<br>"FEBS support for young scientists" |
| 10:10 | OPENING LECTURE<br>**Ivan Đikić** (Germany)<br>"Surprises in science – lessons from COVID-19"<br>*Chairs: Maja Katalinić and Irene Díaz-Moreno* |
| 11:00 | *Practical Exercises*<br>PARALLEL GROUP SESSIONS:<br>**Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CV" **Keith Elliott** (United Kingdom)<br>• "HOW TO WRITE AN EFFECTIVE ABSTRACT" **Duncan Wright** (United Kingdom)<br>• "VALUE OF A WELL-RUN LAB BOOK" **Jason Perret** (Belgium)<br>• Poster session – 1 min poster presentations |
| 12:30 | Coffee break |
| 12:50 | *Practical Exercises*<br>PARALLEL GROUP SESSIONS:<br>**Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CV" **Keith Elliott** (United Kingdom)<br>• "HOW TO WRITE AN EFFECTIVE ABSTRACT" **Duncan Wright** (United Kingdom)<br>• "VALUE OF A WELL-RUN LAB BOOK" **Jason Perret** (Belgium)<br>• Poster session – 1 min poster presentations |

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| 14:15 | SUM UP OF THE DAY<br>Quiz<br>*Chairs: Maja Katalinić and Irene Díaz-Moreno* |

## Wednesday, June 16, 2021

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|---|---|
| *9:15 - 9:30 Signing in* | |
| 9:30 | KEYNOTE LECTURE<br>**Eric Westhof** (France)<br>"Translation Control in Betacoronaviruses: Sequence Signatures in the 5'UTR RNA and NSP1 Protein"<br>*Chair: Nino Sinčić* |
| 10:15 | SHORT TALKS FROM PARTICIPANTS<br>SOP 1<br>**Marko Tijardović** (Croatia)<br>"Intense physical exercise induces an anti-inflammatory change in IgG N-glycosylation profile" |
| | SOP 2<br>**Angela Garcia-Mato** (Spain)<br>"IGF-1 via PI3K/AKT activation promotes survival and anabolic metabolism in HEI-OC1 auditory cells" |
| | SOP 3<br>**Robert Beagrie** (United Kingdom)<br>"Investigating the link between DNA replication, chromatin change and transcriptional regulation during *in vivo* erythroid differentiation"<br>*Chair: Nino Sinčić* |
| 10:40 | *Practical Exercises*<br>PARALLEL GROUP SESSIONS:<br>**Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CV" **Keith Elliott** (United Kingdom)<br>• "HOW TO WRITE AN EFFECTIVE ABSTRACT" **Duncan Wright** (United Kingdom)<br>• "VALUE OF A WELL-RUN LAB BOOK" **Jason Perret** (Belgium)<br>• Poster session – 1 min poster presentations |
| 12:10 | Coffee break |
| 12:30 | *Practical Exercises*<br>PARALLEL GROUP SESSIONS: |

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|---|---|
| | **Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CV" **Keith Elliott** (United Kingdom)<br>• "HOW TO WRITE AN EFFECTIVE ABSTRACT" **Duncan Wright** (United Kingdom)<br>• "VALUE OF A WELL-RUN LAB BOOK" **Jason Perret** (Belgium)<br>• Poster session – 1 min poster presentations |
| 13:55 | Careers – FELLOWSHIP OPPORTUNITIES<br>FEBS - **Alain Krol** (France)<br>MSCA - **Gaetano Castaldo** (Belgium)<br>EMBO - **Kelly Sheehan-Rooney** (Germany)<br>*Chairs: Nino Sinčić and Vlastimil Kulda* |
| 14:40 | SUM UP OF THE DAY<br>Quiz<br>*Chairs: Nino Sinčić and Vlastimil Kulda* |

## Thursday, June 17, 2021

| | |
|---|---|
| *9:15 - 9:30 Signing in* | |
| 9:30 | KEYNOTE LECTURE<br>**Cecília Maria Arraiano** (Portugal)<br>"The amazing new world of RNA"<br>*Chair: Jerica Sabotič* |
| 10:15 | SHORT TALKS FROM PARTICIPANTS<br>SOP 4<br>**Eva Jarc Jovičić** (Slovenia)<br>"Lipid droplets and autophagy cooperate in the protection of cancer cells against metabolic stress" |
| | SOP 5<br>**Anastasiia Sivkina** (Russia)<br>"Role of C-terminal domains of yeast FACT complex in nucleosome unfolding" |
| | SOP 6<br>**Elzbieta Rembeza** (Sweden)<br>"Investigation of functional annotations to enzyme classes reveals an extensive annotation error"<br>*Chair: Jerica Sabotič* |
| 10:40 | *Practical Exercises* |

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|---|---|
| | PARALLEL GROUP SESSIONS:<br>**Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CATCHING ORAL PRESENTATION" **Miguel A. De la Rosa** (Spain)<br>• "PUBLIC ENGAGEMENT IN SCIENCE" **Mark Roberts** (United Kingdom)<br>• "SHAPING YOUR CAREER (ALSO AS AN EDUCATOR) - TIPS AND TRICKS FOR A YOUNG SCIENTIST" **Ferhan G. Sağin** (Turkey)<br>• Poster session – 1 min poster presentations |
| 12:10 | Coffee break |
| 12:30 | *Practical Exercises*<br>PARALLEL GROUP SESSIONS:<br>**Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CATCHING ORAL PRESENTATION"**Miguel A. De la Rosa** (Spain)<br>• "PUBLIC ENGAGEMENT IN SCIENCE" **Mark Roberts** (United Kingdom)<br>• "SHAPING YOUR CAREER (ALSO AS AN EDUCATOR) - TIPS AND TRICKS FOR A YOUNG SCIENTIST" **Ferhan G. Sağin** (Turkey)<br>• Poster session – 1 min poster presentations |
| 13:55 | Careers – SCIENCE FROM ANOTHER POINT OF VIEW<br>**Antonio Ferrer-Montiel** (Spain)<br>"Start-ups and their relation to science"<br>*Chairs: Jerica Sabotič and Anna Jagusiak* |
| 14:20 | SUM UP OF THE DAY<br>Quiz<br>*Chairs: Jerica Sabotič and Anna Jagusiak* |

## Friday, June 18, 2021

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|---|---|
| *9:15 - 9:30 Signing in* | |
| 9:30 | KEYNOTE LECTURE<br>**Nina Vardjan** (Slovenia)<br>"Astroglial excitability in brain health and disease"<br>*Chair: Anja Pišlar* |
| 10:15 | SHORT TALKS FROM PARTICIPANTS<br>SOP 7 |

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|---|---|
| | **Adèle Dramé-Maigné** (France)<br>"Programmable External Network based Compartmentalized Self-Replication (PEN CSR): a new method for *in vitro* directed evolution of enzymes" |
| | SOP 8<br>**Matvey Horetski** (Belarus)<br>"Novel fluorescent BODIPY probe for photoaffinity labeling"<br>*Chair: Anja Pišlar* |
| 10:35 | *Practical Exercises*<br>PARALLEL GROUP SESSIONS:<br>**Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CATCHING ORAL PRESENTATION" **Miguel A. De la Rosa** (Spain)<br>• "PUBLIC ENGAGEMENT IN SCIENCE" **Mark Roberts** (United Kingdom)<br>• "SHAPING YOUR CAREER (ALSO AS AN EDUCATOR) - TIPS AND TRICKS FOR A YOUNG SCIENTIST" **Ferhan G. Sağin** (Turkey)<br>• Poster session – 1 min poster presentations |
| 12:00 | Coffee break |
| 12:20 | *Practical Exercises*<br>PARALLEL GROUP SESSIONS:<br>**Career skills - TIPS AND TOOLS and POSTERS**<br>• "HOW TO PREPARE A CATCHING ORAL PRESENTATION" **Miguel A. De la Rosa** (Spain)<br>• "PUBLIC ENGAGEMENT IN SCIENCE" **Mark Roberts** (United Kingdom)<br>• "SHAPING YOUR CAREER (ALSO AS AN EDUCATOR) - TIPS AND TRICKS FOR A YOUNG SCIENTIST" **Ferhan G. Sağin** (Turkey)<br>• Poster session – 1 min poster presentations |
| 13:45 | Careers – SCIENCE FROM ANOTHER POINT OF VIEW<br>**David Adam** (United Kingdom)<br>"Communication of science by a scientist turned journalist"<br>*Chairs: Anja Pišlar and Morana Dulić* |
| 14:10 | SUM UP OF THE DAY<br>Announcement of the best SOP and the best Poster award winners<br>*Chairs: Anja Pišlar and Morana Dulić* |
| 14:30 | CLOSING REMARKS *Maja Katalinić and Irene Díaz-Moreno* |

# Practical Exercises - Schedule for each Group

## Group LIPA

The **linden tree** (*lipa* in Slovene) is an informal symbol of the Slovenian nation. There are many very old linden trees in Slovenia, some are even supposed to be around 700 years old. In many Slovene villages a linden tree represented an important gathering place and the center of the community. It was customary for people to discuss minor disputes on stone seats under the linden tree, make decisions on mutual tasks, vote, and they also gathered for celebrations, festivities and dances. The tradition also holds that, since time immemorial, people have planted linden trees to mark special occasions. Given the very symbolic importance held by the linden tree, one was planted 30 years ago on Republic Square (Trg republike) in front of the National Assembly building in Ljubljana on the day the Republic of Slovenia proclaimed its independence and sovereignty.

**Tuesday, June 15, 2021**

11:00 *Practical Exercises*
"HOW TO PREPARE A CV" Keith Elliott (United Kingdom)

12:50 *Practical Exercises*
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**Wednesday, June 16, 2021**

10:40 *Practical Exercises*
"VALUE OF A WELL-RUN LAB BOOK" Jason Perret (Belgium)

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Poster session – 1 min poster presentations

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12:20 *Practical Exercises*
Poster session – 1 min poster presentations

## Group MARUN

A large refined variety of **sweet chestnut**, *kesten* in Croatian, named *marun* is abundant on the slopes of Učka mountain, mostly in the vicinity of Lovran. So, in this area it is also called "Lovran marun". The marun is sweeter than ordinary chestnut. Due to its presence in the Lovran region, marun has strongly marked its gastronomy. It has long been used to prepare various soups, sauces, fillings and side dishes, as well as cakes and desserts. Marun is used to stuff ducks, veal steaks and squid. From marun, croquettes, ravioli and other types of pasta are handmade. In the past, people roasted chestnuts and then ground and cooked them as a substitute for coffee, and made chestnut brandy and mead from marun honey. Every year in October, the famous Marunada festival is held in Lovran in honor of the marun. If you want to try some chestnut treat, now you know where you have to be in October.

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## Group ZMAJ

The **dragon** (*zmaj* in Slovene) is the symbol of Ljubljana and part of the city's coat of arms. It exemplifies strength, courage and greatness. It is also depicted on the Dragon Bridge, on the registration plates of Ljubljana, on city buildings, and canal covers. Legend has it that Ljubljana was founded by Jason, the hero of Greek mythology who stole the Golden Fleece from the King of Colchis and then fled aboard the Argo with his band of heroes, known as the Argonauts. While fleeing across the Black Sea they accidentally sailed up the Danube and the Sava until they reached the Ljubljanica, where the waterway ended. The Argonauts built a settlement named Emona. There they dismantled their ship in order to carry it overland to the Adriatic coast, where they rebuilt the vessel and set sail back to Greece. In the immediate vicinity of the source of Ljubljanica stretched vast marshes inhabited by a gigantic monster. Jason fought and defeated the dragon monster. Since ancient times the dragon gradually transformed from a monster to a symbolic protector of the city of Ljubljana.

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Poster session – 1 min poster presentations

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"HOW TO PREPARE A CATCHING ORAL PRESENTATION"
Miguel A. De la Rosa (Spain)

12:20 *Practical Exercises*
"PUBLIC ENGAGEMENT IN SCIENCE" Mark Roberts (United Kingdom)

## Group LOVOR

Lovran is one of the oldest coastal settlements on the eastern coast of the Istrian peninsula. The city was founded when the Roman patrician Marcus Vipsanius Agrippa built his summer residence in this quiet and poetic place in the first century AD. It was named *Lauriana*, which in Latin simply means "The city of laurels". Indeed, the **laurel tree**, *lovor* in Croatian, grows wild in abundance in the Lovran region, contributing to an atmosphere of relaxation and serenity. "Since I am eternally young, neither will you, evergreen leaves, ever fade" said the god Apollo to laurel, making it a symbol of eternity. Maybe it is a reason why the city succeeded to proudly preserve its name through all of the turbulent centuries, as well as laurel trees woven into the very identity of this area. Leaves of laurel tree are used in cooking for their distinctive flavor and fragrance. They are often used to flavor (and to be removed before eating) soups, stews, braises and pâtés.

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12:20 *Practical Exercises*
"HOW TO PREPARE A CATCHING ORAL PRESENTATION"
Miguel A. De la Rosa (Spain)

# Keynote Speakers

**Ivan Đikić** (Frankfurt, Germany)

*Opening Keynote Lecture: 'Surprises in science – lessons from COVID-19'*

Ivan Đikić is professor and director of the Institute of Biochemistry II, Goethe University Frankfurt Medical School, Germany and a Fellow of Max Planck Society. He is a leading expert in the field of ubiquitin biology, which has relevance to diseases such as cancer, neurodegeneration and infection. Most recently, he established two large research consortia at Goethe University (ENABLE and PROXIDRUGS) with an aim to utilize new technologies toward drug development, in particular using proximity-inducing mechanisms for targeted protein degradation. For his scientific work he has received numerous awards, including the Jung Prize for Medicine and the Leibniz Prize, the highest scientific honour in Germany. He is an elected member of the EMBO, the German Academy Leopoldina and the European Academy, as well as an honorary member of the American Academy of Arts and Sciences.

**Eric Westhof** (Strasbourg, France)

*Keynote Lecture: 'Translation Control in Betacoronaviruses: Sequence Signatures in the 5'UTR RNA and NSP1 Protein'*

Eric Westhof, professor emeritus at the University of Strasbourg, France, is at the Institut de Biologie Moléculaire et Cellulaire in the unit Architecture et Réactivité de l'ARN of the CNRS. He is an executive editor of *Nucleic Acids Research*, the *RNA Journal*, the *Journal of Molecular Recognition* and *BBRC*. He is a member of EMBO, Deutsche Akademie der Naturforscher LEOPOLDINA, Academia Europaea, and the French Académie des Sciences. His research activities are centred on the evolution and recognition functions of RNA architectures.

## **Cecília Arraiano** (Lisabon, Portugal)

*Keynote Lecture: 'The amazing NEW world of RNA'*

Cecília Arraiano is coordinating investigator at the Instituto de Tecnologia Química e Biológica (ITQB) of Universidade Nova de Lisboa, Oeiras, Portugal, where she directs the Control of Gene Expression Laboratory. Her principal interests have been ribonucleases, and the understanding of the RNA processing and degradation mechanisms mainly in microbes. She has received numerous distinctions, including the Câmara Pestana/GlaxoSmithKline award and election to the Portuguese Academy of Sciences, EMBO and both the European and the American Academy of Microbiology.

## **Nina Vardjan** (Ljubljana, Slovenia)

*Keynote Lecture: 'Astroglial excitability in brain health and disease'*

Nina Vardjan is assistant professor in Biochemistry and a coordinating director of the Neuroglia Projects in the Laboratory of Neuroendocrinology-Molecular Cell Physiology at Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana and in the Laboratory for Cell Engineering at Celica Biomedical, Slovenia. She studies astroglial physiology in brain health and disease. She is the president of the Slovenian Physiological Society, member of FEPS and IUPS, and a core group member of the COST action European Research Network on Signal Transduction

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# Keynote Speakers' Abstracts

**Surprises in science – lessons from COVID-19**

**Ivan Đikić**
Goethe University and Max Planck Institute for Biophysics, Frankfurt, Germany

The emergence of COVID-19 was a big challenge for the whole world considering that it is a new, unknown virus for which we did not have tests, drugs or vaccines. Science, in the shortest possible time, had to give answers to the questions of how the virus spreads, what can it really cause and what impact does it have on the organization of the health and education system. Meanwhile, the comprehensive efforts of the entire scientific community have gathered a lot of valuable new data, appropriate genetic, antigenic and serological tests have been created, and several vaccines have shown efficacy. In my presentation, I will look at the ongoing efforts to mobilize science and technology to develop new, more effective therapeutic approaches against COVID-19, including specially tailored coronavirus drugs.

Our focus was centred on the action of papain-like protease (PLpro), an essential coronavirus enzyme required for viral replication and spread. We provided biochemical, structural and functional characterization of the SARS-CoV-2 PLpro (SCoV2-PLpro) in controlling host interferon (IFN) and NF-κB pathways. Upon infection, SCoV2-PLpro contributes to the cleavage of ISG15 from interferon responsive factor 3 (IRF3) and attenuates type I interferon responses. Importantly, inhibition of SCoV2-PLpro with GRL-0617 impairs the virus-induced cytopathogenic effect, fosters the anti-viral interferon pathway and reduces viral replication in infected cells. These results highlight a dual therapeutic strategy in which targeting of SCoV2-PLpro can suppress SARS-CoV-2 infection and promote anti-viral immunity.

Taken together, through sharing basic scientific discoveries with our colleagues in Munich, Leiden, Freiburg, and Mainz we showed how cross-border culture of openness has brought some new discoveries. Equally important, such collaborative science has fostered a sense of purpose, inspiration and togetherness among our students and postdocs, showing that science is an incredibly rewarding and important profession.

## Translation Control in Betacoronaviruses: Sequence Signatures in the 5'UTR RNA and NSP1 Protein

**Eric Westhof**
Architecture & Reactivity of RNA, Institute of Molecular and Cellular Biology, University of Strasbourg, France

The 5'UTR segemnt of coronavirus genomes plays key roles in the viral replication cycle and the translation of the viral mRNAs. The first 75-80 nucleotides are identical for the genomic mRNA and for the subgenomic mRNAs. The cooperative actions between the 5'UTR and the non-structural protein NSP1 have been shown to be essential for both the inhibition of host mRNAs and for the specific translation of viral mRNAs[1]. Here, sequence analyses of both the 5'UTR RNA segment and the NSP1 protein have been done for several betacoronaviruses. The conclusions are the following: (i) precise molecular signatures can be found in both the RNA and the protein; (ii) both types of signatures strongly correlate between each other; (iii) definite sequence motifs in the RNA correlate with sequence motifs in the protein so that the identification of one predicts the conserved type of sequence in the other one. Finally, these molecular signatures are (iv) specific to the type of coronavirus and thus (v) the various types of the 5'UTR first 80 nucleotides reproduce the main branches of the various phylogenetic trees deduced from whole genomes comparisons or functional genomic segments of betacoronaviruses. Using rabbit reticulocyte lysates, the efficiency of translation was measured for various reporter constructs containing the 5'UTR upstream of the Renilla luciferase gene in presence or absence of WT and mutant NSP1. The data presented, although limited in genomic sequence length, set strong molecular constraints on the viral sequences for successful translation and replication in specific living cells.

Keywords: Betacoronavirus, 5'UTR, NSP1, Sequence alignments, translation.

[1]Tidu, A., Janvier, A., Schaeffer, L., Sosnowski, P., Kuhn, L., Hammann, P., Westhof, E., Eriani, G., and Martin, F. (2021) The viral protein NSP1 acts as a ribosome gatekeeper for shutting down host translation and fostering SARS-CoV-2 translation. *RNA* **27**:253–26.

## The Amazing New World of RNA

**Cecília Maria Arraiano**
Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Biological processes can not be fully understood without a deep understanding of RNA metabolism. In 2006 and 2009 Nobel prizes were dedicated to research in the field of RNA and more recently the power of small RNAs and CRISPr technology has given a new perspective to Molecular Biology and led to another Nobel in 2020. Recently, RNA based vaccines against SARS-CoV2 RNA virus, have been successful in holding the COVID-19 pandemic crisis, and this brought new interest on RNA in the media.
Our laboratory has been focused in the study of RNA degradation mechanisms and the characterization of enzymes and RNA chaperones that mediate RNA decay. Namely we have studied RNase II family of ribonucleases in the maturation, degradation, and quality control of mRNAs and functional non-coding small RNAs, and we have extended our research to eukaryotes to further understand the role of RNases in global regulation and Disease. Our studies have been also applied to areas of Biotechnological interest and Health, and we have been involved in European Projects on Synthetic Biology to reprogram bacteria for biotechnology use. Recently we have characterized the mechanism of action of the two SARS-CoV2 ribonucleases which have shown to be prominent targets for the development of novel antiviral drugs.
The intent of this talk will be to refresh your knowledge on RNA and to encourage you to learn more about the AMAZING NEW WORLD OF RNA!

**Astroglial excitability in brain health and disease**

Anemari Horvat, Robert Zorec, **Nina Vardjan**

*Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; Celica Biomedical, Ljubljana, Slovenia*

Astrocytes, abundant and functionally heterogeneous cell type of neuroglia in the brain, are essential for the maintenance of brain homeostasis. Although electrically silent cells, astrocytes respond to extracellular signals via receptors, by increasing intracellular levels of second messengers $Ca^{2+}$ and/or cAMP (*i.e.*, cytosolic excitability), what affects astrocyte function. Astrocytes rich in adrenergic receptors (not neurons) were recently recognised as the main target of the *locus coeruleus* (*LC*) noradrenergic neurons. During attention, wakefulness, and stress *LC* noradrenergic system by releasing noradrenalin activates brain to augment brain metabolism, memory formation and learning. *LC* is one of the first areas undergoing degradation in various neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. How this affects astrocyte function, contributing to brain homeostasis, is unclear. We have shown recently by real-time imaging of intracellular fluorescent $Ca^{2+}$ and metabolite sensors that activation of astroglial adrenergic receptors via $Ca^{2+}$ and cAMP signals within minutes attenuates exocytosis of secretory vesicles, alters astroglial morphology, preventing cytotoxic oedema, and augments cell metabolism, including lipid droplet accumulation and aerobic glycolysis with the production of L-lactate. L-lactate is an important energy fuel transported from astrocytes to neurons to support neuronal functions, including learning and memory formation. However, adrenergic signalling, metabolism and L-lactate release were dysregulated in astrocytes that form intracellular protein inclusions, a hallmark of neurodegenerative diseases. This suggests that astroglial adrenergic activation and capacity to homeostatically support neurons are impaired. Thus, astrocytes may importantly contribute to the progression of the disease and cognitive decline, representing a novel target to treat neurological disorders.

# Career Skills Contributors

## **David Adam** (United Kingdom)

*Science communication*

Dr David Adam is a best-selling author and an award-winning journalist, who covers science, environment, technology, medicine and the impact they have on people, culture and society. After nearly two decades as a staff writer and editor at *Nature* and *The Guardian*, he set up as a freelancer in 2019.

## **Gaetano Castaldo** (Belgium)

*MSCA Fellowships*

Dr Gaetano Castaldo is from the European Research Executive Agency (REA) of the European Commission. After 12 years working as scientist with a PhD in Biochemistry, he has served the European Commission first as Scientific and Policy Officer and, since 2017, at REA working as Project Adviser and coordinator of the Life Science for Marie Sklodowska-Curie Actions (MSCA). He coordinates the work on proposal evaluation and the entire project life cycle of MSCA projects including monitoring of the implementation and the assessment of the projects.

## **Miguel A. De la Rosa** (Spain)

*Giving scientific talks*

Prof. Miguel A. De la Rosa has been the FEBS Congress Counsellor since 2015, and before that served as FEBS Executive Committee Vice-chair and Chair in 2013–2014. His research interests are structure–activity relations of biological macromolecules and, in particular, the protein–protein and protein–nucleic acid interactions which are crucial for a very broad range of cell processes and diseases. He uses a clear-cut multi- and interdisciplinary technology, ranging from molecular and cell biology to biochemistry, biophysics, structural biology and computational chemistry. Prof. De la Rosa has published ca. 200 peer-reviewed articles, four books and two patents, and has been invited to deliver more than 150 lectures worldwide. As university professor with over 40 years' experience, he has supervised 22 PhD students and more than 20 postdoctoral fellows.

## **Irene Díaz-Moreno** (Spain)

*Chair, FEBS Working Group on the Careers of Young Scientists*

Dr Irene Díaz-Moreno is Full Professor of Biochemistry and Molecular Biology at the Institute of Chemical Research of the Scientific Research Centre Isla de la Cartuja – cicCartuja, in Seville. She was awarded her PhD by the University of Seville, and was an EMBO postdoctoral fellow (2006–2008) at the NIMR-MRC in London (UK), working on the regulatory mechanisms of mRNA decay by RNA-binding proteins. In 2010, she got a

permanent position at the University of Seville, where she is developing research projects in the biointeractomics field, as well as on the post-translational regulation of biological macromolecules in the crosstalk between the nucleus and mitochondria in human disease. Dr Díaz-Moreno has held the FEBS Executive Committee position of Chair of the 'Working Group on the Careers of Young Scientists' since the start of 2018.

## **Keith Elliott** (United Kingdom)

*CV writing*

Dr Keith Elliott has spent 40 years teaching and researching, mainly in the areas of metabolism and enzymology at the University of Manchester, developing a particular interest in education and career development. He has chaired the Education Committee and been Careers Advisor for the UK Biochemical Society. He was a founder member of the FEBS Education Committee and has run workshops on educational methods and career development in 25 FEBS countries. He has been running CV support sessions at the YSF since 2007 and was awarded the FEBS Diplôme d'honneur in 2014 for his contributions.

## **Antonio Ferrer-Montiel** (Spain)

*Start-ups in science*

Prof. Antonio Ferrer-Montiel is a Professor of Biochemistry and Molecular Biology at the University Miguel Hernández (UMH) and Director of the Institute of Research, Development and Innovation in Healthcare Biotechnology of Elche (IDiBE). Prof. Ferrer-Montiel works in the field of ion channels and their role in the pathophysiology of chronic pain. He has published 150 widely cited papers, led 20 projects, presented 30 patent applications, and mentored 25 doctoral theses. Apart from his research activities, Prof. Ferrer-Montiel is also an entrepreneur who has created 5 spin-off companies, which have brought 20 biotech products to market and 3 compounds to clinical trials. He is currently President of AntalGenics, Fastbase Solutions, and Prospera Biotech.

## **Anna Jagusiak** (Poland)

Dr Anna Jagusiak was a chair of the local organizing committee of the 19$^{th}$ FEBS YSF (2019), which was held in Krakow, Poland. She teaches medical chemistry and biochemistry at Jagiellonian University, Faculty of Medicine in Krakow. Her research activities are focused on drug delivery systems with use of nanotechnology, in particular the use of complexes of carbon nanotubes and self-assembling molecules.

## **Alain Krol** (France)

*FEBS Fellowships*

Prof. Alain Krol is Director of Research emeritus at the CNRS and Professor conventionné at the University of Strasbourg. He has served as a FEBS Fellowships Committee member for four years (2013–2016) and has been the Chair since 2017. From the beginning of his

career, his research interests have been centred on the function and structure of various RNAs involved in RNA maturation and translation.

## **Vlastimil Kulda** (Czech Republic)

*Giving scientific talks*

Dr Vlastimil Kulda was a chair of the local organizing committee of the 18th FEBS YSF (2018), which was held in Prague. He teaches medical chemistry and biochemistry at Charles University, Faculty of Medicine in Pilsen. His research activities are focused on microRNAs in carcinogenesis.

## **Israel Pecht** (Israel)

*FEBS support for young scientists*

Prof. Israel Pecht is a member of the Dept of Immunology of the Weizmann Institute of Science in Rehovot. He has served FEBS in several positions, such as Chair of the FEBS Fellowships Committee (1993–2001) and as FEBS Secretary General (2008–2017), a leading function of that organization. Prof Pecht's research field is biophysical chemistry, where he employed a range of methods, such as fast kinetics to different spectroscopic methods, aiming at resolving elementary steps of key biochemical and immunological processes, from antigen recognition by the immune system and its coupling to effector processes to electron transfer reactions mediated by proteins. He has published over 400 peer-reviewed research articles as well as reviews, patents and books. His many contributions have been recognized through numerous honorary positions and awards.

## **Jason Perret** (Belgium)

*Lab notebooks*

Prof. Jason Perret is a research PI and Professor at the faculty of medicine of the Université Libre de Bruxelles (ULB). He has been teaching Molecular Biology to medical, biomedical and veterinary students for over 20 years, supervising master thesis, PhDs and Postdocs. His research has spanned from GPCRs (TSH, glucagon, VPAC and orphan receptors) to aquaporins and transcriptions factors in various pathologies, and DNA methylation gene regulation as biomarkers. He spent 4 years in industry as head of Baxter Health Care Molecular Biology Research Lab in Belgium (1993–1997). He holds two patents and two invention records. He is a long standing member of the FEBS Education workgroup (2003–2008) and then committee (2008–2012 and 2017–2020). He is also president of the Belgian Society for Biochemistry and Molecular Biology and member of the Belgian French-speaking Interuniversity Biochemistry Doctoral School.

## **Mark Roberts** (United Kingdom)

*Public engagement*

Dr Mark Roberts is a biochemist based at the Universty of Oxford; prior to this he was at Queen Mary University of London. His research area is the cell biology of dental pathogens. He is a member of the FEBS Science and Society Committee, the UK Biochemical Society Education, Training and Public Engagement Committee and the UK Biochemical Society policy advisory panel. He has organized and been involved in numerous science communication activities from science fairs to museum exhibitions.

## **Ferhan G. Sağin** (Turkey)

*Teaching and training*

Ferhan G. Sağin (MD, PhD) is a full professor of Biochemistry at Ege University Medical Faculty. Besides delivering undergraduate courses at the Medical Faculty, she is an active researcher and supervisor at the postgraduate level. Her main research areas include biomarkers, biosensors, biological psychiatry and education. She has seats on a variety of Boards/Committees including the Turkish Biochemistry Society (TBS Executive Board/vice-president and TBS Academy/Chair), International Union of Biochemistry and Molecular Biology (IUBMB/Education Committee Member), and International Association of Medical Science Educators (IAMSE/Public Affairs Committee Member+Educational Scholarship Committee Member). She has served the FEBS Education Committee as a member (2015–2018) and has been the Chair since 2019.

## **Kelly Sheehan-Rooney** (Germany)

*EMBO Fellowships*

Dr Kelly Sheehan-Rooney is Head of the Fellowship Programme at EMBO. She obtained her PhD in Developmental Biology from the University of Manchester, UK, before carrying out a three-year postdoctoral position at the University of Texas in Austin, USA. Now with 10 years' experience in international research development, Kelly has worked with thousands of scientists across the world; supporting them in their various careers, building collaborative networks, and fostering a research environment where scientists can achieve their best work.

## **Duncan Wright** (United Kingdom)

*Writing abstracts*

Dr Duncan Wright is the editorial manager of *FEBS Open Bio*, an open access journal for the publication of technically sound research articles across the molecular and cellular life sciences. He previously worked for the journal *Molecular Oncology* and as developmental editor at Academia Sinica, Taipei, during which time he gave talks on how to write a scientific article across Taiwan.

# Career Skills Lectures Overview

## Career skills - TIPS AND TOOLS

**How to prepare a catching oral presentation**

**Miguel A. De la Rosa**
Institute for Chemical Research, cicCartuja, University of Seville & CSIC, Sevilla, Spain

According to Aristotle, the classical Greek philosopher, the art of rethoric or communication is based on three key pillars: *ethos*, *logos* and *pathos*. They are modes of persuasion used to convince audiences. *Ethos* refers to the authority and credibility of the speaker, i.e. their knowledge on the matter. If you want to make an audience accept anything you say, they have to trust you because of your credibility. *Logos* stands for the logical reasoning used by the speaker to support their authority. You might thus use effective arguments — e.g., evidences, data, testimonials, etc. — to back up your claims. And *pathos* refers to empathy, i.e. the ability of the speaker to transmit their own emotions to the audience, which will be much more receptive if they can identify with you.
In the group discussion, we will try to carefully reflect on what "persuasive communication" means, and will perform a practical exercise to improve our oral skills in the light of Aristotle's rethorical triangle.
Recommended reading:
https://network.febs.org/posts/online-teaching-and-aristotle-s-pillars-of-rhetoric

**Preparing your CV: How to make the most of yourself!**

**Keith Elliott**
FEBS Education Committee, UK

Your *curriculum vitae* will probably be the first information a potential employer has about you. A *curriculum vitae* may also be required when applying for grants and fellowships. It is important to create a good impression and make the most of what you have achieved – making sure that the right information is presented in a logical order, with appropriate emphasis. There is no one correct way to write a *curriculum vitae*, but the talk will give hints on how to approach the task to help ensure that you give yourself the best opportunity to be interviewed, or get the job, fellowship or grant.

**Value of a well-run lab book**

**Jason Perret**

Laboratory of Pathophysiological and Nutritional Biochemistry LBPN, Biochemistry Department, Faculty of Medicine, Campus Erasme, Universite Libre De Bruxelles (ULB), Brussels, Belgium

Keeping a Lab Notebook is one of the most overlooked skills during university studies, be it undergraduate, graduate or postgraduate studies, be you a lab scientist, architect, engineer, medical doctor, nurse. However, astonishingly, all academics and professionals will agree that this is an essential skill at all levels. Failure to write, keep and "upgraded" a Lab Notebook may lead to damageable (scientifically and even legally) and sad situations. Furthermore, in many settings, namely industry, it is mandatory to provide a "Lab Notebook" responding to defined criteria.

The talk(s) and workshops we will share during the YSF will try to make you apprehend the importance, not to say utmost importance, of your Lab Notebook. We will also address the emergence of the digital Lab Notebooks and discuss the issues surrounding this evolution.

It is essential to understand that a Lab Notebook IS NOT "*YOUR PRESS BOOK"*, that "sells you"!

No it is not!!!

A Lab Notebook is your "*BLACK BOX*", your "*LOGBOOK*", it should reflect what you plan to do, what you did (and all changes to the plan), what happened while you were doing it (this is of critical importance), the observations made all along, the outcome(s), remarks and finally the interpretation with "what next".

When the going is smooth, no problem but when the going gets rough and things do not look good it is those details in your lab notebook that will allow you to evaluate where the problems could be and thereby propose solutions. It is also your tool of communication with your PI and colleagues. It allows you (and others) to reproduce or change the way of doing the next experiments, blueprints or care for your patients.

These are just some of the requisites behind the art of consigning the "good science" being done at the bench.

**Public engagement in science**

**Mark Roberts**

University of Oxford, UK

Science does not sit alone from society. Communication and engaging the wider public in your science is an important skill for a scientist. In this session we'll explore public engagement with science, what this means, and explore how you might do this with your own research, including looking at examples of other peoples' engagement.

**Shaping your career (also as an educator) - Tips and tricks for a young scientist**
**Ferhan Sağin**
Ege University Medical Faculty, Izmir, Turkey

Educators' role is to unlock the PhD students' creativity by broadening their vision while students' responsibility is to nurture both practically and intellectually on the career pathway by beginning with the end in mind.
This can only take place if PhD training integrates other skills into the curriculum that students will need in different career paths afterwards.
In most bench sciences, fewer than half of graduate students anticipate an academic career, and fewer still end up in one. Furthermore, the Covid-19 pandemic has gutted the academic job market, and changed the outlook of students and faculty members together. Thus, now is the time for educators' and students' to rethink career-diverse PhD's and to readjust mind-sets for shaping one's career also as an educator.
This session is aimed to raise some awareness in young researchers about the latest scientific research in learning with the hope that participants will continue to follow this interesting area besides their research field.
With engaging activities, participants will also discover the path that leads to be a good educator. Team work and online educational technologies will also be used to provide an enjoyable, friendly and safe learning environment.

**How to write an effective abstract**
**Duncan Wright**
*FEBS Open Bio*, UK

The abstract of your research article will be read more than the article itself. The ongoing growth in global scientific output means that even researchers working in closely related areas may only have time to read the abstract of your paper. In addition, the abstract is the most disseminated part of an article, as it is the only part indexed by PubMed and other search engines. As such, it is worth taking the time to carefully consider how to write your abstract. An effective abstract that clearly and concisely communicates your intent, findings, and conclusions is more likely to encourage other researchers to read and perhaps cite your work, and thus may result in more opportunities and collaborations in future. In this workshop, we will consider best practice when it comes to writing abstracts for research articles and conference submissions.

# Careers – FELLOWSHIP OPPORTUNITIES

**FEBS Fellowships**

**Alain Krol**
Directeur de Recherche émérite au CNRS, Architecture et Réactivité de l'arN, Université de Strasbourg – CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg Cedex, France

FEBS currently offers Short-Term Fellowships, Collaborative Developmental Scholarships and Summer Fellowships. Short-Term Fellowships are for scientists who have obtained their PhD degree within the past six years, and PhD students who have at least one published paper as a main author in an international scientific journal. Collaborative Developmental Scholarships are for PhD students in certain disadvantaged FEBS countries. Summer Fellowships are for PhD students and Master's students. All are for researchers studying or working in a FEBS country and wishing to move to a different country in the FEBS area for the duration of the Fellowship. In addition, candidates for Short-Term Fellowships and Collaborative Developmental Scholarships need to be a member of one of the FEBS Constituent Societies.

Furthermore, FEBS is pleased to announce the launch of a prestigious new scheme from 2021 – the FEBS Excellence Award – to support early-career group leaders in the FEBS area. FEBS Excellence Awards provide funding for research equipment and consumables over a 3-year period. With the launch of the new FEBS Excellence Award scheme in 2021, the FEBS Long-Term Fellowships scheme, previously supporting long-term visits for the purpose of scientific collaboration or advanced training, is now closed to new applications.

The FEBS Fellowships programme and FEBS Excellence Award scheme are overseen by the FEBS Fellowships Committee

**MSCA fellowships**

**Gaetano Castaldo**
European Research Executive Agency (REA) of the European Commission, Brussels, Belgium

Marie Skłodowska-Curie Actions (MSCA) is the EU's reference programme for doctoral education and postdoctoral training. MSCA support the training, career development and mobility of researchers in all scientific domains, at all stages of their careers and from all over the world. It supports both individual researchers and institutions (universities, research organisations, enterprises, and any other relevant organisations). Since 2014, MSCA have supported 65,000 researchers in Europe and beyond, including 25,000 doctoral candidates and more experienced researchers, and have funded over 1000 excellent international doctoral networks. The talk will provide an overview of the main characteristics of the different MSCA funding schemes as defined in the new framework programme Horizon Europe.

**EMBO fellowships**

**Kelly Sheehan-Rooney**

Head of the Fellowship Programme at EMBO, Heidelberg, Germany

EMBO supports outstanding life scientists at all career stages in Europe and around the world. Awardees can benefit from funding, career development support and networking opportunities to help create an environment in which scientists can excel. Several schemes are available for early career scientists such as the EMBO Postdoctoral Fellowships, Scientific Exchange Grants, and the New Venture Fellowships. During this lecture you will hear more details about EMBO, its programmes, evaluation criteria as well as top tips for successful grant writing.

# Careers – SCIENCE FROM ANOTHER POINT OF VIEW

**Start-ups and their relationship with Science**

**Antonio Ferrer-Montiel**
Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Av. De la Universidad s/n, 03202 Elche, Spain

It is currently accepted that scientific knowledge is a pivotal academic, medical and economical asset that increases societal wealth. The real value of knowledge arises from its transformation in innovative products and services that promote the progress of our society. A case in point is represented by biomedical knowledge that has more than double our life expectancy and increase life quality in the past century. Biomedical knowledge has resulted in earlier and better diagnostics and therapies for both highly prevalent and rare diseases. The achieved progress has been possible thanks to the impulse of entrepreneurial activities leaded by scientists that have promoted the foundation of start-up and/or spin-off companies. These biotechs have focused in transforming the knowledge generated by their founders into innovative products and services. Biotech companies have revealed themselves as central stakeholders of science transformation and exploitation. The close relationship of these companies with academic labs has been crucial for their business models that rest in using scientific knowledge as an unlimited source highly competitive products and services. As a result, the triangle of knowledge is being implemented by Universities in their training programs by combining education, R&D and entrepreneurship. My personal 20-year experience as scientist and entrepreneur will be shared for discussion.

**Communication of science by a scientist turned journalist**

**David Adam**
Frelancer, https://www.davidneiladam.com, UK

Through this lecture you will hear about the other aspect of scientific work and how can a career be fulfilled outside the lab. The interesting path led the speaker form a PhD student to editor at the world's best scientific journals (Elsevier Science and Nature), to correspondent writing on the Guardian newspaper, and all the way to a freelancer. All the gathered experience helped shaped a critically-acclaimed and best-selling popular science book on obsessive-compulsive disorder in 2014, as well as a book on a study of the emerging science of cognitive enhancement, published in 2018. Through the lecture you will get the idea how a scientific finding description and significance differ from academic to journalist point of view, and what does it take to write news, features, comment and analysis for both general and specialist audiences - quickly, accurately, with flair and within the deadline. Most of all, you will hear what it means to be an entrepreneur in this scientific environment and to do a step forward in achieving your potentials outside the lab.

# List of Short Oral Presentations

1. **M. Tijardović** P-08.3-02
   Intense physical exercise induces an anti-inflammatory change in IgG N-glycosylation profile
2. **A. Garcia-Mato** P-08.5-03
   IGF-1 via PI3K/AKT activation promotes survival and anabolic metabolism in HEI-OC1 auditory cells
3. **R.A. Beagrie** ShT-01.4-1
   Investigating the link between DNA replication, chromatin change and transcriptional regulation during *in vivo* erythroid differentiation
4. **E. Jarc Jovičić** ShT-02.2-1
   Lipid droplets and autophagy cooperate in the protection of cancer cells against metabolic stress
5. **A. Sivkina** P-04.2-01
   Role of C-terminal domains of yeast FACT complex in nucleosome unfolding
6. **E. Rembeza** ShT-01.3-1
   Investigation of functional annotations to enzyme classes reveals an extensive annotation error
7. **A. Dramé-Maigné** SpT-05-02
   Programmable External Network based Compartmentalized Self-Replication (PEN CSR): a new method for in vitro directed evolution of enzymes
8. **M. Horetski** P-03.2-04
   Novel fluorescent BODIPY probe for photoaffinity labeling

# Short Oral Presentation Abstracts

## SOP 1

P-08.3-02

**Intense physical exercise induces an anti-inflammatory change in IgG N-glycosylation profile**

**M. Tijardović**[I], D. Marijančević[II], D. Bok[III], D. Kifer[I], G. Lauc[IV], O. Gornik[I], T. Keser[I]

*[I]University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia, [II]Endocrinology Laboratory, Department of Oncology and Nuclear Medicine, Sestre milosrdnice University Hospital Center, Zagreb, Croatia, [III]Faculty of Kinesiology, University of Zagreb, Zagreb, Croatia, [IV]Genos Glycoscience Research Laboratory, Zagreb, Croatia*

Exercise is known to improve many aspects of human health, including modulation of the immune system and inflammatory status. Despite the general understanding that exercise reduces inflammation, the relation of the two is not yet fully understood. N-glycosylation of immunoglobulin G (IgG) and total plasma proteins was previously shown to reflect changes in inflammatory pathways, which could provide valuable information to further clarify effects of exercise. In order to better understand the relationship between physical activity and inflammation, we examined the effect of intense exercise, in the form of repeated sprint training (RST), on IgG and total plasma proteins N-glycosylation. Twenty-nine male physical education students were separated into treatment (RST, N = 15) and control (N = 14) groups. The RST group completed a 6-week exercise protocol while the control group was instructed to refrain from organized physical activity for the duration of the study. Three blood samples were taken at different time points: prior to start of the training program, the final week of the exercise intervention, and at the end of the four-week recovery period. Following the recovery period IgG N-glycosylation profiles showed anti-inflammatory changes in RST group compared to the control group, which manifested as a decrease in agalactosylated and an increase in digalactosylated and monosialylated N-glycans. Observed changes show the potential of intense physical exercise to reduce levels of systemic basal inflammation, as well as the potential for IgG N-glycosylation to serve as a sensitive longitudinal systemic inflammation marker.

## SOP 2

P-08.5-03

**IGF-1 via PI3K/AKT activation promotes survival and anabolic metabolism in HEI-OC1 auditory cells**

**A. Garcia-Mato**[I], L. Rodriguez-de la Rosa[I], B. Cervantes[I], I. Varela-Nieto[I]
[I]*Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, Spain*

Hearing loss is the most common sensory deficit in the human population. Mutations in the gene coding for IGF-1 cause sensorineural hearing loss in man and mice (previously published in: Rodríguez-de la Rosa L et al. (2017) Front Aging Neurosci 12,9:411). Actions of IGF-1 are mediated by binding to its high affinity transmembrane receptor, IGF1R. This interaction typically leads to the activation of the PI3K-AKT pathway and of the MAPK-ERK cascade. To gain insight into the molecular mechanisms involved in IGF-1 downstream signaling in the sensory hair cells, we have used the HEI-OC1 cell line derived from the Immortomouse™ hearing receptor (previously published in: Kalinec GM et al. (2003) Audiol Neurootol 4, 177-189), which is the cell line of choice to study the molecular steps occurring during the differentiation to outer hair cell-like cells of auditory progenitors. The study of the relative expression of genes of the IGF system by RT-qPCR showed that IGF system factors and receptors are expressed in both progenitors and differentiated auditory cells. We also studied the actions and main downstream signaling pathways of IGF-1. Apoptosis and cell viability were studied by flow cytometry and XTT assay, respectively, whilst activation of target proteins was measured by Western blotting. The consequences of blockage of IGF-1 actions were also investigated by using specific IGF1R inhibitors and XTT assay. IGF-1 increased survival, proliferation, as well as glucose metabolism and protein synthesis, whereas autophagic flux was decreased and apoptosis inhibited. Our data indicate that HEI-OC1 cells can be used as a model to understand the actions of IGF-1 in hair cells, to identify novel targets and to unravel the molecular mechanisms involved in IGF-1 deficiency-associated otic damage.

## SOP 3

ShT-01.4-1

**Investigating the link between DNA replication, chromatin change and transcriptional regulation during *in vivo* erythroid differentiation**

**R.A. Beagrie**[I,II], A.M. Oudelaar[I], M. Gosden[I], D. Hidalgo[II], J.R. Hughes[I], M. Socolovsky[II], D.R. Higgs[I]

[I]*Weatherall Institute of Molecular Medicine; University of Oxford, Oxford, United Kingdom,* [II]*University of Massachusetts Medical School, Worcester, MA, United States of America*

In mouse red blood cell differentiation (erythropoiesis), early progenitors transition to terminal differentiation by passing through a highly specialised cell cycle[1]. During this cell cycle, DNA is replicated faster than in preceding or following cycles due to accelerated replication forks[2]. We have performed an integrated analysis of matched chromatin accessibility and single-cell expression data, revealing that this cell cycle also coincides with widespread changes in gene expression and chromatin accessibility. We analysed chromatin folding of erythroid gene loci at various stages in red blood cell differentiation and show that specific enhancer-promoter loops are formed concomitantly with both progressive upregulation of gene expression and changes in histone post-translational modifications. We used SeqGL[3], a quantitative model using a k-mer feature representation and group lasso regularization to identify transcription factor motifs enriched in enhancer sequences at each stage of red blood cell differentiation. SeqGL highlighted the erythroid transcription factor GATA1 as highly enriched at enhancers that first become accessible during the transition to terminal differentiation. Using CUT&RUN, we are able to show that this specialised cell cycle is indeed the point at which GATA1 first binds to chromatin. Finally, we inhibit DNA replication and measure the effects of this perturbation on Gata1 recruitment and on chromatin composition. Our findings demonstrate that chromatin architecture and gene activation are tightly linked during development and provide insights into the distinct mechanisms contributing to the establishment of tissue-specific chromatin structures. References: 1. Pop ***et al.*** PLoS Biol. 8, e1000484 (2010). 2. Hwang ***et al.*** Sci. Adv. 3, e1700298 (2017). 3. González ***et al.*** Nat. Genet. 47, 1249–1259 (2015).

## SOP 4

ShT-02.2-1

**Lipid droplets and autophagy cooperate in the protection of cancer cells against metabolic stress**

**E. Jarc Jovičić**[I,II], M. Jusović[II], Š. Koren[I], P. Starič[I], E. Guštin[I], A. Kump[I], D. Lainšček[III], R. Jerala[III,IV], T. Petan[I]

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Lipid droplets (LDs) are lipid storage organelles present in most eukaryotic cells. They are composed of a core of neutral lipids surrounded by a phospholipid monolayer and proteins. LD biogenesis is induced in cells exposed to excess nutrients and lipids and is characteristic of many diseases, such as obesity, diabetes and cancer. Intriguingly, their formation occurs also in cells fully deprived of nutrients and oxygen, suggesting that LDs are an integral part of the cellular stress response. LDs engage in a complex and as yet poorly defined relationship with autophagy, the major cellular recycling machinery and stress response pathway. First, autophagy may drive LD biogenesis by providing lipids recycled from other membranous organelles. Second, autophagy may participate in LD breakdown through a selective form of autophagy named lipophagy. Third, LDs may promote autophagy by providing lipids or signals that support the formation of autophagosomal membranes. We aim to discover the principal ways in which LDs and autophagy cooperate to promote the resistance of cancer cells to stress. We have found that lipid droplets are dynamically synthesized and broken down in cancer cells depending on the length and severity of nutrient deprivation. Autophagy is required for their biogenesis under acute starvation conditions, whereas lipolysis seems to be involved in their breakdown under milder conditions of starvation. By manipulating the activities of the major enzymes involved in LD metabolism in the context of activated or inhibited autophagy, we are currently examining the links between these two processes and their roles in cancer stress resistance. Our work may open new perspectives in cancer research by providing important clues on the function of the recently recognized stress-associated organelle – the lipid droplet.

## SOP 5

P-04.2-01

**Role of C-terminal domains of yeast FACT complex in nucleosome unfolding**

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FACT (facilitates chromatin transcription) is a histone chaperone that is involved in the processes of transcription initiation and elongation, DNA replication and repair [1]. Yeast FACT conducts large-scale ATP-independent nucleosome unfolding [2]; but the detailed mechanism of this process is unknown. Here we determined the role of Nhp6 protein and C-terminal domains of yeast FACT complex in nucleosome unfolding using single-particle Förster resonance energy transfer (spFRET) microscopy, EMSA and FRET-in-gel methods. Nhp6 protein interacts with the C-terminal domains of Spt16 and Pob3 subunits of yFACT, inducing unfolding of FACT in the absence of nucleosomes. Analysis of yeast FACT interaction with nucleosomes and hexasomes suggests that Spt16 subunit of FACT drives nucleosome unfolding, while Pob3 subunit is required for complete unfolding. Thus, FACT-dependent nucleosome unfolding is a tightly coordinated process that requires Nhp6 protein and C-terminal domains of Spt16 and Pob3 subunits of yFACT. Our data suggest a detailed model of FACT-dependent nucleosome unfolding. This work was supported by the Russian Science Foundation grant 19-74-30003. Previously published in: [1] K Gurova et al (2018) BBA – Gene regulatory Mechanism 1861, p. 892-904. [2] ME Valieva et al (2016) Nat Struct Mol Biol 23, p. 1111-1116.

## SOP 6

ShT-01.3-1

**Investigation of functional annotations to enzyme classes reveals an extensive annotation error**

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Only a small fraction of genes deposited to databases has been experimentally characterised. The majority of proteins have their function assigned automatically, which can result in erroneous annotations. The reliability of current annotations in public databases is largely unknown, and we lack experimental attempts to validate accuracy of existing annotations. In our study we performed an overview of functional annotations to the BRENDA enzyme database. We first applied a high-throughput experimental platform to verify functional annotations to an enzyme class of S-2-hydroxyacid oxidases (EC 1.1.3.15). We chose 122 representative sequences of the class and screened them for their predicted function. Based on the experimental results, predicted domain architecture and similarity to previously characterised S-2-hydroxyacid oxidases, we inferred that at least 78% of sequences in the enzyme class are misannotated. We experimentally confirmed four alternative activities among the misannotated sequences and showed that misannotation in the enzyme class increased over time. Finally, we performed a computational analysis of annotations to all enzyme classes in BRENDA database, and showed that nearly 18% of all sequences are annotated to an enzyme class while sharing no similarity to experimentally characterised representatives. We showed that even well-studied enzyme classes of industrial relevance are affected by the problem of functional misannotation.

## SOP 7

SpT-05-02

**Programmable External Network based Compartmentalized Self-Replication (PEN CSR): a new method for *in vitro* directed evolution of enzymes**

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Directed evolution is a well-established method for enzyme engineering. Mimicking the process of Darwinian evolution with iterative cycles of genetic diversification and selection, researchers can find new enzyme variants with enhanced or completely new properties. Despite engineered enzymes are often intended for use in unnatural environments, existing methods allowing high-throughput in vitro selection conditions can only be applied to polymerases replicating their own genetic sequence in microdroplets. Here, we used an external DNA-based artificial network to create a feedback loop linking the activity of a nicking enzyme to the replication of its own gene. Taking the enzymatic activity at the input, the molecular program is producing a correlated amount of specific primers that are necessary for the PCR amplification of the gene. Bacteria carrying and expressing the mutants are co-encapsulated and lysed with the molecular program in individual droplets using microfluidics. The isothermal primers amplification by the network is initiated by raising the temperature to 45°C. The yield of the PCR then launched in each droplet depends on the amount of primers, therefore on the enzyme activity. After emulsion breakage, we retrieve a gene pool enriched in the best mutant genes. We applied the method to select for faster or more thermostable enzymes. After one selection cycle, next generation sequencing using MinION allowed us to detect key mutations involved in the improvement of these two traits. We generated mutants of the nickase by introducing some of these mutations in the wild-type sequence and could confirm that indeed these mutants had improved properties with sometimes additive effects. This work is the first demonstration of the Programmable External Network based CSR (PEN CSR) method. Programs detecting other types of activity can be envisioned and would allow not only to greatly expand the scope of the CSR but also to implement smart selection functions.

## SOP 8

P-03.2-04

**Novel fluorescent BODIPY probe for photoaffinity labeling**

**M. Horetski**[I,II], Y. Faletrov[II], Y. Dichenko[III], N. Sluchanko[IV], M. Rubtsov[V], V. Shkumatov[II]

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Photoaffinity labeling (PAL) has become a popular tool in proteomics and drug discovery[1]. PAL probes act via UV-triggered free radicals generation followed by covalent bonding with a target molecule. Benzophenone (BP) derivatives are among such probes structures[2]. Often PAL probes include a reporter tag allowing better detection of labeled molecules, e.g. using fluorescence. Today boron-dipyrromethenes (BODIPYs) have become widely used as fluorophores due to their photostability and quantum yields. Both BODIPY and phenyl groups have planar cyclic conjugated structure, so BODIPYphenylketones (BPKs) can be considered as potential fluorescent analogs of BP-based PAL probes. However, there is only one research where BPK photoactivity has been mentioned[3]. Thus, we have synthesized a "minimalistic" BPK (1) by acylation of 8-methylBODIPY. Such acylation of a BODIPY been constructed on unsubstituted pyrroles hasn't been described yet. The structure is characterized by NMR, ESI-MS, UV-vis spectrometry and fluorimetry. From quantum-mechanical calculations (PBE, ma-def2-SVP) the HOMO/LUMO gap for 1 (1.78 eV) is smaller than for BP (2.88 eV) assuming that photoactivation of ***1*** can be achieved by less energetic light than BP. The compound was found to give an adduct with cytochrome P450 CYP7B1 after 365 nm-UV exposition according to monitoring of the fluorescent band with the parent protein mobility on SDS-PAGE. Molecular docking demonstrated affine binding of 1 with a set of human P450s (free binding energies up to -15,1 kcal/mol). Compound 1 is a potentially new photoactive fluorescent ligand. Thus, 1 and other BPKs can be developed as facile and powerful proteomic tools to reveal new structure-function features of biomolecules.

Grants BRFFI X19PM-062 / X19PM-062-1 – RFFI 19-54-04009 Bel_mol_a.

References: 1. Yip MSG et al. (2013) Nat Chem Biol 9, 715-720. 2. Smith E et al. (2015) Future Med Chem 7, 159-183. 3. Murale DP et al. (2015) Chem Commun 51, 6643-6646.

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# Poster Abstracts

## Genome structure and regulation

P-01.1-02

**CRISPR/Cas9 mediated knockout of GLI1, GLI2 and GLI3 genes in melanoma cell lines**

**M. Kurtović**[I], N. Rinčić[II], D. Trnski[I], V. Musani[I], P. Ozretić[I], M. Sabol[I]

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GLI transcription factors are the main mediators of Hedgehog-GLI (HH-GLI) signaling pathway. They activate the transcription of many target genes which are involved in various aspects of tumorigenesis. Targeting the HH-GLI signaling pathway is one of the recent approaches in cancer therapy. Our preliminary data suggest that melanoma cells harboring the BRAF mutation show a better response to GLI inhibition than cells with the NRAS mutation, suggesting a differential role for the HH signaling pathway in melanoma cells with different genetic background. In order to elucidate the role of GLI proteins in melanoma with these genetic backgrounds (BRAF mutation, NRAS mutation, no mutation), we are now in phase of constructing GLI1/2/3 knockout melanoma cell lines using CRISPR/Cas9 system. For this purpose, for each GLI protein, we designed two sgRNAs which guide the Cas9 protein to the specific sequences in the genome where they create a double stranded break. The designated sequence was the region near the ATG of GLI1/2/3 and the region near the end of the genes. These two breaks were repaired via homology-directed repair (HDR) with a help of HDR cassette that was transfected along with CRISPR/Cas9. So far we have managed to construct GLI2 knock-outs in two melanoma cell lines. In parallel, we have also over-exressed GLI1/2/3 proteins in the same melanoma cell lines. Each maternal cell line and its over-expressed cell line for GLI1, GLI2 and GLI3 will be analyzed by RNA-seq to determine the changes in transcriptomes. This analysis, in combination with knock-out cell line analysis results, should provide us with the information of which genes are specifically regulated by each of the GLI proteins in each of the genetic background. That may provide insight into the observed differences between the cell lines with different genetic background.

P-01.1-04

**miR-27b modulates insulin resistance in hepatocytes by targeting insulin receptor and repressing insulin signaling pathway.**

**A. Benito**[I], K. Uribe[I], U. Galicia-Garcia[I], S. Jebari[I], A. Larrea-Sebal[I], C. Martin[I]
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Obesity is a global epidemic that has nearly tripled since 1975 and, with more than 1.9 billion overweight adults and 650 million obese people in 2016, constitutes the main risk of cardiovascular disease and type 2 diabetes mellitus (T2DM). Insulin resistance (IR) is one of the key factors in the development of T2DM however; the molecular mechanism leading to disease is still unclear. The implication of microRNAs (miR) in the pathophysiology of multiple cardiometabolic pathologies, including obesity, atherosclerosis heart failure and IR, has emerged as a major focus of interest in recent years. Indeed, upregulation of several miRNAs has been associated to obesity and IR, among them, it has been shown that miR-27b is overexpressed in liver of obese people, but its role in IR has not been deeply explored. The main objective of the present work has been to investigate the possible role of miR-27b in insulin signaling pathway regulation in hepatocytes. Results: The results of the present study demonstrate that mir-27b is able to regulate hepatic insulin sensitivity by directly interacting with INSR and IRS1. Conclusion: This work emphasizes the importance of miRNA modulation studies to determine their functional effects. In fact, our study demonstrates the direct effect of miR-27b on INSR and IRS1 expression and its potential role as insulin signalling regulator.

P-01.1-06

**Characterization of sponge homolog of human metastasis suppressor DRG1**

**S. Beljan**[I,II], K. Dominko[II], A. Talajić[II], M. Radić[III], M. Herak Bosnar[III], K. Vlahovićek[I], H. Ćetković[II]

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Cancer is known as a disease of multicellular animals caused by the errors within the multicellular system, leading to the proliferation of "selfish" cell lines. Research of ancestral homologs of cancer-related genes in humans has gained more popularity in recent years since comparative genomic studies have confirmed that many homologs of human genes were already present in simple metazoans. From an evolutionary point of view, the development of cancer is most likely related to the development of multicellularity and the appearance of true tissues and organs. Despite their simple morphology, with only a few cell types and without true tissues and organs, sponges possess complex genomes harboring many genes highly similar to their vertebrate homologs. Therefore, they provide an excellent model for studying the evolution of different genes that were most possibly present in the genome of the animal ancestor. Our research focuses on metastasis suppressor genes. Metastasis suppressors inhibit metastasis formation without affecting primary tumor growth. Bioinformatics analyses have shown that homologs of metastasis suppressors were probably already present in the last common ancestor of all animals. To better understand the basic role of ancestral metastasis suppressor homolog, we analyzed the sponge homolog of the main metastasis suppressor gene: developmentally-regulated GTP-binding protein 1 (DRG1). Our bioinformatics and phylogenetic analyses showed that these proteins are conserved across animals. Transfection of sponge and human cells revealed the intercellular localization of DRG1 proteins. The proteins were then overexpressed in E. coli and confirmed by Western blot and the protein GTP-binding properties by a GTPase activity assay. Further biochemical and biological characterization is in progress. These results will provide a better understanding of the intracellular processes related to the metastasis suppression and pathology of cancer and metastasis.

P-01.1-07

**Investigation of the gene expression pattern and the regulation of stearoyl-CoA desaturase 5 (SCD5)**

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Elevation of free fatty acid (FA) levels is a key component in the development of severe diseases. The cellular stress caused by saturated FA overload can be reduced by local desaturation. Thus, the stearoyl-CoA desaturase (SCD1) enzyme is an important member in the cellular defense mechanism against lipotoxicity. The function and the regulation of Scd1 are well characterized, but SCD5, the other human isoform is barely been studied yet. The present work aimed to reveal, whether both SCD5 transcriptional variants (A and B) described in NCBI database were transcribed in human tissues and, if so, in what extent. We also aimed to identify the promoter region of SCD5 gene.The total SCD5 gene expression of hepatic and renal cell lines and eight different human tissues was assessed by RT-PCR. Specific primer pairs were designed to quantify the two transcriptional variants separatelly by qPCR. To analyze SCD5 promoter region, four fragments of different length were amplified from human genomic DNA and cloned into the pGL3-Basic luciferase reporter vector. Promoter activities were measured by luciferase assays from transiently transfected HEK293T or HepG2 cells. The SCD5 mRNA was detected in HEK293T cell line, whereas it is not present in HepG2 cells. The 1000 bp length region 5' upstream from start codon has been shown to be the most transcriptionally active in luciferase reporter system, however in cell line specific manner. The transcriptional variant A of SCD5 turned out to be the most abundant in the brain, while the highest expression level of variant B was measured in the pancreas. Variant A was present 10-100 times higher than B in all tissues. Although both transcriptional variants are expressed, the significantly lower expression of the B isoform cannot be explained by the common promoter. Further research is needed to elucidate the mechanism of the observed cell type specificity of SCD5 promoter activity, as well as its potential contribution in human diseases.

## RNA function

P-01.2-01

**small noncoding RNAs as a tool to modulate gene expression**

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Pseudomonas putida is a highly attractive production system for industrial needs. However, for its improvement as a biocatalyst at the industrial level, modulation of its gene expression is urgently needed. We report the construction of a plasmid expressing a small RNA-based system with the potential to be used for different purposes. Due to the small RNAs modular composition, the design facilities and ability to tune gene expression, they constitute a powerful tool in genetic and metabolic engineering. In the tool presented here, customized sRNAs are expressed from a plasmid and specifically directed to any region of a chosen target. Expression of these customized sRNAs is shown to differentially modulate the level of endogenous and heterologous reporter genes. The antisense interaction of the sRNA with the mRNA produces different outcomes. Depending on the particularity of each sRNA-target mRNA pair, we demonstrate the duality of this system, which is able either to decrease or increase the expression of the same given gene. This system combines high specificity with the potential to be widely applied, due to its predicted ability to modulate the expression of virtually any given gene. This plasmid can be used to redesign P. putida metabolism, fulfilling an important industrial gap.

P-01.2-03

**Transcriptional analysis of the human apoptosis-related BOK gene reveals novel, alternatively spliced messenger RNAs, a previously unknown 5′untranslated region (5′ UTR), and two new, shorter 3′ UTRs**

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The BCL2 family includes pro- and anti-apoptotic members, basically differing in the combination of BCL2-homology (BH) domains. BOK is an apoptosis facilitator, yet an anti-apoptotic behavior has been described as well. This ambiguity could be attributed to the existence of multiple alternatively spliced transcripts encoding for distinct BOK protein isoforms. In this study, we describe the discovery of novel transcripts of the human ***BOK*** gene, most of which comprise new open reading frames (ORFs) and probably encode for new BOK protein isoforms. Moreover, we determined a new 5′untranslated region (5′ UTR) and two shorter 3′ UTRs. In brief, we started by performing bioinformatical analysis of publicly available expressed sequence tags (ESTs). Next, total RNA was isolated from 23 cancer cell lines originating from different human tissues and first-strand complementary DNA (cDNA) was synthesized starting from 5 μg of total RNA. PCR primers were designed to amplify only ***BOK*** cDNA-specific sequences and were used in two successive PCRs. Rapid amplification of cDNA ends (RACE) was used to study the 5′ and 3′ UTRs. Nested PCR and RACE products were electrophoresed on an agarose gel; bands of unexpected size were gel-extracted, purified, and sequenced using Sanger sequencing. We also performed next-generation sequencing (NGS) to unravel rare ***BOK*** transcripts. Our results led to the discovery of 21 novel ***BOK*** transcripts, 13 of which have distinct ORFs. ***in silico*** translational analysis revealed the putative existence of 7 novel BOK protein isoforms lacking internal peptides and possessing distinct C-termini. Moreover, we identified a previously unknown 5′ UTR, probably preceded by its own promoter, as well as two novel, shorter 3′ UTRs, with fewer post-transcriptional regulatory regions. Overall, the prospect of novel BOK protein isoforms and alternative UTRs raises questions about the role of this gene in both normal and pathological states and necessitates additional research.

## Epigenetics

P-01.4-04

**Looking for hypoxia fingerprint in malignant tumors**

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Hypoxia, a decreased availability of oxygen, is a feature of most tumors, which increases patients treatment resistance and favors tumor progression. Although several pathways have been identified to regulate hypoxic adaptation of cells, the primary mediators of this response are HIF-1 (hypoxia inducible factor 1) transcription factors. The aim of our research was to identify the hypoxia signature in malignant tumors. For our research we have chosen two types of tumors. Malignant melanoma, developing in constantly hypoxic environment, and multiple myeloma, which develops in an environment where only hypoxic niches occurs. In the first step, cancer cell lines were cultured under normoxic (21%$O_2$) and hypoxic (1%$O_2$) conditions for 16h. The presence of hypoxia was confirmed by the stabilization of HIF-1α subunit and detection of protein-pimonidazole adducts. Next, the panel of HIF-1 target genes, selected based on the literature, was examined using RT-PCR. All the studied genes were confirmed as HIF-1 targets and contained hypoxia response elements (HRE) in their promoters. Next, we determined molecular hypoxia signature using the genes with low basal expression and significant induction in hypoxia. Subsequently, using binominal Bernouli distribution, the molecular signature was verified on patients transcriptomic data to answer the question of whether a group of hypoxic patients is actually present in a given tumor. Our work has shown that hypoxia molecular signatures vary between different types of cancer, and that for each type of tumor the molecular signature must be determined individually. The approach we proposed may constitute an universal tool that will allow for searching for hypoxia fingerprint in transcriptomic data of cancer patients.

P-01.4-05

**Epigenetic biomarkers in precision treatment of obesity: differential methylation levels between responders and non-responders to weight loss diets**

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Epigenetic marks, particularly DNA methylation levels in CpG sites, may be useful in the personalized treatment of obesity, helping to predict a better response to specific weight loss diets. The purpose of the present research was to examine whether DNA methylation levels could be associated with the metabolic response to weight loss diets. For this purpose, DNA methylation was analyzed in responders and non-responders to two different hypocaloric diets (30% energy restriction), at the beginning of the dietary intervention. DNA from white cells of 100 volunteers who participated in the Obekit dietary intervention program for 6 months was used. Volunteers were randomized in two different diets: moderately high-protein (30% protein, 30% lipids and 40% carbohydrates) and hypolipidic (18% protein, 22% lipids and 60% carbohydrates). To analyze the CpGs whose methylation was associated with better response to each intervention, the population of each diet was divided into quartiles according to their weight loss response, and the methylation levels of quartiles 1 and 4 were compared for each diet. DNA methylation levels were quantified using Infinium Methylation EPIC Bead Chip kits (Illumina). This study identified 14 CpG sites with DNA methylation differences greater than 5% between responders and non-responders to hypocaloric diets. Seven genes were associated with a better response to the high-protein diet and five genes with better response to the hypolipidic diet ($p < 0.05$). In summary, epigenetic biomarkers could help to predict the response to a weight loss diet and be used in the personalization of obesity treatment.

## Protein biosynthesis and expansion of genetic code

P-02.1-01

**Biochemical approaches to developing spent media from industrial bioprocesses for new protein production in E. coli fermentation systems.**

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What if we could take waste byproducts from industrial processes and generate new value in secondary processes with that waste? Of the thousands of tonnes of hazardous chemical waste generated from the biopharmaceutical manufacture of protein-based drugs each year, spent cell culture media from bioprocessing represents one potential area for exploitation using applied biochemical approaches. Studies have shown that levels of up to 60% spent media added to cell culture systems helped to increase protein production by mammalian and insect cells. In this poster presentation we describe (i) the proof of principle experiments to determine viability of E.coli cultures using harvested spent media from CHO cell culture (ii) the expression analysis of a novel fusion protein by the cultures and (iii) the empirical approaches taken to optimise the culture conditions in comparison to rich bacterial media and nutrient limiting minimal media controls respectively. Our data shows that expression of a fluorescent mCherry fusion to a novel affinity construct of commercial value is maintained by the bacterial cultures in 100% spent medium from mammalian cell culture. The careful empirical optimisation of biochemical parameters in the expression culture system are presented to show how a spent medium from bioprocess can generate proteins of value in newly designed processes, including the titration of low cost additives such as glucose and glycerol, as well as gradients of temperature and expression time. A key aim of the circular bioeconomy is to maximise the utility of resources by ensuring waste can be used to generate new value and this study confirms the potential for the reuse of spent media from bioprocess as a food source for microbial expression cultures.

* The authors marked with an asterisk equally contributed to the work.

P-02.1-02

**Stress-Induced Modulation of Human Antigen R by the Apoptosis Mediator Cytochrome c and Tyrosine Kinase JAK3**

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The post-transcriptional control of gene expression is mediated by the so-called RNA-binding proteins (RBPs). One of the best studied RBPs is the Human antigen R (HuR), which usually enhances the stability and translation of its mRNA ligands. Moreover, HuR binds to the histone chaperone ANP32B to export cargo mRNAs into the cytoplasm. Our group is currently developing two lines of research on the regulation of HuR function. The first one originated from pull-down assays in which we had detected the association of the apoptosis mediator cytochrome ***c*** (C***c***) with HuR upon DNA damage stimulus. Interestingly, we already had evidence suggesting a physiologically relevant interaction between ANP32B and C***c***. Therefore, our ongoing investigation aims to elucidate whether C***c*** directly binds to HuR or, alternatively, ANP32B acts as a molecular bridge linking the other two proteins. Furthermore, we are also examining the biological significance of the above-mentioned interactions in the context of programmed cell death. On the other hand, our second research line focuses on the role of Janus kinase 3 (JAK3) in the modulation of HuR binding to cognate mRNAs. Indeed, stress-induced phosphorylation of HuR at Tyr200 by JAK3 has been related to a reduced interaction of this RBP with its target transcripts. To get a deeper insight into this observation, we mimicked Tyr200 phosphorylation by co-expressing a tRNA/aminoacyl-tRNA synthetase pair specific for the non-canonical amino acid ***p***-carboxymethyl-L-phenylalanine (***p***CMF) together with an HuR construct. Through several biophysical assays with phosphomimetic Y200***p***CMF HuR and single-stranded DNA oligonucleotides, we want to assess the impact of JAK3 activity on HuR affinity for mRNA. Importantly, HuR is considered an oncoprotein and its dysregulation has been implicated in several diseases. Thus, a better understanding of the molecular mechanisms controlling HuR function could provide valuable data for the design of new therapies.

P-02.1-04

**Isoleucyl-tRNA synthetase editing domain accepts broad range of amino acids that are efficiently discriminated at the synthetic active site**

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Aminoacyl-tRNA synthetases (aaRSs) activate amino acids and transfer them to cognate tRNAs. Some aaRSs cannot establish a required specificity in amino acid recognition and thus may erroneously activate noncognate amino acids with a frequency higher than $10^{-3}$. These enzymes evolved a separate editing domain to hydrolyze formed misaminoacylated tRNAs (post-transfer editing). An initial model of discrimination at the editing domain proposed that binding of the cognate amino acid is prevented by a steric clash. Yet, we showed that the cognate amino acid may bind at the editing site, but unproductively (previously published in Dulic et al. J Mol Biol (2018) 430, 1-16). To understand better what shaped selectivity of the editing domain we have used isoleucyl-tRNA synthetase (IleRS) as a model enzyme in our recent (previously published in Bilus et al. J Mol Biol (2019) 431, 1284-1297; Zivkovic et al. FEBS J (2019) early view) and novel work. We tested a broad range of substrates belonging to i) proteinogenic (Ile, Ala, Val, Leu, Thr, Ser and Met), ii) nonproteinogenic (α-aminobutyrate, norvaline (Nva) and norleucine), and iii) synthetic (di- and tri-γ-fluoro-α-aminobutyrate) amino acids. Among them, only Val and Nva mimic well the cognate Ile and were poorly discriminated (< 200-fold), while the others were well discriminated at the IleRS synthetic site (500- to $10^6$-fold). Nevertheless, we prepared misacylated tRNAs with all tested amino acids and followed their hydrolysis in an independent assay. Surprisingly, all misacylated tRNAs were hydrolyzed by IleRS at similar rates (35-70 $s^{-1}$). Thus, how efficient amino acids were discriminated at the synthetic site and consequently whether these amino acids posed an evolutionary threat to translation fidelity does not determine the efficiency of their post-transfer editing. Only the cognate Ile-$tRNA^{Ile}$ was hydrolyzed slowly (0.058 $s^{-1}$), suggesting that this is the main requirement that shaped specificity of the editing domain.

## Proteolytic processing

P-02.3-02

**The impact of proteasome impairment on microglia function and neuroinflammation**

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Cellular protein homeostasis is maintained by the ubiquitin proteasome system (UPS) via protein ubiquitylation and proteasomal degradation. In response to inflammation, catalytic β-subunits of the standard proteasome (SP) are replaced by the inducible subunits and form an alternative isoform, the immunoproteasome (IP). Proteasome (IP and SP) dysfunction results in accumulation of ubiquitylated proteins, the induction of type I interferons (IFNs), and systemic inflammation including neuroinflammation (Previously published in: Brehm A et al. (2015) J Clin Invest 125, 4196-211). Microglia are immune cells of myeloid origin in the brain which constitutively express IP. Since our understanding of the impact of proteasome impairment on microglia function is very limited, we sought to determine the molecular link between ubiquitin-conjugate accumulation following proteasome dysfunction and neuroinflammation. In order to mimic inflammation, we subjected primary microglia isolated from wild type and LMP7 knockout mice, which harbor a deletion of the ***PSMB8*** gene encoding the IP catalytic subunit LMP7, to treatment with the proteasome inhibitor bortezomib (BTZ) and the toll-like receptor 4 ligand lipopolysaccharide (LPS). BTZ treatment induced type I IFNs dependent on the IRE1-arm of the unfolded protein response in wild type microglia (Previously published in: Studencka-Turski M, Çetin G et al. (2019) Frontiers Immunol doi: 10.3389/fimmu.2019.02900). LPS treatment caused an accumulation of ubiquitylated proteins in primary microglia of both genotypes, however cells with impaired IP function exhibited more of the ubiquitin-conjugates. Moreover, molecular analysis revealed significantly stronger induction of inflammation, as indicated by higher levels of type I IFNs and interferon-stimulated genes in primary microglia with IP impairment. In an attempt to identify the drivers of the inflammation, the ubiquitylated proteins will be characterized further by proteomic analysis.

## Protein folding and misfolding

P-02.4-01

**Biochemical characterization of short collagen-like otolin-1 involved in otoliths and otoconia formation**

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Many distinctive proteins regulate the process of tissue mineralization. These macromolecules control the deposition of biominerals and have significant influence on the nucleation, growth, localization and morphology of growing crystals. Many of biomineralization related proteins are intrinsically disordered (IDP) considered as the major regulators of the process. However, significant role of matrix proteins involved in biomineralization cannot be neglected. These proteins provide an organic scaffold and surface for mineral deposition. In the formation of fish otoliths and higher vertebartes otoconia a short collagen-like protein otolin-1 is involved as the scaffold providing and tethering element of these calcium carbonate inner ear structures. In this work we present the preliminary biochemical characterization of two homologs of otolin-1. ***Danio rerio*** and ***Homo sapiens*** otolin-1 was obtained as a recombinant protein from bacterial culture. The estimation of secondary structure content, the influence of calcium ions on the stability of these proteins and its oligomeric state show many differences in molecular properties between homologous proteins originated from these two species. Further investigation will be mostly focused on the resolution of protein structure. Acknowledgments: This work was supported by the National Science Center (Poland) [UMO-2015/19/B/ST10/02148] and in a part by statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Science and Technology.

## Protein localization and dynamics

P-02.5-01

**Mapping the network regulating transcription factor activity and dynamics by TF-FRAP**

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Cells respond to their environment via an intricate cellular signaling network. Insight into this network regulating cell fate is important for controlling stem cell differentiation, understanding disease pathology and defining better regenerative medicine strategies. Changes in cell fate are characterized by changes in gene transcription. These changes are dictated by changes in (master) transcription factor (TF) activity. SOX9 is the master TF of cartilage development. However, its dysfunction is associated with diseases, such as cancer, osteoarthritis (OA), fibrosis, sclerosis, etc. Here, we present a new method to directly monitor changes in TF activity. We developed Transcription Factor – Fluorescence Recovery After Photobleaching (TF-FRAP) to measure SOX9 dynamics and activity in primary human chondrocytes (hPCs) to understand its role in OA pathology. We found that changes in SOX9 dynamics as measured by TF-FRAP correlated to its transcriptional activity. Higher DNA binding and longer residence time of SOX9 on DNA increased its target gene expression levels and vice versa. SOX9 dynamics studies on hPCs showed that its residence time and DNA binding is significantly lower in OA as compared to healthy hPCs. We cross-validated TF-FRAP data with ChIP-qPCR and quantified gene expression changes with RT-qPCR. Moreover, TF-FRAP also identified subpopulations of cells within a donor, based on distinct dynamic rates of SOX9. Distinct and diffused SOX9 nuclear localization patterns were observed in the healthy and OA hPCs respectively. Distinct nuclear localization patterns correlated to higher DNA binding rate and longer residence times in healthy hPCs as compared to OA hPCs. Our data indicate a differential response of SOX9, depending on the disease state of the hPCs. This may have implications for treatment strategies that aim to restore SOX9 function. We show for the first time that our TF-FRAP method enables monitoring TF activity in real-time in primary cells.

P-02.5-02

**Molecular basis for the DNA damage-induced interaction between cytochrome c and the histone chaperone SET/TAF-Iβ**

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During the DNA damage response, nucleosome eviction by histone chaperones provides access of repair machinery to DNA injuries. The histone chaperone and oncoprotein SET/***template-activating factor***-Iβ (SET) engages DNA repair response. Upon DNA insults, mitochondrial cytochrome ***c*** (C***c***) reaches the cell nucleus, where it binds SET so as to inhibit its histone chaperone activity. SET functions as a homodimer in which each monomer consists on an N-end α-helix dimerization domain (residues 1-80), a globular α/β domain (a.k.a. ***earmuff***; 81-225) and a low-complexity acidic region (LCAR; 226-277). Our previous data demonstrate that SET dimerization and ***earmuff*** domains are sufficient to bind both histones and C***c***. To further characterize the SET-C***c*** complex, we deployed a methodological approach combining Electron Paramagnetic Resonance (EPR) and Nuclear Magnetic Resonance (NMR) with Small-Angle X-ray Scattering (SAXS). For such purpose, we have designed five single cysteine mutants across a SET construct lacking its disordered region, named SET-ΔC (residues 1 to 225). Cysteine residues were bound to either nitroxide spin or $^{19}$F probes. Continuous-wave EPR spectra of the spin probe and chemical-shift perturbations of $^{19}$F resonances were assessed to determine those regions from SET-ΔC interacting with C***c***. Paramagnetic Relaxation Enhancement NMR (PRE-NMR) measurements induced by the SET spin probe onto the C***c*** surface deciphered those residues of the hemeprotein involved in the complex formation. SAXS experiments enabled to obtain a low-resolution model of the SET-C***c*** complex. Altogether, our findings indicate that C***c*** recognizes the globular domains of SET, where histones bind to, so providing a molecular basis for histone chaperone inhibition activity.

P-02.5-07

**Increased sensitivity of MS – based proteomics methods obtained by sample fractionation technique.**

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The Chromosome – centric Human Proteome Project (C – HPP) aims to find high-stringency evidence for all proteins encoded by the human genome, the major splice forms of each protein. The Russian part of this project consists of detecting proteins encoded by the 18th human chromosome. In this work HT-29 human cell line was used as a biological object of interest. Whole-cell protein extract was prepared and digested by trypsin protease. Fifteen and sixty – three proteins encoded by the 18th human chromosome could be detected in complex peptide sample by shotgun mass – spectrometry (MS) and Multiple Reaction Monitoring with stable isotope – labelled standards (MRM SIS) respectively. MRM SIS is 6 folds more sensitive compared to shotgun MS, but due to the high complexity of peptide sample (4359 unique peptides identified by shotgun MS, related to 1200 proteins), some of the identifications detected by MRM SIS were doubtful (low signal, signal interference). To decrease peptide sample complexity and increase the sensitivity of shotgun mass – spectrometry and MRM SIS methods peptide sample fractionation technique was used. It was shown that reversed – phase liquid chromatography (RP – LC) in alkaline conditions performed prior to MS analysis allowed to confirm all the doubtful identifications detected by MRM SIS and to detect 11 additional proteins encoded by the 18th human chromosome with shotgun MS (11750 unique peptides identified, related to 2837 proteins). Thus, using RP – LC in alkaline conditions allowed to detect 63 proteins by MRM SIS and 34 proteins by shotgun MS (18 protein identifications were common for both methods), 45 proteins were detected by MRM SIS only, 16 proteins were detected by shotgun MS only. In summary 79 proteins encoded by the 18th human chromosome were detected. Applying RP – LC in alkaline conditions in combination with shotgun MS and MRM SIS allows deeper proteome coverage in chromosome - centric way.

P-02.5-09

**Atypical antipsychotic clozapine binds fibrinogen and affects fibrin formation**

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Clozapine is an atypical antipsychotic used for the treatment of schizophrenia. Prescribed daily doses of clozapine may reach over 900 mg/day. Some studies reported a connection between clozapine usage and the development of thrombosis. Our ***in vitro*** study aimed to provide insight into molecular bases of this observation, investigating clozapine binding to isolated fibrinogen, the main protein involved in hemostasis. Fibrinogen/clozapine interaction was confirmed by protein fluorescence quenching, with affinity constant calculated to be $1.7 \times 10^5$ $M^{-1}$ and the number of binding sites more than one. Direct interactions do not affect the structure of fibrinogen, as determined by UV-VIS spectrometry and Fourier-transform infrared spectroscopy, nor fibrinogen melting temperature, examined by fluorescence spectroscopy. However, clozapine binding affected fibrin formation, by reducing coagulation speed and thickness of fibrin fibers. This behavior suggests that in the presence of clozapine, fibrinogen may acquire thrombogenic characteristics. Although no difference in fibrin gel porosity was detected, other factors present in the blood may act synergistically with altered fibrin formation to modify fibrin clot, thus increasing the risk for development of thrombosis in individuals on clozapine treatment. By ORAC and HORAC antioxidant assays, we found that clozapine efficiently protects fibrinogen from free-radicals oxidation. Since the effect of clozapine on fibrin formation is dose-dependent, it seems that the dosage of the medication could be the main factor that determines if clozapine will have a more positive or negative effect on fibrinogen and coagulation process ***in vivo***.

P-02.5-11

**New potential role of Vps34 kinase in the control of the cell size**

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Platelets, the smallest blood cells, are produced in the bone marrow by their precursors, megakaryocytes (MKs). One of the most characteristic features of the MK maturation is a substantial increase in size, together with the polyploidization of the nucleus. At the end of the maturation process, MKs generate prolonged cytoplasmic protrusions, termed proplatelets, which extend through the vascular sinusoids of the bone marrow and release platelets into the bloodstream. Phosphoinositides are small membrane phospholipids implicated in cellular signalling, organelle trafficking and cytoskeletal dynamics. Phosphatidylinositol 3-monophosphate (PI3P), which is mainly produced by the Vps34 kinase, is a key component in vesicular trafficking processes, as well as autophagy and mTOR signaling. Nucleolus is nuclear subcompartment rich in RNA and RNA-protein complexes. It is the site of different steps of ribosome biogenesis, including transcription of ribosomal genes (rDNAs) and processing of ribosomal RNAs (rRNAs). In this study we show that in immature small MKs majority of Vps34 kinase localizes in organized structures within nucleolus, in fibrillar center (FC), where transcription of rDNA occurs. Treatment of MKs with RNA Pol I inhibitors abolishes Vps34 localization in nucleolus. In addition, when we specifically inhibit Vps34 in immature MKs, they fail to increase in size, and express lower levels of GPIb, indicating failure in maturation. All together, these data indicate that Vps34 might play an important, still undescribed, role in the nucleolar structure organization and/or in the control of MKs size and maturation via ribosome biogenesis. Additional studies are underway for better understanding of these events. Acknowledgement: this work is supported by American Society of Hematology Global Research Award and University of Rijeka support grant no. 18-188-1343. I.B. is supported by Croatian Science Foundation (HRZZ-09-2016).

P-02.5-13

**Resolving DPC: analysis of repair pathway**

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Common type of DNA lesions are DNA-protein crosslinks (DPC), a result of a covalent interaction of proteins involved in DNA maintenance and DNA molecule, due to endogenous and exogenous environmental conditions (previously published in: Stingele J, Jentsch, S (2015) Nat Rev Mol Cell Biol 16, 455–460). Spartan is one of the rare human proteins whose function is involved in resolving DPC (previously published in: Lopez-Mosqueda J et al. (2016) Elife 5:e21491). Mutations in SPRTN gene in humans cause hepatocellular carcinoma, as well as chromosomal breakage and Ruijs-Aalfs type of progeria (previously published in: Lessel D et al. (2014) Nat Genet 11,1239–1244). We hypothesized that Spartan protein regulates the activation of DPC repair pathway and that cells without Spartan function will have altered expression levels of genes related to DNA maintenance and repair. To test our hypothesis, we have compared cells with endogenous Spartan protein function with cells silenced for Sprtn gene and with or without reconstitution of Spartan with exogenous wild type or mutated Spartan. Cells were exposed to different types and intensities of genotoxic stress and the level of gene expression was measured on mRNA level using $RT^2$ profiler PCR array, as well as RNA sequencing. The results obtained were further confirmed with protein mass spectrometry, pulldown, western blot and flow cytometry analysis. The aim of our study was to determine how Spartan influences the activity of other DNA repair genes expression and to elucidate the mechanism of DPC resolving pathway. * The authors marked with an asterisk equally contributed to the work.

## Neurobiochemistry

P-03.1-01

**Subacute effects on mice central nervous system of dimethyl isophthalate (DMIP)**

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Phthalates are a family of chemical compounds primarily used to make PVC flexible. They are used in many products in homes, hospitals, cars, and businesses. Phthalates are known as toxic substances and they can affect the central nervous system. DMIP is used as a perfume fixative and as a plasticizer to make polyester resins. There is no information on DMIP toxicity. The aim of the study was to determine the histopathological effects and oxidative stress-inducing potential in the brain tissue by subacute exposure of DMIP. In the study, animals were orally fed with 150, 300, 600 mg/kg/day DMIP for 5 consecutive days per week for 28 days (OECD Guideline 407). At the end of the study, no significant changes were observed in body weight gains, absolute and relative organ weights of DMIP treated mice compared with the control. SOD activities in the tissues at the150 and 300 mg/kg DMIP treatment groups were significantly higher than the control ($p < 0.05$). The degrees of lipid peroxidation changed dose-dependently. In GST activities, there is no significant difference in the groups. However, AChE activities significantly decreased dose-dependently in all exposure groups. Moreover, DMIP caused dose-dependent histological changes such as an expansion of capillary vessels in the brain cortex and medullar areas. Also, excessive vacuolization in stromal areas were observed. Pyknotic nucleus and atrophic degenerate cells were observed in most of the granular and pyramidal cells in the brain cortex and in the highest dose, astrocytic infiltration concentrating on the pia mater is noteworthy. These results suggested that DMIP exposure induces oxidative stress in the brain and exposure of DMIP during a long period of time could lead to serious brain damage. In conclusion, DMIP has been shown to have neurotoxic effect on brain tissue. Acknowledgments: Ege University Local Ethical Committee of Animal Experiment (31.08.2016, 2016-073). Funding: Ege University Project No: 2017/FEN/027

P-03.1-02

**Effect of glucosylceramide accumulation on the neuronal homeostasis: a new neuronal in vitro model of Gaucher disease**

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Gaucher disease (GD) is a lysosomal storage disorder due to an impairment of the lysosomal β-glucocerebrosidase (GCase) activity with consequent accumulation of glucosylceramide (GlcCer). Nowadays it is clear that a common feature of all the different phenotypes of GD is the onset of neuronal degeneration; nevertheless, the molecular mechanism underlying the relation between GBA mutations and the onset of neuronal damage in GD remains unclear so far. To figure out which is the possible molecular mechanism linking GCase loss of function with the onset of neuronal damage, we developed an in vitro model of the neuronal form of GD represented by iPSCs-derived dopaminergic neurons, obtained from healthy subjects' fibroblasts and treated for 7, 13 and 29 days with conduritol B epoxide (CBE), a specific inhibitor of GCase. In CBE-treated neurons we found a progressive and time-dependent accumulation of GlcCer. Upon reaching a threshold of GlcCer accumulation, CBE-treated neurons showed: i) a significant neuronal damage as demonstrated by the reduction of the main neuronal markers such as Tau, Synapsin, MAP2 beta3-tubulin, and PSD95, ii) increased volume of intracellular acidic organelles and augmented lysosomal biogenesis, iii) impairment of the lysosomal sphingolipid catabolism, iv) block of the autophagic flow in term of augmented LC3IIB and p62, and v) as occurs in several other lysosomal storage disorders, the secondary accumulation of not catabolized glycosphingolipids. In addition, we found that the accumulated GlcCer is not just confined to the lysosome but affects also the plasma membrane. In conclusion, this in vitro model helps to investigate the onset of cell damage induced by GlcCer accumulation and lysosomal dysfunction. The obtained results let to speculate on the existence of a mechanism involving the plasma membrane in the onset of neuronal degeneration occurring in the brain pathology of GD .

P-03.1-03

**New functional cAMP compartment in neuroprotection organized by AKAP6**

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Neuronal survival and axon growth is known to be regulated by cAMP signaling. Last decades of research showed that cAMP signaling in neurons is highly compartmentalized, however besides dendritic and synaptic regulation, only few functional subcellular compartments in terms of molecular architecture have been identified so far. cAMP signaling is largely orchestrated by multimeric complexes called A-kinase anchoring proteins (AKAPs) that locally target PKA and other signaling molecules to specific compartments. These complexes are thought to serve as nodal points for signaling integration for pro-survival and pro-regenerative upstream stimuli. By using new PKA sensors for live-cell FRET imaging and molecular tools to specifically alter local cAMP level, we demonstrated that perinuclear compartment organized by AKAP6 is necessary for hippocampal neurons outgrowth and survival. Displacement of AKAP6-associated phosphodiesterase 4D3 (PDE4D3) with competing peptide (4D3-mCherry) significantly enhanced local cAMP elevations and promoted neuronal extension in the absence of any additional stimuli. Contrary, increasing PDE4D3 hydrolytic activity by targeting constitutively active enzyme to this compartment dramatically reduced length of axons and neuronal survival. In addition, in vivo delivery of 4D3-mCherry using AAV2 increased retinal ganglion cell survival following optic nerve injury. Our findings provide a demonstration of a new, functionally distinct neuronal compartment that regulate cAMP-dependent neuroprotection and axon growth and may be therapeutically targeted with AAV-based gene therapy. Supported by National Science Center grant no. 2019/33/B/NZ4/00587

P-03.1-04

**Cotinine and 6-hydroxy-L-nicotine attenuates memory deficits and reduce anxiety and oxidative stress in a zebrafish (Danio rerio) model of Alzheimer's Disease**

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Alzheimer's Disease (AD) is a progressive neurodegenerative disorder which affects almost 47 million people worldwide representing thus the most common form of dementia. AD is characterized by progressive cognitive decline and mood changes, accompanied by a loss of cholinergic neurons. Zebrafish (Danio rerio) has been successfully used to simulate AD pathology. In the central nervous system, nicotinic acetylcholine receptors (nAChRs) are involved in higher brain functions, such as memory, cognition and learning. There is considerable interest in modulating nAChRs to treat nervous system disorders, such as AD. Nicotine is a well-known agonist of nAChRs and was reported to improve memory, learning and attention, but the therapeutic use in AD was limited by its cardiovascular and addictive side-effects. Thereby, we focused on two structural related nicotine derivatives, namely cotinine (COT) and 6-hydroxy-L-nicotine (6HLN), that previously showed to improve cognition without exhibiting nicotine's side-effects. We evaluated the impact of COT and 6HLN on memory impairment, anxiety and oxidative stress in a zebrafish model of AD induced by scopolamine (SCOP). For this, COT and 6HLN were acutely administered by immersion to zebrafish that were treated with SCOP before testing. Anxiety was measured using the novel tank diving test (NTT) and memory performances were assessed by Y-maze and novel object recognition test (NOR). The oxidative stress was measured from brain samples. We have shown that 6HLN and COT improve memory performances in Y-maze and NOR tasks and reduce the anxiety level in NTT. Moreover, our data showed that these compounds reduce SCOP-induced oxidative stress. These findings support the premise that COT and 6HLN could be used as therapeutic agents in AD. * The authors marked with an asterisk equally contributed to the work.

P-03.1-05

**Donepezil exerts cytotoxic effect on glioma cells**

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Donepezil is a highly selective reversible inhibitor of acetylcholinesterase (AChE) that has been used to treat Alzheimer's disease (AD) due to its neuroprotective effects. There are a few studies that show its cytotoxic effect on cancer cells, but the mode of its action is poorly understood. In the present study, we investigated the mechanisms of potential cytotoxic effect of donepezil on C6 (rat glioma) and U251 (human glioma) cell lines. The viability rate of C6 and U251 cell lines was determined with the Cristal violet and MTT assay after 24h. Morphological changes were followed using light microscopy. Production of the reactive oxygen species (ROS), caspases activity, externalization of phosphatidylserine and the presence of the acid cytoplasmic vesicles were measured by Flow cytometry using specific fluorochromes (DHR, apostat, annexin-propidium iodide, and Acridine orange, respectively). In order to silence autophagy, U251 cells were transfected shRNA targeting human LC3II and AMPKα 1/2 genes. Donepezil decreased viability of both cell lines in dose dependent maner. When applied in its IC50 concentration donepezil triggered oxidative stress which has led to the caspase activation and the increased number of double-positive Ann/PI cells indicating the induction of apoptosis. In addition, donepezil induced autophagy since it increased the presence of the acid cytoplasmic vesicles (quantified as an increase of the orange-FL3/FL1 fluorescence, compared to the control). However, the production of ROS was decreased in AMPK and LC3II knockout cells, pointing out that oxidative stress triggers autophagy in U251 cell line. Based on the given results, it could be concluded that donepezil exerts its cytotoxic effect by inducing oxidative stress that causes both, apoptotic and autophagic cell death of C6 and U251 cells.

P-03.1-27

**T3 thyroid hormone regulates the actin cytoskeleton dynamics during hypoxia through avb3 integrin in differentiated PC-12 cell.**

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Integrins mediate a lot of cellular responses to extracellular signals. Hypoxia induces the F actin accumulation and actin filament rigidness, that initiate the neurodegeneration and reduce the plasticity during hypoxia. It was hypothesized that thyroid hormones via avb3 integrin maintain the actin filament rearrangement ability during hypoxia and increase the viability of neuronal cells. We investigated the action of 3,5,3'-triiodo-L-thyronine (T3) and L-thyroxine (T4), and anti-avb3-integrin antibody (for integrin inhibition) on the actin cytoskeleton dynamics and analysed the possible integrin-mediated downstream signalling pathway under hypoxic conditions in differentiated pheochromocytoma (PC-12) cells. It was found that T3 deprivation alters the G/F actin ratio reducing the actin filament rearrangement ability during hypoxia. Whereas the presence of thyroid hormones maintains the dynamic ability of actin filament inducing the partial activation of Fyn through avb3-integrin that and alters G/F actin ratio via Rac1/NADPHoxidase/cofilin-1 pathway and survive the neuronal cells during hypoxia. We propose that presence of T3 during hypoxia maintains the neuronal actin filament dynamics via avb3 integrin and this way increases the viability of the cells during hypoxia and elevates the recovery ability of cells after hypoxia. Keywords: Integrin; cofilin-1; actin filament; Fyn; hypoxia; PC-12. Significance of the Study. To our knowledge, these observations may be important for understanding the effects of thyroid hormone in the hypoxia-induced alterations and avb3 integrin participation in the recovery ability of neurons due to increasing the G actin pools, alteration in G/F ratio and actin filament dynamics during hypoxia. Acknowledgments: This research is supported by the Shota Rustaveli National Science Foundation of Georgia SRNSF Georgia grant #: PHDF-19-751. The author would like to thank the World Federation of Scientist.

## Receptor–ligand interactions

P-03.2-02

**Characterization of short neuropeptide F (sNPF) and its specific receptors in the hard tick Ixodes ricinus**

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Neuropeptides are by far the largest and most diverse group of signalling molecules in multicellular organisms. They are acting as neurotransmitters, neuromodulators and are involved in the regulation of a diverse array of biological processes including reproduction, growth, circadian clock, ecdysis, feeding activity and metabolism. However, we have an insufficient information about their identity, function, and expression level in pathogen-carrying vectors like the hard ticks Ixodidae. In this study, we focused on sNPF signalling in ***I. ricinus***. The gene coding for sNPF was cloned, and the identified nucleotide and peptide sequences used for development of the specific hybridization probe and peptide-specific antiserum. In situ hybridization and fluorescent immunohistochemistry disclosed cells producing sNPF in the central nerve system and in peripheral tissues like gut, and salivary glands. Homology-based search for sNPF receptors revealed two putative G protein-coupled receptors (sNPFR1 and sNPFR2) in the ***I. ricinus*** genome. Ligand binding assay showed that both receptors interacted specifically with sNPF. High expression levels of sNPFR1 and sNPFR2 were observed at the end of blood feeding in salivary glands and gut using quantitative PCR. All these findings suggest that sNPF and its receptors control feeding and digestion of blood in ***I. ricinus***.

P-03.2-03

**Adamantane based derivatives as reversible inhibitors of human AChE and BChE**

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Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two related enzymes responsible to control the level of neurotransmitters. Acetylcholine (ACh) is removed from cholinergic synapse by enzymatic hydrolysis with AChE, which leads to termination of impulse transmission. Both, AChE and BChE, are identified as targets in the treatment of neurodegenerative disorders, such as Alzheimer's disease (AD). The most used approach in finding new drugs for the treatment of AD is the inhibition of AChE. We synthesized a series of 4-aminoquinoline derivatives of adamantane with different linkers between two subunits, based on the highly active and selective cholinesterase inhibitor with adamantane.[1] Compounds were tested as inhibitors of human AChE and BChE, with acetylthiocholine (ATCh) as the substrate for enzyme activity measurements. AChE and BChE activity toward ATCh decreased in the presence of all tested compounds. Inhibition potency of tested compounds expressed as enzyme-inhibitor complex dissociation constant ($K_i$), ranged from 0.1 to 4.2 μM for AChE and from 0.1 to 7.8 μM for BChE. Changing the length of the linker, structural isomerization and their conformation freedom, we influenced the inhibition potency of compounds, as well as their BChE / AChE selectivity. Increasing of steric demand of molecule resulted in a decrease of inhibitory activity. The compound with ***n***-octyl alkyl chain between quinoline and adamantyl fragments has the lowest $K_i$ values which are approximately 8 and 39 times lower for AChE and BChE respectively than previously tested derivative.[1] Docking simulations showed that adamantyl fragment achieved additional interactions with amino acids residues indicating that adamantane scaffold is important for inhibition of cholinesterase activity, aside from aminoquinoline pharmacophore. [1]Previously published in: Bosak A et al. (2019) Chem -Biol Interact 308, 101-109.

P-03.2-04

**Novel fluorescent BODIPY probe for photoaffinity labeling**

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Photoaffinity labeling (PAL) has become a popular tool in proteomics and drug discovery[1]. PAL probes act via UV-triggered free radicals generation followed by covalent bonding with a target molecule. Benzophenone (BP) derivatives are among such probes structures[2]. Often PAL probes include a reporter tag allowing better detection of labeled molecules, e.g. using fluorescence. Today boron-dipyrromethenes (BODIPYs) have become widely used as fluorophores due to their photostability and quantum yields. Both BODIPY and phenyl groups have planar cyclic conjugated structure, so BODIPYphenylketones (BPKs) can be considered as potential fluorescent analogs of BP-based PAL probes. However, there is only one research where BPK photoactivity has been mentioned[3]. Thus, we have synthesized a "minimalistic" BPK (1) by acylation of 8-methylBODIPY. Such acylation of a BODIPY been constructed on unsubstituted pyrroles hasn't been described yet. The structure is characterized by NMR, ESI-MS, UV-vis spectrometry and fluorimetry. From quantum-mechanical calculations (PBE, ma-def2-SVP) the HOMO/LUMO gap for 1 (1.78 eV) is smaller than for BP (2.88 eV) assuming that photoactivation of ***1*** can be achieved by less energetic light than BP. The compound was found to give an adduct with cytochrome P450 CYP7B1 after 365 nm-UV exposition according to monitoring of the fluorescent band with the parent protein mobility on SDS-PAGE. Molecular docking demonstrated affine binding of 1 with a set of human P450s (free binding energies up to -15,1 kcal/mol). Compound 1 is a potentially new photoactive fluorescent ligand. Thus, 1 and other BPKs can be developed as facile and powerful proteomic tools to reveal new structure-function features of biomolecules. Grants BRFFI X19PM-062 / X19PM-062-1 – RFFI 19-54-04009 Bel_mol_a. References: 1. Yip MSG et al. (2013) Nat Chem Biol 9, 715-720. 2. Smith E et al. (2015) Future Med Chem 7, 159-183. 3. Murale DP et al. (2015) Chem Commun 51, 6643-6646.

## Membranes

P-03.3-02

**Characterization of the binding of Rabphilin-3A to membranes.**

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Rabphilin 3A is a membrane traffic protein that contains a tandem C2AB-domain located in its C-terminal that is responsible for the Ca2+-dependent phospholipid binding and mediates interactions with regulatory proteins like SNAP25, CASK, Anexin A4 and Miosin V. We report here functional analyses to characterize the molecular determinants of the Rabphilin3A interaction with membranes. By using Isothermal Titration Calorimetry we have determined the affinities and thermodynamic properties of these interactions and the results indicate that the C2AB domain binds preferentially to membranes containing PIP2 and phosphatidylserine in the presence of Ca2+. This is an exothermic reaction driven mainly by enthalpy changes. Dynamic Light Scattering assays have demonstrated that the main aggregation capacity resides in the C2B domain. Site-directed mutagenesis of key residues located at the different interacting surfaces of the C2AB domain shows that each one plays differential roles in the tandem. These is due to a collection of conserved key functional residues, but at the same time each one possess differential amino acids that confer them special abilities to interact with the membrane and with other proteins. These findings provide functional explanation about how these domains are regulated by a dual-target mechanism and reveal how this family of proteins can employ subtle structural changes to modulate their sensitivity and specificity to various celular signals.

P-03.3-18

**Biophysical and biochemical studies on mitochondrial potassium channels using polymer nanodiscs**

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Several potassium channel proteins are located in the inner mitochondrial membrane. They play role in the regulation of mitochondrial respiration, synthesis of reactive oxygen species, and cell death. One of them is the ATP-regulated potassium channel (mitoK$_{ATP}$), which seems to be comprised of the ROMK2 protein. Another channel is the large-conductance calcium-activated potassium channel (mitoBK$_{Ca}$). To investigate these channels we used a novel approach, in which membrane proteins are extracted by copolymers of styrene-maleic acid (SMA). Commonly, detergents are used for this purpose. However, in detergents proteins often lose activity and their complexes are unstable. This can happen, because necessary for protein function lipids have been stripped away. SMA polymers produce membrane protein-containing particles (SMALPs) without the need for the use of detergents. Therefore, proteins isolated by this method maintain their native lipid and protein environment exhibiting higher stability and activity. Using SMA we carried out isolation of human ROMK2 protein expressed in ***Escherichia coli*** cells. The channel electric activity, similar to activities of mitoK$_{ATP}$ described previously, was observed after fusion of ROMK2-SMALPs with planar lipid bilayers. The impact of known mitoKATP channel modulators, such as ATP/$Mg^{2+}$, VU591 (ROMK channel blocker), and diazoxide (mitoK$_{ATP}$ opener) on the observed activity was investigated. In addition, we examined the impact of other potential modulators. Previously, it was shown that polymer nanodiscs can be used for the isolation and stabilization of protein complexes. I found that it is possible to solubilize by SMA potassium channels as large SMALPs of megadalton size with mitochondrial proteins. The isolation of these complexes allows for their more detailed biochemical characteristics.

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## Molecular machines

P-04.2-01

**Role of C-terminal domains of yeast FACT complex in nucleosome unfolding**

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FACT (facilitates chromatin transcription) is a histone chaperone that is involved in the processes of transcription initiation and elongation, DNA replication and repair [1]. Yeast FACT conducts large-scale ATP-independent nucleosome unfolding [2]; but the detailed mechanism of this process is unknown. Here we determined the role of Nhp6 protein and C-terminal domains of yeast FACT complex in nucleosome unfolding using single-particle Förster resonance energy transfer (spFRET) microscopy, EMSA and FRET-in-gel methods. Nhp6 protein interacts with the C-terminal domains of Spt16 and Pob3 subunits of yFACT, inducing unfolding of FACT in the absence of nucleosomes. Analysis of yeast FACT interaction with nucleosomes and hexasomes suggests that Spt16 subunit of FACT drives nucleosome unfolding, while Pob3 subunit is required for complete unfolding. Thus, FACT-dependent nucleosome unfolding is a tightly coordinated process that requires Nhp6 protein and C-terminal domains of Spt16 and Pob3 subunits of yFACT. Our data suggest a detailed model of FACT-dependent nucleosome unfolding. This work was supported by the Russian Science Foundation grant 19-74-30003. Previously published in: [1] K Gurova et al (2018) BBA – Gene regulatory Mechanism 1861, p. 892-904. [2] ME Valieva et al (2016) Nat Struct Mol Biol 23, p. 1111-1116.

## Imaging for life: From molecules to organisms

P-04.3-01

**Fluorescein-labeled derivative for study of cytotoxicity mechanism of iron (II) clathrochelates.**

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We synthesized iron (II) clatrochelate complex labeled with fluorescent dye, characterized them in vitro and tested their subcellular localization in cancer cells. Clatrochelates are complex compounds with central metal ion incapsulated in tridimensional macrobicyclic organic ligand. Such complexes could be easily functionalized – their eight positions could be modified with different substituents, which opens a large potential variety of biological activity depending on the nature and geometry of those substituents. Recently, the high toxicity of functionalized iron (II) clatrochelate complexes (IC50 about 1 mkM) against cancer cells (line HL-60) was discovered. However, the mechanism of their activity is still unknown. To get hint about their mode of action we studied accumulation and redistribution of clatrochelates in cancer cells. For that purpose we synthesized a labeled with fluorescein clatrochelate complex. Due to low stability of clatrochelates in alkaline environment, during the synthesis we used enzymatic catalysis for the deprotection of acetyl group of the acetylated fluorescein. CD spectroscopy showed that labelled clatrochelate binds with serum albumins, similarly to unlabeled model complex. Fluorescence polarization studies also confirmed binding of labeled clathrochelate to albumin. Despite of clatrochelate ability to quench fluorescence, due to high quantum yields of fluorescein such complexes possess sufficient emission intensity allowing its visualization in cells. Study on human ovarian cancer A2780 cell line has shown that the clathrochelate is able to penetrate through cell membrane, it doesn't enter into the nucleus and accumulates in cytoplasm. No accumulation in any intracellular organelle was apparent. Now we continue structure optimization of fluorescently labeled clathrochelate for its use in the thorough study of the mechanism of cytotoxicity of these compounds. This work was supported by the grant H2020-MSCA-RISE 778245.

## Structures of nucleic acids

P-04.4-01

**PARP1, PARP2 and PARP3 interaction with nucleosomes containing DNA-lesions**

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The regulation of DNA repair is one of the most studied topics in molecular biology, not least because of the possibility of using these data for the development of anti-cancer agents. Poly(ADP-ribose)polymerase1 (PARP1) is an enzyme that involved in regulation DNA repair. It has been shown to be prospective target for anticancer monotherapy and anti-inflammatory therapy. However, cytotoxicity of current drugs does not allow to widespread using. This problem requires not only the developing of novel inhibitors, but also fundamental studies of PARP1. To this end we have developed a test-system, which allows analyzing of PARP1 activity in real time [1]. This approach is based on the detection of fluorescence anisotropy of nucleic acid-protein complexes. The fluorescein-labeled DNA duplex (31b.p.) is used as a PARP1 cofactor. The PARP1-DNA complex formation leads to increase of anisotropy due to decreasing of FAM rotation. Under PARylation conditions the PARP-DNA complex dissociates leading to decrease of the anisotropy level. In current work we applied this method using nucleosome instead of short naked DNA, which is more similar to ***in vivo*** conditions. In this system the anisotropy is increased when PARP proteins bind to nucleosome in close proximity of FAM-labelled DNA-ends. We reconstituted nucleosomes with AP-site or gap in two positions with different proteins accessibility. We have shown the applicability of our method to detect PARP1, PARP2 and PARP3 interaction with nucleosome. Shielding of lesions in nucleosome particle leads to decrease of their recognition and binding capacity by PARPs. The reported study was funded by RFBR, project number 20-34-90095 and by RSF 17-74-20075. 1. Kurgina T.A., Anarbaev R.O., Sukhanova M.V., Lavrik O.I. A rapid fluorescent method for the real-time measurement of poly(ADP-ribose) polymerase 1 activity. Anal.Biochem. 2018.

## Bionanotechnology

P-05.2-01

**Engineering fluorescent probiotic bacteria against vaginal infections**

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Vaginal infections are among the most frequent health problems in women and can be caused by several microorganisms, including bacteria, yeasts and parasites. One of the efficient ways to treat vaginal infections is with probiotic bacteria. Probiotics are live microorganisms that can confer health benefits to the host when administered in adequate amounts. Their antimicrobial properties are related to the production of hydrogen peroxide, lactic acid and bacteriocins. In order to better understand the mechanisms of action of probiotics it is important to track them inside the host, and one of the ways to achieve this is through imaging the bacteria with fluorescent proteins. We have successfully transformed the most important vaginal species ***Lactobacillus gasseri***[1], ***Lactobacillus plantarum***[2] and ***Lactobacillus crispatus***[3] with three different plasmids that enable expression of fluorescent proteins with different spectral properties (IRFP, GFP and mCherry). The transformation into the bacteria was performed by electroporation using specific cell wall weakening agents. The genes for the fluorescent proteins were cloned under the control of the strong LDH promoter by direct fusion of the promoter with fluorescent genes using overlap extension PCR. The purpose of the engineered lactobacilli expressing different fluorescent proteins is to allow observation of their cell distribution. This will allow tracking of the lactobacilli ***in vivo*** and learning more about their effects and characteristics. Furthermore, to enhance the viability of lactobacilli and to protect them from the environment, we plan to incorporate them into electrospun synthetic polymer nanofibers. Thereby, the nanofibers would serve as an efficient intravaginal delivery system for probiotic lactobacilli.

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P-05.2-04

**Nanoparticle-mediated delivery of Cpf1 for the generation of improved gene editing tools**

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The CRISPR/Cas technology allows for the efficient manipulation of DNA sequences. It consists of a Cas nuclease in complex with a guide RNA complementary to a target DNA. Following guide recognition, the nuclease generates a double strand break on the DNA that can be repaired through non-homologous end joining and homology directed repair, less efficient but required for precise editing. Although to date, research has mainly focused on Cas9, its analogous, Cpf1 (or Cas12a), is receiving increasing attention due to its higher specificity[1]. The correction of disease-causing mutations through gene editing has a great therapeutic potential, however the efficient delivery of CRISPR molecules remains a major challenge[2]. This project aims to design CRISPR nanostructures able to overcome current delivery issues that can be used for the efficient in vivo editing of oncogenic mutations. These nanostructures consist of Cpf1 nuclease conjugated with biocompatible nanoparticles (NPs) including magnetic and albumin NPs and albumin-coated gold nanoclusters. Cpf1-NP binding was carried out through two main strategies: electrostatic interaction and covalent conjugation. For the latter, the NPs were previously modified with a smart linker for the controlled intracellular release of Cpf1. All the NPs tested showed Cpf1-binding capacity. Additionally, HeLa cells were shown to internalize the conjugates. Moreover, Cpf1 was engineered for improved delivery. One of the main obstacles to nanoparticle-mediated cell delivery is endosomal entrapment. Thus, we produced Cpf1 variants fused to peptides known to enhance endosomal escape. Cpf1 activity remained intact upon modification as shown by in vitro tests. [1] A.C. Komor et al. CRISPR-based technologies for the manipulation of eukaryotic genomes, Cell (2017) [2] C. Liu et al. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications, J. Control. Release (2017)

P-05.2-07

**Delivery and targeting of fibrinolytic agents to blood clots**

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Cardiovascular disease (CVD) accounts for nearly one-third of deaths worldwide. There are increasing evidences for a consistent association between denser fibrin clot structure, more resistant to degradation (fibrinolysis), with CVD and (athero)thrombotic disorders. Fibrin polymerization starts by thrombin-mediated cleavage of fibrinopeptides A and B from the N-terminal of Aα- and Bβ-chains of fibrinogen, respectively. This exposes small residue sequences, knobs A and B, that interact with their respective binding pockets on the C-terminal region of the Aα- and γ-chains of another fibrinogen molecule, leading to the formation of fibrin fibres. Liposome nanoparticles have drawn interest as pharmaceutical nanocarriers, due to their stability and content release in a controlled manner. The aim of the work is to develop an encapsulated fibrinolytic nanoparticle strategy with lower bleeding risk, to be incorporated in the clot structure. We studied the impact of the empty liposome nanoparticle on blood clot formation and lysis and demonstrated that it does not affect haemostasis properties, by recording clot polymerization and lysis kinetics. Using dynamic light scattering and zeta potential assays, we concluded that the nanoparticle is stable over time, without any measurable aggregation or change in its surface charge for 28 days. Turbidimetry studies showed that the presence of the nanoparticles was associated to a non-significant small increase in fibrin fiber radius, protofibril packing and protein content with increasing lipid concentrations. The fibrinolytic agent tissue plasminogen activator (tPA) was added as liposome cargo, achieving 60-80% encapsulation efficiency. Preliminary results demonstrated a controlled release of tPA in a solid emulation of a clot, without activity loss. The work is now focused on optimizing the nanocarrier by surface decoration with a targeting element toward fibrin clots.

P-05.2-08

**The efficacy of designed anti-measles virus peptides depends on the stability of self-assembled clusters.**

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The resurgence of several infectious diseases, like measles, has driven the search for new chemotherapeutics to prevent and treat viral infections. Self-assembling antiviral peptides are a promising class of entry inhibitors capable of meeting this need. Fusion inhibitory peptides derived from the heptad repeat of the C-terminal (HRC) of the measles fusion protein, dimerized and conjugated with lipophilic groups, were found to be efficacious against measles virus. The structures of the self-assembled nanoparticles formed by these peptides modulated their activity. Based on the analysis of a L454W mutation in the fusion protein of a naturally occurring measles viral isolate, HRC peptides bearing the tryptophan residue at position 454 (HRC-L454W) were synthesized with the goal of improving membrane anchoring and manipulating self-assembly. Monomeric and dimeric peptides, whether conjugated or not to a single lipophilic group, reduced infection in vivo. Bis-conjugation with lipophilic groups, in contrast, abrogated activity. Based on the physicochemical properties of self-assembly and membrane insertion kinetics of the HRC-L454W peptides we show that bis-conjugation increases the stability and order of the inner core of the spontaneously self-assembled nanoparticles, resulting in their compaction. The presence of the tryptophan residue also increases steric hindrance effects in the nanoparticle of the dimeric peptides, contributing to inter-peptide cluster meshing, but the same level of compaction is not achieved. We propose that the highly ordered packing and stability of molecular clusters forming the inner core of self-assembled nanoparticles prevent efficient dissociation of the peptides in vivo, hindering their release and therefore eliminating their antiviral efficacy.

## Designed regulatory circuits and genome editing

P-05.3-01

**Ultrafast Circulating Breast Tumor DNA Detection in Blood by CRISPR/dCas9 Biosensor**

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Extracellular nucleic acids, a biomarker evaluated in the context of liquid biopsy, are small nucleic acids that are not associated with the cell or cell fragments. The increase in the latter, called the circulating tumor DNA (ctDNA), can be used for diagnostic purposes in blood. Till recently, extracellular DNA was analysed with quantitative real-time polymerase chain reaction (PCR)-based, fluorescent-based or spectrophotometry-based approaches. We aimed to develop an electrochemical biosensor based on a very specific and innovative measurement approach that targets the ctDNA sequence in plasma. Biosensors are a combination of a biorecognition receptor and a physicochemical transducer. The hallmark in our study is the use of a very specific biorecognition receptor, CRISPR/dCas9 complex, that specifically binds to the mutated sequence in the ctDNA carrying genetic mutations. To develop the biosensor, gold electrodes were modified by Cysteamine (Cys) to form a self-assembly monolayer. Then, PAMAM-G5 dendrimers were covalently immobilized on Cys layer via glutaraldehyde. The next step is the immobilization of dCas9 on AuE-Cys-PAMAM modified electrode via glutaraldehyde followed by the immobilization of sgRNA on the dCas9 layer. All modifications were characterized by electrochemical impedance spectroscopy (EIS). EIS parameters were set as 10 000-0.05Hz, 10 mV AC and 180 mV DC current. Afterwards, a calibration curve was prepared between 10 to 640 pM (R2=0.9811). LOD and LOQ were calculated as 8.19 pM and 24.8 pM, respectively. The newly developed biosensor with the CRISPR/dCas9 complex based biorecognition receptor can determine ctDNA in 25 seconds successfully. With its highly selective, practical and cost-effective characteristics, this biosensor is a promising tool for the determination of ctDNA in cancer patients.

P-05.3-02

**The role of Tyrosyl-DNA-phosphodiesterases in the repair of DNA-protein crosslinks in vivo**

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DNA protein crosslink (DPC) is a type of DNA lesion which occurs when protein becomes irreversibly covalently bound to DNA. If not repaired, DPCs interfere with all DNA transactions, thus causing genomic instability which can lead to cancer, accelerated aging and neurodegeneration. The orchestration of DPC repair pathway is still unknown, especially ***in vivo***. Recently, it has been shown that metalloproteases Wss1 in yeast and SPRTN in mammals play central role in the repair of DPCs. These proteases cleave crosslinked proteins, thus leaving protein remnants of unknown size in the DNA backbone. While proteases act ubiquitously, cleaving wide variety of general DPCs, different set of proteins are involved in the repair of enzymatic DPC. Enzymatic DPCs are formed when proteins which are reversibly bound to DNA in order to preform their function become crosslinked due to endogenous and/or exogenous inducers. Tyrosyl phosphodiesterase 1 and 2 (TDP1 and 2) play crucial role in the removal of these enzymatic DPCs. TDP1 has been shown to remove protein remnant of TOPO1cc by separating DPC from DNA backbone through esterase activity, most probably after SPRTN mediated proteolytic cleavage of TOPO1 crosslinked to DNA. TDP2 can act (a) downstream of SPRTN mediated proteolysis of TOPO2 DPC or (b) together with ZATT protein to remove TOP2 DPC independent of SPRTN proteolysis. We aim to show the function of TDPs in DPC removal ***in vivo*** using the zebrafish model using CRISPR/Cas9 mediated mutagenesis of the TDPs active site via knock-in technology and fluorescent reporter. We have identified zebrafish TDPs and compared them to human orthologs in regard to phylogenetics, synteny and mRNA and protein expression, while functional studies are on the way. Our study will reveal actual contribution of TDP1 and 2 in the DPC repair pathway at the organismal level.

## Plant biotechnology

P-05.4-02

**Stress specific regulation of a monocot chromatin factor under inducible and constitutive promoters in Arabidopsis**

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Chromatin dynamics is one of the key factors funneling cellular regulation during stress in plants. Manipulations in an abiotic-stress responsive chromatin factor is thus promising to reach sustainable plant production levels. Yet, its modulation might differ under different functional promoters while plants processing environmental cues. Here we characterized the metabolic changes of ABA inducible (pAsr11875) and constitutive (CaMV p35S) promoters in drought and salt stressed Arabidopsis transgenics over-expressing an actin depolymerizing factor (ADF) of a Louisiana native monocot halophyhte Spartina alterniflora ADF2. Transgenic lines bearing both p35s and pAsr1 which is carrying a combination of cis-regulatory elements showed improved physiological and phenotypic response under salt and drought stress attributed to healthy siliques with robust shoot systems, better germination capacity, water homeostasis and membrane stability. The growth of the transgenics expressing the gene under pAsr1 was better compared to p35S under non-stressed control conditions affirming the idea that using inducible promoters maximize the benefits of transgenes and avoid responses that may adversely affect the plant performance under specific stresses. Since dicot halophytes like Arabidopsis wild relative T. halophila differs extensively than monocots due to the different morpho-physiological features, elucidation of stress adaptations by manipulating transcription factor expressions using wild relatives of monocot crops such as rice is also advantageous to transfer stress inducible promotor knowledge to other cereal species.

## New frontiers in medicinal chemistry

P-06.2-01

**DSC of blood plasma: A complementary technique for following efficacy of disease treatment**

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Clinicians exploit the human plasma proteome routinely as an indicator of patient health and as a source for biomarkers of human disease. A number of FDA-approved plasma /serum diagnostic assays are routinely used. These include serum plasma electrophoresis and a variety of immunochemical assays that can monitor the concentrations of specific proteins in plasma. Recently, it was suggested that differential scanning calorimetry (DSC) could be used as a new disease-monitoring application. DSC is a quick and simple technique, which does not require a complicated sample preparation. Surveys already proved that DSC can distinguish between plasma from healthy person and sick person. Not only that the thermograms of blood plasma obtained from sick individuals are different from the thermograms of blood plasma obtained from healthy individuals but also several types of diseases display a characteristic thermogram. Our goal was to evaluate potential correlation between changes in DSC profile of blood plasma and treatment progress of patients with multiple myeloma (MM). For each included patient we performed DSC analysis of blood plasma for several patients that were diagnosed with MM for the first time or had a relapse. Alongside DSC analysis, we obtained the information on heavy chain class (IgG, IgE, IgA, IgD, and IgM) and the light chain proteins together with major diagnostic and prognostic markers (b2 microglobulin, lactat dehydrogenase, serum albumine). During and after the intensive treatment of patients, we analyzed blood plasma in regular time intervals (every 2 or 3 days) and evaluated correlation between DSC thermogram profile changes and monoclonal protein/clonal free light chain concentration. The collected data suggests that changes in DSC thermograms are in correlation with changes in biochemical tests (usually the main marker is M spike – heavy chain class). We will discuss these results in more detail.

P-06.2-02

**Effects of cold atmospheric plasma on cancer and normal cells in vitro**

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Cold atmospheric plasma (CAP) has been shown as potential anticancer tool. CAP is an ionized gas consisting of charged particles, active uncharged particles, an electric field and UV radiation. Here, we analyzed the cell death mechanism after CAP treatment using various cell lines. The biological effect of plasma-activated cultural medium on the epidermoid carcinoma cells A431, and normal human embryonic kidney cells HEK-293T has been investigated. The medium was exposed to CAP irradiation generated in argon gas for 2–8 min at a voltage of 4.9 kV and then added to the cells. The proliferation of the treated cells in real time mode was measured using iCELLigence RTCA. The rate of apoptotic and necrotic cells were analyzed by the flow cytometry. The levels of intracellular reactive oxygen (ROS) and nitrogen (RNS) species, which are known to be the main CAP affectors, were measured with 2',7'–dichlorofluorescin diacetate fluorescent dye. To revel the conditions when the selectivity of CAP against tumor cells can be achieved we used a pair of lung cell lines: adenocarcinoma cells A549 and normal fibroblasts Wi-38. The viability of A549 and Wi-38 cells after direct CAP treatment were examined. Under optimized CAP conditions (duration 60 c, voltage amplitude 4.2 kV, 3 L/min in helium) Wi-38 stayed alive and A549 cancer cells were killed. The expression profiles of treated cells were evaluated by using RNA-seq. Functional analyses were employed to reveal the difference in normal and cancer cell response. The data obtained could be a basis for the development of selective CAP treatment of cancer cells. This study was supported by the RSF grant # 19-19-00255 and by the RFBR grant # 20-34-90021.

P-06.2-03

**Anticancer peptide RL2 – a promising tool for intracellular delivery of therapeutics and diagnostic molecules**

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Understanding of mechanisms by which biologically active compounds penetrate into cells is necessary for development of antitumor drugs and overcoming multidrug resistance. RL2 is a fragment of human kappa-casein, which induces apoptosis of cancer cells and suppresses tumor growth in vivo. It efficiently penetrates into cancer and normal cells, but it has no cytotoxic effect towards the latter. However, the mechanism of RL2 penetration into cells remained unclear. We have shown that RL2 efficiently enters into cells partly through lipid raft-mediated pinocytosis and partly by direct penetration through the plasma membrane which described for Cell-penetrating Peptides (CPPs) – peptides are known for their ability to effective deliver cargo molecules into the cells. The study of RL2 structure by circular dichroism and NMR spectroscopy showed that RL2 is an intrinsically disordered peptide, capable of partial folding of 31-40 a.a. fragment in hydrophobic environment. Such folding is also usual for CPPs, it is believed to allow them to penetrate cell membranes. Thus, RL2 structure and cell interaction characteristics allow to classify this peptide as CPP. So far as RL2 is CPP, it was interesting to investigate its ability to form noncovalent complexes with nucleic acids and deliver these molecules into human cells. We found that RL2 is capable of delivering different nucleic acids (plasmid DNA expressing the green fluorescent protein EGFP, siRNA against EGFP and cytotoxic small nucleolar RNA) as non-covalent complexes as well as covalently bound paramagnetic label into human cells. So, anticancer peptide RL2 is a promising tool for intracellular delivery of therapeutics and diagnostic molecules.

P-06.2-05

**New synthesized ferrocenyl trizole nucleobase derivatives induced cell death by mitochondria- mediated apoptosis in tumor cells in vitro**

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Taking into consideration the biological importance of ferrocene and nucleoside analogues, the synthesis of twenty mono- and bis-ferrocene conjugated nucleobases that are bridged by triazole linker was undertaken with the aim to evaluate their antioxidant potential and activity against various tumor cell lines. Radical scavenging capacity, measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and expressed as a % of DPPH reduction after 30 and 60 min, was between 62 and 90 %. Antiproliferative capacities of ferrocene derivatives were tested by MTT test on adenocarcinoma, leukemia, lymphoma, and normal fibroblast like cells. According calculated IC50 values, selectivity index, and no cytostatic effect on normal Madin-Darby canine kidney fibroblast like cells (MDCK1), four derivatives were selected for further testing on effects on intracellular ROS accumulation, mitochondrial membrane potential dysfunction, as well as apoptosis and autophagy induction in treated tumor cells. Slight changes in ROS intracellular accumulation and activated autophagy were observed. Tested new synthesised derivatives induced disruption in mitochondrial membrane potential (ΔΨm) and mitochondrial dysfunction, resulting in mitochondria swelling accompanied by activation of apoptosis in treated cells. Early apoptosis induction was determined in colon adenocarcinoma (CaCo-2), Burkitt lymphoma (Raji) and acute lymphoblastic leukemia (CCRF-CEM) cells after treatment with selected derivatives. In conclusion, obtained results contribute to better understanding of antiproliferative activity of new ferrocenyl triazole nucleobase derivatives. Key words: bis-ferrocene conjugated nucleobases, antiproliferative activity, apoptosis, mitochondrial potential disruption, ROS accumulation

P-06.2-45

**Anti-HIV-1 activity and mode of action of a viral-derived and proteolysis-resistant peptide CXCR4 antagonist**

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We report the anti-HIV-1 peptide pepRF1, a human serum-resistant peptide derived from the Dengue virus capsid protein. In vitro, pepRF1 shows a 50% inhibitory concentration ($IC_{50}$) of 1.5 nM with a potential therapeutic window higher than 53,000. This peptide is specific for CXCR4-tropic HIV-1 strains, preventing viral entry into target cells by binding to the viral co-receptor CXCR4. Upon binding, neither internalization nor intracellular $Ca^{2+}$ influx are triggered, showing that pepRF1 is an antagonist of this chemokine receptor. pepRF1 is more effective than T20, the only peptide-based HIV-1 entry inhibitor approved by FDA for clinical use, and excels in inhibiting an HIV-1 strain resistant to T20 (HIV-1NL4.3 DIM) with an IC50 of 2.8 nM. Overall, our study led to the discovery of a peptide highly active against HIV-1, serum-stable, and with low toxicity, that acts as a CXCR4 antagonist. Potentially, pepRF1 can be used alone or in combination with other anti-HIV drugs to fight AIDS. Furthermore, one can also envisage its use as a novel therapeutic strategy for other CXCR4-related diseases.

## Pharmacogenomics and biomarkers

P-06.3-01

**Potential platelet's miRNA biomarkers of Acute Coronary Syndromes**

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MicroRNA microarray technology provides a high-throughput tool for analysis of thousands of miRNAs in a single experiment, allowing to identify an altered expression and distinguish signatures associated with diseases. Modifications of gene expression are a frequent phenomenon marking the existing health disorder. The discovery of those changes helps in early diagnosis and prognosis of patients and allows to understand the pathophysiological mechanisms of disease. Due to high resistance to harmful conditions, stability in physiological states, and easy, non-invasive collection method of miRNA, they are considered as ideal potential biomarkers. The general aim of our work was to find the miRNA signatures that could help to define human predisposition to the Acute Coronary Syndromes (ACS). For this purpose we performed for the first time a screening and comparative analysis of platelet miRNA transcriptome from patients with diagnosed ACS in comparison to healthy donors. Data obtained from microarrays showed altered expression of 172 platelet miRNAs which are associated with more than 2,000 mRNA molecules. In the next stage we performed selection of miRNA's based on their significant function in the molecular pathways of platelet activation. Altered expression ($p < 0.05$) profile of selected miRNA's: miR-21-5p, miR-222-3p, miR-98-5p and miR-4306 can influence on translation process of mRNAs that encode the most abundant glycoprotein on the platelet surface – GPIIb/IIIa (ITGA2 and ITGB3), whereas miR-103-a-3p, miR-107, miR-19a-3p, miR-19b-3p, miR-15a, miR-16-5p, miR-195-5p, miR-497-5p, miR-424-5p and miR-15b-5p are associated with ITPR1, PIK3R1, PRKG1 and PLCB1, which are linked with intracellular signalling of platelet activation. Our results provide a new insight in the molecular mechanism of intracoronary thrombus formation and indicate the direction for our further studies whose primary goal is to look for a molecular markers of human predisposition to ACS.

## Stem cells and regenerative medicine

P-06.4-02

**Studying the dynamics of the development of the therapeutic effect of initial and preconditioned MSCs on a model of acute abdominal inflammation of mice**

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The aim of this research was to compare the therapeutic effects of initial and preconditioned MSCs from human umbilical cord based on a model of acute inflammation in mice. We studied the dynamics of sterile inflammation in mice after intraperitoneal injection of a 3% solution of proteose peptone. The experiment showed that 20 min. after the introduction of peptone, the number of peritoneal macrophages increased compared with the control and after 24 hours the number of macrophages significantly increased compared with the control. Comparison of the effectiveness of transplantation of different doses of MSCs (from 5 $x10^3$ cells / mouse to 100 $x10^3$ cells / mouse) showed that the injection of MSCs 20 min. after proteose peptone leads to almost complete disappearance of inflammatory signs in mice. When transplanting high-doses of initial MSCs (25 - 100 $x10^3$ cells/mouse) 24 hours after the injection of peptone that is in conditions of acute inflammation, the inflammatory signs almost completely disappeared within 1 hour after injection of MSCs. The transplantation of low doses of MSCs (5 - 10 $x10^3$ cells /mouse) led to a 2-fold decrease in the number of peritoneal macrophages compared to proteose peptone. High doses of MSCs reduced the phagocytic activity of peritoneal macrophages almost to control and low doses of MSCs reduced the phagocytic activity by 1.5 times in 30 min after transplantation MSCs. The injection of low doses of MSCs, which don't lead to the complete clearance of inflammation, allows comparing the therapeutic efficacy of initial MSCs and preconditioned MSCs. The expression of the IL-10 gene decreased after the injection of proteose peptone to mice, and increased after transplantation of the initial and preconditioned MSCs. The date showed that the use of low doses of MSCs more clearly demonstrates the difference between MSCs variants preconditioned by various factors. * The authors marked with an asterisk equally contributed to the work.

## Bioinformatics and computational biology

P-06.5-02

**Evaluation of the therapeutic potential of dihydroartemisinin and its dimer as β-amyloid aggregation and β-secretase inhibitors**

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Alzheimer's disease is characterized by the formation of plaques in the brain, which are commonly composed of insoluble forms of amyloid peptides as a result of aspartyl protease β-secretase expression. The interaction of artemisinin derivatives — dihydroartemisinin and its dimer, with the amyloidogenic peptides $5A\beta_{17-42}$, $12A\beta_{9-40}$ and β-secretase was studied in silico. The comparison was made with curcumin, which is in phase II of clinical trials. The presented work shows that the interaction of investigated ligands with all targets is characterized by fairly high binding energy values, with a maximum for dihydroartemisinin dimer. Studied ligands directly interact with amino acids, which are responsible for the formation, growth and stabilization of the peptides. So, dihydroartemisinin can prevent the formation of $5A\beta_{17-42}$, while dihydroartemisinin dimer and curcumin can affect its growth. All ligands can prevent the formation of the $12A\beta_{9-40}$, dihydroartemisinin dimer and curcumin can suspend its stabilization. For all ligands interactions with important amino acids of β-secretase are observed, dihydroartemisinin and curcumin interact with critical amino acids of the catalytic center of β-secretase. In this work, pharmacological characteristics, such as HIA and BBB were analyzed for the studied compounds. They can cross the BBB to one degree or another and have a very high absorbability. Based on our results, it can be concluded that dihydroartemisinin dimer can prevent the formation, growth and stabilization of amyloid peptides, and can modulate β-secretase activity. It interacts with peptides and β-secretase with higher affinity compared to other ligands. Thus, dihydroartemisinin dimer can be considered as a possible candidate for the treatment of Alzheimer's disease.

P-06.5-04

**Molecular mechanism of cationic antiseptics action revealed by coarse-grained molecular dynamics**

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Antiseptics are antimicrobial compounds used in a clinical setting. Positively charged cationic antiseptics bind strongly to the bacterial cell walls and membranes because of their opposite negative charge. The bacterial plasma membrane is supposed to be the main target of antiseptic action. We created molecular dynamics (MD) coarse-grained models of four cationic antiseptics of different classes, including biguanides (chlorhexidine and picloxydine), pyridine derivatives (octenidine) and quaternary amines (miramistin). The model plasma membrane consisted of 180 POPE and 60 POPG molecules. To study the molecular mechanism of action of antiseptics at various concentrations, we designed molecular models of the membrane with antiseptic molecules, with antiseptic to lipid ratio of 1/24 (low concentration), 1/8 (middle concentration) and 1/4 (high concentration). The systems with low and middle concentration were simulated for 3 microseconds and the systems with high concentration – for 30 microseconds. To analyze the interaction of antiseptics with the model membrane, we estimated macroscopic parameters such as area per lipid, density profiles, order parameters and bilayer thickness for all systems. We have shown that octenidine, in comparison with other compounds, had the most pronounced disintegrating effect on the bacterial plasma membrane due to significant change in the lipid area and bilayer thickness. However, in addition to changing macroscopic parameters, one of the effects of antiseptics on the bacterial plasma membrane is its depolarization. This effect was revealed in molecular dynamics calculations with an extra electric potential applied to the membrane in the presence of antiseptics. We demonstrated that antiseptics induce faster membrane pore formation even at low values of electric potential. Acknowledgements: The reported study was funded by RFBR, project number 19-34-90045.

P-06.5-07

**Investigating whole-transcriptomics alterations in early and proliferative stages of brain regeneration**

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While mammals have a very limited capacity to regenerate their brain after an injury, the extensive regeneration capacity of the adult zebrafish brain makes zebrafish a useful model to understand the molecular programs that are necessary for central nervous system regeneration. Although several studies have been performed on zebrafish brain regeneration at the cellular level using histological methods, the way how underlying molecular mechanisms are controlled at the RNA level has not yet been elucidated. In this study, we used molecular biology methods and bioinformatics techniques together to investigate alterations on mRNA molecules in brain regeneration at its very early versus proliferative stages. For this aim, we generated stab lesions in adult zebrafish telencephalon to trigger brain regeneration. Next, we dissected lesioned and unlesioned hemispheres separetely and we performed RNA-Sequencing after isolating their RNAs. DESeq2 package of Bioconductor was used to analyze differentially expressed genes (DEGs). Disease (DO) and gene ontology (GO) enrichment and pathway analyses were performed based on the obtained DEG lists. Also, we performed quantitative PCR to check the expression levels of particular early and mature neuronal marker genes in samples before and after RNA sequencing. According to our results, while cell signaling, regeneration and development related GO terms were enriched in early stage, differentiation and homeostasis related terms were predominantly enriched in the proliferative phase. P53 and MAPK signaling and apoptosis related pathways were enriched in both stages. We believe that these results will greatly extend our knowledge about molecular mechanisms involved in brain regeneration and will pave the way to the development of new therapeutic approaches of traumatic brain injuries or neurodegenerative diseases.

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P-06.5-08

**The catalytic mechanism of the human aldehyde oxidase**

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The human aldehyde oxidase (hAOX) plays a crucial role in the metabolism of diverse compounds, either endogenous or xenobiotics. For that reason, the hAOX interferes in the pharmacokinetics of many drugs what makes it one of the enzymes with highest interest for the scientific and pharmaceutical communities. In our work, we studied the structure and the dynamics of this key metabolic[1] enzyme and unveiled, with atomistic detail, its full catalytic mechanism of oxidation of the ***N***-heterocycle phthalazine to phthalazin-1(2H)-one, along with its associated energy profile. Moreover, the puzzling variations of the oxidation state of the molybdenum ion of hAOX throughout the catalytic cycle were carefully examined. We applied state of the art computational methods, namely a quantum mechanics/molecular mechanics scheme (QM/MM) within the ONIOM methodology. This methodology has been successfully applied to study catalytic mechanisms of many enzymes. The final energies were calculated at the B3LYP-D3/6–311++G(2df,2pd)/SDD:FF99SB//B3LYP/6–31G(d)/SDD:FF99SB. Our results indicate that the Lys893 has a fundamental role for the activity of the enzyme, for the favorable positioning of the substrate within the catalytic site and on the catalytic mechanism as well, as it must donate a proton to the substrate for the catalytic reaction to proceed. We found that the rate limiting step of the mechanism correspond to the transfer of a hydride from the substrate to the molybdenum sulfur ligand with a concomitant reduction of Mo from $Mo^{VI}$ to $Mo^{IV}$. In addition, the unbinding of the products from the molybdenum cofactor seem to be more favorable with the Mo ion in its $Mo^{V}$ state together with FADH•. In short, this work provided insights on the mechanism of oxidation of ***N***-hetorcycles by the hAOX which is a valuable information for the study of the interaction of this enzyme with drugs and for the design of inhibitors. [1] Ferreira, P et al. (2019) Phys. Chem. Chem. Phys., 21 (25), 13545-13554.

P-06.5-12

**In silico screening of flavones and its derivatives as potential inhibitors of quorum-sensing regulator LasR of Pseudomonas aeruginosa**

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Antibiotic resistance is a global problem nowadays and in 2017 the World Health Organization published the list of bacteria for which treatment are urgently needed, where ***Pseudomonas aeruginosa*** is of critical priority. Current therapies lack efficacy because this organism creates biofilms conferring increased resistance to antibiotics and host immune responses. The strategy is to "not kill, but disarm" the pathogen and resistance will be developed slowly. It has been shown that LasI/LasR system is the main component of the quorum sensing system in ***P. aeruginosa.*** LasR is activated by the interaction with its native autoinducer. A lot flavones and their derivatives are used as antibacterial drug compounds. The purpose is to search compounds that will inhibit LasR. This leads to the inhibition of the synthesis of virulence factors thus the bacteria will be vulnerable and not virulent. We performed virtual screening using multiple docking programs for obtaining consensus predictions. The results of virtual screening suggest benzamides which are synthetical derivatives of flavones as potential inhibitors of transcriptional regulator LasR. These are consistent with recently published experimental data, which demonstrate the high antibacterial activity of benzamides. The compounds interact with the ligand binding domain of LasR with higher binding affinity than with DNA binding domain. Among the selected compounds, by conformational analysis, it was found that there are compounds that bind to the same amino acids of ligand binding domain as the native autoinducer. This could indicate the possibility of competitive interaction of these compounds. A number of compounds that bind to other conservative amino acids ligand binding domain have also been discovered, which will be of interest for further study. Selected compounds meet the criteria necessary for their consideration as drugs and can serve as a basis for conducting further ***in vitro / in vivo*** experiments.

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**A transcriptomic-based drug repurposing strategy for the identification of new SMN-independent skeletal muscle treatments for spinal muscular atrophy**

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Spinal muscular atrophy (SMA) is a neuromuscular disorder (NMD) caused by loss of the ***survival motor neuron 1*** (***SMN1***) gene. We have previously shown that administering prednisolone, a synthetic glucocorticoid (GC) to SMA mice improved muscle health and survival. As chronic use of prednisolone can lead to adverse effects in muscle, we wanted to determine its molecular targets to develop more selective therapeutic approaches. We performed RNA-sequencing on triceps from symptomatic untreated and prednisolone-treated SMA mice and healthy control littermates. The single RNA reads were then aligned to an mm10 ***Mus musculus*** genome through HISAT2 and gene read summarisation was calculated by FeatureCounts. DeSeq2 analysis estimated 3056 significant differentially expressed genes (DEG) (Log2 fold change >0.6, p-adj < 0.05) in prednisolone-treated SMA mice muscle compared to untreated counterparts. Importantly, further comparison with untreated healthy littermates showed that prednisolone treatment in SMA muscle normalised the expression pattern of a large subset of DEG to healthy levels. Further analysis through iPathwayGuide of the gene targets in prednisolone treated SMA muscle, revealed several biological pathways ($p < 0.05$) associated with muscle metabolism, structure and function. Using various drug databases and published literature, we selected 2 clinically approved drug candidates (metformin and oxandrolone) that are predicted to similarly target the DEG and biological pathways in SMA muscle. Although metformin is commonly used for type 2 diabetes and oxandrolone for burn patients, both drugs have previously shown muscle-specific benefits in NMD, metabolic and muscle-wasting disorders. Our ***in silico*** work has thus identified potential new drugs that will be assessed in cellular and animal models. Importantly, this transcriptomic-based drug repurposing approach provides a less expensive and faster alterative for the development of new muscle-specific treatments for SMA.

## Biochemistry of toxins

P-07.1-02

**The inhibitory effect of Moroccan cobra Naja haje legionis' venom and its toxic fraction on Hedgehog-dependent medulloblastoma DAOY cell growth**

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The cobra Naja haje legionis (Nhl) is the only representative of the elapid family in Morocco. It belongs to the Naja haje species which can be prominently found in North Africa and is mainly known for its composition in various molecules especially enzymes and toxins responsible for neurotoxic, cardiotoxic and myotoxic effects. In the present work, we investigated the proteome of Naja haje legionis' crude venom and isolated toxic fraction for their potential antiproliferative activity on hedgehog-dependent human medulloblastoma cancer cells (DAOY) as well as a non-cancerous cell line (MEF) as control. To this end, we conducted proliferation and mortality assays using increasing concentrations of the Nhl crude venom and its toxic fraction (6.25, 12.5, 25, 50 and 100 µg/mL). The results were observed after 24, 48 and 72 hours. The results showed that the Nhl crude venom had an effect on cell growth in both cell lines from the lowest concentration (6.25µg/ml) as well as inducted a fast increase in cell mortality rates as soon as 24 hours into the experiment. As for the toxic fraction, no growth inhibition was observed on the non-cancerous MEF cells as opposed to the DAOY cancer cells where a decrease in cell growth was achieved from the lowest concentration (6.25 µg/ml). As of yet, this study is the first report showing the potential of the Naja haje legionis venom as an alternative therapeutic approach for cancer therapy and will prospectively further explore the venom to specifically identify the molecules responsible for the antitumoral activity.

P-07.1-04

**Structure-function relationship of broad-range phospholipase C from Listeria monocytogenes**

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Broad-range phospholipase C (PC-PLC) is a metalloenzyme and a crucial virulence factor of ***Listeria monocytogenes*** (***Lm***). Crucial step in ***Lm*** pathophysiology is the escape from the lipid encased phagosome after internalization. Disintegration of the phagosome membrane is facilitated by three key bacterial proteins: pore-forming cholesterol dependent cytolysin listeriolysin O (LLO), and two listerial phospholipases C: PC-PLC and PI-PLC. PC-PLC can hydrolyse a variety of lipid substrates, including those having phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine headgroups as well as sphingomyelin. We recombinantly expressed the enzyme and purified PC-PLC to high concentrations, enabling us crystallisation and determination of its crystal structure at 2.0 Å resolution. With structure solved, we searched for mutants retaining wild-type protein fold, while exhibiting lower enzymatic activity. The prime candidate was N-terminal Trp1 residue, which coordinates one of the $Zn^{2+}$ ions and anchors the N-terminus in the hydrophobic core of the protein. We prepared mutants where W1 was replaced by A, E, F, or K or was deleted (dW1S2). While all mutated proteins retained wild-type fold, they had mostly reduced enzymatic activity: wild-type W1 ~ W1F >> W1A > W1E ~ W1K ~ dW1S2. To decipher the interplay between LLO and PC-PLC, we pre-incubated POPC/SM/cholesterol lipid vesicles with PC-PLC. PC-PLC caused significant increase in LLO binding to liposomes and LLO induced vesicle leakage, while PC-PLC on its own did not cause any permeabilization. Preincubation with less active PC-PLC mutants resulted in reduced LLO binding and lower vesicle leakage. Those findings suggest that activity of PC-PLC may increase the availability of membrane cholesterol. Further structure-based functional studies of PC-PLC with LLO are in progress, aiming towards a better understanding of the mechanism and interplay between these two toxins of ***Lm***.

P-07.1-06

**Chronic low-dose exposure of human granulosa cells to the mixture of endocrine disruptors alters steroidogenesis in human granulosa cells in vitro**

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Endocrine disrupting chemicals are present in numerous human body fluids, including follicular. In the past decades, the individual effects of those chemicals were studied and there are evidences that they have negative influence on ovarian granulosa cells' function. However, the prevalence of some disorders cannot be explained by the influence of only one endocrine disruptor. Studying effects of chronic exposure to low doses of chemical mixtures is necessary to mimic the real-life exposure. The aim of this study was to investigate the effects of chronic exposure to chemicals at concentrations found in human follicular fluid on the function of human granulosa cells (HGrC1). The mixture (MIX) was made of bisphenol A [2 ng/mL], polychlorinated biphenyl 153 [70pg/mL], benzo[a]pyrene [1 ng/ml] and perfluorooctanesulfonate [100 pg/ml]. Treatment lasted for 4 weeks, and the granulosa cells' function was evaluated every week. The results showed that chronic exposure to MIX had no effects on cell morphology, but transiently decreased cell viability after 3 weeks of exposure, which returned to control values by the end of treatment. Production of estradiol decreased after 2 and 3 weeks, returning to the control level after 4 weeks of exposure. The mRNA levels of CYP19A1, enzyme responsible for estradiol biosynthesis, increased after 2 weeks of MIX treatment. However, CYP19A1 protein levels decreased after 2 weeks, explaining decline in the estradiol production in the MIX-exposed HGrC1. MIX increased the production of progesterone after 3 and 4 weeks of treatment, without affecting the mRNA or protein levels of steroidogenic acute regulatory protein (StAR), protein that regulates rate-limiting step in the production of progesterone. These results indicate that chronic exposure to low doses of endocrine disruptors mixture leads to imbalance in estradiol and progesterone production, which could have negative impact on ovarian function and fertility.

## Cellular organization

P-07.3-01

**MKK6-p38γ/δ signaling is essential to control cardiac electrical activity**

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Maladaptive cardiac hypertrophy can progress to heart failure, a worldwide leading cause of mortality. However, knowledge about the signaling pathways implicated in how cardiomyocyte growth could induce ventricular arrhythmias remains incomplete. Our group recently showed that p38MAPK signaling pathway has been implicated in the promotion of cardiac hypertrophy. p38MAPKs are activated by the upstream kinases MKK3 and MKK6. However, the specific function and the control of the activation of the different p38MAPK isoforms by these kinases in cardiomyocytes remains elusive. To further explore this, we generated MKK6-KO and MKK3-KO mice. We saw that MKK3 is the main activator of p38γ/δ in the heart, whereas MKK6 activates cardiac p38α. Echocardiography, histology, and immunoblot show that lack of MKK6-p38α activation leads to MKK3-p38γ/δ hyperactivation and increased mTOR signaling, resulting in physiological cardiac hypertrophy. In addition, on patch-clamping experiments ventricular cardiomyocytes from MKK6-KO mice showed spontaneous calcium release events, formation of early afterdepolarization and increased arrhythmia susceptibility on programmed electrical stimulation, particularly in the presence of β-adrenergic stimulation. We also demonstrate that p38γ/δ control several pathways involved in heart failure and arrhythmogenesis: they promote hyperphosphorylation of RyR2 and the scaffold protein SAP97, together with increased expression and activation of CaMKII. Using co-immunoprecipitation experiments, we define a macromolecular complex comprising RyR2, p38γ/δ, PKA and CaMKII together with SAP97 and AKAP79. We show that p38γ/δ phosphorylation of RyR2 increases its binding to PKA, SAP97, p38γ/δ and AKAP79 resulting in RyR2 hyperphosphorylation. In conclusion, this work shows for the first time the importance of cardiomyocyte MKK6-p38γ/δ signaling in cardiac hypertrophy and how p38γ/δ hyperactivation predisposes to stress-induced arrhythmias and sudden death.

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P-07.3-02

**3D hepatic tumor cell culture plasticity mediated by YAP-mTOR axis**

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Cell culture in monolayer on standard plastic dish (so-called 2D cell culture) is a well-established system, which is inexpensive and easy to analyze. Unfortunately, current 2D cell culture systems do not represent real cell conditions; and especially in drug testing are not always predictive. However, so-called 3D cell culture systems offer more physiologically relevant and so far more reliable model than 2D cell culture. Furthermore, with 3D cell cultures we can get more realistic view on tumor cells growth and behavior. Thus, adding one dimension extra provides environment, which could better mimic natural in vivo conditions. In this study, we used collagen scaffold as a model of soft 3D environment to alter extracellular conditions and study the mechanotransduction of hepatic tumor cell lines (HepG2 and Alexander). We found that cell mechanics, guided by the physical constraints of 3D collagen scaffolds greatly affect cellular size and morphology, proliferation, cytoskeleton organization and molecular signaling. Additionally, we identified YAP-mTOR pathways as a downstream effector in 3D cell culture mechanotransduction.

## Molecular interactions of plants with the environment

P-07.4-01

**Effect of increased temperature on seed germination and DNA methylation in Arabidopsis thaliana, Brassica rapa and Solanum lycopersicum**

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Temperatures vary geographically and are predicted to rise with global warming. For dormant seeds, temperature and light are two main cues required for germination. Therefore, appropriate germination timing and response to temperature cues are necessary for plant development and later growth and reproduction. Plants have a very rich epigenetic machinery that enables them to readily adapt to environmental conditions. Understanding the epigenetic basis of sensitivity to temperature is therefore required for understanding interactions between plants and environment. Here, we will examine how environmental temperature impacts germination and DNA methylation of three plant species, model plant ***Arabidopsis thaliana*** and two globally important crop species ***Brassica rapa*** and ***Solanum lycopersicum***. For germination tests, seeds were exposed to a set of rising temperatures ranging from 28 to 40 °C and global DNA methylation level was assessed by immunospecific detection. DNA methylation in plants was shown to be temperature dependent.

P-07.4-02

**Monitoring phytoplasma infection in tomato during two growing seasons**

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Phytoplasma is a wall-less worldwide pathogenic microorganism affecting a large number of economically important crops. The aim of the study was to identify '***Candidatus*** Phytoplasma solani' in tomatoes during the growing season. The study was carried out on four genotypes (varieties) of tomato over two years (2019 and 2020). Molecular diagnosis revealed a significant difference in plant infection rates between two years of research. In whole, the infection spread was significantly lower in 2020 than in 2019. The total proportion of plants infected with phytoplasma in July of 2019 was 37.5%; it reached 69.6% at the end of the growing season (mid-September). In July of 2020, '***Ca***. P. solani' infection was absent in the tomato field. In September of 2020, the percentage of infected plants was no more than 14.5%. Such contrasting results obtained over two years can be explained by the influence of different climatic conditions of these years in combination with specificity of agricultural techniques. In addition to different levels of infection during the years of research, it was found that the sensitivity of tomato plants to phytoplasma infection varied depending on the genotype. Thus, the tomato variety Mary Gratefully showed a highest sensitivity to '***Ca***. P. solani' in both growing seasons (92% of infected plants in September of 2019 and 33% in 2020). The tomato varieties Elvira and Desteptarea had intermediate indicators of susceptibility to phytoplasma infection in both years. On the contrary, the Cerasus variety can be characterized as the most resistant to '***Ca***. P. solani' compared with three other studied varieties. Namely, in July and August of 2019, the percentage of infected plants of Cerasus was 25% and reached 58% only at the end of the growing season. In 2020 this variety demonstrated complete immunity to phytoplasma during the growing season. Obtained results can be used in the breeding and creating tomato varieties resistant to phytoplasma infection.

## Cancer immunology and immunotherapy

P-08.1-02

**Herpesvirus-based vectors for oncolytic biotherapy**

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Herpesviruses are attractive for cancer therapy because of several characteristics including their cytolytic nature and well-characterized genome; some herpesviruses, namely C-strains of herpesvirus saimiri, are also able to immortalize T-lymphocytes. The challenge in designing a new recombinant HVS vector that maintains its specificity without being harmful to normal cells represent a huge obstacle to come over as it requires performing a homologous recombination in virus-permissive cell line that are tricky to transfect. The aim of this work is to design a new recombinant herpes virus that can replicate in vitro and comprise an effective way to deliver virus particles to tumor cells. Achieving this goal needs to fulfill several tasks: design the new recombinant virus, demonstrate an effective way of replication in vitro and developing a new method of delivery in vivo. Our results showed a successful recombination of herpes virus genome with transgenic cassette expressing Orange Fluorescence Protein (OFP) in infected OMK cells with forming syncytia with another type of cells. We were able to clone the cells and see the cytopathic effect of the monoclonal virus stocks prepared from single OMK cells. Infecting T-lymphocytes with the recombinant HVS-OFP resulted in immortalization of these cells with expressing functional T cells markers in comparison with control uninfected cells. These results lead to the successful preparation of recombinant HVS in vitro in order to experiment it in vivo as well. The cytopathic effect of this virus was confined to simian OMK cells which means it conserves the species specificity of the original virus. The immortalization of T-lymphocytes after infecting with HVS-OFP means that it maintains the ability to transform human lymphoid cells and utilized for cellular immunotherapy. Further application by targeting HVS-immortalized T-cells to cancer antigens and by using them as carriers to precisely deliver oncolytic viruses to tumor site.

P-08.1-05

**Cystatin F Is Expressed in Glioblastoma cancer and cancer stem like cells**

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Glioblastoma is the most aggressive type of brain tumors, composed of heterogenous cell populations with great invasive potential. Tumor tissue contains not only differentiated and stem-like cancer cells but also activated microglia and other immune cells. Cystatin F, member of cystatin family of endogenous inhibitors of lysosomal cysteine peptidases is generally present in immune cells. Its expression is regulated by C/EBPα. Although cystatin F can be secreted in dimeric form it is only active intracellularly. In monomeric form it inhibits activity of cathepsins L, S, H and C. In some pathological conditions cystatin F can be upregulated, like in cancer and neurodegeneration diseases. The aim of this study is to examine the expression of cystatin F in glioblastoma. Imunohistochemical detection was used to determine cystatin F expression and localisation in formalin-fixed paraffin-embedded tissue. Gene expression analysis of cystatin F and C/EBPα was performed with glioblastoma and low grade glioma samples, as well as glioblastoma tumor cells. ***In trans*** action of cystatin F was tested using U-251 MG glioblastoma and U-937 pro-monocyte cell lines by non-reducing SDS PAGE and western blot analysis. Impact of cystatin F internalisation on cathepsin L activity was also checked. We found that cystatin F is present in glioblastoma tissue but not in healthy brains. It is localised in cancer cells, cancer stem cells and microglia cells expressing GFAP, SOX2 and Iba-1, respectively. Its expression is increasing with glioma progression and is elevated in cancer stem cells in comparison to differentiated cancer cells. The same trend is true for C/EBPα. Cystatin F was able to internalize U-251 MG cells after their exposure to U-937 cells. The internalisation affected cathepsin L activity in glioblastoma cells and the interaction was confirmed by co-immunoprecipitation of cystatin F and cathepsin L. Our future aim is to elucidate the role of cystatin F expression in glioblastoma.

## Cancer initiation and progression

P-08.2-01

### Evaluation of Anticancer Efficacy of Temozolomide and Resveratrol in Human Glioma Cells

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Cancer is one of the leading causes of human death. In vitro studies are very important to understand the molecular mechanisms of tumor formation in cancer research. Besides this, glioblastoma is the most common and aggressive primary malignant brain tumor. These types of tumors are highly resistant to chemotherapy and the average life expectancy is not more than 14 months. The alkylating agent temozolomide (TMZ) is a chemotherapy drug to treat glioblastoma. Although it is widely used in patients with brain cancer, its therapeutic effect is very limited due to its resistance to glioblastoma. Resveratrol (RSV), a natural polyphenolic molecule, has anticarcinogenic potential and it is an efficacious compound for cancer prevention and treatment. Thus, RSV could enhance the sensitivity of glioblastoma cells to TMZ therapy. Heat shock proteins (Hsps) have been found to be elevated in many cancer types and they have been shown to be a biomarker. Their overexpression in brain tumors has been associated with cell proliferation, apoptosis inhibition and chemoresistance. Hsps have become significant anticancer targets and the development of Hsp inhibitors is important for cancer treatment. In this study, we aimed to investigate the molecular mechanisms of TMZ and RSV in U87 MG human glioma cells and we carried out in vitro experiments to evaluate the combination of these compounds. The results show that the combined therapy of RSV with TMZ suppressed Hsps and induced apoptosis in U87 MG cells. This combination exhibited an increase in DNA breaks, protein carbonyl content and intracellular reactive oxygen species. Conversely, there was no this kind of significant results in noncancerous HEK 293 human embryonic kidney cells. In conclusion, these findings indicate that this combined therapy is a promising candidate for cancer therapy and provides a viable strategy to achieve better therapeutic efficacy by avoiding possible toxicity and side effects to noncancerous cells.

P-08.2-03

**The Effects of Metabolic Drugs on Tumor Behaviour in Oral Squamous Cell Carcinoma**

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Oral squamous cavity cancer (OSCC), highly aggressive malignant tumor, is the sixth leading cancer and current treatment strategies for this cancer include surgical resection, radiotherapy, and/or platinum-based chemotherapy. Because of its highly aggressive biology, it is crucial to search for innovative therapeutic strategies to reposition FDA-approved drugs, and thus to develop treatment options with lower costs and fewer side effects for OSCC patients. Metformin is an anti-diabetic agent that inhibits the complex-1 of mitochondria. Dichloroacetate (DCA), used for the treatment of lactic acidosis, is a small inhibitor of pyruvate dehydrogenase kinase. In this context, Metformin and DCA have the potential to be used in cancer treatment because of their effects on tumor metabolism. The aim of this study is to determine the effects of Metformin, DCA and their combination (Met+DCA) on cell viability, cell proliferation and cell death in oral squamous cell carcinoma (UPCI-SCC-131 cell line) under normoxia/hypoxia. The IC50 values of Metformin and DCA were determined with WST-1 cell viability assay. Colony formation assay was used to assess the long term effects of these drugs on cell viability. Cell proliferation was analyzed using the BrdU assay. Cell death and cell cycle assays were examined with flow cytometry. Metformin and DCA reduced UPCI-SCC-131 cell viability and cell proliferation. Consistent with our results flow cytometry results showed that apoptosis was increased in Metformin and DCA treated cells. As regards tumor behavior, Metformin and Met+DCA decreased 3D spheroid formation in UPCI-SCC-131 cells. In conclusion, there is an urgent need for novel agents that increase the chemosensitivity, enable the use of lower doses and have a synergistic effect. Metformin and DCA may take a step forward as anti-tumorigenic agents in the treatment of patients with oral cavity cancer. This study was supported by a grant (no. 118S576) from TUBITAK.

P-08.2-04

**New antitumor unsymmetrical bisacridines derivatives affect c-myc and K-Ras level leading to cell death and accelerated senescence in human lung and colon cancer cells**

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Unsymmetrical bisacridines (UAs) are new antitumor derivatives patented in Europe (EP 3070078 B1) and USA (US 10202349 B2). In their structure they consist previously synthesized in our Department drugs: C-1311 and C-1748. Importantly, UAs exhibit different properties than their monomer components. They do not intercalate to dsDNA, but interacts with quadruplex DNA. G-quadruplex structures are present in promoter regions of oncogenes, such as: MYC, KIT, RAS genes, BCL2, VEGF and in telomeric repeats. The aim of the studies was to evaluate whether UAs can influence the expression and protein level of c-myc and K-Ras in living cells and what is the consequence for the cellular effects of UAs treatment. The action of four UAs: C-2028, C-2041, C-2045 and C-2053 were examined in human lung H460 and colon HCT116 cancer cells. All compounds exhibited high cytotoxicity against tumor cells and the $IC_{90}$ dose ranged from 0.04 to 0.4 µM and were similar for both cell lines. In H460 cells all studied drugs at $IC_{90}$ concentration decreased expression and completely inhibited protein level of c-myc since 72 h of incubation and slightly increased K-Ras. In HCT116 cells UAs did not cause remarkable difference in expression and protein level of c-myc, only weak increase of K-Ras. H460 and HCT116 cell exposed to UAs underwent apoptosis what was confirmed by changes in nucleus morphology, cytometric analysis of cell cycle, active caspase-3 and mitochondrial transmembrane potential. Importantly, the apoptosis was induced by UAs earlier and to a greater extent in H460 (especially in cells exposed to C-2045 and C-2053) compared to HCT116 cells (except C-2041). Furthermore, accelerated senescence was induced by UAs only in H460 cells. Concluding, strong c-myc inhibition by UAs in H460 cells seems to contribute to induction of apoptosis and accelerated senescence in these cells. These studies were supported by the National Science Center, Poland, No. UMO-2016/23/B/NZ7/03324.

P-08.2-05

**Involvement of P2X7 receptor in glioma cell growth and spreading**

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The glioma tumor microenvironment is characterized by an abundant amount of ATP, released from stressed or dying cells as a result of chemotherapy or radiotherapy. A high concentration of extracellular ATP leads to the stimulation of P2X7 nucleotide receptor which acts as an non-selective ion channel. P2X7 regulates many crucial functions like inflammasome formation, ATP release, immune response and cell death in somatic and cancer cells. Role of P2X7 in glioma tumors, receptor is however still poorly characterized and requires a more detailed study. The microarray analysis data demonstrate that P2X7 is upregulated in human low grade gliomas. Interestingly, P2X7 expression was significantly lower in IV grade glioma samples compare to low grade gliomas and healthy brain tissue. Additionally, P2X7 receptor expression is significantly lower in glioma cell lines compared to primary astrocytes in vitro. P2X7 activation did not induce cell death in studied glioma cells. On the contrary, stimulated glioma cells proliferation and adhesion to ECM components. The convergent results were obtained after P2X7 inhibition in glioma xenograft tumors in vivo. Administration of selective P2X7 inhibitor, brilliant blue G (BBG), leads to significant tumor mass reduction and decreases tumor cells spreading. Namely, we observed a decrease in matrix metalloproteinase 2 activity in BBG treated tumor mass. We observed also a decrease of β-catenin expression and total reduction of N-cadherin and vimentin levels in treated tumors compared to control tumors. Summarizing, our results suggest that activation of P2X7 receptor via extracellular ATP can be engaged in shaping glioma tumor microenvironment and promotion of glioma tumor growth. Acknowledgements: This work has been supported by National Science Centre research grant no. 2015/17/B/NZ3/03771

P-08.2-06

**Challenges in translating miRNA into clinics**

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Prostate cancer (PCa) represents a most diagnosed neoplasm among men, with incidence rates steadily rising. There is a need for more sophisticated biomarkers for diagnosis, prognosis and management. MicroRNAs (miRNAs) in liquid biopsies and tissue emerged as potential biomarkers for PCa that could help better distinguish aggressive vs. non-aggressive tumors, and malignant vs. non-malignant conditions. However, conflicting data was reported for certain miRNAs and published papers often lack certain information which is necessary to contextualize the results. With growing body of evidence, questions are being raised regarding diverse (pre)analytical factors influencing miRNA analysis and hinder translation of miRNA into clinical practice. In order to analyze and address current problems in miRNA clinical research on PCa, a PubMed-based literature search was conducted with the last update in May 2019. Diverse (pre)analytical factors influencing miRNA analysis were studied and compared across the studies. By examining published papers, we conclude that studies widely differ in study design parameters: control groups, serum and plasma comparison, sample storage, quality control steps, data normalization. There is an immense lack of data on methodology framing parameters used in published studies. There is a lack of consistency and reproducibility, largely due to a missing consensus on preferred sample collection, sample handling, RNA extraction and miRNA analysis. Other aspects such as different control groups and sample storage also influence variable results obtained in miRNA research. Of outmost importance seems to be reaching a consensus on guidelines and widely accepted protocols for sample processing and quality control. After identifying critical variations in designs and protocols that undermined clear-cut evidence acquisition, we propose specific guidelines for critical steps that should be considered in future research of miRNA as biomarkers especially in PCa.

## Immune and inflammatory disorders

P-08.3-02

**Intense physical exercise induces an anti-inflammatory change in IgG N-glycosylation profile**

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Exercise is known to improve many aspects of human health, including modulation of the immune system and inflammatory status. Despite the general understanding that exercise reduces inflammation, the relation of the two is not yet fully understood. N-glycosylation of immunoglobulin G (IgG) and total plasma proteins was previously shown to reflect changes in inflammatory pathways, which could provide valuable information to further clarify effects of exercise. In order to better understand the relationship between physical activity and inflammation, we examined the effect of intense exercise, in the form of repeated sprint training (RST), on IgG and total plasma proteins N-glycosylation. Twenty-nine male physical education students were separated into treatment (RST, N = 15) and control (N = 14) groups. The RST group completed a 6-week exercise protocol while the control group was instructed to refrain from organized physical activity for the duration of the study. Three blood samples were taken at different time points: prior to start of the training program, the final week of the exercise intervention, and at the end of the four-week recovery period. Following the recovery period IgG N-glycosylation profiles showed anti-inflammatory changes in RST group compared to the control group, which manifested as a decrease in agalactosylated and an increase in digalactosylated and monosialylated N-glycans. Observed changes show the potential of intense physical exercise to reduce levels of systemic basal inflammation, as well as the potential for IgG N-glycosylation to serve as a sensitive longitudinal systemic inflammation marker.

P-08.3-05

**Effects of high-fat diet on functional and molecular metabolic parameters in rats with constitutionally altered serotonin homeostasis**

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Serotonin (5HT) is bioamine implicated in many fundamental biological functions including maintaining of energy homeostasis. Recent studies in mice suggest that pharmachological inhibition of peripheral 5HT synthesis protectes against obesity and type 2 diabetes, however little is known about role of endogenous 5HT tone in development of obesity and obesity-related health conditions. Here we investigated the relationship between endogenous 5HT activity and body weight regulation using sublines of rats with constitutionally up-regulated (high-5HT) or down-regulated (low-5HT) blood 5HT leves. Male rats from both sublines were fed a high fat diet (HFD, 45% kcal from fat) for 11 weeks. Control diet (CD)-fed high 5HT rats had elevated body weight as compared to CD-fed low 5HT rats, however only low-5HT rats showed significantly increased body weight in response to HFD-feeding. In line, impaired glucose and insulin tolerance in response to HFD-feeding was observed only in low-5HT animals. Blood cholesterol, trygliceride and leptin levels were in both sublines increased by HFD-feeding while blood levels of insulin and glucagon were affected by HFD-feeding only in low-5HT rats. HFD had no effect on platelet 5HT levels, but it significantly increased 5Ht uptake rates in platelets of low-5HT rats. Brown adipose tissue thermogenesis, as measured by infrared thermography, was in both sublines similarily elevated by HFD-feeding while expression levels of multiple energy homeostasis-related genes in hypothalamus and adipose tissue were affected by HFD-feeding only in low-5HT rats. Our results suggest that low-5HT rats are more susceptible to deletorious effects of HFD than high-5HT animals. Research was funded by Croatian Science Foundation, grant no IP-2014-09-7827

P-08.3-07

**Ubiquitin signalling in inflammation**

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It is commonly accepted that tight regulation of proteostasis is key to ensure intact cellular function. Posttranslational modifications (PTMs) such as ubiquitination give rise to a complex layer of regulation. PTMs regulate protein function by orchestrating maturation, stability, localization and (in)activation. Ubiquitin regulatory versatility arises through the formation of ubiquitin chains upon ubiquitination of one of the 7 internal lysin residues or the N-terminal methionine. Specificity of chain types allows for a specific ubiquitin code comprised of at least eight different types of ubiquitin chains: Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, Lys63 and Met-1. In addition to its well-studied function as a marker for proteasomal degradation, linear ubiquitin-chains, arising from Met-1 coordinated linkage, are required for activation of NFkB signalling during innate immune response. Dedicated writers, readers and erasers (dis)assemble the code targeting various substrates. Since the discovery of Met-1 chain dependence of innate immune signalling, the mode of action of the respective ubiquitin modifying enzymes has been readily studied, leading to the discovery of several important regulators of inflammatory response whose absence is connected to chronic inflammatory disease. Using an integrated structural, biochemical and biophysical approach, we aim to understand, at the mechanistic level, how the ubiquitin code is regulated and how the ubiquitin signal controls inflammatory signalling pathways. Structure guided point mutations can be generated to dissect complicated biological pathways without abrogating the general function in the overall physiological context. The overall aim is to unravel regulation of protein function within the cell. By adding to the understanding of ubiquitin signalling in inflammation we aim to contribute towards deciphering how dysregulation leads to disease.

## Aging stress and neurodegeneration

P-08.4-05

**Neurotoxic effect of extracellular alpha-synuclein can be alleviated by AMPK and autophagy**

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Alpha-synuclein (ASYN) is regarded as one of the key culprits in pathogenesis of synucleinopathies, including Parkinson's disease, and impaired regulation of autophagy is associated with the ASYN aggregation. Autophagy is regulated by complex mechanisms, including AMP activated protein kinase (AMPK), a key energy sensor regulating cellular metabolism to maintain energy homeostasis. The aim of our study was to investigate the role of AMPK and autophagy in neurotoxic effect of secreted ASYN, as well as dopamine-modified and nitrated recombinant wild-type ASYN oligomers, on retinoic acid (RA)-differentiated SH-SY5Y cells. The culture supernatant from neuroblastoma cells stably expressing wt ASYN was collected and used as conditioned medium (CM). The presence of wt ASYN in CM was confirmed by immunoblot, following lyophilisation. The CM, as well as recombinant dopamine-modified or nitrated ASYN, all reduced viability in differentiated SH-SY5Y cells. This decrease in viability was accompanied by reduced AMPK activation, increased conversion of LC3-I to LC3-II and increase in Beclin-1 level, as demonstrated by immunoblot. Pharmacological activators of AMPK and autophagy (metformin and AICAR) significantly increased the cells' viability in the presence of CM and modified ASYN forms. Level of AMPK-activated pULK was reduced in presence of CM, but pharmacological activators of AMPK reversed that effect. Pharmacological inhibitors of autophagy, further reduced cell viability in the presence of extracellular ASYN. The shRNA-mediated LC3 downregulation, as well as the RNA interference-mediated knockdown of ATG7 gene, both important for autophagosome biogenesis/maturation, increased sensitivity of SH-SY5Y cells to the extracellular ASYN-induced toxicity. These data demonstrate the protective role of AMPK and autophagy against the toxicity of extracellular ASYN, suggesting that their modulation may be a promising neuroprotective strategy in Parkinson's disease.

## Redox biology – oxidative stress signalling

P-08.5-02

**The role of mitochondrial phospholipase A2γ in the regulation of cellular redox homeostasis and oxidative stress signalling**

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Redox-dependent regulations play an essential role in a wide range of biological activities. Mitochondria in numerous tissues represent a primary source of superoxide and subsequent downstream oxidants, notably $H_2O_2$ and lipid hydroperoxides. However, the understanding of the role of mitochondrial oxidant production in pathology and normal physiology is limited. Mitochondrial calcium-independent phospholipase A2γ (iPLA2γ) belongs to a family of enzymes that participate in cellular signalling by simultaneously producing free fatty acids (FA) and lysophospholipids. Here we hypothesise that iPLA2γ plays an antioxidant role in various tissues by contributing to FA-dependent dissipation of the protonmotive force and subsequent attenuation of mitochondrial oxidant production. Following the respiration of mitochondria isolated from selected tissues of wild-type (WT) and iPLA2γ-KO mice, we demonstrated an increase in respiration following the addition of extrinsic hydroperoxides, including $H_2O_2$. The oxidant-induced increase in respiration was prevented by (1) R-bromoenol lactone, a selective inhibitor of iPLA2γ, (2) carboxyatractyloside, an inhibitor of adenine nucleotide translocase (ANT), and (3) thiol reducing agents. The oxidant-induced changes in respiration were absent in mitochondria isolated from iPLA2γ KO mice. To confirm our hypothesis, that the oxidant-induced increase in respiration is due to the release of FA, we have analyzed the samples obtained from the respiration assay by HPLC-MS. The data show an oxidant-dependent increase in relative concentrations mainly of docosahexaenoic, oleic, and arachidonic acid, which was prevented by R-BEL but not by ANT inhibitor. These results suggest that the oxidant-induced activity of iPLA2γ leads to a release of free fatty acids, which promote ANT-dependent $H^+$ transport, leading to a decrease in the mitochondrial protonmotive force and subsequent attenuation of mitochondrial superoxide production.

P-08.5-03

**IGF-1 via PI3K/AKT activation promotes survival and anabolic metabolism in HEI-OC1 auditory cells**

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Hearing loss is the most common sensory deficit in the human population. Mutations in the gene coding for IGF-1 cause sensorineural hearing loss in man and mice (previously published in: Rodríguez-de la Rosa L et al. (2017) Front Aging Neurosci 12,9:411). Actions of IGF-1 are mediated by binding to its high affinity transmembrane receptor, IGF1R. This interaction typically leads to the activation of the PI3K-AKT pathway and of the MAPK-ERK cascade. To gain insight into the molecular mechanisms involved in IGF-1 downstream signaling in the sensory hair cells, we have used the HEI-OC1 cell line derived from the Immortomouse™ hearing receptor (previously published in: Kalinec GM et al. (2003) Audiol Neurootol 4, 177-189), which is the cell line of choice to study the molecular steps occurring during the differentiation to outer hair cell-like cells of auditory progenitors. The study of the relative expression of genes of the IGF system by RT-qPCR showed that IGF system factors and receptors are expressed in both progenitors and differentiated auditory cells. We also studied the actions and main downstream signaling pathways of IGF-1. Apoptosis and cell viability were studied by flow cytometry and XTT assay, respectively, whilst activation of target proteins was measured by Western blotting. The consequences of blockage of IGF-1 actions were also investigated by using specific IGF1R inhibitors and XTT assay. IGF-1 increased survival, proliferation, as well as glucose metabolism and protein synthesis, whereas autophagic flux was decreased and apoptosis inhibited. Our data indicate that HEI-OC1 cells can be used as a model to understand the actions of IGF-1 in hair cells, to identify novel targets and to unravel the molecular mechanisms involved in IGF-1 deficiency-associated otic damage.

P-08.5-04

**Association between oxidative stress parameters (Malondialdehyde, Superoxide dismutase, Pro-Oxidant Antioxidant Balance) and obesity in the pediatric population**

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Introduction. Obesity, as one of the biggest threats to public health, is a condition that is reflected in the increased accumulation of body fat. As a complex disease, obesity can be associated with the emergence of multiple serious conditions such as oxidative stress. Oxidative stress represents an imbalance between prooxidants and antioxidants causing increased amounts of reactive oxygen and nitrogen species. The aim of this study was to investigate the association between measured oxidative stress parameters (MDA - lipid peroxidation marker, SOD - protects us from dangerously reactive forms of oxygen, PAB) and obesity in the pediatric population. Materials and methods. The study included 135 obese and overweight children (BMI above the 85th percentile) and 86 healthy controls (BMI less than 85th percentile) aged 2-19, who underwent an anthropometric evaluation. All subjects included in the study were recruited at the Clinical Centre University of Sarajevo and were free of evidence of chronic problems (infections, surgery, thyroid disease, polycystic ovarian syndrome), active liver and kidney damage and were not using any hormonal therapy. The oxidative stress parameters (MDA, SOD and PAB) were determined by spectrophotometric methods, according to the protocols. Results. In our study, results demonstrated a significant increase in the MDA levels in obese children compared to the healthy controls ($p< 0,001$). Also, obese children had a significant increase in the SOD levels compared to the control group ($p=0.002$). On the other hand, results demonstrated a significant decrease in the PAB levels in obese children compared to the healthy controls ($p< 0.001$). Conclusion. Our study demonstrated that measured oxidative stress parameters show a strong association with obesity in the pediatric population. This study also suggests that adequate control of body weight could improve the quality of life and prevent possible pathological conditions caused by oxidative stress.

## Structural and functional glycobiology

P-09.1-01

**Heparin-binding activity of extracellular vesicles from human seminal plasma**

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Prostasomes are extracellular vesicles (EVs) originating from the prostate and abundantly present in human seminal plasma (SP). They have intrinsically heterogeneous molecular composition and ultrastructure. Surface glycans specifically contribute to their properties in terms of marking distinct prostasome populations and were, also, found to change during different physiological conditions. In this study, heparin-binding activity is aimed as an adjunct parameter to additionally annotate prostasome populations already established on the basis of a specific pattern of glycosylated markers. Heparin-binding proteins (HBP) are found on different membranous structures, but not studied in the context of association with prostasomes. Resolving this issue could be also of biomedical importance since HBP may attribute prostasomes their various biological activities some of which are known to correlate with fertility status. Heparin-affinity chromatography was probed for separation of prostasomes isolated by ultracentrifugation and gel filtration, from normozoospermic- (sPro-N) and oligozoospermic- (sPro-O) men. EVs recovered in non-bound and bound fractions were examined by transmission electron microscopy and characterized according to total protein/glycoprotein composition. Both sPro-N and sPro-O consisted of one major and at least three minor charge-resolved EVs populations differing in the presentation of mannosylated and sialylated glycans. Prostasomal patterns of glycoproteins and tetraspanin markers remained mainly in the non-bound fraction, whereas heparin-binding activity was annotated to vesicles exhibiting different morphology and low activity of surface-associated gamma-glutamyl transferase. Addressing both glycosylated and matching carbohydrate-binding molecules on prostasomal surface is of general importance since they make the first contact with interacting molecules during exerting putative role as a communication tool in reproduction-related processes.

## Lipidomics

P-09.3-01

**Ancestral paternal exposure to high-fat diets causes testicular metabolic and functional disturbances in mice up to two generations**

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The onset of overweight and type 2 diabetes occurs at ever younger age, raising concerns regarding the metabolic health of the offspring due to possible transgenerational effects of high-fat diet (HFD). Herein, we describe the effects of HFD, even if transient, in glucose homeostasis and in testicular metabolome, lipidome and function of the offspring (sons and grandsons). 3 groups of 12 mice/each were randomly assigned to distinct diet regimen after weaning: CTRL–standard chow; HFD–high-fat diet; HFDt–HFD for 60 days, then replaced by standard chow. Animals had unrestricted access to water and food until sacrifice, at 200 days post-weaning. Body weight was monitored. Testis, serum and sperm were collected after sacrifice. Glucose homeostasis was assessed according to HOMA2 metrics. Sperm quality, testicular metabolome ($^1$H-NMR) and lipidome (GC-MS) were characterized. At 120 days post-weaning, males were mated with normoponderal females (1:1) to obtain the $F_1$ generation (sons), later used to obtain the $F_2$ generation (grandsons), in the same conditions. The descendants were all fed with standard chow and procedures were repeated. No evidence of metabolic syndrome was found in sons and grandsons of HFD-fed mice. However, the grandsons of mice fed with HFD, even transiently, had lower sperm concentration and higher prevalence of sperm defects than the grandsons of mice fed with standard chow. Using sPLS-DA, we found distinct testicular metabolome and lipidome profiles between the 3 diet regimens, notably in the diet-challenged mice and their grandsons. The differences are mostly explained by metabolites related to energy production and lipid remodelling (glutamine, acetate, inosine) and, in grandsons of lifelong HFD mice, to an increased proportion of ω6 fatty acids. Therefore, ancestral HFD causes testicular metabolic signatures in the progeny up to two generations which are correlated to testicular dysfunction, expressed as poorer sperm parameters.

## Autophagy and Protein Recycling

ShT-02.2-2

**Mitophagy is downregulated upon thermogenic stimulus in human beige adipocytes**

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Adipocytes are classified into white, brown and beige. Brown and beige adipocytes are important targets to combat obesity, as they are capable to dissipate energy in the form of heat, while the white adipocytes are primarily for energy storage. UCP1, an inner mitochondrial membrane protein mediates thermogenesis by uncoupling the mitochondrial respiratory chain from ATP synthesis. Hence, mitochondria are important for the thermogenic and metabolic functions of adipocytes. $UCP1^{+}$ mitochondria in human adipocytes are mostly fragmented (Pisani et al, 2017). Mitophagy plays a vital role in beige to white adipocyte transition in mouse (Altshuler-Keylin et al, 2016). We intend to characterize the role of mitophagy in the thermogenic activation of primary human abdominal subcutaneous adipocytes and SGBS cells. Isolated preadipocytes were differentiated into white and browning adipocytes, which were treated with dibutyryl-cAMP (6,10 and 14hours). Genes related to parkin-dependent and independent mitophagy were downregulated upon thermogenic stimulus; the parkin dependent ones being the most downregulated. The lipidated form of LC3, LC3-II is recruited on the outer membrane of the autophagosome which indicates ongoing autophagy. TOM20 is an outer mitochondrial membrane protein and marks the mitochondria. LC3 and TOM20 immunostaining were performed, followed by quantification of LC3 punctae, which was high in untreated control adipocytes but decreased significantly upon thermogenic stimulus, suggesting repressed autophagy/mitophagy. Colocalization of TOM20 and LC3 can indicate mitophagy. Decreased colocalization was observed upon thermogenic stimulus, which further proved repressed mitophagy. TOM20 quantification showed increased number of fragmented mitochondria upon thermogenic stimulus suggesting prompt inhibition of mitophagy, thereby protecting many fragmented mitochondria from degradation and boosting thermogenesis. This work was supported by GINOP-2.3.2-15-2016-00006.

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ShT-02.2-1

**Lipid droplets and autophagy cooperate in the protection of cancer cells against metabolic stress**

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Lipid droplets (LDs) are lipid storage organelles present in most eukaryotic cells. They are composed of a core of neutral lipids surrounded by a phospholipid monolayer and proteins. LD biogenesis is induced in cells exposed to excess nutrients and lipids and is characteristic of many diseases, such as obesity, diabetes and cancer. Intriguingly, their formation occurs also in cells fully deprived of nutrients and oxygen, suggesting that LDs are an integral part of the cellular stress response. LDs engage in a complex and as yet poorly defined relationship with autophagy, the major cellular recycling machinery and stress response pathway. First, autophagy may drive LD biogenesis by providing lipids recycled from other membranous organelles. Second, autophagy may participate in LD breakdown through a selective form of autophagy named lipophagy. Third, LDs may promote autophagy by providing lipids or signals that support the formation of autophagosomal membranes. We aim to discover the principal ways in which LDs and autophagy cooperate to promote the resistance of cancer cells to stress. We have found that lipid droplets are dynamically synthesized and broken down in cancer cells depending on the length and severity of nutrient deprivation. Autophagy is required for their biogenesis under acute starvation conditions, whereas lipolysis seems to be involved in their breakdown under milder conditions of starvation. By manipulating the activities of the major enzymes involved in LD metabolism in the context of activated or inhibited autophagy, we are currently examining the links between these two processes and their roles in cancer stress resistance. Our work may open new perspectives in cancer research by providing important clues on the function of the recently recognized stress-associated organelle – the lipid droplet.

## Molecular Evolution and Phylogenetics

ShT-01.3-1

**Investigation of functional annotations to enzyme classes reveals an extensive annotation error**

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Only a small fraction of genes deposited to databases has been experimentally characterised. The majority of proteins have their function assigned automatically, which can result in erroneous annotations. The reliability of current annotations in public databases is largely unknown, and we lack experimental attempts to validate accuracy of existing annotations. In our study we performed an overview of functional annotations to the BRENDA enzyme database. We first applied a high-throughput experimental platform to verify functional annotations to an enzyme class of S-2-hydroxyacid oxidases (EC 1.1.3.15). We chose 122 representative sequences of the class and screened them for their predicted function. Based on the experimental results, predicted domain architecture and similarity to previously characterised S-2-hydroxyacid oxidases, we inferred that at least 78% of sequences in the enzyme class are misannotated. We experimentally confirmed four alternative activities among the misannotated sequences and showed that misannotation in the enzyme class increased over time. Finally, we performed a computational analysis of annotations to all enzyme classes in BRENDA database, and showed that nearly 18% of all sequences are annotated to an enzyme class while sharing no similarity to experimentally characterised representatives. We showed that even well-studied enzyme classes of industrial relevance are affected by the problem of functional misannotation.

## Proteolytic Processing

ShT-02.3-1 *Participation in the FEBS YSF and Congress supported by the Biochemical Society (UK)*

**From ubiquitin-independent degradation to ubiquitin degradation – A signature activity of emergency 20S proteasome in hypoxia**

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Eukaryotic cells harbor fully assembled free 20S proteasome in addition to active 30S/26S proteasomes. 20S proteasome efficiently degrades unstructured proteins ***in vitro*** however; its cellular proteolytic activity has been a long-standing debate. Although partial evidences support the ***in vivo*** function of 20S proteasome, indistinct contribution from both 26S and 20S proteasomes blur this hypothesis due to common core enzymes. In this study, we unravel the unique contribution of 20S proteasome towards intracellular degradation and proteostasis. By following series of systematic approaches including chemical synthesis of a set of synoptic ubiquitin-conjugates, single particle cryo-EM analysis and intracellular peptidomics, we defined "Four signature activities" of 20S proteasome that are distinct from 26S proteasome. Under condition of hypoxia or human Failing heart, we discovered these signature activities of 20S proteasome due to its elevated levels. Taking Cyclin B1 as a model substrate and a genetically modified Hi20S mammalian cell model, we revealed that 20S proteasome cleavage activity and product outcomes are different from 26S proteasomes. One of the signature is the participation of 20S proteasome in degradation of polyubiquitin conjugates under hypoxic stress. Further single particle Cryo-EM study, elucidated the ubiquitin conjugate driven asymmetric conformational changes for 20S proteasome function. In conclusion, we could determine 20S proteasome signature function in mammalian cells that can be exploited as a prognostic marker for hypoxia associated diseases. Moreover, Hi20S state under hypoxia might be an adaptive response for emergency proteasome activity to alleviate proteotoxic-load and provide better survival.

## Epigenetics

ShT-01.4-1

**Investigating the link between DNA replication, chromatin change and transcriptional regulation during in vivo erythroid differentiation**

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In mouse red blood cell differentiation (erythropoiesis), early progenitors transition to terminal differentiation by passing through a highly specialised cell cycle[1]. During this cell cycle, DNA is replicated faster than in preceding or following cycles due to accelerated replication forks[2]. We have performed an integrated analysis of matched chromatin accessibility and single-cell expression data, revealing that this cell cycle also coincides with widespread changes in gene expression and chromatin accessibility. We analysed chromatin folding of erythroid gene loci at various stages in red blood cell differentiation and show that specific enhancer-promoter loops are formed concomitantly with both progressive upregulation of gene expression and changes in histone post-translational modifications. We used SeqGL[3], a quantitative model using a k-mer feature representation and group lasso regularization to identify transcription factor motifs enriched in enhancer sequences at each stage of red blood cell differentiation. SeqGL highlighted the erythroid transcription factor GATA1 as highly enriched at enhancers that first become accessible during the transition to terminal differentiation. Using CUT&RUN, we are able to show that this specialised cell cycle is indeed the point at which GATA1 first binds to chromatin. Finally, we inhibit DNA replication and measure the effects of this perturbation on Gata1 recruitment and on chromatin composition. Our findings demonstrate that chromatin architecture and gene activation are tightly linked during development and provide insights into the distinct mechanisms contributing to the establishment of tissue-specific chromatin structures. References: 1. Pop ***et al.*** PLoS Biol. 8, e1000484 (2010). 2. Hwang ***et al.*** Sci. Adv. 3, e1700298 (2017). 3. González ***et al.*** Nat. Genet. 47, 1249–1259 (2015).

ShT-01.4-2

**Novel approach for genome-wide high-resolution profiling of 5-hydroxymethylcytosine and its application for neuroblastoma analysis**

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The epigenetic DNA modification 5-hydroxymethylcytosine (5hmC) has a crucial role in development and gene regulation and is associated with complex human diseases including cancer. Neuroblastoma (NB) is the most common solid extracranial pediatric tumor. Its low mutational burden points out the need to find new epigenetic markers for better diagnosis and treatment monitoring. Analysis of 5hmC changes in tumor samples requires cost-effective high-resolution techniques. We developed a bisulfite-free approach for 5hmC profiling at single-nucleotide resolution, named hmTOP-seq (5hmC-specific tethered oligonucleotide-primed sequencing) (Gibas et al. (2020) PLoS Biol 18(4):e3000684), which is based on the direct sequence readout primed at covalently labeled 5hmC sites from an in situ tethered DNA oligonucleotide. hmTOP-seq was validated on a model bacteriophage genome and mouse embryonic stem cells, indicating quantitative, single-base resolution detection of 5hmC. We showed that hmTOP-seq is capable to detect subtle differences in the strand-specific CG hydroxymethylation and also allows 5hmC identification in a non-CG context. After extensive validation we employed this new approach for genome-wide 5hmC profiling of different NB cells grown under atmospheric or hypoxic conditions. A combined analysis of 5hmC and transcriptome demonstrated hypoxic signatures of NB cells and defined a link between gene expression and hypoxic 5hmC changes, suggesting epigenetic 5hmC functions in response to oxygen deprivation. Furthermore, we demonstrated that 5hmC well characterizes cell identities of various NB cells, proposing their different malignant transformations and diverse involvement in NB progression. Altogether, hmTOP-seq is a valuable cost-effective technique for detection of 5hmC profiles in various tissues and cell types that could help explore tumor heterogeneity. * The authors marked with an asterisk equally contributed to the work.

## Molecular Genetics, Education

SpT-01-01

**Rearrangement of nuclear lamina complexes during stress – identification and quantification of lamin- associated proteome after heat shock induction.**

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One of the most important structural component of the nuclear envelope in the cell nucleus is nuclear lamina. The major component of the nuclear lamina are lamins (type V intermediate filaments). They are playing a key role in nuclear assembly, chromatin organization and regulation of cytoskeleton organization. Two types of lamins are distinguished due to their structural features: A- and B-type. In this study, we focused on the identification of potential lamin protein partners and the rearrangement of those complexes after stress induction. The best-known stress is heat shock response which is based on activation of a single transcription factor - HSF. For studies, we chose the Drosophila melanogaster model system, since it has only one isoform of each lamin (C and Dm, which corresponds to A- and B-type respectively). Experiments were performed on Drosophila Kc cell line. To investigate the lamin- associated proteome the co-immunoprecipitation of cross-linked cell extracts against specific antibodies against lamin was performed followed by tandem mass spectrometry analysis. We observed several differences in the quantitative and qualitative analysis of the protein composition after stress induction. A significant increase in the number of interacting proteins has been noticed after heat-shock. Functional analysis of those identifications showed that proteins that occurred only after heat shock induction are mainly responsible for RNA binding, nucleic acid binding and ATP activity. Moreover, we demonstrated that heat shock increases the solubility of lamin Dm and associated proteins such as topoisomerase II. This finding led us to a suggestion that heat shock indicates changes in the lamin phosphorylation rate. In conclusion, we believe that lamins may play a key role in the regulation of transcription after stress induction either by changes in interaction with chromatin or changes in lamin- associated composition.

## Protein Turnover, Organisms-Molecules Interactions

SpT-02-02

**Altered autophagy-dependent c-Src/Fyn degradation in Huntington's Disease – impact on NMDAR activity**

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Huntington's Disease (HD) is an autosomal dominant progressive neurodegenerative disorder that affects the striatum and later the cortex, with no effective therapies. Mutant huntingtin (mHTT), the main HD hallmark, participates in reactive oxygen species (ROS) formation, mitochondrial dysfunction and modified NMDAR activity. Importantly, c-Src and Fyn, two members of the Src Kinase Family activated by ROS, are enriched in striatal neurons, and are implicated in brain neuronal transmission, synaptic regulation of NMDAR activity and mitochondrial function. These observations favor a common inter-player between mHTT and HD-related neuronal dysfunction, suggesting a relevant role for c-Src/Fyn-regulated pathways in HD pathogenesis, which has been largely unexplored. Thus, in this study, we analyzed the levels/activation of c-Src/Fyn in different HD models as well as the influence of autophagy on c-Src/Fyn regulation. We also investigated the role of these tyrosine kinases on NMDAR regulation in HD context. Our data showed consistent decreased c-Src/Fyn levels and activation in several models, namely in human postmortem brain samples, brain tissue and primary neurons derived from YAC128 transgenic mice and STHdhQ111/Q111cells,along with augmented c-Src/Fyn degradation by autophagy in HD, which can partially explain c-Src/Fyn decreased levels. Moreover, YAC128 mouse primary striatal neurons evidenced decreased c-Src/Fyn levels in distal neurites and postsynaptic density, as well as diminished PSD-95 levels and puncta, suggesting a role of c-Src/Fyn on synapse number in HD neurons. Concordantly, decreased c-Src/Fyn in YAC128 mice was accompanied by decreased Tyr1472 phosphorylation of GluN2B-composed NMDAR and by decreased NMDAR-mediated intracellular Ca2+levels. Thus, we demonstrate for the first time a direct involvement of c-Src/Fyn tyrosine kinases in HD pathogenesis, supporting that c-Src/Fyn-related pathways may constitute potential neuroprotective targets in HD.

SpT-02-01

**Conformational flexibility and allosteric modulation in a heme-binding resurrected ancestral glycosidase**

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Resurrected ancestral proteins generally display unusual properties which reflect ancestral adaptations to conditions that differed from the conditions in which extant proteins operate. In this work, we present the remarkable biophysical and biochemical properties of a putative resurrected ancestor of bacterial and eukaryotic family GH1 glycosidases. The ancestral protein conserves the typical TIM-barrel fold of GH1 glycosidases, with a rigid core barrel that enables an optimum enzymatic activity temperature within the experimental range of thermophilic GH1 glycosidases. However, the rest of the structure displays large regions with greatly enhanced conformational flexibility as determined by experimental and computational approaches. Remarkably, the ancestral protein binds a heme molecule tightly and stoichiometrically in a well defined buried site. Heme binding triggers an increase in the catalytic power through an allosteric modulation of enzymatic activity likely linked to the rigidification of the protein structure. These results suggest a complex molecular evolutionary history for family GH1 glycosidases, and demonstrate the capability of ancestral protein resurrection to reveal novel and valuable biomolecular features. The potential of the ancestral glycosidase as a flexible scaffold for custom catalysis and biosensor engineering is discussed.

## How to Improve Immunotherapy of Cancer?

EB-02-1

**An enhanced CRISPR tool for treating chronic myelogenous leukemia**

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The CRISPR/Cas system is a highly potent tool which has revolutionized genome engineering and regulation of gene transcription in various cells and organisms. This gene-editing tool consists of a guide RNA (gRNA) and Cas9 endonuclease. Cas9 catalyzes the formation of double-strand DNA breaks, which are then repaired by different cell mechanisms. Error-prone Non-homologous end joining occurs, resulting in random indel (insertion-deletion) mutations, which can lead to functional gene inactivation by either frameshift or deletions. To achieve greater indel mutations, CRISPR system can be coexpressed in cells with DNA exonucleases, which cause increased recessions of DNA following DNA breaks. We show that joint action of the CRISPR system with different exonucleases significantly increases the percentage of indel mutations at various targeted genes. Of the different exonucleases tested, the E.coli-derived exonuclease III (EXOIII) exhibited the best performance in terms of indel formation. To further improve the rate of indel mutations, Cas9 and EXOIII were brought into the proximity via coiled-coil forming heterodimeric peptides (CCExo). This resulted in increased indel formation compared to the classical CRISPR/Cas system as well as more efficient than cointroduction of non-interacting and genetically fused Cas9-EXOIII. We performed a case study for the use of the CRISPR-EXO system as a potential anti-cancer therapeutic tool. The Philadelphia chromosome, which occurs in leukemic cancer cells, is the result of characteristic the reciprocal genome translocation t(9:22) and is responsible for higher proliferation of tumorous cells. Using the CCExo system, we achieved a higher degree of indel mutations at the translocation site, which resulted in greater killing of cancer cells, thus providing a useful potential anti-cancer therapeutic tool.

## Neurobiochemistry

ShT-03.1-1

**Altered Levels of Cholinesterase Splice Variants in Parkinson's and Alzheimer's Disease Brains**

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Alzheimer's and Parkinson's diseases (AD, PD) are the most common neurodegenerative disorders, and both are accompanied by imbalanced acetylcholine signaling. Here, we report that the cholinergic imbalance in AD and PD brains associates with post-transcriptional, but not inherited variations in the brain-expressed cholinesterase genes controlling acetylcholine hydrolysis. Briefly, we sought genetic variants and transcript levels of the butyrylcholinesterase (BChE) gene and quantified alternatively spliced acetylcholinesterase (AChE) transcripts in the Amygdala (AMG) and Substantia Nigra (SN) of PD brains and in the Superior temporalis gyrus (STG) of AD brains. Both the coding sequence BCHE-K allele (rs1803274) and the single nucleotide polymorphisms (SNPs) in the 5'UTR (rs1126680) and intron 2 (rs55781031) BCHE alleles showed similar incidence in diseased and healthy brains. However, compared with control brains, BChE levels increased in AD brains but were unchanged in the PD SN and AMG. Furthermore, quantitative qPCR tests revealed brain region-specific decline of the 'synaptic' membrane-bound AChE-S variant, the soluble stress and increases in the anxiety-related Readthrough AChE-R variant and the N-terminally extended AChE-Next variant. Specifically, the major AChE-S variant declined in the PD SN and the AD STG, but not in the PD AMG, indicating association with the loss of neurons in the affected brain regions. Inversely, the soluble AChE-R variant was elevated ($p < 0.05$) in the AD STG and the PD AMG, but not the SN, compatible with stress-related roles for this neuronal AChE splice variant in the deteriorating brains; whereas the AChE-Next variant showed elevated levels ($p < 0.05$) in the PD AMG, but not in the neurons-deprived PD SN and the AD STG. The AMG-specific AChE-R increases in AD and PD brains and the AChE-Next excess in the PD, but not AD brains may reflect disease-specific post-transcriptional modifications, compatible with patients' stress symptoms.

## Imaging for Life: From Molecules to Organisms

ShT-04.3-1

**Early fluorescent imaging of de novo synthesized proteins based on coiled-coils heterodimerization**

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The fluorescent proteins, which are covalently fused to target proteins, are commonly used for imaging in living cells. However, their use for imaging of de novo synthesized proteins is hindered by a slow maturation of fluorophore. Here we report a novel method of imaging the nascent proteins with transiently interacting artificial helices (K/E-coils). We have recently shown that K/E-coils despite their small size (21-28 amino acids) and low affinity are specific enough to be used for imaging intracellular proteins (Perfilov MM et al. (2020) Cell Mol Life Sci 77, 4429–4440). To achieve the labeling, one has to express in the cell simultaneously protein of interest tagged by one coil and a fluorescent protein tagged by the complementary coil. Furthermore, an excess of the pre-matured fluorescent protein is maintained in the cytoplasm, readily available to bind and therefore mark the location of the nascent protein of interest. We tested this idea by simultaneous labeling of caveolae with red fluorescent protein mCherry via K/E-coils and fused with green fluorescent protein EGFP (mCherry-K + caveolin-EGFP-E). After the appearance of the red signal in the cytoplasm, we triggered the synthesis of caveolin, using doxycycline-dependent system. Following the induction, we observed the translocation of mCherry-K to structures resembling caveolae, followed by a delayed appearance of a green signal from caveolin-EGFP-E, consistent with maturation time of EGFP. Also, our preliminary data shows the increased labeling contrast of nascent proteins when using multi-coils: for example, caveolin-EGFP-E-E-E recruits more mCherry-K than caveolin-EGFP-E, therefore increasing the structure/cytoplasm signal ratio. To conclude, we developed a simple approach for imaging of de novo synthesized proteins, bypassing the limit imposed by chromophore maturation of fluorescent proteins. Work was supported by Ministry of Science and Higher Education of Russian Federation (grant 075-15-2020-773).

## Membranes

ShT-03.3-1

**The unique story of Pleurotus aegerolysins: From specific lipid binding to potential biopesticides**

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Aegerolysins from the fungal genus ***Pleurotus***, namely ostreolysin A (OlyA), pleurotolysin A2 (PlyA2) and erylysin A (EryA), were recently found to interact strongly with lipid vesicles that contained ceramide phosphoethanolamine (CPE), the major sphingolipid in invertebrate cell membranes and an analog of sphingomyelin, which dominates in vertebrates. Furthermore, OlyA6, PlyA2 and EryA were shown to combine with pleurotolysin B (PlyB) or erylysin (EryB), 59-kDa protein partners with a MACPF domain, to form multimeric bi-component, A2B-type, transmembrane cytolytic complexes. We provide insights into the molecular mechanisms of the interaction of ***Pleurotus*** aegerolysins with membranes containing CPE as well as their specificity to this sphingolipid. Moreover, we characterized their pore formation in the presence of PlyB or EryB. Interestingly, spectral FRET analysis showed that monomers of fluorescently labeled OlyA pack closely together only on CPE-containing membranes. These aegerolysins bind to insect cells and artificial lipid membranes at physiologically relevant CPE concentrations. Moreover, aegerolysins permeabilize these membranes when combined with PlyB. OlyA/PlyB, PlyA2/PlyB and EryA/PlyB complexes have shown a selective toxic effect on Colorado potato beetle (CPB) larvae and Western corn rootworm (WCR), and not to other tested insect pests. Using brush border membrane vesicles (BBMVs) from WCR and CPB larvae, we evaluated the presence of other potential aegerolysin receptors. The current study highlights the unique binding of aegerolysins to CPE-containing membranes and their ability to form transmembrane pores with MACPF protein partner, thus suggesting their possible use as biopesticides for controlling selected insects.

## Signals, Membranes, Glycans and Lipids

SpT-03-03

**Tebuconazole induces apoptosis through ROS-mediated endoplasmic reticulum stress pathway**

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Tebuconazole (TEB) is an effective systemic fungicide that belongs to the triazoles family. It has been widely used in both agricultural and medical sectors for the control of fungal diseases. This study investigated the mechanism of TEB-induced toxicity in human embryonic kidney cells (HEK293). Our results showed that TEB activated endoplasmic reticulum (ER) and unfolded protein response as evidenced by up-regulation of GRP78, GRP94 and GADD34, splicing of XBP1 mRNA, and expression of the proapoptotic factor CHOP. This ER stress response was accompanied by the induction of the mitochondrial apoptotic pathway. Apoptosis occurred with ROS production, drop in mitochondrial membrane potential and caspase activation. Pretreatment of cells with the chemical chaperone 4-phenylbutyrate (PBA), known to alleviate ER stress, prevented significantly the apoptotic process triggered by TEB. The treatment of cells with the ROS scavenger N-acetyl cysteine inhibits the ER stress response and prevents mitochondrial apoptosis. Taken together, these results suggest that TEB induces cytotoxicity through a ROS-dependent mechanism involving ER stress and activation of mitochondrial apoptotic pathway in human kidney cells.

## Plant Biotechnology

ShT-05.4-1

**Developing insect sex pheromone production in plants with the support of transcriptomic data**

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Use of insect sex pheromones has become an important part of integrated pest management in agriculture as they provide a species-specific control of insect pests and contribute to reduction in conventional insecticide use. Despite of the great potential, their widespread use is still limited due to unsustainable and not cost-effective manufacturing by chemical synthesis. A green alternative, biomanufacturing in plants, is a goal of the SUPSHIRE project, which aims to upgrade the current proof-of-concept pheromone producing plant lines, called the SexyPlants, which successfully synthetize moth (Lepidoptera) pheromones. Our goals are twofold: to improve the plant chassis by removing molecular bottlenecks that cause growth penalty in lines with high pheromone yields and to develop new biosynthetic pathways, enabling synthesis of unique and chemically complex monoterpenoid pheromones of insects from the Coccoidea family. So far, we have used transcriptomic data to identify differentially expressed genes between the high and low producing SexyPlants, which where visualised in the MapMan tool and used for gene set enrichments analysis. This enabled us to pinpoint the cellular processes that are most affected by higher pheromone production, e.g. gibberellin synthesis. We are now working on network analysis to more specifically identify key molecular pathways and genes that lead to growth arrest. To develop the new biosynthetic pathway for production of Coccoidea pheromones, we have generated RNA-seq expression data from citrus mealybug (***Planoccoccus citri***) and combined it with functional predictions of contigs in the prepared ***de novo*** transcriptome assembly to extract candidate genes responsible for key conversions in the sex pheromone synthesis. Genes with confirmed desired enzymatic activity are used as baits in coexpression network analysis to find the full synthetic pathway that could be implemented in the plant chassis.

## Pharmacogenomics and Biomarkers

ShT-06.3-1

**Insights into the biological role of thiopurine S-methyltransferase**

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Thiopurine ***S***-methyltransferase (TPMT) is an important enzyme involved in deactivation of thiopurine drugs. Its activity represents a major determinant of thiopurine-related toxicities. However, the endogenous role of TPMT is still unknown. Our genome-wide expression population studies suggested a close relation of TPMT to genes involved in oxidoreductive processes. Since selenium is a representative micronutrient involved in oxidoreductive reactions in biological systems and certain selenium-containing organic compounds have chemical properties similar to thiopurines, we investigated the role of this enzyme in selenium metabolism. By STD NMR spectroscopy and tryptophan fluorescence measurements, we showed that selenocysteine (Sec) binds ***in-vitro*** to human recombinant TPMT. After the incubation of Sec with TPMT and methyl donor, ***S***-adenosylmethionine, a methylated product, ***Se***-methlyselenocysteine, was formed. To explore the biological relevance of this finding, we performed experiments on lymphoblastoid cell lines (LCLs) from different individuals. LCLs were genotyped for TPMT genetic variants and TPMT activity was measured in all cells. We observed genotype-activity correlation – LCLs with at least one variant TPMT allele had TPMT activity significantly decreased. When evaluating the sensitivity of the cells to selenium compounds with proliferation assay, we found that LCLs with wild-type TPMT were less sensitive to Sec and sodium selenite compared to LCLs with heterozygous or double-variant genotype. Applying similar approach, results were further confirmed on knock-out Hap1 cell model and Hek293T cells overexpressing TPMT. When inducing oxidative stress, the production of reactive oxygen species was lower, if cells were pre-treated with Sec2 and had low TPMT activity. This study revealed Sec as the first known biological compound acting as a substrate for TPMT. Further studies are needed to elucidate the exact role of TPMT in selenium-mediated oxidative reactions.

## Biochemistry of Toxins

ShT-07.1-1

**Molecular characterization of the ΦX174-E mediated cell lysis pathway**

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The rapidly emerging microbial resistances become a serious threat to global public health and the discovery and development of new antibiotics experiences a new peak of interest. In particular, drugs addressing enzymes located in the inner cell membrane are rare and highly desired. Bacteriophages such as ΦX174 efficiently lyse bacterial cells by small membrane bound toxins. The ΦX174-E toxin requires activation by the chaperone SlyD and inhibits cell wall precursor forming enzyme MraY. However, the final cell lysis by large pore formation may require close interaction with additional targets. Functional and structural details of the lysis mechanism could not be studied so far due to its high toxicity in conventional cell-based expression systems. We combine cell-free expression systems with nanoparticle technologies to synthesize the functionally folded full-length ΦX174-E toxin and engineered derivatives. By this new approach, the toxins are co-translationally inserted into preformed nanodiscs contaning defined membrane compositions. This strategy allowed studying the role of SlyD in ΦX174-E activation as well as subsequent mechanisms of membrane insertion and cell lysis at the molecular level. We could show for the first time that SlyD keeps the hydrophobic toxin in solution and catalyzes its membrane insertion. Furthermore, by implementing native mass spectrometry techniques, we could show that ΦX174-E forms higher oligomeric assemblies in the membrane. By co-insertion of MraY enzymes together with ΦX174-E toxins into the same nanodisc particles, we could demonstrate the inhibition of the cell-wall precursor formation. The established fully defined synthetic system allows to address key questions of bacteriophage toxin mediated lysis of bacterial cells. Detailed functional and structural studies including NMR spectroscopy and electron microscopy will reveal underlying mechanisms of toxin function such as target selectivity and pore forming mechanisms.

## Bioinformatics and Computational Biology

ShT-06.5-1

**A rational approach for structure design based on a new architectural level**

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Computational Protein Design is a challenging and emerging field of computational biology. Its goal is to create new proteins with new functions. While most work are focused on the discovery of the appropriate sequence to fold in a given architecture, some aim to define original and new structures. But despite recent breakthroughs in the field, designed proteins are small or display simple conformations, thus limiting the possibilities. A simple way to build new complex objects consists in the combination of some more simple parts. Identify and use such parts of protein architecture could ease and rationalise the creation of new proteins. Well-known protein structure organisation levels are secondary structures and domains, respectively too simple or too complex to be efficiently reassembled in new ensembles. Since the birth of structural biology field, the existence of an intermediate level have been discussed and recurrent structural motifs called supersecondary structures have been identified. These intermediate-sized motifs are mandatory to explain protein evolution and folding. Still, only a few have been discovered so far. We thus systematically and exhaustively characterized suitable sub-regions of proteins with complexity and size between secondary structures and domains that we denominated Protein Units. We demonstrated that these 3.000 recurrent structural motifs identified without a priori correspond to a new level of structural organisation and would be useful objects to build artificial proteins. We also studied their implication in protein evolution, genesis and folding.

## Synthetic and System Biology, Bioinformatics and Biomarkers

SpT-05-02

**Programmable External Network based Compartmentalized Self-Replication (PEN CSR): a new method for in vitro directed evolution of enzymes**

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Directed evolution is a well-established method for enzyme engineering. Mimicking the process of Darwinian evolution with iterative cycles of genetic diversification and selection, researchers can find new enzyme variants with enhanced or completely new properties. Despite engineered enzymes are often intended for use in unnatural environments, existing methods allowing high-throughput in vitro selection conditions can only be applied to polymerases replicating their own genetic sequence in microdroplets. Here, we used an external DNA-based artificial network to create a feedback loop linking the activity of a nicking enzyme to the replication of its own gene. Taking the enzymatic activity at the input, the molecular program is producing a correlated amount of specific primers that are necessary for the PCR amplification of the gene. Bacteria carrying and expressing the mutants are co-encapsulated and lysed with the molecular program in individual droplets using microfluidics. The isothermal primers amplification by the network is initiated by raising the temperature to 45°C. The yield of the PCR then launched in each droplet depends on the amount of primers, therefore on the enzyme activity. After emulsion breakage, we retrieve a gene pool enriched in the best mutant genes. We applied the method to select for faster or more thermostable enzymes. After one selection cycle, next generation sequencing using MinION allowed us to detect key mutations involved in the improvement of these two traits. We generated mutants of the nickase by introducing some of these mutations in the wild-type sequence and could confirm that indeed these mutants had improved properties with sometimes additive effects. This work is the first demonstration of the Programmable External Network based CSR (PEN CSR) method. Programs detecting other types of activity can be envisioned and would allow not only to greatly expand the scope of the CSR but also to implement smart selection functions.

SpT-05-01

**The impact of repetitive scuba dives on the cardiovascular, muscular and immune system function and integrity biomarkers**

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Recreational SCUBA (self-contained underwater breathing apparatus) diving is a special form of physical activity, which due to specific environmental conditions, triggers a stress response of the organism. To explore whether repetitive recreational SCUBA (rSCUBA) diving triggers an adaptive response of the cardiovascular (CV), muscular, and immune system, we measured the cardiac damage (NT-proBNP, hs-TnI, and CK-MB), muscle damage (myoglobin (Mb), galectin-3, CK, and LDH), vascular endothelial activation (ET-1 and VEGF), and inflammatory (leukocyte count (Lkc), CRP, and IL-6) biomarkers. A longitudinal intervention study included divers (N = 14) who conducted one dive per week over 5 weeks at the depth of 20-30 m for 30 min after a non-dive period of 5 months. The blood samples were collected before and after the 1$^{st}$, 3$^{rd}$, and 5$^{th}$ dives and specific biomarkers were measured in plasma or serum by the standard laboratory methods. The concentrations of the majority of measured biomarkers increased after every single dive; the exception was ET-1 concentration that decreased. The cumulative effect of five dives has been reflected in diminishing changes in hs-TnI, Mb, galectin-3, ET-1, VEGF, and IL-6 levels, and more pronounced increases in NT-proBNP and hs-CRP levels. The median values of all measured biomarkers in all time points, except Mb, remained within the corresponding reference range. This study showed that rSCUBA diving causes changes in specific biomarkers that reflect (patho)physiological changes in CV, muscular, and immune system after the dive. However, it is shown for the first time that contiuously preformed SCUBA diving caused decrease of specific biomarkers highlighting possibly positive effect of diving on CVS.

## Molecular Medicine

SpT-06-01

**Human aquaporin-5 selectivity and peroxiporin activity modulate cell survival and cancer cell migration**

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H2O2 is the main reactive oxygen species (ROS) involved in oxidative sensing and signaling. This molecule has a dual effect: at low concentrations can act as a second messenger in redox signaling, while at high concentrations contributes to oxidative stress. A few mammalian aquaporins (AQPs) called peroxiporins, are known to facilitate H2O2 permeation through membranes, controlling its intracellular concentration and participating in tumorigenesis. Both AQP3 and AQP5 are overexpressed in cancer tissues and AQP3 peroxiporin activity has been related with cancer cell migration. Here, we report that human AQP5 also facilitates H2O2 diffusion through membranes and has a role in modulating cell growth and resistance to oxidative stress. By mutagenesis studies we found that His173 located in AQP5 selectivity filter is crucial for AQP5 permeability, and its proximity to phosphorylated Ser183 may impair permeability through pore constriction. Moreover, AQP5-silenced pancreatic cancer cells showed impaired cell migration. Our data disclose the important residues for AQP5 water conductance and reveal a major role of this channel in the fine-tuning of intracellular H2O2 concentration with impact in cell survival under oxidative stress, suggesting that human AQP5 is a promising target for cancer therapeutics.

## Immune and Inflammatory Disorders

ShT-08.3-2

**Mice lacking DNA repair factor XLF and MRI show leaky severe combined immunodeficiency (SCID)**

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Non-homologous end-joining (NHEJ) is a DNA repair pathway required to detect, process, and ligate DNA double-stranded breaks (DSBs) throughout the cell cycle. The NHEJ pathway is necessary for V(D)J recombination in developing B and T lymphocytes. During NHEJ, Ku70 and Ku80 form a heterodimer that recognizes DSBs and promotes recruitment and function of downstream factors PAXX, MRI, DNA-PKcs, Artemis, XLF, XRCC4, and LIG4. Inactivation of ***Ku70*** or ***Ku80*** genes in mice results in severe combined immunodeficiency phenotype (SCID) and high levels of genomic instability. Deletion of the ***Dna-pkcs*** gene results in SCID, while inactivation of ***Xlf***, ***Paxx***, or ***Mri*** results in viable mice with no or modest phenotypes. A combined deficiency of XLF and PAXX, or XLF and MRI, results in embryonic lethality in mice, which correlates with extensive apoptosis in the central nervous system. These findings indicate important overlapping functions between PAXX, MRI and XLF. To determine if the embryonic lethality is ***Trp53***-dependent, we intercrossed mice homozygous for ***Xlf*** null allele and heterozygous for both ***Mri*** and ***Trp53*** (***Xlf***$^{-/-}$***Mri***$^{+/-}$***Trp53***$^{+/-}$). We demonstrated that inactivation of pro-apoptotic factor p53 rescues embryonic lethality of ***Xlf***$^{-/-}$***Mri***$^{-/-}$ double knockout mice. Similarly, we rescued the embryonic lethality of ***Xlf***$^{-/-}$***Paxx***$^{-/-}$ mice. We demonstrated that the triple-deficient mice possessed reduced body weight, size of spleens and thymi, lack of mature B cells in the spleen, and dramatically reduced numbers of T cells in both spleen and thymus. Moreover, there was an accumulation of progenitor B cells in the bone marrow of triple deficient mice. Overall, we concluded that X***lf***$^{-/-}$***Mri***$^{-/-}$***Trp53***$^{+/-}$ and ***Xlf***$^{-/-}$***Paxx***$^{-/-}$***Trp53***$^{+/-}$ mice possess leaky SCID phenotype.

## Cancer Initiation and Progression

ShT-08.2-1

**Phenotype switching in melanoma cells resistant to targeted therapy**

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Melanoma is an aggressive malignancy that is, despite being a rare type of cancer, responsible for the vast majority of skin cancer-related deaths. Recent advances in melanoma therapy, like targeted therapy and immunotherapy, contributed profoundly to the increased overall survival of patients. Nevertheless, the development of resistance to therapy remains a major clinical issue. Targeted therapy, like the BRAF inhibitor (BRAFi) therapy for melanoma patients harboring the V600E mutation, is initially highly effective, but the majority of patients develop resistance and relapse within a few months. To better understand the mechanisms of resistance to the BRAFi targeted therapy, we generated cell lines resistant to vemurafenib, a BRAF inhibitor used for the treatment of late-stage melanoma with the common BRAFV600E mutation. Vemurafenib-resistant human melanoma cell lines were generated by growing primary melanoma, WM793B cell line, and metastatic melanoma, A375M cell line, both harboring the BRAFV600E mutation, in the vemurafenib-enriched medium. The occurrence of resistance was confirmed by MTT assay. Newly generated resistant cell lines showed immense phenotype changes in terms of cell migration and proliferation. Our results indicated partial EM transition, which is known to increase invasive cell properties, promoting resistance to anti-cancer drugs. We performed mass-based parallel cell mRNA sequencing (RNA-seq) and found that the mechanism of resistance differs between the two cell lines. Furthermore, we have demonstrated a significant downregulation of metastasis suppressor genes, NME1 and NME2 and the p53 protein isoform Δ133p53β, which was shown to promote cancer cell invasion. A number of previous studies suggested several mechanisms of resistance and phenotype switching in targeted therapy. Our results set a new direction for further research in therapy resistance that needs to be elucidated.

## Aging Stress and Neurodegeneration

ShT-08.4-1

**Influence of the BCHE gene polymorphism on the inhibition of butyrylcholinesterase by biscarbamates, a potential Alzheimer's disease drugs**

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In the middle and late stages of Alzheimer`s disease (AD), the activity of butyrylcholinesterase (BChE), a co-regulator of acetylcholine levels in brain and muscles increases, indicating that selective inhibition of BChE can represent a new pathway in treating AD. The ***BCHE*** gene is highly polymorphic and thus far, 66 isoforms of BChE have been discovered. Clinically, the most interesting is atypical BChE because people with it are not able to hydrolyse succinylcholine, a positively charged muscule relaxant and can experience prolonged apnea if it is administered. The efficiacy of any drug that targets BChE activity can be affected by human ***BCHE*** gene polymorphisms. We designed and synthesized six biscarbamates with a modified carbamoyl and amine part of the molecule with the aim of determining their BChE inhibition potency toward usual and atypical BChE. The order of magnitude of the carbamylation rate constants $k_i$ of all six biscarbamates for usual BChE was $10^3$ $M^{-1}min^{-1}$, which makes these carbamates fast inhibitors for usual BChE. The carbamylation rates of atypical BChE were 400 to 1,500 time slower compred to usual BChE, proving to the fact that new carbamates are very discriminative to usual BChE. The most discriminative were bisdiethylcarbamates indicating that the carbamylation rate of atypical BChE depends on the substituents on the carbamoyl part of the molecule. In order to evaluate synthesized compounds as potential drugs, their cytotoxicity on human hepatic and neuronal cell was determined. The determined $IC_{50}$ values showed that adamantyl in the amine part of the molecule decreases carbamate drugability since these carbamates are toxic in concentration ranges used for the inhibition of both types of BChE.

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ShT-08.4-2

**Inhibition of cathepsin X as a novel strategy for the treatment of neuroinflammation-associated diseases**

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Inflammation is closely implicated in the pathogenesis of several neurodegenerative disorders, including Parkinson's disease (PD) and multiple sclerosis (MS), where the hallmark of neuroinflammation is activated microglia. Microglia-derived lysosomal cathepsins have been increasingly recognized as important inflammatory mediators that trigger signalling pathways that aggravate neuroinflammation. In the past, a contribution to neuroinflammation processes has been shown for cathepsin X in vitro, however; the expression patterns and functional roles of cathepsin X in neuroinflammatory brain pathology remained elusive. Our recent studies revealed a strong neuroinflammation-induced upregulation of cathepsin X expression and activity using in vivo models that mimic the pathology of PD and MS, respectively. Unilateral injection of lipopolysaccharide into the rat striatum induced strong upregulation of cathepsin X expression and its activity in the ipsilateral striatum and in other brain areas such as cerebral cortex, corpus callosum, subventricular zone and external globus pallidus, whereas the upregulation was mainly restricted to activated microglia and reactive astrocytes. Similarly, a marked increase in expression and activity of cathepsin X was observed in spinal cord in rat model of experimental autoimmune encephalomyelitis. Additionally, cathepsin X upregulation was observed in injured peripheral nerve, localized in the inflammatory cell type, M1 macrophages. Nevertheless, continuous administration of the cathepsin X inhibitor showed moderate protective effects against neuroinflammation-induced degeneration; further indicating that cathepsin X plays a role as a pathogenic factor in neuroinflammation-induced neurodegeneration and represents a potential therapeutic target for neurodegenerative diseases associated with inflammation.

# Metabolic Engineering: Emerging Technologies for Industrial Process Development

ShT-05.1-1

**Dye-decolorizing peroxidases from Streptomyces coelicolor show organosolv lignin remodeling activity**

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Lignin is the second most abundant biopolymer on earth and makes up about 15–30% of plant biomass. Due to its complex and highly phenolic and heterogeneous nature, lignin is by many seen as a green alternative to petrochemicals. The chemically recalcitrant nature of lignin provides plant biomass with structural rigidity and resistance against degradation by the elements. Several groups of organisms have been identified that can use the lignin portion of lignocellulose as a carbon source. Almost four decades ago some soil bacteria in the actinomycete phylum were identified as lignin degraders by Crawford and coworkers. Thanks to availability of full genome sequences, we now know that these organisms lack a type of the redox enzymes typically associated with fungal biochemical pathways that lead to lignin mineralization – manganese peroxidases (MnPs). Although streptomycetes and other soil bacteria are devoid of MnPs, they encode a different class of heme-containing peroxidases – dye-decolorizing peroxidases (DyPs). We have cloned and heterologously expressed the three DyP-type peroxidases from ***Streptomyces coelicolor*** – SCO2276, SCO3963 and SCO7193. All three enzymes are able to oxidize the classical peroxidase substrates, such as 2,6-dichlorophenol. However, using HPLC techniques, we also show that all three DyP-type peroxidases can use organosolv lignin as a substrate. We thus hypothesize that next to the small laccase of ***S. coelicolor***, the DyP-type peroxidases fulfill the second oxidative enzyme class category required by that organism to break down lignin.

## Structural and Functional Glycobiology

ShT-09.1-2

**The plasticity of the beta-trefoil-type lectins from higher fungi**

**J. Sabotič**[I], J. Kos[I,II]

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Lectins are a diverse group of proteins that specifically bind carbohydrates and act as recognition molecules through binding to glycosylated ligands playing essential cellular and biological functions. We have described lectins isolated from fruiting bodies of different higher fungi, including clouded agaric (***Clitocybe nebularis***) and parasol mushroom (***Macrolepiota procera***). These exceptionally stable proteins share low sequence similarity but similar biochemical properties, which are summarized in the common 3D structure, the beta-trefoil fold. It is formed by the core six-stranded beta-barrel which supports 11 loops of different shapes and composition that provide a versatile surface for different types of interactions. These lectins display extraordinary versatility of carbohydrate-binding specificity, the position of glycan-binding sites and dimerization plasticity. Their unique features are reflected also in their biological activity showing immunomodulatory properties and high specificity and selective toxicity against different cancer cell lines. Lectins from higher fungi offer a wide variety of possible applications in the fields of biotechnology and medicine. Previously published in: Sabotič J and Kos J. (2019) Molecules 24, 4204.

# List of Participants

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| 1. | **Abelyan Narek** | Russian-Armenian University | Yerevan, Armenia | Lipa |
| 2. | **Abramović Irena** | University of Zagreb, School of Medicine | Zagreb, Croatia | Marun |
| 3. | **Adam David** | Freelancer | United Kingdom | Speaker |
| 4. | **Aldag Ceyda** | Ege University, Faculty of Science, Department of Biology | Izmir, Turkey | Marun |
| 5. | **Ancic Anja** | University of Split, School of Medicine | Split, Croatia | Lovor |
| 6. | **Antičević Ivan** | Ruđer Bošković Institute, Division for Marine and Environmental Research, Laboratory for molecular ecotoxicology | Zagreb, Croatia | Zmaj |
| 7. | **Apura Patrícia** | ITQB-NOVA | Lisboa, Portugal | Lovor |
| 8. | **Arraiano Cecília** | Universidade Nova de Lisboa, Instituto de Tecnologia Química e Biológica (ITQB) | Oeiras, Portugal | Keynote |
| 9. | **Artemaki Pinelopi I.** | National and Kapodistrian University of Athens, School of Science, Faculty of Biology, Department of Biochemistry and Molecular Biology | Athens, Greece | Zmaj |
| 10. | **Bahsiev Aighiuni** | Institute of Genetics, Physiology and Plant Protection | Chisinau, Moldova | Marun |
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| 12. | **Baltanas Copado Jesus** | Dpt. Bioquimica-A, Facultad de Veterinaria, Universidad de Murcia, | Murcia, Spain | Zmaj |
| 13. | **Beagrie Robert** | University of Oxford, Weatherall Institute of Molecular Medicine | Oxford, United Kingdom | Lovor |
| 14. | **Beljan Silvestar** | University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology | Zagreb, Croatia | Lipa |
| 15. | **Ben Othmène Yosra** | University of Monastir, Faculty of Dental Medicine of Monastir | Monastir, Tunisia | Lipa |
| 16. | **Benito Asier** | Biofisika Institute (CSIC-UPV/EHU) | Leioa, Spain | Lipa |
| 17. | **Bertović Ivana** | University of Rijeka, Department of Biotechnology | Rijeka, Croatia | Marun |
| 18. | **Bielak Klaudia** | Wrocław University of Science and Technology, Faculty of Chemistry, Department of Biochemistry | Wrocław, Poland | Marun |
| 19. | **Boiangiu Razvan Stefan** | Alexandru Ioan Cuza University of Iasi | Iasi, Romania | Marun |
| 20. | **Carsana Emma Veronica** | L.I.T.A | Segrate, Italy | Lovor |

| No. | Surname and Name | Affiliation | City and Country | YSF Role or Group |
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| 22. | **Castaldo Gaetano** | European Research Executive Agency (REA) of the European Commission | Brussels, Belgium | Speaker |
| 23. | **Castaneda Zegarra Sergio Miguel** | Norwegian University of Science and Technology (NTNU), Department of Clinical and Molecular Medicine | Trondheim, Norway | Lipa |
| 24. | **Çetin Gonca** | University of Greifswald | Greifswald, Germany | Marun |
| 25. | **Chakir Salma** | Faculty of Sciences and Technology of Mohammedia, Morocco | casablanca, Morocco | Zmaj |
| 26. | **Chinak Olga** | Siberian Branch of the Russian Academy of Sciences (ICBFM SB RAS), Institute of Chemical Biology and Fundamental Medicine | Novosibirsk, Russia | Marun |
| 27. | **Crisóstomo Luís** | University of Porto, Abel Salazar Institute of Biomedical Sciences (UMIB-ICBAS), Unit for Multidisciplinary Research in Biomedicine | Porto, Portugal | Zmaj |
| 28. | **Cuevas Sierra Amanda** | University of Navarra | Pamplona, Spain | Lovor |
| 29. | **De La Rosa Miguel A.** | Scientific Research Center Isla de la Cartuja (cicCartuja) | Seville, Spain | Speaker |
| 30. | **Demirci Yeliz** | Izmir Biomedicine and Genome Center | Izmir, Turkey | Lipa |
| 31. | **Díaz-Moreno Irene** | Institute of Chemical Research of the Scientific Research Centre Isla de la Cartuja – cicCartuja | Seville, Spain | Organiser |
| 32. | **Domingues Marco** | Universidade de Lisboa, Faculty of Medicines, Instituto de Medicina Molecular (IMM) | Lisbon, Portugal | Marun |
| 33. | **Dramé-Maigné Adèle** | Institut de la Vision | Paris, France | Marun |
| 34. | **Dulić Morana** | University of Zagreb, Faculty of Science | Zagreb, Croatia | Organizer |
| 35. | **Đikić Ivan** | Goethe University Frankfurt Medical School, Institute of Biochemistry II | Frankfurt, Germany | Keynote |
| 36. | **Elliott Keith** | University of Manchester | United Kingdom | Speaker |
| 37. | **Escalona-Noguero Carmen** | IMDEA-Nanoscience | Madrid, Spain | Lipa |
| 38. | **Fão Lígia** | CNC - Center for Neuroscience and Cell Biology and IBILI - Institute for Biomedical Imaging and Life Sciences | Coimbra, Portugal | Lovor |
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| No. | Surname and Name | Affiliation | City and Country | YSF Role or Group |
|---|---|---|---|---|
| 40. | **Ferrer-Montiel Antonio** | University Miguel Hernández (UMH); Institute of Research, Development and Innovation in Healthcare Biotechnology of Elche (IDiBE) | Alicante, Spain | Speaker |
| 41. | **Frtús Adam** | Czech Academy of Sciences, Institute of Physics | Prague, Czech Republic | Zmaj |
| 42. | **Garcia Mato Angela** | Instituto de Investigaciones Biomédicas Alberto Sols | Madrid, Spain | Zmaj |
| 43. | **Gok Muslum** | Hacettepe University, Faculty of Medicine, Department of Medical Biochemistry | Ankara, Turkey | Marun |
| 44. | **Govindaraj Kannan** | University of Twente | Enschede, Netherlands | Lovor |
| 45. | **Gutierrez Rus Luis Ignacio** | University of Granada, Faculty of Sciences, Department of Physical Chemistry | Granada, Spain | Lovor |
| 46. | **Hamad Azzam** | MIPT - Moscow Institute of Physics and Technology | Dolgoprudny, Moscow Region, Russia | Zmaj |
| 47. | **Hambardzumyan Yelena** | Russian-Armenian University | Yerevan, Armenia | Lovor |
| 48. | **Hoolachan Joe** | Keele University | Newcastle-under-Lyme, United Kingdom | Lipa |
| 49. | **Horetski Matvey** | Belarusian State University | Minsk, Belarus | Marun |
| 50. | **İnanç-Sürer Şeniz** | Dokuz Eylul University | Izmir, Turkey | Lovor |
| 51. | **Jagusiak Anna** | Medical Biochemistry Jagiellonian University – Medical College | Kraków, Poland | Guest |
| 52. | **Janković Tamara** | IInstitute for the Application of Nuclear Energy INEP, Univesity of Belgrade | Belgrade, Serbia | Zmaj |
| 53. | **Jarc Jovičić Eva** | Jozef Stefan Institute, Department of Molecular and Biomedical Sciences | Ljubljana, Slovenia | Marun |
| 54. | **Jeremic Marija** | University of Belgrade, School of Medicine, Institute of Medical and Clinical biochemistry | Belgrade, Serbia | Lovor |
| 55. | **Jukić Marijana** | Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Department of Medicinal Chemistry, Biochemistry and Clinical Chemistry | Osijek, Croatia | Lipa |
| 56. | **Juteršek Mojca** | National Institute of Biology | Ljubljana, Slovenia | Lovor |
| 57. | **Katalinić Maja** | Institute for Medical Research and Occupational Health | Zagreb, Croatia | Organizer |
| 58. | **Kholina Ekaterina** | M.V.Lomonosov Moscow State University, Faculty of Biology | Moscow, Russia | Lovor |

| No. | Surname and Name | Affiliation | City and Country | YSF Role or Group |
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| 59. | **Komatović Katarina** | University of Belgrade, Faculty of Chemistry | Belgrade, Serbia | Marun |
| 60. | **Krajewska Milena** | Nencki Institute of Experimental Biology | Warsaw, Poland | Lipa |
| 61. | **Krol Alain** | CNRS; University of Strasbourg | Strasbourg, France | Speaker |
| 62. | **Kulda Vlastimil** | Charles University, Faculty of Medicine in Pilsen | Pilsen, Czech Republic | Speaker |
| 63. | **Kurgina Tatiana A.** | The Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences | Novosibirsk, Russia | Lovor |
| 64. | **Kurtović Matea** | Rudjer Boskovic Institute | Zagreb, Croatia | Marun |
| 65. | **Kvergelidze Elisabed** | Ilia State University, Faculty of Natural Sciences and Medicine | Tbilisi, Georgia | Lovor |
| 66. | **Lainšček Duško** | National Institute of Chemistry, Department of Synthetic Biology and Immunology | Ljubljana, Slovenia | Zmaj |
| 67. | **Lisek Malwina** | Medical University of Lodz, Department of Molecular Neurochemistry | Lodz, Poland | Zmaj |
| 68. | **Lynch Ciara** | University College Dublin | Dublin, Ireland | Marun |
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| 70. | **Matysniak Damian** | Nencki Institute of Experimental Biology PAS | Warsaw, Poland | Zmaj |
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| 72. | **Megušar Polona** | University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology | Ljubljana, Slovenia | Marun |
| 73. | **Mendonça Diogo** | Universidade de Lisboa, Faculdade de Medicina, Instituto de Medicina Molecular | Lisboa, Portugal | Lipa |
| 74. | **Mertoğlu Elif** | Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics | Istanbul, Turkey | Zmaj |
| 75. | **Meseldzic Neven** | University of Sarajevo, Faculty of Pharmacy, Department of Biochemistry and Clinical Analysis | Sarajevo, Bosnia and Herzegovina | Zmaj |
| 76. | **Mezhyrova Julija** | Goethe-University Frankfurt, Institute for Biophysical Chemistry & Centre for Biomolecular Magnetic Resonance | Frankfurt am Main, Germany | Lipa |
| 77. | **Narmontė Milda** | Vilnius University, Life Sciences Center, Institute of Biotechnology, Department of Biological DNA Modification | Vilnius, Lithuania | Lipa |
| 78. | **Pałka Marta** | University of Wroclaw, Faculty of Biotechnology | Wrocław, Poland | Lovor |

| No. | Surname and Name | Affiliation | City and Country | YSF Role or Group |
|---|---|---|---|---|
| 79. | **Panevska Anastasija** | University of Ljubljana, Biotechnical faculty, Department of Biology, Chair of Biochemistry | Ljubljana, Slovenia | Lipa |
| 80. | **Patrakova Ekaterina** | The Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences | Novosibirsk, Russia | Lipa |
| 81. | **Petrišić Nejc** | National Institute of Chemistry | Ljubljana, Slovenia | Marun |
| 82. | **Pawłowska Monika** | Gdańsk University of Technology, Faculty of Chemistry, Department of Pharmaceutical Technology and Biochemistry | Gdansk, Poland | Lipa |
| 83. | **Pecht Israel** | Weizmann Institute of Science, Department of Immunology | Rehovot, Israel | Speaker |
| 84. | **Pehlivan Necla** | Recep Tayyip Erdogan University | Rize, Turkey | Lovor |
| 85. | **Perfilov Maxim** | Russian Academy of Sciences, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry | Moscow, Russia | Zmaj |
| 86. | **Périn Charlotte** | DSIMB, INSERM | Paris, France | Zmaj |
| 87. | **Perret Jason** | Université Libre de Bruxelles (ULB), Faculty of Medicine | Brussels, Belgium | Speaker |
| 88. | **Pikus Polina** | Institute of Molecular Biology and Genetics NASU | Kyiv, Ukraine | Marun |
| 89. | **Pimpão Catarina** | University of Lisbon, Faculty of Pharmacy | Lisbon, Portugal | Lipa |
| 90. | **Pišlar Anja** | University of Ljubljana, Faculty of Pharmacy | Ljubljana, Slovenia | Organizer |
| 91. | **Pruchova Pavla** | The Czech Academy of Sciences, Institut of Physiology, Department of Mitochondrial Physiology | Prague, Czech Republic | Lovor |
| 92. | **Pupart Hegne** | Tallinn University of Technology, Institute of Chemistry and Biotechnology | Tallinn, Estonia | Lipa |
| 93. | **Radic Martina** | Ruđer Bošković Institute, Division of Molecular Medicine | Zagreb, Croatia | Lovor |
| 94. | **Rembeza Elzbieta** | Chalmers University of Technology | Gothenburg, Sweden | Lovor |
| 95. | **Roberts Mark** | University of Oxford | Oxford, United Kingdom | Speaker |
| 96. | **Romero Becerra Rafael** | Spanish National Center for Cardiovascular Research Carlos III (CNIC) | Madrid, Spain | Marun |
| 97. | **Sabotič Jerica** | Jožef Stefan Institute, Department of Biotechnology | Ljubljana, Slovenia | Organizer |
| 98. | **Sağin Ferhan G.** | Ege University Medical Faculty | Izmir, Turkey | Speaker |
| 99. | **Sahu Indrajit** | Technion—Israel Institute of Technology, Faculty of Biology | Haifa, Israel | Zmaj |

| No. | Surname and Name | Affiliation | City and Country | YSF Role or Group |
|---|---|---|---|---|
| L00. | **Samardzija Nenadov Dragana** | University of Novi Sad, Faculty of Sciences | Novi Sad, Serbia | Lovor |
| L01. | **Schonewolff Maribel** | University of Oxford, Department of Biochemistry | Oxford, United Kingdom | Zmaj |
| L02. | **Selin Roman** | IGIC NAS Ukraine | Kiev, Ukraine | Lipa |
| L03. | **Senjor Emanuela** | Jožef Stefan Institute, Department of Biotechnology | Ljubljana, Slovenia | Zmaj |
| L04. | **Shaw Abhirup** | University of Debrecen, Faculty of Medicine, Department of Biochemistry and Molecular Biology | Debrecen, Hungary | Lovor |
| L05. | **Sheehan-Rooney Kelly** | EMBO | Heidelberg, Baden-Württemberg, Germany | Speaker |
| L06. | **Sinčić Nino** | University of Zagreb, School of Medicine | Zagreb, Croatia | Organizer |
| L07. | **Sivkina (maiden Kozlova) Anastasiia** | Lomonosov Moscow State University | Moscow, Russia | Marun |
| L08. | **Stojanov Spasoje** | Jožef Stefan Institute, Department of Biotechnology | Ljubljana, Slovenia | Lovor |
| L09. | **Szelenberger Rafał** | University of Lodz, Department of General Biochemistry | Lodz, Poland | Lovor |
| L10. | **Tasić Jelena** | University of Belgrade, School of Medicine, Institute of Medical and Clinical biochemistry | Belgrade, Serbia | Lipa |
| L11. | **Tijardović Marko** | University of Zagreb, Faculty of Pharmacy and Biochemistry | Zagreb, Croatia | Marun |
| L12. | **Tokić Mirta** | University of Zagreb, Faculty of Science | Zagreb, Croatia | Zmaj |
| L13. | **Trojan Sonia Elzbieta** | Jagiellonian University - Medical College, Faculty of Medicine, Chair of Medical Biochemistry | Krakow, Poland | Marun |
| L14. | **Urbančič Dunja** | University of Ljubljana, Faculty of Pharmacy | Ljubljana, Slovenia | Lipa |
| L15. | **Uygun Zihni Onur** | Ege University | Izmir, Turkey | Zmaj |
| L16. | **Vardjan Nina** | University of Ljubljana, Faculty of Medicine, Laboratory of Neuroendocrinology-Molecular Cell Physiology at Institute of Pathophysiology; Laboratory for Cell Engineering at Celica Biomedical | Ljubljana, Slovenia | Keynote |
| L17. | **Vasović Tamara** | University of Belgrade, Faculty of Chemistry, Department of Biochemistry | Belgrade, Serbia | Zmaj |
| L18. | **Vavilov Nikita** | Institute of Biomedical Chemistry | Moscow, Russia | Lipa |

| No. | Surname and Name | Affiliation | City and Country | YSF Role or Group |
|---|---|---|---|---|
| L19. | **Veiga Ana Salome** | Universidade de Lisboa, Faculdade de Medicina, Instituto de Medicina Molecular | Lisboa, Portugal | Zmaj |
| L20. | **Velázquez-Cruz Alejandro** | Institute of Chemical Research (IIQ) – cicCartuja; University of Seville - CSIC | Seville, Spain | Marun |
| L21. | **Westhof Eric** | University of Strasbourg | Strasbourg, France | Keynote |
| L22. | **Wright Duncan** | FEBS Open Bio | United Kingdom | Speaker |
| L23. | **Zámbó Veronika** | Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry | Budapest, Hungary | Lipa |
| L24. | **Žarak Marko** | Dubrava University Hospital, University Department for Laboratory Diagnostics | Zagreb, Croatia | Zmaj |
| L25. | **Živković Igor** | University of Zagreb, Faculty of Science, Department of Chemistry | Zagreb, Croatia | Lovor |

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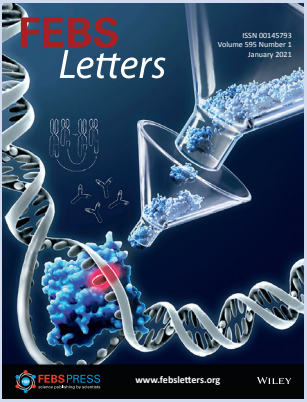


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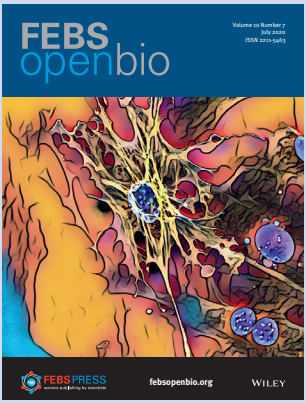


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