FEBS3+ MEETING

EXPLORING MOLECULAR FRONTIERS

Croatia · Finland · Sweden

September 25-28, 2024
Hotel Park Plaza Histria, Pula
Croatia

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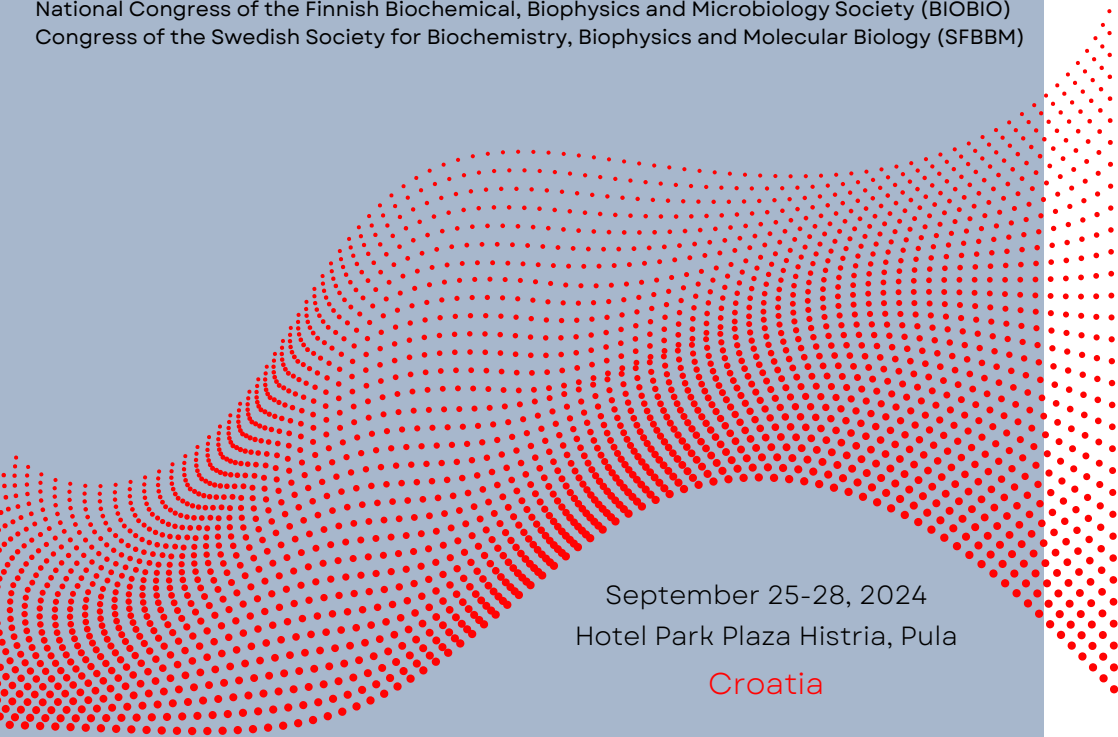
EXPLORING MOLECULAR FRONTIERS

BOOK OF ABSTRACTS

International Scientific Congress supported by the FEBS3+ Meeting Programme
National Congress of the Croatian Society of Biochemistry and Molecular Biology (HDBMB)
National Congress of the Finnish Biochemical, Biophysics and Microbiology Society (BIOBIO)
Congress of the Swedish Society for Biochemistry, Biophysics and Molecular Biology (SFBBM)



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Book of Abstracts of the International Scientific Congress supported through the FEBS3+ Meeting Programme, National Congress of the Croatian Society of Biochemistry and Molecular Biology (HDBMB), National Congress of the Finnish Biochemical, Biophysics and Microbiology Society (BIOBIO) and Congress of the Swedish Society for Biochemistry, Biophysics and Molecular Biology (SFBBM)

FEBS3+ MEETING: Exploring Molecular Frontiers

HDBMB
CROATIAN SOCIETY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

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BOOK OF ABSTRACTS

Foreword

Dear colleagues,

Welcome to the International Scientific
Congress FEBS3+ Meeting: Exploring Molecular Frontiers
supported through the FEBS3+ Meeting Programme. This conference is the National Congress of the Croatian Society of Biochemistry and Molecular Biology (HDBMB), the National Congress of the Finnish Biochemical, Biophysics and Microbiology Society (BIOBIO), and a Congress of the Swedish Society for Biochemistry, Biophysics and Molecular Biology (SFBBM). We are pleased that our Congress is being held in Pula, Istria County, one of the most beautiful coastal regions of Croatia.

The congress is organized under the auspices of the Ministry of Science, Education and Youth of the Republic of Croatia, the University of Rijeka, the University of Osijek, and the University of Zagreb. We use this opportunity to express our gratitude to them all and to all of the exhibitors, industry and institutional partners as well as donors whose support is invaluable to the overall success of the Congress.

The Scientific Programme is focused on important recent developments in a wide range of molecular life sciences including biochemistry, molecular biology, biotechnology, and biomedicine, but also on education in science.

The program is headed by five outstanding plenary lecturers: Ivan Ahel (UK), Johanna Ivaska (Finland), Simon Newstead (UK), Boris Turk (Slovenia) giving FEBS National Lecture, and Robert Harris (Sweden). In addition, the programme comprises 14 invited lectures, 19 short presentations, and 86 posters. The best short oral presentation will be awarded by Avantor sciences, and the best poster prize by FEBS Open Bio.

Framing this international environment, we hope to provide an excellent opportunity to exchange ideas and experiences with colleagues all around Europe, establish new acquaintances, and renew old ones.

Enjoy a successful and stimulating FEBS3+ Meeting and have a very pleasant stay in Pula!

Morana Dulić
Chair of the Scientific Committee

Nino Sincic
Chair of the Organising Committee

ORGANISERS

Croatian Society of Biochemistry and Molecular Biology (HDBMB), www.hdbmb.hr

Finnish Biochemical, Biophysics and Microbiology Society (Biobio), www.biobio.org

Swedish Society for Biochemistry, Biophysics and Molecular Biology (SFBBM), www.kemisamfundet.se

SUPPORTER

Federation of European Biochemical Societies (FEBS), www.febs.org

AUSPICES

Ministry of Science, Education and Youth of the Republic of Croatia
University of Rijeka
University of Osijek
University of Zagreb

SCIENTIFIC COMMITTEE

Morana Dulić, Chair
Members: Ronnie Berntsson, Hannu Koistinen, Vesa Hytönen, Magnus Wolf-Watz, Antonija Jurak Begonja, Vedrana Filić Mileta

ORGANISING COMMITTEE

Nino Sincic, Chair
Maja Katalinić, Vice-Chair
Members: Ronnie Berntsson, Ruusu-Maaria Kovanen, Igor Stuparević

LOCAL ORGANISING COMMITTEE

Members: Dora Raos, Maria Bošković, Antonio Zandona

CONGRESS SECRETARIAT

febs3pula@filidatravel.hr
febs3pula.com

VENUE

Hotel Park Plaza Histria
Verudela 17, HR-52100, Pula, Croatia

SUPPORTED BY

Ministry of Science and Education of the Republic of Croatia

FEBS - Federation of European Biochemical Societies

Institute for Medical Research and Occupational Health, Zagreb

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SPONSOR PAGE

SPONSOR PAGE

TABLE OF CONTENT

INFORMATION

01 REGISTRATION

Registration will take place at the **Registration desk in the Meeting lobby** of the Hotel Park Plaza Histria from 14:00 on Wednesday, September 25th.

Registration fee for participants includes admission to lectures and exhibitions, Book of Abstracts, congress materials, lunches, refreshments during the congress and admission to all social events including congress dinner and excursion.

Registration for accompanying persons includes all social events including congress dinner and excursion.

The certificate of attendance will be provided online after the congress.

02 LANGUAGE

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

03 LECTURES

A standard personal computer and LCD projector will be available. **The speakers are kindly asked to deliver their presentations (USB stick) in advance to our Local Organizig Committee member at the Registration desk, at least one hour before the session start.** The use of personal laptops is discouraged due to potential compatibility and timing issues. **Avantor sciences SOP Award** will be given to the best short oral presentation at the closing ceremony.

04 PLENARY ROOM

Lectures and oral presentations will be possible to follow in the "Ulika" plenary congress hall in the F2F mode. The main congress hall will accommodate the lecturers, session chairs, and up to 200 participants. Audience interaction will be enabled directly by the microphones in the congress room.

05 POSTER SESSIONS

Poster presentations will take place in the Ulika Hall in the designated area. **Posters should be mounted till 08:30 on Thursday 26th September** according to the List of posters in the Book of Abstracts, and be removed before the end of the conference. **All posters have to be displayed on the panels during the whole congress.**

Presenters of the posters with **even numbers** have to stand in front of their poster during the poster session on Thursday 26th September. Presenters of the posters with **odd numbers** have to stand in front of their poster during the poster session on Thursday 27th September.

Poster panels and mounting accessories will be available at the site. All posters will be evaluated by the Committee. **FEBS Open Bio Poster Award** will be given to the best presenter at the closing ceremony.

06 SOCIAL EVENTS

All official social events stated in the congress programme are included in the registration fee. For all services or goods outside the official contract with the congress organiser, additional hotel fees may apply. If you have any question on services and goods included in the registration fee, contact the organisers or the PCO stuff at the registration desk.

07 EXCURSION AND CONGRESS DINNER

Congress excursion and Congress dinner are organised as a single social event. Both are included in the registration fee for all registered participants.

Excursion will start with the **gathering** of the participants **in front of the Hotel Park Plaza Histria main entrance between 13:30 and 14:00**. All participants will be provided with the **lunch boxes**.

The **Excursion buses will leave the meeting point at 14:00 sharp.** No additional transportation will be available after 14:00 to join the Excursion and the Congress dinner.

During excursion guided tours in smaller groups will be organised. Personal free time is planed during the excursion. Therefore we advice all participants to take small amount of money and credit cards with them.

Congress dinner is scheduled for 19:30.

The **return to the Hotel Park Plaza Histria will be organised for all participants and scheduled for 23:00.**

***** The organiser or the PCO will not provide any variation in the scheduled times or additional transportation possibilities.

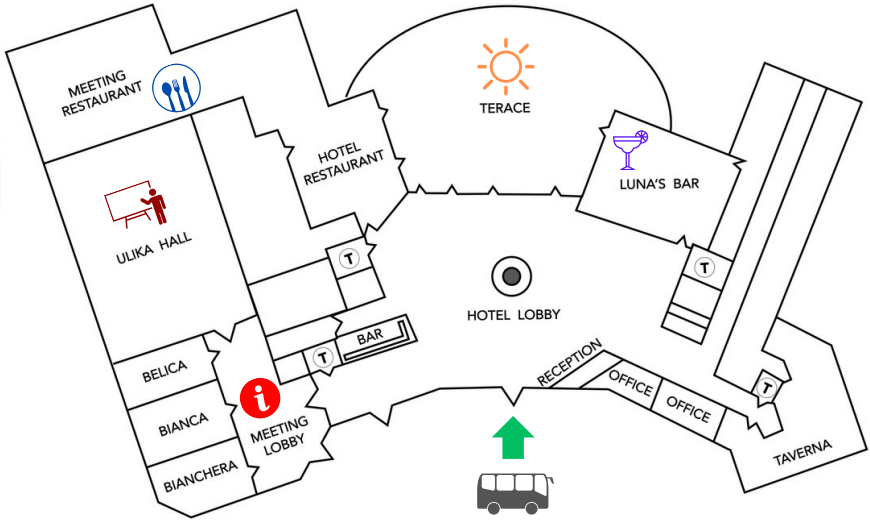
08 VENUE

The **Hotel Reception** is located on the right in the hotel Lobby. Check-in (after 14:00) and check-out (before 12:00) are carried out by the participants themselves at the hotel reception on a first-come, first-served basis.

Conference centre is located immediately on the left in the hotel Lobby. **Registration desk** is located in the **Meeting lobby** and will be open during the conference programme. **Exhibitions** of industry partners and **coffee breaks** will be held in the Meeting lobby.

All **conference rooms** are accessible from the Meeting lobby only.

Planned **meals** will be served in the hotel restaurant in the designated area i.e. **Meeting restaurant.**



PROGRAMME

SEP 25 WEDNESDAY

14:00 - 16:00 **Registration**
Meeting lobby

16:00 - 16:30 **Opening ceremony**
Ulika Hall
Chairs: Morana Dulic and Nino Sincic

16:30 - 17:15 **Plenary lecture 1**
Ulika Hall
Chair: Damjan Franjevic

Ivan Ahel (Oxford, UK)
ADP-ribosylation signalling in genome stability and beyond

17:15 - 17:45 **Coffee break**
Meeting lobby

17:45 - 19:20 **Session 1**
Ulika Hall
Chairs: Igor Weber and Ita Gruic-Sovulj

17:45 - 18:10 **Gemma Atkinson (Lund, Sweden)**
Discovery of novel bacterial immune systems using high-throughput sequence and structural bioinformatics

18:10 - 18:35 **Pontus Nordenfelt (Lund, Sweden)**
Discovery and engineering of protective human antibodies against viral and bacterial pathogens

18:35 - 18:50 **Wei-Sheng Sun (Umeå, Sweden)**
Breaking Barriers: pCF10 Type 4 Secretion System Relies on a Self Regulating Muramidase to Modulate the Cell Wall

18:50 - 19:05 **Juliane John (Stockholm, Sweden)**
A novel version of the metal-free class Ie ribonucleotide reductase

19:05 - 19:20 **Labena Mini Symposium**
Cancer in liver or the story of many-faced god

20:00 – 21:00 **Dinner**
Meeting restaurant

21:00 – 22:00 **Welcome party**
Histria Plaza Hotel Bar & Terrace

SEP 26 THURSDAY

08:30 – 09:15 **Plenary lecture 2**
Ulika Hall
Chair: Vesa Hytönen

Johanna Ivaska (Turku, Finland)
Cancer Cells Crossing Borders

09:15- 10:10 **Session 2**
Ulika Hall
Chairs: Vesa Hytönen and Antonija Jurak Begonja

09:15 - 09:40 **Igor Weber (Zagreb, Croatia)**
The IQGAP-related RasGAP IqgC regulates cell-substratum adhesion in Dictyostelium

09:40 - 09:55 **Shrikant B. Kokate (Helsinki, Finland)**
Calponins: guardians of stress fiber dynamics in non-muscle cell architecture

09:55 - 10:10 **Darija Putar Brajkovic (Zagreb, Croatia)**
IqgC negatively regulates macropinocytosis by deactivating RasG, but RasG is required for its recruitment to macropinocytic cups

10:10 - 10:40 **Coffee break**
Meeting lobby

10:40 - 12:30 **Session 3**
Ulika Hall
Chairs: Jasmina Rokov Plavec and Christian Reynolds

10:40 - 11:05 **Sesilja Aranko (Aalto, Finland)**
Post-translational protein editing for advanced protein-based biomaterials

11:05 - 11:30 **Ita Gruic-Sovulj (Zagreb, Croatia)**
Unexpected alteration in the signature motif of a class I aminoacyl-tRNA synthetase confers antibiotic hyper-resistance

11:30 - 11:45 **Dina Franic (Zagreb, Croatia)**
Protein quality control in quiescent yeast cells requires parallel activity of the proteasome and a novel form of selective autophagy

11:45 - 12:00 **Igor Zivkovic (Zagreb, Croatia)**
(Recipient of the HDBMB Annual Award to Young Scientists for 2022)
How did negative catalysis tailor the specificity of isoleucyl-tRNA synthetases's editing domain?

12:00 - 12:15 **Bojan Zunar (Zagreb, Croatia)**
(Recipient of the HDBMB Annual Award to Young Scientists for 2022)
Retooling yeast *Saccharomyces cerevisiae* into a next-generation eukaryotic whole-cell copper biosensor and deploying it as a living component of the pluronic f127-based bioink

12:15 - 12:30 **NANOTEMPER Mini Symposium**
Unlocking difficult interactors: insights into structure and interactions with Nanotemper technologies platforms

12:30 - 14:00 **Lunch**
Conference restaurant

14:00 - 14:45 **Plenary lecture 3**
Ulika Hall
Chair: Ronnie Berntsson

Simon Newstead (Oxford, UK)
Understanding the role of solute carriers in health & disease

14:45 - 15:40 **Session 4**
Ulika Hall
Chairs: Ronnie Berntsson and Maja Katalinic

14:45 - 15:10 **Sara Liin (Linköping, Sweden)**
Fatty acids as activators of the cardiac potassium channel KV7.1/KCNE1

15:10 - 15:25 **Monika Oberer (Graz, Austria)**
Evolutionary conservation reveals key residues governing activation of ATGL, a central player in intracellular lipid degradation

15:25 - 15:40 **Snjezana Juric (Ljubljana, Slovenia)**
Elucidating the movement-defective mutations in florigen

15:40 - 16:10 **Coffee break**
Meeting lobby

16:10 - 17:10 **Poster session (Even numbers)**
Ulika Hall

17:10 - 18:40 **Session 5**
Ulika Hall
Chairs: Dubravka Svob Strac and Marija Pinteric

17:10 - 17:35 **André Mateus (Umeå, Sweden)**
Functional proteomics of human gut microbiome species

17:35 - 18:00 **Minna Liisa Änkö (Tampere, Finland)**
RNA structures shape oncogenic gene expression

18:00 - 18:25 **Anamaria Brozovic (Zagreb, Croatia)**
Pan-tubulin β and class III β-tubulin-crossing the carboplatin and paclitaxel resistance of ovarian cancer

18:25 - 18:40 **Marco Cavaco (Lisbon, Portugal)**
Tackling triple-negative breast cancer with new peptide-based protein-protein inhibitors via the suppression of the WNT/B CATENIN pathway

18:40 - 18:55 **Dubravka Hranilovic (Zagreb, Croatia)**
Orphan nuclear receptor Nurr1 – A possible link between perinatal hypoxia and behavioral aberrations in adolescent rats

18:55 - 19:10 **Altium Mini Symposium**
Label-free quantitative phase imaging (QPI) — transforming live cell analysis in biomedical research

20:30 - 22:00 **Quiz night**
Ulika Hall

SEP 27 FRIDAY

08:30 - 09:15 **Plenary lecture 4**
Ulika Hall
Chair: Hannu Koistinen

Boris Turk (Ljubljana, Slovenia)
(FEBS National Lecture)
Cysteine cathepsins in inflammation-associated diseases

09:15 - 10:10 **Session 6**
Ulika Hall
Chairs: Hannu Koistinen and Marta Popovic

09:15 - 09:40 **Caglar Elbuken (Oulu, Finland)**
Nonlinear microfluidics: From large molecule focusing to complex dynamic networks

09:40 - 09:55 **Cecile Otten (Zagreb, Croatia)**
Repair of DNA protein crosslinks by Tyrosyl-DNA phosphodiesterase 2

09:55 - 10:10 **Mateo Glumac (Split, Croatia)**
(Recipient of the HDBMB Annual Award to Young Scientists for 2023)
SPRTN: A crucial player in cellular proteostasis

10:10 - 10:40 **Coffee break**
Meeting lobby

10:40 - 11:40 **Poster session (Odd numbers)**
Ulika Hall

11:40 - 13:15 **Session 7**
Ulika Hall
Chairs: Dubravka Hranilovic and Max Renner

11:40 - 12:05 **Ilija Brizic (Rijeka, Coatia)**
IFN-γ impairs synapse formation in the cytomegalovirus-infected developing brain

12:05 - 12:30 **Giray Enkavi (Helsinki, Finland)**
Modulation of BDNF Signaling Through TrkB Transmembrane Domain

12:30 - 12:45 **Alexander Röntgen (Cambridge, UK)**
Splice isoforms of alpha-synuclein enhance its aggregation

12:45 - 13:00 **Dubravka Svob Strac (Zagreb, Croatia)**
DHEA(S) and BDNF in dementia: from cellular and animal models to human research

13:00 - 13:15 **Biosistemi Mini Symposium**
Elevating research with the latest applied biosystems QuantStudio absolute Q Digital PCR

14:00 - 23:00 **Conference excursion and dinner**
Gathering in front of the hotel

SEP 28 SATURDAY

08:30 - 10:00 **Scientific challenge**
Ulika Hall

10:00 - 10:30 **Coffee break**
Meeting lobby

10:30 - 11:15 **Plenary lecture on Education and Training**
Ulika Hall
Chair: Ferhan Sagin

Robert Harris (Stockholm, Sweden)
Is there a reason to be afrAId how TECHnology will affect the future of university research and education?

11:15 - 12:05 **Session on Education and Training**
Ulika Hall
Chairs: Nino Sincic and Igor Stuparevic

11:15 - 11:40 **David Smith (Sheffield, UK)**
The Power GenAI in Bioscience Education

11:40 - 12:05 **Damjana Kastelic (Barcelona, Spain)**
Applications of Virtual Reality in Research and Training

13:15 - 14:00 **Closing ceremony**

POSTER LIST

P1

EXPLORING THE DYNAMICS OF SPRTN PROTEASE AND TYROSYL-DNA PHOSPHODIESTERASE 1 IN DNA-PROTEIN CROSSLINK REPAIR

Ivan Anticevic, Cecile Otten, Luka Vinkovic, Luka Jukic, Marta Popovic

P2

SALVAGING dNTPs FOR mtDNA RESCUE

Ololade Awoyomi, Michael Gorospe, Sushma Sharma, Anna Karin Nilsson, Olena Diachenko, Pradeep Mishra, Phong Tran, Paulina Wanrooij, Andrei Chabes

P3

DEEP INTO THE PROTEOMIC NETWORK OF HEMOZOIN FORMATION IN THE MALARIA PARASITE

Vitória Baptista, Miguel Silva, Bruno Freitas, Susana O. Catarino, Angél Vizoso-Vázquez*, Maria Isabel Veiga*

*Shared last authorship

P4

FUNCTIONAL IMPACTS OF PROMOTER EVOLUTION IN *Cyprinus carpio carpio* REVEALED BY HIGH-RESOLUTION CAGE ANALYSIS

Damir Baranašić, Bojan Žunar, Marta Bošnjaković, Ada Jimenez-Gonzalez, Yavor Hadzhiev, Ferenc Müller, Boris Lenhard

P5

THE INFLUENCE OF DIPEPTIDYL PEPTIDASE 3 OVEREXPRESSION AND SILENCING ON SH-SY5Y CELL MIGRATION

Lea Barbarić, Nikolina Stojanović, Mihaela Matovina

P6

COMPARISON OF STRUCTURALLY DIFFERENT LIGANDS' ABILITY TO PREVENT AMYLOID FORMATION IN ALZHEIMER'S DISEASE

Marija Bartolić, Dajana Sokač Gašo, Valentina Bušić, Alma Ramić, Ines Primožič, Anita Bosak

P7

A CELL- AND DEVELOPMENTAL STAGE-SPECIFIC EXPRESSION OF METABOLIC AND SEROTONIN REGULATING GENES IN MURINE PLACENTA – THE EFFECTS OF MATERNAL DIET AND FETAL SEX AT TERM PREGNANCY

Ivona Beceheli, Alejandro A. Candia, Jonas Zaugg, Samantha Lean, Amanda Sferruzzi-Perri

P8

FUNCTIONAL ANNOTATION OF A DIVERGENT GENOME USING SEQUENCE STRUCTURE-BASED SIMILARITY

Dennis Svedberg*, Rahel R. Winiger*, Alexandra Berg*, Himanshu Sharma, Christian Tellgren-Roth, Bettina A. Debrunner-Vossbrinck, Charles R. Vossbrinck, Jonas Barandun

*These authors contributed equally to this work.

P9

TANDEM MASS SPECTROMETRY IN COMBINATION WITH ION MOBILITY FOR CHARACTERIZATION OF DIET IMPACT ON CARDIOLIPIN MOLECULAR DIVERSITY

Nada Birkic, Ana Maria Ivanov, Angelo Novak, Christian A. Reynolds

P10

ROLE OF Arf GAP PROTEINS IN MURINE CYTOMEGALOVIRUS INFECTION

Gordana Blagojević Zagorac, Natalia Jug Vučko, Valentino Pavišić, Hana Mahmutefendić Lučin, Igor Štimac, Tamara Gulić, Pero Lučin

P11

YEAST CELLS SUBJECT TO ABRUPT GLUCOSE DEPRIVATION RESUME ATP-CONSUMING PROTEIN QUALITY CONTROL BY THE UBIQUITIN-PROTEASOME SYSTEM

Mihaela Pravica, Dina Franić, Klara Zubčić, Antonio Bedalov, Mirta Boban

P12

WHOLE EXOME SEQUENCING OF A FAMILY WITH A HISTORY OF EARLY-ONSET HASHIMOTO'S THYROIDITIS

Vesna Boraska Perica, Dean Kaličanin, Robert Belužić, Ana Barić, Marko Vuletić, Sanda Gračan, Vedrana Čikeš Ćulić, Ante Punda, Veselin Škrabić, Maja Barbalić

P13

CARBAMATES – A PRIVILEGED PHARMACOPHORE IN THE DESIGN OF POTENT CHOLINESTERASE INHIBITORS FOR TREATING ALZHEIMER'S DISEASE

Anita Bosak, Ana Matošević, Ines Primožič

P14

UNRAVELING SPRTN FUNCTION IN DNA METHYLATION MAINTENANCE

Maria Boskovic, Ines Tomaskovic, Vincent Cahais, Thorsten Mosler, Cristian Pietro-Garcia, Felicia Fei Lei Chung, Cyrille Cuenin, Zdenko Herceg, Janos Terzic, Ivan Dikic

P15

THE NEUROIMMUNOMODULATORY POTENTIAL OF CD26 IN CROHN'S DISEASE

Sunčica Buljević, Iva Vukelić, Sara Borovac, Ivana Marić, Dijana Detel

P16

PHOTOSYNTHETIC PERFORMANCES AND PHOTO-PROTECTION STRATEGIES IN THE HAPLOID AND DIPLOID STAGES OF THE COCCOLITHOPHORE *Calcidiscus leptoporus*

Frederic Chaux, Jelena Godrijan

P 17

THE RESPONSE OF HUMAN GINGIVAL FIBROBLASTS TO METAL IONS ELUTED FROM ORTHODONTIC APPLIANCES

Lara Dežulović, Iva Suman, Ksenija Durgo, Vito Kovač, Željka Fiket, Gordana Čanadi Jurešić

P18

QUALITY, QUANTITY AND NEXT GENERATION SEQUENCING ANALYSIS OF CIRCULATING CELL-FREE DNA OBTAINED FROM LIQUID BIOPSY SAMPLES OF PATIENTS WITH COLORECTAL CANCER

Andrea Čeri, Martha Koržinek, Alma Cvetković, Ivana Ćelap, Marija Grdić Rajković, Neven Ljubičić, Neven Baršić, Donatella Verbanac, Karmela Barišić

P19

UNRAVELING SEX-SPECIFIC PATTERNS IN HNSCC: EXPLORING THE CLINICAL SIGNIFICANCE OF SEX HORMONE RECEPTORS DIFFERENTIAL EXPRESSION IN HNSCC

Josipa Čonkaš, Janja Josić, Jacqueline-Katrin Kranjčević, Nina Milutin Gašperov, Ozren Vugrinec, Ivan Mumlek, Ana Kvolik Pavić , Dinko Leović , Petar Ozretić

P20

THE EFFECT OF SMALL MOLECULE MIF ENOLASE INHIBITOR TE-91 ON ACTIVATION AND METABOLIC REPROGRAMMING OF MACROPHAGES

Péter Deák, Eszter Vámos, Viola Bagóné Vántus, Tamás Loránd, Ferenc Gallyas Jr., Balázs Radnai

21

BIOAVAILABILITY AND INTESTINAL ABSORPTION OF BIOACTIVE MOLECULES FROM PLANT EXTRACTS AND FOOD BORNE PESTICIDE RESIDUES IN 2D CULTURE OF CaCo2 CELLS

Domagoj Đikić, Irena Landeka Jurčević, Dyana Odeh, Ivana Šola, Verica Dragović Uzelac, Zoran Zorić, Gordana Jurak, Jasna Bošnir

P22

METABOLIC SHIFT IN HEPATIC CANCER CELLS CULTURED IN 3D COLLAGEN SCAFFOLD

Adam Frtús, Barbora Smolková, Šárka Kubinová

P23

IDENTIFICATION AND CHARACTERIZATION OF PROTEINS RELATIVELY RESISTANT TO CARBONYLATION IN *E. coli* AND MAMMALIAN CELL PROTEOMES

Andrea Gelemanović, Guillaume F. Combes, Jeffrey N Savas, Miroslav Radman, Katarina Trajković

P24

OLIVE LEAF POLYPHENOLS ALTER THE EXCITOTOXICITY OF GLUTAMATE BY MODULATING GLUTAMATE TRANSPORT IN THE RAT BRAIN AFTER INDUCED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

Jasminka Giacometti, Marta Peruč, Petra Vargić, Tamara Šoić-Vranić, Marina Nikolić, Tanja Grubić Kezele

25

MONITORING THE CELL CYCLE DYNAMICS OF PROMOTER ACTIVITY WITH NET-SLIC-CAGE REVEALS NOVEL RULES OF CELL CYCLE STAGE-SPECIFIC TRANSCRIPTIONAL REGULATION

Elena Gómez-Marín, Bojan Žunar, Ekjot Kaur, Fabio D'Orazio, Damir Baranašić, Alexis R. Barr, Boris Lenhard

P26

THE ROLE OF NON-CODING RNAS IN THE PROGRESSION OF IDH-MUTANT ASTROCYTOMAS TO GRADE 4 AFTER TREATMENT

Anja Hartewig, Serafiina Jaatinen, Matti Annala, Pauli Helén, Joonas Haapasalo, Wei Zhang, Hannu Haapasalo, Matti Nykter, Xin Lai, Kirsi J. Rautajoki

P27

NME6 - STED MICROSCOPY VISUALIZATION AND IMPORT TO MITOCHONDRIA

Bastien Proust, Valentina Štimac, Nikolina Škrobot Vidaček, Iva Marija Tolić, Maja Herak Bosnar

P28

INFUSE vs. OSTEOGROW: BIOLOGICAL ACTIVITY, RELEASE PROFILE AND EARLY CELLULAR RESPONSE

Natalia Ivanjko, Nikola Stokovic, Natasa Kovacic, Danka Grcevic, Slobodan Vukicevic

P29

EXPLORING THE MECHANICAL CHARACTERISTICS OF TUMOUR MICROENVIRONMENT IN COLORECTAL CANCER

Jenni Keränen, Austin Donnelly Evans, Rolle Rahikainen, Toni Seppälä, Oommen Oommen, Vesa Hytönen

P30

THE MyD88 SIGNALING ACCOMPANIED BY MICROBIOTA CHANGES SUPPORTS URINARY BLADDER CARCINOGENESIS

Dora Knezović, Blanka Milić Roje, Katarina Vilović, Lucija Franković, Jelena Korac-Prlic, Janoš Terzić

P31

BIOSCAVENGING CAPACITY OF BUTYRYLCHOLINESTERASE REDUCES INHIBITION IMPACT OF ORGANOPHOSPHATE COMPOUNDS IN HUMAN BLOOD

Dora Kolić, Goran Šinko, Ines Primožič, Zrinka Kovarik

P32

EFFECT OF INTERLEUKIN-1 ALPHA ON MEGAKARYOPOIESIS AND PLATELET FORMATION IN VITRO

Robert Kolman, Ivana Bertović, Josip Peradinović, Nikolina Mohović, Ivana Munitić, Antonija Jurak Begonja

P33

PLASTICS IN FOCUS: THE INFLUENCE OF POLYSTYRENE ON THE FITNESS OF FRESHWATER ALGAE

Bruno Komazec, Biljana Balen, Nino Dimitrov, Petra Peharec Štefanić

P34

CHOLESTEROL OXIME OLESOXIME ASSESSED AS A POTENTIAL LIGAND OF HUMAN CHOLINESTERASES

Dora Kolić, Goran Šinko, Zrinka Kovarik

P35

UNIQUE RECOGNITION OF tRNAIle BY ISOLEUCYL-tRNA SYNTHETASE TYPE 2 IN *Priestia megaterium*

Petra Kozulić, Alojzije Brkić, Marc Leibundgut, Nenad Ban, Ita Gruić Sovulj

P36

STRUCTURE AND pH STABILITY OF THE MINIMAL PROGENITOR TOXIN COMPLEX B

Ajda Krc, Sara Kosenina, Pål Stenmark

P37

ALTERNATIVE SPLICING OF BPM2 AS A MECHANISM FOR ESTABLISHING A FUNCTIONAL DIVERSITY OF MATH-BTB PROTEIN FAMILY IN *Arabidopsis thaliana*

Dunja Leljak-Levanić, Mateja Jagić, Paula Štancl, Gaj Keresteš, Marta Frlin, Rosa Karlić, Nataša Bauer

P38

EXPLORING THE INTERACTIONS OF MANGANESE-DEPENDENT TRANSCRIPTION REGULATORS SloR AND ScaR WITH DNA – AN EXPERIMENTAL STUDY

Ivana Leščić Ašler, Katarina Radman, Branimir Bertoša

P39

DOMAIN SCOUTING AND EXPRESSION OPTIMISATION OF HUMAN PNPLA7 PROTEIN IN *E. coli* USING ALPHAFOLD2 GUIDANCE

Ana-Marija Lulić, Laura Gödl, Jovica Lončar, Tvrtko Smital, Monika Oberer, Maja Katalinić

P40

VIMENTIN EXPRESSION IN KIDNEY AND CHANGES CAUSED BY CISPLATIN NEPHROTOXICITY IN WISTAR RATS

Marija Ljubojević, Vedran Micek, Mirela Pavić Vulinović, Ivana Vrhovac Madunić

P41

ATYPICAL ARGINYL-tRNA SYNTHETASE OF PREDATORY BACTERIA *Herpetosiphon aurantiacus* AND ITS POTENTIAL PROTEIN INTERACTORS

Ana Madunić, Igor Živković, Marko Močibob, Ita Gruić Sovulj

P42

GENOME EDITING OF THE VvPDS GENE IN GRAPEVINE (*Vitis vinifera* L.) SOMATIC EMBRYOS VIA BIOLISTICS

Tomislav Mamić, Filip Španić, Dunja Leljak-Levanić, Nenad Malenica

P43

THE ROLE OF UvrD IN PRESPACER PREPARATION FOR CRISPR-Cas IMMUNITY

Tomislav Mamić, Ivana Ivančić-Baće

P44

MAPPING OF BARTH SYNDROME PATHOGENIC VARIANTS LINKS TAFAZZIN STRUCTURAL ALTERATIONS AND MONOLYSOCARDIOLIPIN BINDING

Irma Markesina, Jurica Novak, Christian A. Reynolds

P45

IMPACT OF PROTEIN DELETIONS IN GPI ANCHOR SYNTHESIS PATHWAY ON YEAST SURFACE DISPLAY EFFICIENCY

Tea Martinić Cezar, Bojan Žunar, Mateja Lozančić, Vladimir Mrša, Renata Teparić

P46

BCHE POLYMORPHISM EFFECT ON THE EFFICIENCY OF 5-(2-AMINO-1-HYDROXYETHYL) PHENOL CARBAMATES AS POTENTIAL NEW DRUGS FOR ADVANCED ALZHEIMER`S DISEASE TREATMENT

Ana Matošević, Nikola Maraković, Zrinka Kovarik, Anita Bosak

P47

DIPEPTIDYL PEPTIDASE 3 KNOCKOUT IN 293FT CELLS DOWNREGULATES NRF2 IN THE BASAL CONDITIONS

Mihaela Matovina, Ana Tomašić Paić, Filip Rokić, Robert Belužić, Marijana Popović Hadžija

48

THE IMPACT OF ENDOPLASMIC RETICULUM STRESS ON THE MODULATION OF THE UROKINASE PLASMINOGEN ACTIVATION SYSTEM

Maja Matulić, Diana Culej Bošnjak, Doris Janjić, Mariastefania Antica

P49

ASSOCIATION OF LIVER ENZYME LEVELS WITH COVID-19 SEVERITY

Neven Meseldžić, Maja Malenica, Tanja Dujić, Una Galmočlija, Selma Imamović-Kadrić, Lejla Prnjavorac, Omer Bedak, Besim Prnjavorac, Damir Marjanović, Tamer Bego

P50

RESOLVING THE MYTH OF GANGLIOSIDE PRESENCE IN MITOCHONDRIA: CHARACTERIZATION OF MITOCHONDRIAL GANGLIOSIDOME

Kristina Mlinac Jerković, Borna Puljko, Nikolina Maček Hrvat, Raluca Ica, Mirela Sarbu, Alina Zamfir, Svjetlana Kalanj Bognar

P51

ENHANCING SENSITIVITY IN ALPELISIB AND FULVESTRANT QUANTITATION BY SWEEPING MEKC-MS/MS

Zvonimir Mlinarić, Lu Turković, Biljana Nigović, Miranda Sertić

P52

DEVELOPMENT OF A COMPUTATIONAL PIPELINE FOR THE DESIGN OF MONOBODY BINDERS

Pedro Moreira, Shuhao Xiao, Madalena C. Marques, Mariana Parada, Carlota J. B. Conceição, Manuel N. Melo, Miguel Rocha, Isabel Abreu, João B. Vicente, Bruno E. Correia, Cláudio M. Soares, Diana Lousa

P53

DIVERSITY IN THE BIVALENT AND MULTIVALENT INTERACTIONS OF THE LC8 HUB PROTEIN

Eszter Nagy-Kanta, Zsófia E. Kálmán, Zoltán Gáspári

P54

HUMAN TRYPSIN-ISOENZYME INHIBITORS FROM FINNISH CYANOBACTERIA

Xiaodan Ouyang, Muhammad N. Ahmed, Matti Wahlsten, Jouni Jokela, David P. Fewer, Hannu Koistinen

P55

FINE-TUNING THE PRODUCTION OF RECOMBINANT PROTEINS USING tRNA POST-TRANSCRIPTIONAL MODIFICATIONS

Jenni Pedor, Matea Radesic, L. Peter Sarin

P56

SKIN MICROBIOTA IN SUBJECTS WITH HAND ECZEMA AFTER PROBITECT-C EMOLLIENT PLUS APPLICATION

Mihaela Peric, Dora Hrestak, Hana Cipic Paljetak, Mario Matijasic, Nika Filipovic, Liborija Lugovic Mihic, Mirta Mikulecky, Mladen Meglaj, Suzana Ljubojevic Hadzavdic

P57

INTERPLAY BETWEEN ONTOGENY AND REGULATORY EVOLUTION OF OHNOLOGUES FOLLOWING AUTOPOLYPLOIDIZATION IN SALMONIDS

Diego Perojil Morata, Manu K. Gundappa, Marie-Odile Baudement, Damir Baranašić, Gareth Gillard, Lars Grønvold, Pooran Dewari, Tomasz Podgorniak, Rose Ruiz-Daniels, Sigbjørn Lien, Boris Lenhard, Daniel J. Macqueen

P58

CREATING A MODEL SYSTEM FOR STUDYING DNA-PROTEIN CROSSLINK REPAIR IN HUMAN CELLS USING CRISPR/dCas9 TECHNOLOGY

Loren Petrušić, Ivan Antičević, Marta Popović

P59

SIRT3 AFFECTS SEX-SPECIFIC DIFFERENCES IN OXIDATIVE AND ANTIOXIDANT PARAMETERS IN KIDNEY AND BRAIN TISSUE IN MICE

Marija Pinterić, Robert Belužić, Marta Kaloper, Ena Šimunić, Iva I. Podgorski, Marijana Popović Hadžija, Tihomir Balog, Sandra Sobočanec

P60

EFFECTS OF BEP CHEMOTHERAPY ON AMINO ACID EXCRETION IN TESTICULAR CANCER PATIENTS

Alica Pizent, Tanja Živković Semren, Blanka Tariba Lovaković, Marija Gamulin

P61

N-GLYCANS OF BOTH FC AND FAB REGIONS OF IgG ARE CHANGED IN CHILDREN WITH TYPE 1 DIABETES

Branimir Plavša, Najda Rudman, FlemmingPoicot, Olga Gornik

P62

DECIPHERING DNA-PROTEIN CROSSLINK REPAIR IN VIVO USING CRISPR-CAS GENE EDITING IN A ZEBRAFISH MODEL

Ivan Anticevic, Cecile Otten, Marin Kutnjak, Marta Popovic

P63

AN INTEGRATED LANDSCAPE OF GANGLIOSIDES, NEUROPLASTIN, AND PLASMA MEMBRANE Ca2+-ATPase IN HUMAN GLIOBLASTOMA

Borna Puljko, Anja Bukovac, Ana Karla Vodanović, Anja Kafka, Mario Stojanović, Niko Njirić, Svjetlana Kalanj Bognar, Nives Pećina-Šlaus, Kristina Mlinac Jerković

P64

EVALUATING THE INFLUENCE OF tRNA POST-TRANSCRIPTIONAL MODIFICATIONS ON TRANSLATION

Matea Radešić, Jenni Pedor, Peter Sarin

P65

UNVEILING THE MOLECULAR DANCE: EXPLORING SloR AND ScaR PROTEIN-DNA INTERACTIONS THROUGH MD SIMULATIONS

Katarina Radman, Branimir Bertoša

P66

OPTICAL CONTROL OF COVALENT PROTEIN ASSEMBLY TO INVESTIGATE EARLY CELL ADHESION FORMATION

Rolle Rahikainen, Susan K. Vester, Paula Turkki, Chasity P. Janosko, Alexander Deiters, Vesa P. Hytönen, Mark Howarth

P67

IDENTIFICATION OF POTENTIAL NOVEL GLI2 TRANSCRIPTIONAL TARGETS IN PROSTATE CANCER BY PROTEIN ARRAY

Dora Raos, Josipa Čonkaš, Maja Sabol

P68

DISULFIDE LINK IN PLANT CYTOSOLIC AMINOACYL-tRNA SYNTHETASE IS IMPLICATED IN OXIDATIVE STRESS RESPONSE

Jasmina Rokov-Plavec, Valentina Ević, Marko Močibob, Ružica Šoić, Ivana Kekez

P69

CfDNA ANALYSIS IN LIQUID BIOPSY OF PATIENTS WITH TESTICULAR GERM CELL TUMOR

Jure Krasic, Dinko Soic, Lucija Skara Abramovic, Davor Jezek, Dinko Mitrecic, Monika Ulamec, Olga Gornik, Nino Sincic

P70

p53 FAMILY PROFILE IN PATIENT-DERIVED MELANOMA CELL LINES RESISTANT TO TARGETED THERAPY

Anđela Horvat, Ignacija Vlašić, Ana Tadijan, Neda Slade

P71

ERK INHIBITORS AS A NOVEL THERAPY IN KIDNEY MITOCHONDRIAL DYSFUNCTION

Iva Suman, Dorotea Dobrić, Antonijo Grčić, Roberta Ogrizović, Robert Domitrović

P72

EXAMINATION OF VIRAL PepH3 PEPTIDE-FUNCTIONALIZED NANOPARTICLES ON A CULTURE MODEL OF THE BLOOD-BRAIN BARRIER

Anikó Szecskó, Mária Mészáros, Vera Neves, Marco Cavaco, Beatriz Simões, Catarina Chaparro, Miguel A.R.B. Castanho, Mária A. Deli, Szilvia Veszelka

P73

INTERPLAY OF ISOLEUCINE MISTRANSLATION AND OXIDATIVE STRESS FROM A PROTEOMIC PERSPECTIVE

Mateo Šerić, Marko Močibob, Jasmina Rokov Plavec

P74

SEX-DEPENDENT MODULATION OF OXIDATIVE AND ANTIOXIDATIVE STATUS IN NOD MICE: POTENTIAL CONTRIBUTORS TO THE DEVELOPMENT OF DIABETES

Kate Šešelja, Ena Šimunić, Marija Pinterić, Iva I. Podgorski, Marijana Popović Hadžija, Robert Belužić, Tihomir Balog, Sandra Sobočanec

P75

ANTICANCER EFFECTS OF BETULINIC ACID ON HCT116 COLORECTAL CANCER CELLS: ROLE OF OXIDATIVE STRESS, AUTOPHAGY, AND SIGNALING PATHWAYS

Lidija Šimić Ševerdija, Iva Suman, Lara Batičić, Robert Domitrović

P76

EFFECTS OF SIRTUIN 3 ON METABOLIC AND REDOX HOMEOSTASIS IN MOUSE LIVER DURING AGEING

Ena Šimunić, Marija Pinterić, Iva I. Podgorski, Marijana Popović Hadžija, Robert Belužić, Tobias Madl, Hansjörg Habisch, Tihomir Balog, Sandra Sobočanec

P77

SEQUENCE OF ECTOPIC OSTEOGENESIS IN RATS INDUCED BY rhBMP6 DELIVERED IN AUTOLOGOUS BLOOD COAGULUM WITH SYNTHETIC CERAMICS

Nikola Štoković, Natalia Ivanjko, Marina Milešević, Ivona Matić Jelić, Viktorija Rumenović, Slobodan Vukičević

P78

TYROSINE PHOSPHORYLATION OF CYTOCHROME C MODULATES ITS BINDING TO NUCLEAR SET/TAF-IB

Joaquín Tamargo-Azpilicueta, Miguel Á Casado-Combreras, Rafael L. Giner-Arroyo, Adrián Velázquez-Campoy, Miguel A. De la Rosa , Irene Díaz-Moreno

P79

ANALYSIS AND ENGINEERING OF KIT.METRNL SIGNALLING TO BOOST HEART TISSUE REGENERATION AFTER MYOCARDIAL INFARCTION

Martin Toul, Kenneth Verstraete, Savvas N. Savvides

P80

EXPRESSION PATTERNS OF TIGIT AND LAG-3 IMMUNE REGULATION RECEPTORS ACROSS MOLECULAR SUBTYPES OF MUSCLE-INVASIVE BLADDER CANCER

Monika Ulamec, Sarunthon Sujitwanit, Matej Knežević, Silvija Mašić, Igor Tomašković, Marija Miletić, Božo Krušlin

P81

PROTECTION OF BRAIN ENDOTHELIAL CELLS AS A THERAPEUTIC TARGET IN CENTRAL NERVOUS SYSTEM DISEASES

Szilvia Veszelka, Mária Mészáros, Anikó Szecskó, Gergő Porkoláb, Koppány Párdi, Janet Adegbite, Mária A. Deli

P82

DECODING ITIH5 FUNCTION IN MELANOMA RESISTANCE TO TARGETED THERAPY

Ignacija Vlašić, Anđela Horvat, Maja Jazvinšćak Jembrek, Petar Ozretić, Martina Radić, Doris Stepić, Ana Tadijan, Neda Slade

P83

TUMOR PROTEIN p53 (TP53) MUTATIONS IN CROATIAN POPULATION – PRELIMINARY RESULTS

Marko Žarak

P84

SEED TREATMENT WITH COLD PLASMA IN COMBINATION WITH PLASMA-ACTIVATED WATER AND AEROPONICS CULTIVATION FOR PLANT IMPROVEMENT

Rasa Žūkienė, Augustė Judickaitė, Emilija Jankaitytė, Laima Degutytė-Fomins, Zita Naučienė, Vida Mildažienė, Gediminas Kudirka, Liutauras Marcinauskas, Mindaugas Aikas, Rolandas Uscila, Andrius Tamošiūnas, Kazunori Koga, Masaharu Shiratani

P85

***Saccharomyces cerevisiae* Mub1, A SUBSTRATE ADAPTOR OF E3 UBIQUITIN LIGASE, LINKS CELL WALL ROBUSTNESS TO THE UBIQUITIN-PROTEASOME SYSTEM**

Bojan Žunar, Nada Šupljika, Ana Novačić, Tea Martinić Cezar, Antonia Paić, Renata Teparić, Igor Stuparević

P86

EARLY SAFETY EVALUATION OF N-ALKYL QUATERNARY QUINUCLIDINES AS POTENTIAL DRUG CANDIDATES USING IN VITRO TESTS ON HepG2 CELLS

Suzana Žunec, Donna Vadlja, Antonio Zandona, Alma Ramić, Ines Primožič, Maja Katalinić

PLENARY LECTURE ABSTRACT

PL1
ADP-RIBOSYLATION SIGNALLING IN GENOME STABILITY AND BEYOND

Ivan Ahel

Sir William Dunn School of Pathology, University of Oxford, UK

Reversible modification of macromolecules by ADP-ribosylation has been associated with control of many key biological processes, such as DNA-damage repair, transcription, cell division, signal transduction, stress and infection responses, microbial pathogenicity and aging. However, our understanding of ADP-ribosylation signalling is still in its infancy, as can be seen from the current rapid rate of discoveries of previously unknown pathways and molecular targets regulated by ADP-ribosylation. Here, I will discuss mechanisms through which specificity of reversible ADP-ribosylation reactions is regulated in various cellular pathways. I will focus on three different enzymatic systems for reversible ADP-ribosylation we recently identified: the DarTG toxin-antitoxin system that controls ADP-ribosylation of DNA in bacteria, the PARP/HPF1 complex that ADP-ribosylates serine residues in eukaryotic proteins involved in the maintenance of genome stability, and the PARP14, dual function writer/eraser of ADP-ribosylation involved in protection against variety of viruses, including the coronavirus.

PL2
CANCER CELLS CROSSING BORDERS

Johanna Ivaska

University of Turku, Turku Bioscience Center, Finland

Tissue homeostasis is dependent on the spatially controlled localization of specific cell types and the correct composition of the extracellular stroma. Integrin-mediated adhesions, in conjunction with the actin cytoskeleton and signaling by receptor tyrosine kinases, regulate cell fate and identity and allow cells to migrate and invade the surrounding extra-cellular matrix (ECM). We have previously uncovered key differences between normal and cancer-associated stroma, whereby the mechanical and architectural features of normal stroma inhibit tumour growth and may epigenetically reprogram aggressive breast cancer cells towards a more benign phenotype. Recently, we turned our attention to other putative crosstalk mechanisms between cancer cells and the tumor microenvironment as well as tumor cell interactions with distinct tissue borders during systemic dissemination in the body. I will describe different control mechanisms guiding cancer cell invasion across physiological borders and their relevance to cancer progression and metastasis.

PL3
UNDERSTANDING THE ROLE OF SOLUTE CARRIERS IN HEALTH & DISEASE

Simon Newstead

Department of Biochemistry, University of Oxford, UK

Transporters belonging to the Solute Carrier (SLC) family are integral membrane proteins that play essential roles in human physiology. They predominantly function to regulate the transport of small molecules into and out of cells and regulate the availability of amino acids, ions, sugars, lipids and vitamins within the body. SLC transporters also function within intracellular signalling networks, particularly with respect to amino acid and nutrient sensing. Recently, the role of solute carriers in protein trafficking has revealed the close link between transport, cellular homeostasis and signalling in the cell. My talk will focus on recent work from my group that reveals new insights into clinically important solute carrier systems: how they function as drug transporters, the mechanisms used to regulate their function, their role in organelle homeostasis and how their function in the cell is likely far more widespread than previously thought.

PL4
CYSTEINE CATHEPSINS IN INFLAMMATION-ASSOCIATED DISEASES

Boris Turk

Department of Biochemistry and Molecular and Structural Biology, Jozef Stefan Institute, Slovenia
Faculty of Chemistry and Chemical Engineering, University of Ljubljana, Slovenia

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

PL Education and Training
IS THERE A REASON TO BE afrAId HOW TECHnology WILL AFFECT THE FUTURE OF UNIVERSITY RESEARCH AND EDUCATION?

Robert Harris

Karolinska Institute, Sweden

There is currently a technological revolution in biomedical research in which multiple forms of omics and Big Data are used to characterize molecular pathways. In clinical disciplines, medical device and Ai-assisted technologies can now make diagnoses and medical imaging more accurately and consistently than human medics. Education has an ever-increasing portfolio of digital tools (VR, AR, Ai) available to promote pedagogical development and student learning. But how will these technologies be implemented in research and education activities at universities in a responsible and ethical manner? With the premise that Together, Everything Can Happen (TECH), and that collaboration will be central to teaching and learning practices, we will predict the future of researchers and educators alike in this ever-developing academic landscape.

LECTURE ABSTRACT

L1

DISCOVERY OF NOVEL BACTERIAL IMMUNE SYSTEMS USING HIGH-THROUGHPUT SEQUENCE AND STRUCTURAL BIOINFORMATICS

Gemma Atkinson

Lund University, Sweden

Toxin-antitoxin (TA) systems are ubiquitous, diverse and highly mobile gene pairs of microbes. They consist of a gene encoding a a toxin that dramatically inhibits bacterial growth and an adjacent gene encoding an antitoxin that protects against, and neutralises the toxic effect. In addition to the two gene TA systems, there are also single gene TAs where the toxin and antitoxin are fused and expressed as a single protein, and three gene systems where the TA genes are accompanied by a gene encoding a SecB-like chaperone that stabilises the antitoxin (toxin-antitoxin-chaperone TAC systems). We have recently identified fused TA (CapRel) and TAC (HigBAC and CmdTAC) systems that are encoded on prophages in *Escherichia coli* genomes, and function as innate immune systems, protecting against infection by incoming phages. As variable regions of prophages are rich seams of novel biology, we are mining these regions with high-throughput bioinformatics methods to discover new microbial immune systems that we then validate experimentally.

L2

DISCOVERY AND ENGINEERING OF PROTECTIVE HUMAN ANTIBODIES AGAINST VIRAL AND BACTERIAL PATHOGENS

Pontus Nordenfelt

Lund University, Sweden

Antibodies are essential for immunity against viruses and bacteria. Biotechnological development has, in the past decades, allowed for many new treatments using monoclonal antibodies, but so far, few are available against pathogens. We use B cells from convalescent patients to discover potential therapeutic monoclonals, with careful characterization of biophysical and serological function. With this approach, we have discovered protective monoclonals against both group A streptococci and SARS-CoV-2, including novel functional mechanisms. We have also enhanced their protective function through molecular engineering and by combining them in oligoclonal cocktails. The benefit of exploiting human immunity evolved specifically against a pathogen, in this case, the induced B cell response, is that any functional antibody identified will have a high likelihood of being both safe and effective if it reaches clinical use. We hope that our antibodies and findings on antibody biology will aid the development of new treatments against important human pathogens.

L3

THE IQGAP-RELATED RASGAP IQGC REGULATES CELL-SUBSTRATUM ADHESION IN *DICTYOSTELIUM*

Igor Weber

Ruđer Bošković Institute, Croatia

Proper adhesion of cells to their environment is essential for the normal functioning of single cells and multicellular organisms. To attach to the extracellular matrix (ECM), mammalian cells form integrin adhesion complexes (IACs), which consist of many proteins that collectively link the ECM and the actin cytoskeleton. Similar to mammalian cells, the amoeboid cells of the protist *Dictyostelium discoideum* also use multiprotein adhesion complexes to control their attachment to the underlying surface. However, the exact composition of the multiprotein complexes and the signaling pathways involved in the regulation of adhesion in *D. discoideum* have not yet been elucidated. Here we show that the IQGAP-related protein IqgC is important for normal attachment of *D. discoideum* cells to the substratum. Mutant *iqgC*-null cells have impaired adhesion, whereas overexpression of IqgC promotes directional migration. A RasGAP C-terminal (RGCt) domain of IqgC is sufficient for its localization in the ventral adhesion focal complexes, while RasGAP activity of a GAP-related domain (GRD) is additionally required for the proper function of IqgC in adhesion. We identify the small GTPase RapA as a novel direct IqgC interactor and show that IqgC participates in a RapA-regulated signaling pathway targeting the adhesion complexes, which include talin A, myosin VII and paxillin B. Based on our results, we propose that IqgC is a positive regulator of adhesion, responsible for the strengthening of ventral adhesion structures and for the temporal control of their subsequent degradation.

Keywords: cell attachment, DdIQGAP3, amoeboid locomotion, RasG, focal adhesions, cell migration

L4

POST-TRANSLATIONAL PROTEIN EDITING FOR ADVANCED PROTEIN-BASED BIOMATERIALS

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Although there has been some impressive progress in producing recombinant protein-based materials, we have not yet reached their full potential. The mechanical properties and stability of the engineered materials are still compromised and not comparable to those of their natural counterparts. Furthermore, protein-based materials have the potential to be much more than mere substitutes for the currently used oil-based materials, because they can harbor important additional functionalities, such as stimuli-responsiveness, self-healing properties, and adhesiveness.

Two major limitations have hampered the use of recombinant proteins in the design of biomaterials. First, nearly all structural proteins in nature are post-translationally modified, most of them heavily. These modifications can both radically affect the mechanical properties and stability of the resulting materials, along with mediating many of the interesting additional functionalities. However, structural proteins produced in microbes typically do not have any modifications or they are very different to the native ones, due to the differences in the cellular machineries. Second, structural proteins are typically exceptionally long and unstructured, making them challenging to produce in the microbial production systems.

We take advantage of post-translational protein editing of silk proteins to overcome these limitations. We show engineering *Escherichia coli* to enable the production of controlled post-translational modifications of silk proteins. In addition, we apply biomolecular click-reactions to overcome the size limitation in the microbial production of spider silk proteins and to enable site-specific functionalization of silk fibers. The results have the potential to advance the design of protein-based biomaterials with improved properties and additional functionalities.

L5

UNEXPECTED ALTERATION IN THE SIGNATURE MOTIF OF A CLASS I AMINOACYL-tRNA SYNTHETASE CONFERS ANTIBIOTIC HYPER-RESISTANCE

Ita Gruic-Sovulj

Department of Chemistry Faculty of Science University of Zagreb, Croatia

Translation of genetic information into functional proteins relies on aminoacyl-tRNA synthetases (AARSs) and their ability to read the genetic code by linking amino acid to its cognate tRNA. AARSs are divided into two evolutionary distinct classes, I and II, whose members catalyse the same two-step reaction in which the ATP-dependent amino acid activation is followed by the transfer of aminoacyl moiety to the tRNA. Class I AARSs use HXGH (X stands for hydrophobic amino acid) and KMSK motifs for ATP binding in the transition state. As expected, these catalytic motifs are highly conserved and even serve as the class I signature motifs. However, recently, we found that some bacterial isoleucyl-tRNA synthetases (IleRS) have the GXHH sequence instead of the canonical HXGH motif. This unexpected alteration challenges the canonical mechanism in which the 3rd position is sterically constrained while the 1st position participates in catalysis. However, the GXHH-IleRS enzymes are fully active and keep a housekeeping role. Interestingly, the GXHH motif is found only in type 2 IleRS, a branch of bacterial IleRS resistant to micromolar concentrations of antibiotic mupirocin. The motif alteration in IleRS2 increased the inhibitory constant for this clinically relevant antibiotic to the millimolar range, bringing hyper-resistance. In contrast, the other IleRS branch, type 1, is sensitive to mupirocin inhibition and we could not find GXHH-IleRS1 enzymes in databases. We introduced the GXHH motif into *Priestia megaterium* IleRS1 and IleRS2 which naturally have the canonical HXGH motif. The motif alteration inactivated IleRS1 and increased resistance to mupirocin in IleRS2 by 400-fold. Structural data explains why the motif alteration inactivates IleRS1 and not IleRS2. Our data shows the unexpected plasticity of the IleRS2 active site under strong evolutionary pressure. In sharp contrast, IleRS1 is restrained from gaining (hyper)resistance likely because its evolution was shaped under a requirement to keep high aminoacylation turnover to support rapid translation in fast-growing bacteria.

L6

FATTY ACIDS AS ACTIVATORS OF THE CARDIAC POTASSIUM CHANNEL Kv7.1/KCNE1

Sara I Liin

Linköping University, Sweden

The voltage-gated potassium (Kv) ion channel Kv7.1/KCNE1 plays an important role in tuning cardiac excitability. Kv7.1/KCNE1 has therefore been highlighted as a promising new pharmacological target to treat conditions like cardiac arrhythmia. In this overall project, we aim to understand how the Kv7.1/KCNE1 channel is activated by endogenous and synthetic fatty acids and related compounds and how to utilize gained insights in rational drug development. We use electrophysiology techniques in combination with molecular dynamics simulations and synthetic chemistry to determine the molecular mechanisms underlying activating effects and how activation of Kv7.1/KCNE1 induces potentially anti-arrhythmic effects. We anticipate that the molecular understanding of how ligands like polyunsaturated fatty acids and endocannabinoids activate the Kv7.1/KCNE1 channel will open new avenues for future development of more effective anti-arrhythmic drugs utilizing similar binding sites and mechanisms of action.

L7
FUNCTIONAL PROTEOMICS OF HUMAN GUT MICROBIOME SPECIES

André Mateus

Department of Chemistry, Umeå University, Sweden

The human gut microbiome plays a key role in human health, with imbalances in species composition being associated with multiple diseases. Yet, the molecular mechanisms for the majority of currently described associations are largely unknown. Even when molecular mechanisms are proposed, they are difficult to validate or to act upon to restore a healthy state, as we generally do not have molecules to specifically modulate the activity of those proteins, or to deplete a specific species.
Our lab uses a systems biology approach based on proteomics to provide suggestions of protein function for further biochemical characterization. This involves a scalable bottom-up approach of studying one species at a time, currently focused on a collection of bacteria that are representative of the most common species of the human gut microbiome. In particular, we aim to understand what are the targets of drugs that limit growth of microbiome species, and how these species metabolize complex carbohydrates (fibers) from our diet. We combine this information with mechanistic information of how species affect the growth of each other in a community.
The goal of our research is to be able to rationally design strategies to manipulate species composition towards desired setpoints, e.g., depleting a species associated with disease or promoting the growth of a beneficial commensal. This would open the door to preventive or curative approaches.

L8
RNA STRUCTURES SHAPE ONCOGENIC GENE EXPRESSION

Minna Lisa Anko

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RNA molecules in cells are not naked single-stranded stretches of nucleic acids as depicted in textbook cartoons but RNAs interact with proteins in cells forming ribonucleoprotein complexes (RNPs) that fold into secondary and tertiary structures. We have identified RNA folding guided by RNA-protein interactions as a key element in determining oncogenic gene expression in colorectal cancer cells. Recently, we demonstrated that Serine-arginine rich splicing factor 3 (SRSF3) controls the processing OncomiR-1 microRNA cluster by guiding the folding of OncomiR-1 through RNA-protein and protein-protein interactions. At the functional level, SRSF3 binding favours the processing of two paralog miRNAs, miR-17 and miR-20a, over other miRNAs of the cluster, that in turn promotes the tumorigenic properties of cancer cells. Analysis of patient cohorts demonstrated that SRSF3-regulated pathway operates in colorectal cancer, linking SRSF3-mediated RNP structures to cancer pathogenesis. We currently investigate RNA folding in cancer cells more broadly to identify distinct oncogenic RNA structures. The importance of defining oncogenic RNA structures in the cellular context is twofold. Firstly, the interest in RNA-based cancer medicines has exploded but we have very limited understanding of oncogenic 'RNA structuromes'. The determination of RNA structures and their impact on gene expression in cancer cells facilitates the design of RNA targeting cancer medicines by revealing how oncogenic RNAs behave in their cellular context as a folded RNP. Secondly, altering RNA structures is a powerful means to regulate gene expression as evidenced by our work. Understanding oncogenic RNA structures may open a new avenue for the design of RNA structure targeting cancer medicines that have broad applications beyond colorectal cancer.

L9

PAN-TUBULIN β AND CLASS III β-TUBULIN-CROSSING THE CARBOPLATIN AND PACLITAXEL RESISTANCE OF OVARIAN CANCER

Anamaria Brozović

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Resistance to chemotherapy represents a major obstacle to long-term survival in ovarian cancer (OC) patients. We studied the interplay between acquired carboplatin (CBP) resistance using two OC cell models, MES-OV CBP and SK-OV-3 CBP, and non-P-glycoprotein-mediated cross-resistance to paclitaxel (TAX) observed only in MES-OV CBP cells. Decreased platination, mesenchymal-like phenotype, and increased expression of α- and γ-tubulin were observed in both drug-resistant variants compared with parental cells. Both variants revealed increased protein expression of class III β-tubulin (TUBB3) but differences in TUBB3 branching and nuclear morphology. Transient silencing of TUBB3 sensitized MES-OV CBP cells to TAX and, surprisingly, to CBP. This phenomenon was not observed in the SK-OV-3 CBP variant, probably due to the compensation by other β-tubulin isotypes. Reduced TUBB3 levels in MES-OV CBP cells affected DNA repair protein trafficking and increased whole-cell platination level. Furthermore, TUBB3 depletion augmented therapeutic efficiency in additional OC cells, showing a *vice versa* drug-resistant pattern, lacking β-tubulin isotype compensation visible at the total β-tubulin (TUBB) level *in vitro* and *ex vivo*. In summary, the level of TUBB in OC should be considered together with TUBB3 in therapy response prediction.

L10

NONLINEAR MICROFLUIDICS: FROM LARGE MOLECULE FOCUSING TO COMPLEX DYNAMIC NETWORKS

Caglar Elbuken

University of Oulu, Finland

Microfluidics has established itself as an enabling technology in biochemical analysis. The exquisite control on fluid flow at microscale is at the core of many analysis techniques such as flow cytometry, single-cell omics, digital PCR and point-of-care biosensors. Still, these systems use very simple fluid flow principles and rely on the simplified Stokes flow regime. Lately, nonlinear microfluidics has emerged as a field where inertial forces, non-Newtonian fluids or compliant materials were used to explore higher complexity fluidic patterns and applications. In this talk, examples of nonlinear microfluidic systems will be shown ranging from single molecule separation and complex dynamic network analysis. Emphasis will be given to a microfluidic technique we have been developing using viscoelastic medium under externally applied electric field to focus/separate nanoparticles in a carrier fluid. The use of viscoelastic fluids adds the element of elasticity as another parameter to generate normal stress difference in the fluid flow that substantially change the pattern and strength of innate lateral forces applied in colloidal systems. We coined the term electroviscoelastic migration (EVM) for this new technique. Using EVM, we can separate nanoparticles at high throughput. The technique is also responsive to particle surface charge, hence it can be further developed as a new methodology for particle zeta potential, size or diffusion constant analysis.

L11

IFN- γ IMPAIRS SYNAPSE FORMATION IN THE CYTOMEGALOVIRUS-INFECTED DEVELOPING BRAIN

Ilija Brizić

University of Rijeka, Center for Proteomics, Croatia

Congenital cytomegalovirus infection (cCMV) is a major viral cause of developmental abnormalities in newborns in developed countries, with no effective treatment available to limit CMV replication or treat the sequelae. CMV infection in the brain is associated with a strong inflammatory response, characterized by activation of microglia, infiltration of peripheral immune cells, and production of proinflammatory cytokines. Using infection of newborn mice with mouse cytomegalovirus (MCMV) as a reliable model that recapitulates many aspects of cCMV infection, including central nervous system (CNS) infection, altered neurodevelopment, and sensorineural hearing loss, we have previously shown that mitigation of inflammatory response prevented neurodevelopmental delay. Furthermore, we have identified NK and ILC1 cells as the major mediators of alterations in cerebellar development in response to virus infection in the developing CNS. Consequently, mice infected as newborns have impaired locomotor abilities throughout their lifetime. We have found that the compromised locomotor abilities are due to the loss of glutamatergic synapses in the cerebellum. Interestingly, only male, but not female mice, displayed compromised locomotor abilities and loss of synapses. Finally, we identify that IFN-γ is the major culprit for the observed immunopathology, which can be prevented by anti–IFN-γ antibodies.

L12

MODULATION OF BDNF SIGNALING THROUGH TrkB TRANSMEMBRANE DOMAIN

Giray Enkavi

University of Helsinki, Finland

The neuronal receptor tropomyosin receptor kinase B (TrkB), a member of the receptor tyrosine kinase family, plays a critical role in brain-derived neurotrophic factor (BDNF) signaling, essential for neuronal plasticity and survival. Our research demonstrates that antidepressant drugs, including typical and rapid-acting variants, function as allosteric potentiators of BDNF signaling through their interaction with TrkB's transmembrane domain (TMD). This binding stabilizes TrkB dimers, enhancing autophosphorylation and downstream signaling pathways. Cholesterol further modulates TrkB structure and function, influencing its signaling efficacy. Using molecular dynamics (MD) simulations and computational studies, we elucidate the detailed mechanisms of these interactions, providing critical insights into the modulation of TrkB signaling. These findings suggest that targeting the TrkB TMD holds promise for developing novel therapeutic strategies for neurodegenerative and psychiatric disorders by allowing precise modulation of BDNF signaling.

L13 Education and Training
THE POWER GenAI IN BIOSCIENCE EDUCATION

David Smith

Sheffield Hallam University, UK

Assessment in education is transforming with the help of generative artificial intelligence (GenAI), including GPT4o and other large language models (LLMs), which are becoming more prevalent in our daily lives. In this context, we will examine how AI has already been used in bioscience education, both inside and outside the classroom. GenAI provides a fast and efficient way to support research, learning and assessment, develop soft skills and evaluate biological data. During this session, we will share pedagogical approaches and assessment strategies successfully designed and implemented to incorporate GenAI into assessment practices and digital skills development. When used well, AI can be a valuable learning tool for students and an effective teaching assistant for educators.

L14
APPLICATIONS OF VIRTUAL REALITY IN RESEARCH AND TRAINING

Damjana Kastelic

Centre for Genomic Regulation (CRG), Spain

Virtual reality (VR) is poised to be a major technological advancement with countless applications across a wide range of professional fields. It offers entirely new methods for scientific collaboration, providing far more engaging and versatile possibilities compared to traditional video conferencing and online collaboration tools. As a result, VR has the potential to significantly reduce travel and production costs, promoting a more sustainable approach to work. Talk will highlight innovative virtual tools that facilitate more immersive learning, scientific networking, communication, research, and training, surpassing the capabilities of traditional platforms.

SHORT PRESENTATION ABSTRACTS

SL1

BREAKING BARRIERS: PCF10 TYPE 4 SECRETION SYSTEM RELIES ON A SELF-REGULATING MURAMIDASE TO MODULATE THE CELL WALL

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The combination of hospital acquired infections and antimicrobial resistance is a large threat to public health. It is therefore of high interest to understand how antibiotic resistance genes spread between bacteria. Mobile genetic elements (MGEs) are regarded as the major carriers of resistance genes, and conjugation machinery known as the Type 4 Secretion Systems (T4SS) is one of the major routes of spreading MGEs. T4SSs are membrane spanning megadalton-sized protein complexes that facilitate delivery of MGEs from donor to recipient cells. The assembly of the T4SS channel requires a regional lesion in the cell wall, which is introduced by peptidoglycan remodelling enzymes that are usually part of the T4SS. In contrast to their Gram-negative counterparts, Gram-positive T4SSs are less well-characterized, with very limited structural and functional data available.

PrgK, the cell wall remodelling enzyme of the enterococcal pCF10 T4SS, is essential for efficient conjugation and plasmid transfer. According to primary sequence, PrgK is predicted to have a transmembrane helix and three extracellular enzymatic domains: LytM, SLT and CHAP. In this study, we present the structure of these three domains of PrgK by a combination on crystallography and AlphaFold modelling. Our crystal structure show that the LytM domain has a degenerate active site. The other two domains, SLT and CHAP, on the other hand, have conserved active sites. Furthermore, AlphaFold modelling predicted that PrgK interacts with another critical protein of pCF10 T4SS, namely PrgL, that is thought to be involved in channel assembly. This PrgK-PrgL interaction was proven by size-exclusion chromatography coupled to multi angle light scattering (SEC-MALS). *In vitro,* only the SLT domain was active against cell wall extracts of *E. faecalis*. Furthermore, we show that this domain does not have its predicted lytic transglycosylase activity, but rather muramidase activity. The CHAP domain, as the LytM domain, shows no significant catalytic activity *in vitro*. Instead, these two domains regulate the activity of the SLT domain. Surprisingly, this regulatory effect of LytM and CHAP is lost when SLT acts on peptidoglycan from *Vibrio cholerae*, a Gram-negative bacterium. Our

finding here provides new pieces to the puzzle of how the structure, function and assembly of Gram-positive T4SSs.

Key words: T4SS, conjugation, peptidoglycan, muramidase

SL2

A NOVEL VERSION OF THE METAL-FREE CLASS IE RIBONUCLEOTIDE REDUCTASE

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The reduction of ribonucleotides to deoxyribonucleotides under aerobic conditions is catalysed by class I ribonucleotide reductase (RNR). Class I RNRs consist of a smaller subunit R2 that generates a radical that is shuttled to a larger subunit R1 and used there for catalysis. Based on the cofactor used in the radical generation in R2 class I is divided into different subclasses. Class Ib uses a di-nuclear manganese metal centre[1], while class Ie does not require a metal cofactor for radical generation[2,3]. Class Ib is the evolutionary ancestral class. Three of the conserved metal-binding carboxylate ligands of the R2 subunit are substituted in class Ie. Sequences of two different versions of R2e have been found[2]; the evolutionary newer R2e has a valine, proline and lysine in place of the three glutamates in R2b (EEE-> VPK). The focus of recent research has been on this variant. Activity of class Ie_{VPK} requires the posttranslational hydroxylation of the typically radical-harbouring tyrosine close to the active site in R2 to a 3,4-dihydroxyphenylalanine or DOPA[2].
The evolutionary link between the $R2e_{VPK}$ and R2b is $R2e_{QSK}$ with glutamine, serine and lysine (EEE->QSK) in the active site. Here we describe the QSK version of class Ie R2 for the first time. We analyse the taxonomic distribution of the two R2e variants, which each form a distinct clade. We show that in the $R2e_{QSK}$ of the human pathogen *Gardnerella vaginalis* the radical harbouring residue is likewise a DOPA. Using LC-MS we demonstrate that the amount of hydroxylated protein depends on the coexpression of R2 with R1, NrdI and NrdH, the other proteins of the RNR operon. We solve the first structures of unmodified and modified $R2e_{QSK}$ protein. The structures show the absence of metals bound in the active site, which is confirmed by spectroscopic investigations. Finally, we can demonstrate the *in vitro* activity of the class Ie QSK RNR and find it dependent on both the DOPA modification and presence of NrdI.

1. John, J. *et al.* Redox-controlled reorganization and flavin strain within the ribonucleotide reductase R2b–NrdI complex monitored by serial femtosecond crystallography. *eLife* **11**, (2022).

2. Srinivas, V. *et al.* Metal-free ribonucleotide reduction powered by a DOPA radical in Mycoplasma pathogens. *Nature* **563**, 416–420 (2018).
3. Lebrette, H. *et al.* Structure of a ribonucleotide reductase R2 protein radical. *Science* **382**, 109–113 (2023).

SL3 (Mini Symposium)
CANCER IN LIVER OR THE STORY OF MANY-FACED GOD

Uršula Prosenc Zmrzljak[1], Kaja Blagotinšek Cokan[1], Ana Marentič[1], Iva Sabolić[2], Radoslav Atanasoski[1], Barbara Jenko Bizjan[1,3] , Emanuela Boštjančič[4], Tina Drašković[4], Branislava Ranković[4], and Nina Hauptman[4]

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Bia Separations CRO, operating under the umbrella of Labena d.o.o., has a registered research group. We collaborate with researchers from various fields: from implementing new technological processes in pharmaceuticals to research and development in diagnostics. In this presentation, we will introduce our research in cooperation with University of Ljubljana, Faculty of Medicine, Department of Pathology, in the field of liver cancer, which we are conducting using the Xenium platform (10x Genomics). This is an in-situ spatial transcriptomics analysis method that enables localization of transcripts based on histology. Thus, we simultaneously obtain information about spatial distribution and gene expression at the subcellular level.

Liver tumors are common and include primary and metastatic tumors. Accurate determination of the tumor type is a crucial step in selecting the optimal treatment. The most challenging task is to differentiate between metastatic adenocarcinomas of different origins and between metastatic adenocarcinomas and cholangiocarcinoma. This distinction is sometimes difficult, despite the use of the most comprehensive clinical, laboratory, radiological, endoscopic, and pathological examinations. Such a tumor is called a cancer of unknown primary origin.

With the help of spatial transcriptomics tools, it is possible to define markers that, upon appropriate validation, could contribute to the diagnosis of liver cancers and significantly improve the prognosis of treatment for patients.

SL4

CALPONINS: GUARDIANS OF STRESS FIBER DYNAMICS IN NON-MUSCLE CELL ARCHITECTURE

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The actin cytoskeleton is a complex three-dimensional meshwork that generates force for cellular processes, including cell division, motility, morphogenesis, and endocytosis. Consequently, the actin cytoskeleton is essential for the development and normal physiology of multicellular organisms, and abnormalities in the actin cytoskeleton are linked to diseases, such as cancer progression. In non-muscle cells (NMCs), stress fibers (SFs) are the most prominent actomyosin structures. In addition to actin and non-muscle myosin II, SFs are composed of a large array of proteins that regulate their assembly and contractility. While functions of few SF components have been characterized, our understanding on other proteins, including the Calponin (CNN) family proteins, have remained elusive. By depleting the three Calponin isoforms (CNN1, CNN2 and CNN3) individually and together with each other from USOS cells, we revealed Calponins are not negative regulators of myosin II activity as previously proposed, but that they instead are critical regulators of SF architecture and integrity. Consequently, depletion of Calponins resulted in decreased thickness and increased fragility of SFs, and consequent diminished traction forces, as well as defects in cell morphogenesis and migration. Interestingly, our results also revealed that the non-muscle cell Calponin isoform CNN3 displays much more rapid dynamics in SFs as compared to the smooth muscle specific CNN1, and that expression of the smooth muscle CNN1 in non-muscle cells leads to smooth muscle -like thick and static stress fibers. Thus, our data also provides the rationale for the presence of smooth and non-muscle specific CNN isoforms.

SL5

IqgC NEGATIVELY REGULATES MACROPINOCYTOSIS BY DEACTIVATING RasG, BUT RasG IS REQUIRED FOR ITS RECRUITMENT TO MACROPINOCYTIC CUPS

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Macropinocytosis is a bulk nonselective uptake of extracellular fluid that is conserved from unicellular eukaryotes to mammals. However, while mammalian cells use macropinocytosis for specialised functions, the amoeba *Dicytostelium discoideum* uses macropinocytosis as a way of feeding. Interestingly, cancer cells also frequently use macropinocytosis for macromolecule uptake in order to thrive in an amino acid-poor microenvironment. Increased Ras activity has been shown to promote macropinocytosis in both cancer and *D. discoideum* cells. This is one of the many reasons why this amoeba has become a valuable model for biomedical research.

Ras activity promotes macropinosome formation and RasG is one of the most important positive regulators of macropinocytosis in *D. discoideum*. Previous research has shown that an IQGAP-related protein IqgC functions as RasGAP (*Ras GTPase activating protein*), which specifically deactivates RasG during macropinocytosis. Unexpectedly, the deletion of *iqgC* did not lead to an upregulation in macropinocytosis. This was tested in the axenic strain of *D. discoideum*, which already exhibits significantly increased fluid uptake due to the deletion of another RasGAP NF1 which normally strongly suppresses macropinocytosis in wild strains. Therefore, we investigated the effect of IqgC depletion in the NF1+ background of the *D. discoideum* wild strain. Indeed, in this strain we demonstrated that IqgC negatively regulates growth in liquid culture medium and macropinocytosis by restricting the size of macropinosomes. Next, we showed that the recruitment of IqgC to macropinosomes depends on the interaction with its cognate GTPase RasG and that the RasG-binding RasGAP domain of IqgC is required to specifically direct IqgC to the macropinosome membrane. Finally, we have shown that IqgC colocalises and interacts directly with an active form of another small GTPase Rab5A, but does not stimulate its GTPase activity. Furthermore, we observed that an excessive amount of Rab5A reduces the RasGAP activity of IqgC *in vitro* and is associated with premature dissociation of

IqgC from the internalised macropinosome *in vivo*. Therefore, we presume that Rab5A may influence the binding of IqgC to RasG. However, the biological significance of the interaction between IqgC and Rab5A requires further investigation.

SL6

PROTEIN QUALITY CONTROL IN QUIESCENT YEAST CELLS REQUIRES PARALLEL ACTIVITY OF THE PROTEASOME AND A NOVEL FORM OF SELECTIVE AUTOPHAGY

Dina Franić [1,#], Mihaela Pravica [1,#], Klara Zubčić [1], Antonio Bedalov [2], Mirta Boban [1,]

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Accumulation of misfolded proteins can disrupt essential cellular processes and has been implicated in aging and disease such as Alzheimer's, Parkinson's, ALS and others. Under normal circumstances, cells prevent the accumulation of misfolded proteins through the protein quality control (PQC) system, a network of pathways comprising protein refolding, selective degradation and spatial sequestration. PQC has mainly been studied in growing cells, however, many cells, such as microorganisms and metazoan stem cells spend a significant portion of their lifetime in quiescence, a reversible non-dividing state characterized by a distinct cellular organization and metabolism. For example, glucose-depleted quiescent yeast cells induce autophagy and a large fraction of proteasomes relocalize to the cytoplasmic granules thought to contain inactive particles. Here we investigated how quiescent cells manage misfolded proteins by using yeast *Saccharomyces cerevisiae*, a unicellular eukaryote with evolutionarily conserved PQC pathways. We found that misfolded proteins were targeted for selective degradation that required functional proteasomes, indicating that a substantial fraction of the proteasomes remain active in quiescent cells. Misfolded proteins were degraded in a E3 ubiquitin ligase Ubr1- and San1-dependent manner, similar to growing cells, however, upon prolonged substrate expression, degradation became almost exclusively Ubr1-dependent, indicating the inability of substrate modification by San1. Furthermore, degradation of certain substrates required a parallel involvement of autophagy, which occurred independently of the only known ubiquitin-binding autophagy receptor in yeast, Cue5, and required intact nucleus-vacuole junction. This suggests a novel mechanism for selective autophagy of misfolded proteins in quiescent yeast. Taken together, our data shed new light on protein quality control in cell quiescence and point towards importance of misfolded protein elimination during this state.

SL7 (Holder of the HDBMB Annual Award to Young Scientists for 2022)
HOW DID NEGATIVE CATALYSIS TAILOR THE SPECIFICITY OF ISOLEUCYL-tRNA SYNTHETASES'S EDITING DOMAIN?

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Aminoacyl-tRNA synthetases (AARS) catalyse the formation of aminoacyl-tRNAs for ribosomal protein biosynthesis. Aminoacylation occurs in the enzyme's synthetic active site by the amino acid activation and its subsequent transfer to the tRNA. Some AARSs cannot achieve the required level of amino acid selectivity in the activation reaction and thus may incorrectly couple the non-cognate amino acid-tRNA pair. To secure the errorless protein biosynthesis, those AARS evolved a separate editing domain to hydrolyse misaminoacylated tRNAs (post-transfer editing). To understand what shaped the selectivity of the editing site we used *Escherichia coli* isoleucyl-tRNA (IleRS) synthetase as a model enzyme. We investigated the kinetics of synthetic and editing reactions of IleRS using a wide range of amino acids, from proteinogenic to synthetic: Val, Nva, Leu, Thr, Met, Ser, Ala, α-aminobutyrate, norleucine, γ,γ-difluoro-α-aminobutyrate and γ,γ,γ-trifluoro-α-aminobutyrate. We observed that among the tested amino acids only Val and Nva were poorly discriminated in the IleRS synthetic active site (i.e. they were well activated and transferred to the tRNA) and hence pose a threat to the fidelity of translation in the absence of editing. However, tRNAs misaminoacylated with all tested amino acids were rapidly hydrolysed at the editing domain, showing that, surprisingly, neither the amino acid's physicochemical properties nor the level of amino acid discrimination at the synthetic site particularly influence the selectivity of the editing domain. Only the hydrolysis of cognate Ile-tRNAIle (misediting) was slow, suggesting that the need to keep the cognate product out of editing (i.e. preserving the substrate of protein biosynthesis) strongly shaped the evolution of specificity of the editing domain. More detailed kinetic analysis uncovered that IleRS uses Thr246 and His333 for specific destabilization of Ile-tRNAIle hydrolysis, a strategy also known as negative catalysis. Such design enabled IleRS to have a broad substrate acceptance at the editing site whilst maintaining a high specificity towards preventing the futile post-transfer editing cycles. This is the first observation of such broad substrate specificity paired with negative catalysis for some AARS, thus marking a unique point in understanding these fundamental enzymes.

SL8 (Holder of the HDBMB Annual Award to Young Scientists for 2022)
RETOOLING YEAST *Saccharomyces cerevisiae* INTO A NEXT-GENERATION EUKARYOTIC WHOLE-CELL COPPER BIOSENSOR AND DEPLOYING IT AS A LIVING COMPONENT OF THE PLURONIC F127-BASED BIOINK

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Although an essential micronutrient and a metal indispensable for modern technology, copper is toxic at high concentrations. However, traditional methods for quantifying copper are costly, requiring sophisticated equipment and specialised personnel. We address this challenge by leveraging the intrinsic copper response of the yeast *Saccharomyces cerevisiae* and thus retooling this microorganism into a next-generation eukaryotic whole-cell copper biosensor. By combining a dual-reporter fluorescent ratiometric system with an engineered *CUP1* promoter and an overexpressed Cup2 transactivator, we constructed a biosensor able to detect 10^{-8} to 10^{-3} M of bioavailable copper. This biosensor exhibits unparalleled specificity, sensitivity, and a broad detection range while disregarding non-bioavailable copper and other heavy metals. The constructed biosensor is relatively independent of the cell's physiological status and was validated on real-world samples which contained interfering substances. To expand the application of this biosensor, we incorporated it into a Pluronic F127-based bioink. This bioink transforms from a liquid at 4°C to a biocompatible hydrogel at room temperature, making it ideal for 3D bioprinting and engineering of living materials. To ensure the stability of the bioink, we thoroughly characterised cell-hydrogel interactions in such a material. By combining genetically encoded ratiometric biosensors with confocal imaging, we measured intracellular ATP and cytosolic pH at a single-cell level. This approach revealed that cells embedded in hydrogels were ATP-rich, in the exponential or stationary phase. They also formed microcolonies, which sometimes merged into larger superstructures. The hydrogels supported (micro)aerobic conditions and induced a nutrient gradient that limited microcolony size. Furthermore, the hydrogels provided a protective environment for the biosensors, shielding them from contaminations and providing them with nutrients. These findings underscore the potential of Pluronic F127-based hydrogels as effective scaffolds for 3D-bioprinted engineered living materials and highlight their practicality for deploying yeast whole-cell copper biosensors in real-world scenarios.

SL9 (Mini Symposium)
UNLOCKING DIFFICULT INTERACTORS: INSIGHTS INTO STRUCTURE AND INTERACTIONS WITH NANOTEMPER TECHNOLOGIES PLATFORMS

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In order to study the interactome of a given group of proteins requires understanding of their nature and physicochemical character to optimally prepare a given biological system to exert its proper, physiological function. Many proteins are notorious for causing complications in the sample preparation and analysis, especially in the context of biophysical methods. For example; membrane proteins, due to their amphipathic nature as well as lipid bilayer surrounding not only are complicated to stay soluble, but also to maintain their tertiary structure and function intact. To assess both the structural integrity and to determine the strength of various interactions of different proteins we have created a workflow consisting of two of our platforms- Monolith X and Prometheus Pana.
The Monolith X provides the flexibility to work with all types of molecules. The method is immobilization free, requires a few microliters of sample, and experiments can be done in any buffer, including lysate or serum. For the interaction analysis, Monolith X features the latest innovation for measuring binding: Spectral Shift technology. This method is based on a well-known phenomenon where organic fluorophores report changes in their chemical microenvironment by slight shifts of their emission spectrum, e.g., changes in their overall fluorescence intensity or blue- or red-wavelength shifts.
The Prometheus Panta enables scientists to assess the stability and colloidal properties of any protein of interest and to adequately adjust the buffering conditions to both enable and predict the functional behavior of the protein. A combination of four detectors within a parallel readout comprises differential scanning fluorimetry (nanoDSF), turbidity detector, dynamic light scattering (DLS), and static light scattering (SLS), grants researchers unmatched data precision and parameters complexity.
During our talk you will learn the details of our platforms, see an ample of examples coming from our customers as well as get to know the span of potential applications matching your own research. Visit also our booth during the meeting and speak with our expert to find out more about our unmatched products!

SL10

EVOLUTIONARY CONSERVATION REVEALS KEY RESIDUES GOVERNING ACTIVATION OF ATGL, A CENTRAL PLAYER IN INTRACELLULAR LIPID DEGRADATION

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Objective: Adipose triglyceride lipase (ATGL), the principal enzyme in intracellular lipid degradation, plays a critical role in energy homeostasis through the hydrolysis of stored triacylglycerol (TG). Human patients with mutations in the gene coding for ATGL suffer from TG accumulation in multiple tissues in addition to severe cardiomyopathy. ATGL inhibition holds promise for improving metabolic health such as insulin resistance and cardiovascular disease. This study investigates the modulation of ATGL activity by its key coactivator protein termed comparative gene identification-58 (CGI-58).

Methods: Through a comparative genomic approach, we examined the conservation of ATGL across various species within the animal kingdom to identify potential interaction sites. Artificial intelligence–based structural modeling was employed to predict the complex formation between ATGL and CGI-58. Site-directed mutagenesis, activity assays and interaction studies combined with integrated structural biology approaches (HDX-MS, state-of-the-art bioimaging systems) were utilized to assess the importance of the identified residues in ATGL concerning coactivation by CGI-58.

Results: Mutant ATGL at residues N209, I212, and N215 manifested normal basal activity but revealed substantially reduced activation by CGI-58. These findings underscore the crucial involvement of these amino acids in ATGL's functional interaction with CGI-58, indicating that their evolutionary conservation may underpin a critical coactivation interface. Additionally, we will present novel integrated structural biology approaches confirming residues R299, G328, and D334 of CGI-58 at the interface.

Conclusion: This study expands our molecular understanding of ATGL, emphasizing the evolutionary conservation and functional importance of its interaction sites with CGI-58, and sheds light on the regulatory dynamics underpinning intracellular fat catabolism. Recognizing the central role of ATGL in lipid metabolism permits insights into the mechanistic aspects that could be targeted for therapeutic advancements in metabolic disorders involving lipid dysregulation.

SL11

SL11
ELUCIDATING THE MOVEMENT-DEFECTIVE MUTATIONS IN FLORIGEN

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The key developmental switch for the flower/fruit bearing plants is the change from vegetative to reproductive growth, and the timing is crucial. The climate changes have been already impacting the flowering, as several perennial and annual crops have flowered earlier by about 2 days per decade, during the last 50 years. The flowering locus T (FT; florigen), is a plant protein highly recognized as a part of the florigen complex, a mobile protein that is produced in the leaf companion cells and transported to the plant shoot apex to induce flowering. The long-distance delivery of FT is active and requires interactions with at least two proteins, as well as with the lipids. We have demonstrated that FT binds to the negatively charged phospholipids, such as phosphatidylglycerol (PG), over other lipid species, in the temperature-dependent manner [1]. Low ambient temperature promotes sequestration of FT onto the intracellular membranes, diminishing the free FT protein pool from translocating to the sieve elements. With the temperature increase to 22 degrees Celsius (the range of ambient temperature for the Arabidopsis plants, as well as for several crops), FT is deployed from the membranes, travelling to the plant shoot apex to induce the flowering process. We concentrated on the movement-defective mutations in FT [2], aiming to produce and characterize the FT mutant proteins with enhanced lipid-binding properties. The super sticky FT would be difficult to remove from the membrane, which could have a potential industrial application. Moreover, we aim to produce FT with diminished affinity towards anionic phospholipids. Our preliminary protein structure predictions, using ColabFold [3], suggest that the florigen's N- and C-terminal domains might be candidates to interact with the negatively charged phospholipid membranes. The protein species mutated in these regions will be screened using the lipid overlay assay and liposome sedimentation assay [4], whereas the kinetics and affinity of the protein – liposome interaction will be quantified using microscale thermophoresis (MST), and the surface plasmon resonance (SPR) technique.

References:

[1] H. Susila, S. Jurić, *et al*. Florigen sequestration in cellular membranes modulates temperature-responsive flowering. *Science*, 373(6559):1137-1142, 2021

[2] S.C. Yoo *et al*. Phloem long-distance delivery of FLOWERING LOCUS T (FT) to the apex. *The Plant J.,* 75(3), 456 - 468, 2013

[3] M. Mirdita *et al*.ColabFold:making protein folding accessible to all. Nat. Methods, 19: 679 - 682, 2022
[4] H. Susila, S. Jurić, *et al*. In vitro Assays to Evaluate Specificity and Affinity in Protein-phospholipid Interactions. *Bio Protoc.,* 12(10):e4421, 2022

SL12

TACKLING TRIPLE-NEGATIVE BREAST CANCER WITH NEW PEPTIDE-BASED PROTEIN-PROTEIN INHIBITORS VIA THE SUPPRESSION OF THE Wnt/β -CATENIN PATHWAY

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Triple-negative breast cancer (TNBC) represents 10-15% of all breast cancer (BC) cases and it is characterized by the lack of therapeutically targeted receptors, which makes chemotherapy the main therapeutic strategy. However, it possesses a high chemoresistance and metastization rate, which is associated to a small subset of cells in the tumor microenvironment, designated as cancer stem cells (CSCs). Among others, TNBC is enriched in Wnt pathway genes, particularly frizzled homolog 7 (FZD7). Preclinical studies have revealed that the downregulation of FZD7 significantly suppresses tumor formation through reduced cell proliferation. We have been developing antibodies towards the FZD7 receptor, which have shown high binding affinities and efficacy in inhibiting the Wnt/β-catenin pathway. Nevertheless, their production and stability have been an issue that is challenging to tackle, as for many antibodies.

Among other biologics, bioactive peptides have great application potential as multifunctional players in cancer. Peptides are easy to synthesize, have lower immunogenicity and toxicity, and have high selectivity. Considering the overexpression of the FZD7 receptor, peptide-based inhibitors can potentially inhibit tumor progression and metastasis. Therefore, we proposed to 1) characterize the overexpression of FZD7 receptor in TNBC cell lines (monolayer and spheroids), tumor biopsies, and animal model; 2) identify the complementary determining regions (CDRs) of our antibodies; 3) study the binding capacity towards FZD7 receptor using computational simulations; 4) synthesize the most promising peptides; and 5) evaluate their capacity to inhibit cell proliferation and migration.

The data obtained shows the peptides' therapeutic potential. Firstly, the histological characterization validated the overexpression of the FZD7 receptor in

all different TNBC samples. Secondly, using computational modeling, we were able to identify the peptide sequences of the antibodies' CDRs and validate their binding capacity towards the FZD7 receptor *in silico*. Thirdly, among the synthesized peptides, some demonstrated *in vitro* high binding affinity, high antiproliferative capacity, decreased cell migration, and downregulation of downstream proteins of the Wnt/β-catenin pathway. Additionally, they also demonstrated low toxicity and high stability.

SL13

ORPHAN NUCLEAR RECEPTOR Nurr1 – A POSSIBLE LINK BETWEEN PERINATAL HYPOXIA AND BEHAVIORAL ABERRATIONS IN ADOLESCENT RATS

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Mild to moderate hypoxic events during gestation can affect normal brain development, which may pass unnoticed at birth but manifest later as cognitive or behavioral deficit. Findings on human neonates and animal models indicate dopaminergic (DA) dysfunction as one of the consequences of perinatal hypoxic injury. DA system bears a crucial role in behavioral adaptation, and its meso-diencephalic signaling pathway modulates the action of thalamus – a structure bridging sensory perception and cognition to identify behaviorally relevant environmental signals. To examine the link between an early-life hypoxic insult and adolescent behavioral aberrations, we recently established a rat model of mild perinatal hypoxia, by exposing postnatal-day-1 (P1) neonates to 8% O_2 for 2h, and observed altered exploratory behavior accompanied with cognitive impairments in juvenile rats. In this study we focused on hypoxia-induced alterations in the thalamic genes' expression. Thalamus and midbrain samples were collected from 16 hypoxia-exposed and 15 control rats on P50. Differential gene expression analysis performed by DESeq2 on the representative RNA samples, using |fold change| > 0.5 and Benjamini-Hochberg adjusted p-value < 0.05 as designated thresholds, found 27 downregulated and 96 upregulated genes in the hypoxia-exposed group. Among the top 10 upregulated genes we identified *Nurr1 (*also known as *Nr4a2)*, a transcriptional regulator considered important for differentiation and maintenance of meso-diencephalic dopaminergic neurons during development, whose mutations have been associated with DA-related disorders. Indeed, qPCR performed on the entire sample of 31 thalami revealed hypoxia-induced upregulation in the expression of genes for DA receptors D1 and D2, and their down-stream targets (DA- and cAMP-regulated phosphoprotein Mr-32-kDa, the regulatory subunit of protein kinase A, and inhibitor-5 of protein phosphatase 1), paralleled by a threefold increase in the midbrain DA concentrations, as measured by ELISA. We can conclude that *Nurr1* upregulation may represent one of the consequences of a mild perinatal hypoxic insult, potentially affecting development of the meso-diencephalic dopaminergic

neurons, with long-lasting effects on meso-thalamic DA signaling and, consequently, DA-related behavior. This proves our model as suitable for studying mild prenatal hypoxic events in humans, and opens a possibility for exploring new approaches for treating their long-term behavioral outcomes.

SL14 (Mini Symposium)
LABEL-FREE QUANTITATIVE PHASE IMAGING (QPI) — TRANSFORMING LIVE CELL ANALYSIS IN BIOMEDICAL RESEARCH

Lisa Bodily

Phase Holographic Imaging (PHI), Lund, Sweden

Quantitative Phase Imaging (QPI) has emerged as a non-invasive imaging technique that is transforming the landscape of live cell analysis in biomedical research. This method leverages the subtle phase shifts of light passing through living cell cultures, which are then algorithmically converted into digital cell images and quantitative single-cell data without the use of damaging light intensities or invasive labels. As a result, QPI allows for continuous, real-time monitoring and analysis of living cells – even in the incubator, making it an indispensable tool for applications ranging from cancer research, stem cell studies, and *in vitro* drug development to enabling critical cell quality control in regenerative medicine biomanufacturing.

The versatility of QPI is evident in its wide range of applications. For instance, it facilitates the study of cell growth, differentiation, motility and migration, and individual cell morphology changes, providing vital data on cell health and behavior without compromising cell integrity. This capability is particularly crucial for regenerative medicine, where maintaining the viability and functionality of cells for therapy is key. Moreover, QPI's ability to provide high-content quantitative data and time-lapse videos enhances its utility in both research and industrial settings.

The HoloMonitor® live cell analysis system is a pioneering QPI-based tool developed by Phase Holographic Imaging (PHI). It is placed inside standard cell culture incubators and leads the application of non-invasive QPI live cell analysis in providing sophisticated yet user-friendly solutions for live cell researchers and labs globally.

This talk will cover various application examples where HoloMonitor® has been key to advancing our understanding of cellular dynamics or enhancing therapeutic approaches. It will highlight QPI technology and demonstrate the HoloMonitor® system's seamless integration into research workflows as a robust platform for non-destructive, quantitative live cell analysis. Attendees will gain insights into the future possibilities of cell imaging and the significant advantages that label-free, real-time cellular monitoring brings to the scientific community.

SL15
REPAIR OF DNA PROTEIN CROSSLINKS BY TYROSYL-DNA PHOSPHODIESTERASE 2

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DNA-protein crosslinks (DPCs) are frequent lesions that can hamper replication and transcription, thus leading to impaired cell function. Specific, efficient repair of DPCs is essential for the preservation of genomic integrity. Despite recent advances in the study of DPC repair mechanisms, many aspects, especially at the organismal level, remain elusive. Here we show for the first time *in vivo* that the zebrafish orthologs of TDP2 (tyrosyl-DNA phosphodiesterase 2), Tdp2a and Tdp2b, are key players in DPC repair. Both orthologs have conserved sequence and domain architecture compared to human TDP2. They are expressed in zebrafish embryos and in adult tissues, including high expression levels in gonads. Silencing of *tdp2a or tdp2b* in zebrafish embryos did not result in overall gross morphological defects, but enzymatic activity assays showed impaired phosphodiesterase activity in *tdp2b* morphants only. Interestingly, activity assays further revealed that both Tdp2a and Tdp2b, when provided in excess in the embryos, resulted in strongly increased enzymatic activity. Silencing of *tdp2b*, but not of *tdp2a,* resulted in double strand breaks accumulations and increased DPC levels in zebrafish embryos. These effects were specific, as verified by rescue experiments. Taken together, our results show that *tdp2b* is dominantly expressed in zebrafish embryos and is the main contributor to overall endogenous Tdp2 activity during vertebrate development, that it repairs DPCs and protects against double strand breaks.

SL16 (Holder of the HDBMB Annual Award to Young Scientists for 2023)
SPRTN: A CRUCIAL PLAYER IN CELLULAR PROTEOSTASIS

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SPRTN is the first described protease involved in DNA repair. It resolves a specific type of DNA lesion created by covalent attachment of proteins to DNA molecule - DNA-protein crosslinks (DPCs). Mutations in the SPRTN gene lead to segmental progeroid features and the development of hepatocellular carcinoma. DPCs are among the most common types of DNA damage, making SPRTN crucial for cellular homeostasis. We investigated the role of SPRTN-dependent proteolytic degradation of DPCs and its relationship with downstream signaling and DNA repair. In this study, we developed a new assay for the isolation of DPCs, which allowed for the direct detection of DPC degradation, proving SPRTN as the main protease involved in DPC proteolysis. We showed that SPRTN is also crucial for activating downstream signaling through the ATM/H2Ax axis, leading to the recruitment of classical DNA repair enzymes such as PCNA, VCP, PARP1, XRCC3, etc. Failure to activate this signaling cascade prevents the repair of the underlying DNA damage, such as DNA breaks leading to mutations, chromosomal instability, and cell death. Following the discovery of this crucial DNA repair cascade, we switched our attention to the involvement of SPRTNs in the development of hepatocellular carcinoma. We found that a reduction in SPRTN expression led to activation of the ER stress response, which is usually caused by the accumulation of misfolded proteins within the ER lumen. We characterized the interaction between SPRTN and GRP78, which is the main sensor of misfolded proteins in the ER lumen and an activator of the ER stress response. It has previously been shown that ER stress leads to DNA damage. We showed that DNA damage occurring during ER stress directly results from a reduction in SPRTN protein levels in the nucleus under stress conditions. Our findings demonstrate how disruption of ER proteostasis could lead to the accumulation of mutations, chromosomal instability, and tumorigenesis. As both DPCs and misfolded proteins in the ER are different forms of proteotoxicity, we propose that SPRTN could be a general protease involved in preserving cellular proteostasis which is the subject of our current research.

SL17
SPLICE ISOFORMS OF ALPHA-SYNUCLEIN ENHANCE ITS AGGREGATION

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The misfolding and subsequent aggregation of the intrinsically disordered protein α-synuclein (αSyn) is associated with a family of neurodegenerative diseases termed synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). PD alone currently affects more than 6 million people worldwide and is characterised by prominent motor symptoms, such as slowness of movements and tremor, as well as cognitive decline at advanced stages of the disease. αSyn is highly enriched in synaptic terminals and is predominantly expressed as a 140-amino acid protein (αSyn-140) in its most abundant form. However, the advent of long-read RNA sequencing methods is revealing that a variety of αSyn isoforms could be generated via alternative splicing, leading to vastly different sequence compositions. Increasing evidence suggests that these variants are present in the brain and significantly involved in the overall aggregation process. Hence, in the first part of this study, we recombinantly expressed and purified a selection of these isoforms and characterised their biophysical properties *in vitro*. Using computational solubility predictions, thioflavin T aggregation assays, chemical kinetics analysis and transmission electron microscopy, we elucidated the mechanism of aggregation of these isoforms into amyloid fibrils, as well as their effect on the overall aggregation. We found that the variants we studied possessed marked differences in aggregation propensity and morphologies of the formed aggregates, as well as their capacity to accelerate the aggregation of αSyn-140 (Röntgen *et al.*, 2024, *PNAS*). To further elaborate on these results and understand the mechanism through which αSyn isoforms may interact, we are currently investigating the interaction of these disordered protein isoforms at the molecular level using a combination of solution-state and surface-based techniques. Moreover, we are also currently conducting experiments to determine the role of αSyn isoforms in tissue and animal models, using *in vivo* and *ex vivo* approaches. Together, these studies have the potential to unravel the contribution of alternative splicing to the pathological aggregation of αSyn and, thus, to better understand the development of PD and other synucleinopathies.

SL18

DHEA(S) AND BDNF IN DEMENTIA: FROM CELLULAR AND ANIMAL MODELS TO HUMAN RESEARCH

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Dementia is a syndrome associated with a progressive decline in memory, other cognitive abilities and changes in behavior, predominantly affecting older individuals, with Alzheimer's disease (AD) and vascular dementia (VaD) as the most common types. More than 55 million people have dementia worldwide, while nearly 10 million new cases are diagnosed every year. Currently available treatment options can only temporarily slow the worsening of dementia symptoms and improve quality of life, and therefore ongoing research aims to develop disease-modifying therapies. Neurosteroids dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), as well as neurotrophin brain derived neurotrophic factor (BDNF) have gained attention of scientists for their potential beneficial effects in dementia. Specifically, both DHEA(S) and BDNF have been involved in various brain functions such as neural survival, plasticity, cognition and behavior, and are altered in various neuropsychiatric disorders. Therefore, in order to better understand the complex role of DHEA(S) and BDNF in dementia, our research combined cellular and animal models, as well as human patients. To model AD and VaD *in vitro,* we have exposed primary mouse neurons and human neuroblastoma cells to toxic Aβ oligomers or oxygen-glucose deprivation (OGD), respectively. In animal studies, we have utilized triple-transgenic (3xTg-AD) mice and C57BL/6 mice intracerebroventriculary injected with Aβ oligomers, as genetic and pharmacologically induced model of AD, respectively. Finally, our research also enrolled patients with dementia and individuals with mild cognitive impairment (MCI), as comparative group. In addition to cognitive and behavioral evaluation in mice and humans, changes in the gene and protein expression of selected markers, associated with cell death and survival, have been studied in cellular and animal models. Moreover, we have investigated the associations of *SULT2A1* and *BDNF* gene polymorphisms with DHEAS and BDNF concentrations, respectively. Despite differences observed between different research approaches, obtained findings demonstrated potential neuroprotective effects of DHEA(S) and BDNF, suggesting that these compounds should be further studied as new options for the prevention and/or treatment of dementia.

The research has been supported by HrZZ project IP-2019-04-6100.

SL19 (Mini Symposium)
ELEVATING RESEARCH WITH THE LATEST APPLIED BIOSYSTEMS QUANTSTUDIO ABSOLUTE Q DIGITAL PCR

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Digital PCR (dPCR) is a nucleic acid quantification technique that allows absolute quantification without the need for standard curves. When digitizing a reaction, it's important to analyze as much of the loaded reaction as possible to maximize sensitivity and accuracy of measurement. The QuantStudio Absolute Q Digital PCR System analyzes over 95% of the sample, delivering more accurate data to help researchers find the information they're seeking. Using Microfluidic array plate (MAP) plates with proprietary technology, QuantStudio Absolute Q Digital PCR System uses microinjection molded plate technology to overcome common reagent distribution challenges of inconsistency and high dead volume. With the ability to multiplex using up to four optical channels, this system accommodates 16 samples per plate and boasts 20,000 micro-chambers per reaction. Key applications of this system include areas of research such as oncology, reproductive health, as well as applications in infectious and inherited diseases, gene editing, rare target quantification, genotyping, copy number variation, gene expression, and absolute quantification.

POSTER ABSTRACT

P1

EXPLORING THE DYNAMICS OF SPRTN PROTEASE AND TYROSYL-DNA PHOSPHODIESTERASE 1 IN DNA-PROTEIN CROSSLINK REPAIR

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Since DNA-protein crosslinks (DPCs) occur very frequently (more than 6000 per cell per day), they pose a significant threat to genome stability. Among the most abundant endogenous cellular DPCs are topoisomerases and histones. - Topoisomerase 1 (TOP1) resolves torsional stress in the DNA by generating single-strand DNA breaks, which can be stabilized and targeted by various drugs. However, the emergence of drug resistance in cancer cells requires a deeper understanding of TOP1-DPC repair mechanisms, especially at the organismal level. Our study reveals the crucial role of Tyrosyl-DNA phosphodiesterase 1 (TDP1) and SPRTN protease in the repair of TOP1- and histone H3-DPCs in human cells and in zebrafish animal model. Using the CRISPR-Cas system, we created a Tdp1-deficient zebrafish strain and optimized *sprtn* silencing using a morpholino-based approach. Additionally, we optimized RADAR (Rapid Approach to DNA Adduct Recovery) to enhance the reproducibility and specificity of cellular DPC isolation from human RPE1 cells and zebrafish embryos. Total and specific DPC analysis was preformed within the context of physiological deficiency of TDP1 and SPRTN, as well as following DPC induction by formaldehyde (FA) and camptothecin (CPT). These findings unveil a novel TDP1-mediated repair pathway for histone H3-DPCs, characterized by SPRTN proteolysis followed by TDP1-mediated 3' DNA end processing. In contrast, SPRTN and TDP1 operate within distinct pathways for the repair of endogenous TOP1-DPCs and total DPC resolution, yet converge in the same pathway for repairing DPCs induced by CPT and FA. This comprehensive study brings to light the complex mechanisms governing DPC repair in an animal model closely resembling human biology. These insights highlight the potential of TDP1 and SPRTN as promising drug targets in cancer therapy, while also providing a strong basis for understanding diseases associated with TDP1 and SPRTN deficiency.

P2
SALVAGING dNTPs FOR mtDNA RESCUE

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Ribonucleotide Reductases (RNRs) catalyse the committed step in the de novo synthesis of deoxyribonucleoside triphosphates (dNTPs). The small subunit of mammalian RNR exists in two alternative forms: RRM2 and RRM2B. While RRM2 is found in cycling cells, RRM2B is prevalent during quiescence. Consequently, RRM2B deficiency leads to impaired synthesis of dNTPs essential for mitochondrial DNA (mtDNA) replication in quiescent cells. Unfortunately, there is presently no cure for the spectrum of mtDNA Depletion Syndromes (MDDS) associated with RRM2B mutations, ranging from neonatal lethality in autosomal recessive cases to milder, later-onset autosomal dominant forms which mainly affect sense organs.
We have developed a murine RRM2B knockout (KO) model and observed that defective de novo synthesis primarily impacts purine dNTP production; we are therefore exploiting dNTP salvage pathways for therapy. Salvage is a recycling system whereby dNTPs are degraded into deoxynucleosides (dNs) and rephosphorylated back into dNTPs based on cellular metabolic needs. Although the precise dynamics of dN uptake and degradation in mammalian cells remain incompletely understood, our preliminary findings indicate that intravenous injection of exogenous dNs directly increases intracellular dNTP levels in various mouse organs.
We have also discovered that inactivating SAMHD1, a key salvage enzyme that degrades dNTPs into dNs, significantly elevates intracellular dNTP levels and extends the survival of RRM2B KO mice, albeit with a less striking impact on mtDNA copy number.
Our results demonstrate the potential for utilizing dNTP salvage pathways to ameliorate RRM2B deficiency, offering hope for patients afflicted with MDDS linked to RRM2B mutations.

P3

DEEP INTO THE PROTEOMIC NETWORK OF HEMOZOIN FORMATION IN THE MALARIA PARASITE

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Malaria is an infectious disease caused by *Plasmodium* parasites transmitted to and between people by mosquitoes. Despite progress in its control, malaria still threatens half of the world's population yearly, owing to the capacity of the parasite to develop resistance to antimalarial drugs treatment. Thus, innovative intervention measures are urgent and strictly depend on our better understanding of the parasite's complex biology. Throughout their lifecycle, malaria parasites repeatedly invade human host erythrocytes, causing the symptoms of the disease. Within erythrocytes, parasites endocytose hemoglobin into an acidic digestive vacuole. Hemoglobin degradation causes the release of toxic free heme that the parasite sequesters into hemozoin crystals to avoid cell damage. Despite not being well resolved, with different theories proposed for hemozoin formation, this process is vital for the parasite and exploited in antimalarial chemotherapy. Recently, a lipocalin was identified in *P. falciparum* and its conditional knockout demonstrated hemozoin crystal malformation and consequently parasite death. Thus, being lipocalin somehow involved in hemozoin formation, we sought to study its interactors, aiming to identify the players in hemozoin formation. For this, we leverage the yeast to hybrid approach with lipocalin as a bait, in addition to mass spectrometry of gene-edited parasite lines overexpressing lipocalin. This work will improve the knowledge around hemozoin formation and possibly provide a repertoire of targets for the development of new malaria therapies.

P4

FUNCTIONAL IMPACTS OF PROMOTER EVOLUTION IN *Cyprinus carpio carpio* REVEALED BY HIGH-RESOLUTION CAGE ANALYSIS

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Promoters are regulatory regions of DNA at the beginning of every gene, responsible for their timely expression. Core promoter elements define the exact position of the transcription start sites (TSSs) and the shape governing various promoter architectures. However, determining core promoter elements and their interplay and cooperation is not always straightforward. Without understanding the rules of transcription initiation, we cannot aspire to learn the laws controlling the activity of genes in their specific biological context. To probe promoter function genomewide, we employ cap analysis of gene expression (CAGE), currently the most unbiased method for assaying TSSs on a single basepair resolution. As a model of choice, we used the cyprinid fish common carp, *Cyprinus carpio carpio*, not only because of its close relation to the popular model organism zebrafish *Danio rerio* but also because of its recent whole genome duplication, which makes it an excellent system to study genome evolution. By performing CAGE on the developmental time course of the common carp, we discovered that the promoters of developmental genes tend to remain conserved between the two subgenomes. In comparison, promoters of genes involved in primary metabolism show more heterogeneity between the two subgenomes, suggesting their functional divergence compared to the ancestral genomes. Moreover, different promotor grammar between maternally inherited transcripts and zygotic transcripts previously described in zebrafish is mainly observed in one of the duplicated gene copies (ohnologs), meaning that the other ohnolog lost its function in one of the physiological states. Finally, we discovered that the changes from a canonical pyrimidine-purine initiator to a non-canonical initiator between the subgenomes drastically lower the initiator activity, pointing that mutations in promoters are the source of transcriptional divergence of two ohnolog genes. Overall, our results

clearly show an active role of core promoters in the evolutionary adaptation of gene expression.

P5

THE INFLUENCE OF DIPEPTIDLY PEPTIDASE 3 OVEREXPRESSION AND SILENCING ON SH-SY5Y CELL MIGRATION

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Dipeptidyl Peptidase 3 (DPP3) is a zinc-dependent aminopeptidase found across various species and tissues, participating in multiple physiological processes, including protein turnover, oxidative stress response, pain, and inflammation[1]. DPP3 is notably expressed in cardiovascular organs like the heart and blood vessels and is linked to cardiovascular diseases such as hypertension and heart failure. Additionally, it has been identified as a potential therapeutic target for these diseases due to its role in regulating blood pressure via the renin-angiotensin system and involvement in pain signaling[2]. Elevated DPP3 expression has been shown to promote cell proliferation, migration, and survival in vitro, as well as tumor growth and invasion in vivo, particularly in cancers such as esophageal carcinoma and colorectal cancer[3].

In this study, we investigated the influence of DPP3 on cell migration using SH-SY5Y cells, a human neuroblastoma cell line, as our model system. To assess the impact of DPP3 overexpression and silencing on these cells, we utilized the wound healing migration assay, a widely accepted method for studying cell migration. In this assay, a linear wound was created in a confluent monolayer of SH-SY5Y cells, and then cells were exposed to conditions promoting either DPP3 overexpression or silencing.

Preliminary results indicated that DPP3 overexpression enhanced the migration rate of SH-SY5Y cells, suggesting a role in promoting cell motility. Conversely, silencing DPP3 expression reduced the migration rate, indicating that DPP3 is a positive regulator of cell movement. These findings support the hypothesis that DPP3 plays a significant role in cell migration, which could have implications for understanding its function in physiological and pathological processes, including cancer metastasis and wound healing.

References

1. Malovan G. (2022). FEBS J., 290, 2246.
2. Ye, P. (2022). *Front. Cardiovasc. Med*, 9, 974035.
3. Liu, J. K., et al. (2023). *Oncol. Rep*, 49, 9.

P6

COMPARISON OF STRUCTURALLY DIFFERENT LIGANDS' ABILITY TO PREVENT AMYLOID FORMATION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common cause of dementia. AD predominately affects people over 65 years of age. With the rapid growth and general aging of world population, increasing prevalence of AD is becoming a worrisome burden on healthcare systems. For this reason, there is urgency among scientific community to develop effective treatment which would prevent the onset of the disease and/or slow down its progress. One of the major pathophysiological hallmarks of AD is the deposition of insoluble amyloid plaques in the extracellular space of the brain. These plaques, which are responsible for degeneration of neurons and consequent cognitive defects, largely consist of amyloid-β peptides composed of 42 amino acids (Aβ42) which are produced by proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases. Inhibition of β-secretase (beta-site APP cleaving enzyme 1, BACE1) and Aβ self-aggregation presents a promising strategy for the development of drugs which would slow down the disease through combating amyloid burden. In our research, we investigated the potential of three structurally different groups of compounds (hydrazone derivatives of pyridoxal and pyridine-4-carbaldehyde, quinuclidinium *O*-alkyl oximes and 4-aminoquinolines) to act as inhibitors of BACE1 and amyloid self-aggregation. Hydrazone-based compounds stood out as the most promising dual acting inhibitors of both BACE1 and amyloid self-aggregation. The lowest potency to inhibit the action of BACE1 was that of *O*-alkyl oximes, but which displayed considerable amyloid self-aggregation inhibition potency. Aminoquinolines displayed modest BACE1 and anti-amyloid self-aggregation potency. Our results show that all three structural groups have potential to be optimized and developed for targeting amyloid self-aggregation, of which hydrazone-based structures showed promising results to be further developed as exclusively BACE1 inhibitors as well. The CSF no. HrZZ-IP-2020-02-9343 for funding and the ERDF project KK.01.1.1.02.0007 "ReC IMI" for facilities and equipment.

P7

A CELL- AND DEVELOPMENTAL STAGE-SPECIFIC EXPRESSION OF METABOLIC AND SEROTONIN REGULATING GENES IN MURINE PLACENTA – THE EFFECTS OF MATERNAL DIET AND FETAL SEX AT TERM PREGNANCY

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The placenta is a temporary fetal organ with a primary role in the exchange of gasses and metabolites between mother and fetus [1]. Appropriate placental function depends on precisely controlled proliferation, differentiation, and metabolic activity of placental trophoblasts [2]. The serotonin in placenta may play a role in regulating trophoblast functions, including in response to adverse gestational environments, such as maternal obesity [3, 4]. Indeed, changes in placental function have been associated with maternal obesity in both humans and mice [5, 6]. The aim of this study was to determine the expression of serotonin signalling, cell metabolism and growth related genes in specific placental trophoblast types at different developmental stages, and if this may be impacted by maternal obesity and fetal sex.

To investigate this, we analysed publicly available single nuclei RNA-seq data of the mouse placenta obtained across gestation [7] and placental RNA-seq data from a mouse model of diet-induced obesity [8]. We assessed the expression of serotonin-regulating genes, namely the serotonin metabolic enzymes, *Maoa* and *Tph1,* and serotonin transporter *Slc6a4,* as well as genes involved in the regulation of cellular energy balance, lipid and glucose metabolism, and fetal growth, specifically *Pparγ, Lpl, Prkaa2, Akt1, Apoe, Cpt2, Irs3* and *Igf2*.

Our analyses revealed cell type- and gestational age-dependent expression of selected target genes in the murine placenta. For instance, *Maoa* was predominantly expressed in cells of the outer syncytiotrophoblast layer (SynT1) throughout the pregnancy, but only in the inner layer (SynT2) towards term. The observed pattern may reflect increased MAOA activity due to higher diffusion capacity of syncytial membranes towards term. The *Pparγ* gene was ubiquitously expressed but the most abundant in differentiated trophoblast lineages, while *Lpl* was expressed mainly in trophoblast progenitors (LaTp, LaTp2).

Furthermore, maternal obesity was found to significantly upregulate placental *Maoa* ($p=0.016$) and *Lpl*, ($p=0.035$) and downregulate *Apoe* ($p<0.001$) *Cpt2* ($p=0.017$) and *Irs3* ($p<0.001$).

We noted higher *Slc6a4* and *Apoe* expression in female versus male placentas that did not reach statistical significance ($p=0.052$ for both).

Maternal obesity-induced changes in the placental expression of metabolism- and serotonin-regulating genes could play a role in placental physiology and fetal development [9, 10, 11, 12, 13]. These data may be important for understanding the mechanisms underlying developmental disorders programmed in the offspring as a result of adverse intrauterine conditions.

P8

FUNCTIONAL ANNOTATION OF A DIVERGENT GENOME USING SEQUENCE STRUCTURE-BASED SIMILARITY

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Microsporidia are obligate intracellular parasites with extremely compacted genomes and an unusually high sequence divergence. This degree of divergence limits functional genome annotation using traditional, sequence-based methods as they result in numerous genes of unknown function. Compared to primary sequence, protein structure is generally more conserved as it is usually tightly linked to protein function. Therefore, with the current software for fast and accurate protein structure prediction and comparison, structure-based similarity searches can serve as a valuable, complementary approach to traditional functional annotation.
In this study, we combined traditional, sequence-based, and structure-based functional annotation and visualize the results in a ChimeraX plugin called ANNOTEX (Annotation Extension for ChimeraX). We applied this approach on our newly sequenced, high-quality genome of the microsporidian *Vairimorpha necatrix*, a parasite of Lepidoptera. First, we predicted protein-coding DNA sequences and confirmed 89% by RNA sequencing data. For the structural data, we folded the *V. necatrix* proteome using ColabFold and performed structural searches with FoldSeek against the PDB and AlphaFold databases. Both the structural and the sequence-based hits are then manually inspected and curated in ANNOTEX. The curation and the addition of structural matching enhanced the quality and accuracy of the *V. necatrix* genome annotation compared to when blindly relying on sequence similarity. With this approach we can present a comprehensive annotation of the *V. necatrix* genome and highlight the most prevalent protein folds in this understudied organism.

Taken together, we established a workflow for functional genome annotation of divergent, non-model organisms and used it to elucidate the underlying biology of a divergent lepidopteran parasite. We believe that the complementation of functional annotation through structural similarity is a valuable addition for gene function prediction in microsporidia and other divergent organisms.

P9

TANDEM MASS SPECTROMETRY IN COMBINATION WITH ION MOBILITY FOR CHARACTERIZATION OF DIET IMPACT ON CARDIOLIPIN MOLECULAR DIVERSITY

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Cardiolipin (CL) is a unique class of membrane phospholipid distributed almost exclusively in the mitochondrial inner membrane of eukaryotic cells. Extensive remodeling of CL occurs via a highly-conserved deacylation – transacylation process, and deficiency of the CL transacylase leads to Barth syndrome. Due to their unique structure, comprehensive characterization of CL molecular diversity is analytically challenging. Coupling traveling wave ion mobility and high-resolution tandem mass spectrometry (IM-MS/MS) enables a simple and rapid multidimensional characterization CL molecular diversity. Using IM-MS/MS we confirm that S*accharomyces cerevisiae* mutants completely devoid of CL remodeling (taz1Δcld1Δ) exhibit a shift towards CL species with more saturated and shorter acyl chains. We further demonstrate that CL acyl chain composition of WT and taz1Δcld1Δ cells is strongly influenced by the presence of exogenously supplemented fatty acids in the culture media. IM-MS/MS provides a powerful tool for the structural identification of CLs in complex biological samples.

P10
ROLE OF Arf GAP PROTEINS IN MURINE CYTOMEGALOVIRUS INFECTION

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Cytomegaloviruses (CMVs) are widespread DNA viruses that can cause severe clinical presentations in immunocompromised individuals. Murine CMV infection (MCMV) is used as a model of human CMV infection due to a much shorter replication cycle. We have previously shown that Arf proteins have important role in pathogenesis of MCMV infection. The function of Arf GTPases depends on activity of two groups of regulatory proteins: guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), which enable Arfs to cycle between active (GTP) and inactive (GDP) form. The aim of this study was to determine relevance of Arf GAP proteins in establishment and progression of immediate early and early phase of MCMV infection. Balb 3T3 cells were infected with MCMV and at different times post infection expression of Arf GAPs was analyzed by Western blot, while their intracellular localization was determined by confocal microscopy. Balb 3T3 cells transfected with siRNAs targeting different Arf GAPs were infected with MCMV, and viral load and percentage of infected cells were determined by flow cytometry while expression of immediate early and early viral proteins was determined by Western blot. The expression of Smap1 increases, while the expression of ACAP1, ACAP2, ARAP1, ASAP2 and Git1 remains practically unchanged during MCMV infection. The expression of ADAP1 and AGAP1 slightly increases at a late stage, and the expression of ArfGAP1 increases slightly in the early phase but in the late phase returns to baseline. Knockdown of ARAP1, AGAP1 and Git1 didn't have influence neither on establishment of MCMV infection neither on expression of early phase viral proteins, while ADAP1 knockdown reduced all of the above. In cells with knockdown of ArfGAP1 expression of early phase viral proteins (E1, m06 and m25) was increased, while knock down of ASAP2 increased only expression of E1. ACAP2 knockdown doesn't block the establishment of infection or expression of E1 but reduces m25 and m06 expression and therefore could have an impact on the formation of the virion assembly compartment.

P11

YEAST CELLS SUBJECT TO ABRUPT GLUCOSE DEPRIVATION RESUME ATP-CONSUMING PROTEIN QUALITY CONTROL BY THE UBIQUITIN-PROTEASOME SYSTEM

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To prevent the accumulation of misfolded proteins and the formation of protein aggregates, cells have developed protein quality control system, a network of evolutionarily conserved pathways that involve protein refolding, spatial sequestration of misfolded proteins into inclusions, and selective degradation by the ubiquitin-proteasome system and selective autophagy. A recent study has shown that yeast cells subjected to an abrupt glucose deprivation accumulate inclusions of Hsp104-GFP, an ATP-dependent protein disaggregase known to facilitate the disaggregation and clearance of misfolded proteins. This finding raised the possibility that protein quality control mechanisms in glucose-deprived cells switch from ATP-consuming selective degradation to spatial sequestration, but selective degradation of misfolded proteins has not been examined. Here we investigated degradation of model misfolded proteins expressed in yeast cells subjected to four hours of glucose deprivation. Surprisingly, our results show that these cells resume selective degradation of misfolded proteins. Furthermore, by using GFP-Atg8 in a free GFP assay, we show that glucose-deprived cells induce autophagy, but degradation of misfolded proteins was unaffected in the *atg1* deletion mutant, which lacks a key component of the autophagy pathway, suggesting that misfolded proteins are not targeted for autophagy under the conditions of acute glucose deprivation. In contrast, chemical inhibition of the proteasomes resulted in stabilization of misfolded proteins, demonstrating the involvement of the ubiquitin-proteasome system. Taken together, our results indicate that glucose-deprived cells resume selective degradation of misfolded proteins via ubiquitin-proteasome system, which is an ATP-consuming process, thus indicating the importance of misfolded protein elimination for the cell.

P12

WHOLE EXOME SEQUENCING OF A FAMILY WITH A HISTORY OF EARLY-ONSET HASHIMOTO'S THYROIDITIS

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Background/Objectives: Hashimoto's thyroiditis (HT) is a complex polygenic disorder of thyroid gland. To date, only one monogenic form of HT has been identified. Our objective was to explore a possible monogenic form of HT in a family with a history of overt hypothyroidism and early-onset HT.
Methods: We performed clinical assessment and thorough phenotypic characterization of family members. We chose nine participants for whole-exome sequencing (WES), comprising six diagnosed with HT and three unaffected individuals. Among these six patients, four had distinctive features: a) early onset HT (occurring between 5 and 12 years of age); b) overt hypothyroidism, c) markedly elevated levels of thyroid antibodies (TPOAb and/or TgAb). Subsequent to WES analysis, we quantified serum levels of candidate protein using enzyme-linked immunosorbent assay (ELISA). Lastly, we conducted whole-genome sequencing (WGS) on a trio sample (mother, father, and daughter) from this family.
Results: Under an autosomal dominance model, we identified a rare missense variant (rs17110563, MAF_CEU=0.0017), also known as the Pro206Ser mutation, within the tryptophan hydroxylase 2 (*TPH2*) gene. The *TPH2* gene encodes an enzyme responsible for converting L-tryptophan to 5-hydroxytryptofan (5-HTP), a precursor of serotonin. We measured 5-HTP levels in all family members carrying the mutation and 19 non-carriers (control individuals). Although carriers had lower 5-HTP levels compared to controls, the difference was not statistically significant (P=0.362). This indicates a mild dominant negative effect of the Pro206Ser mutation, as previously suggested by functional study of TPH2 enzyme. Of importance, another promising candidate gene implicated in regulation of the hypothalamus–pituitary–thyroid axis, TRHDE (thyrotropin-releasing hormone degrading enzyme), is located in proximity to the *TPH2* gene (within 65 kb). We therefore performed WGS in trio sample to resolve this question (analyses are currently in progress).
Conclusion: Investigating potential monogenic forms of HT holds promise for advancing our understanding and management of this common thyroid disorder. Our present findings imply a role of serotonin metabolism and HT. However, the

proximity of the thyroid-axis regulator *TRHDE* gene has prompted us to perform WGS to elucidate the genetic landscape of this genomic region.

P13

CARBAMATES – A PRIVILEGED PHARMACOPHORE IN THE DESIGN OF POTENT CHOLINESTERASE INHIBITORS FOR TREATING ALZHEIMER'S DISEASE

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Inhibition of the enzymes responsible for the hydrolysis of neurotransmitter acetylcholine, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), represents the first choice in the symptomatic treatment of Alzheimer's disease (AD). As the ratio of AChE and BChE concentrations changes during AD's progression, with BChE concentrations increasing in advanced stages of the disease, treatment using compounds that can inhibit both enzymes represents a promising therapeutic strategy in the regulation of acetylcholine levels in the brain and, consequently, long-term stabilization of cognitive and behavioural symptoms in advanced stages of AD. Due to their very good chemical and proteolytic stability and ability to penetrate the cell membranes, carbamate derivatives earned an important role in drug discovery and medicinal chemistry. Mechanistically, carbamates react with cholinesterases in a way analogous to the action of acetylcholine, but with a prolonged process of decarbamylation due to which carbamates are considered potent covalent inhibitors of cholinesterases. Our study was focused on the design of non-selective or BChE-selective carbamate inhibitors able to act also as chelators of Cu^{2+}, Zn^{2+} and Fe^{2+}, the bio-metals responsible for oxidative stress by enhancing ROS production and neuronal cell death. We synthesised two groups of carbamates, biscarbamates designed using a known selective BChE inhibitor as a structural scaffold and evaluated them as possible drugs in the treatment of late stages of AD, and quinuclidinium compounds as non-selective cholinesterase inhibitors, which can be used in all stages of the disease. The study has singled out bisdimethyl carbamate with piperidine in the hydroxyaminoethyl chain and carbamate with piperidine in the carbamoyl and hydroxyaminoethyl chain as the most promising compounds for the treatment of AD. They strongly and preferentially inhibit BChE, are non-toxic with the potential to cross the BBB and, compared to rivastigmine, possess the ability to chelate bio-metals. Non-quaternized quinuclidine carbamates and quaternized phenyl carbamates can be considered potential candidates for further evaluation as potential cholinesterase-based drugs, or as a good starting point in the design of new CNS-active drugs with improved inhibition potency toward cholinesterases.

P14
UNRAVELING SPRTN FUNCTION IN DNA METHYLATION MAINTENANCE

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Repair of covalent DNA-protein crosslinks (DPCs) by metalloprotease SPRTN is essential for genome maintenance as cells deficient in SPRTN are not viable. DPCs can arise from various sources such as formaldehyde, UV light, ionizing radiation and chemotherapeutic agents. Using the $Sprtn^{Y118C/Y118C}$ mouse model, we demonstrated that homozygosity for this missense mutation can replicate the features of human segmental progeroid syndrome Ruijs-Aalfs (RJALS). Our study linked defective DPC repair, accelerated ageing with the accumulation of epimutations with a significant increase in DNA methylation age of $Sprtn^{Y118C/Y118C}$. We proposed the putative mechanism where SPRTN facilitates DNA methylation maintenance via DNMT1/UHRF1 and defective biosynthesis in S-adenoslymethionine due to formaldehyde accumulation. Additionally, our *in vivo* experiments discovered that genetic depletion of cGAS in the RJALS-mouse model results in a reprogrammed DNA methylome, mitigated inflammageing, and alleviated RJALS-phenotype. Altogether, our findings uncover a previously unknown link between the repair of DNA-protein lesions and DNA methylation.

P15

THE NEUROIMMUNOMODULATORY POTENTIAL OF CD26 IN CROHN'S DISEASE

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Defects in the mechanisms that control inflammation and mucosal healing play a significant role in amplifying and perpetuating the intestinal immune response in Crohn's disease (CD). CD26, also known as dipeptidyl peptidase 4 (DP4), is expressed on intestinal epithelial and immune cells where it acts as a co-stimulatory molecule in the activation of T cells and is involved in the control of the cellular response through enzymatic processing of various mediators. Peptide YY (PYY) and substance P (SP) are its substrates whose receptor affinity is altered by its catalytic action. Since the interaction of these neuropeptides with their receptors is a crucial step in the initiation of the EGFR/MAPK or TGF-β1/SMAD signaling pathways which directly affect the initiation of antiproliferative effects, inhibition of cell growth, and induction of cell differentiation, the question arises as to how CD26 deficiency affects them in CD. Research was performed in wild-type and CD26 deficient mice with 2,4,6-trinitrobenzenesulfonic acid (TNBS) colitis, whose colon was dissected and used for protein and RNA isolation or embedded in paraffin. The expression of PYY, SP, their receptors and other candidate genes in the examined pathways was analyzed by qPCR. Protein levels in colon tissue was analyzed by western blot, ELISA and immunohistochemistry. We found significant alterations in gene and protein expression of both pathways as a result of CD26 deficiency in inflammation: Y1 receptor is increased, while Y2 is decreased, as are EGFR and PKCα, while ERK is increased, suggesting alternative MAPK routing in deficient mice. CD26 inactivates SP thereby inhibiting the SP/NK-1 receptor axis, but surprisingly, increases TGF-β1 and TGF-β receptor II which do not appear to be driven by SMADs, as their expression was not significantly different between strains. We concluded that CD26 deficiency alters neuropetide-mediated signaling and further studies in this area should be conducted to evaluate the potential application of DP4 inhibiting drugs in the treatment of CD.

P16

PHOTOSYNTHETIC PERFORMANCES AND PHOTOPROTECTION STRATEGIES IN THE HAPLOID AND DIPLOID STAGES OF THE COCCOLITHOPHORE *Calcidiscus leptoporus*

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Coccolithophores are an important group of calcifying unicellular algae that play a significant role in the global carbon cycle, oceanic ecology, and climate regulation. In contrast with most eukaryotes, they thrive through a haplo-diplontic life cycle: both haploids and diploids can undergo rounds of mitosis. Moreover, within a given species, haploid and diploid cells are generally enclosed in distinct calcareous plates (holo- and heterococcoliths, respectively) and it has been proposed that they display different physiological features to accommodate distinct ecological niches of the ocean. In this work, we compare physiological differences of haploid and diploid stages of *Calcidiscus leptoporus*, represented by strains from various locations around the globe. We investigated their (i) growth rates in a variety of light, temperature and nutrient conditions and (ii) photosynthetic electron transfers *in vivo* using room-temperature chlorophyll fluorescence. Since the latter method was hardly used before in coccolithophores, we introduce settings for pulse-amplitude modulated measurements, accounting for peculiarities such as the presence of holo- or heterococcoliths and cell densities lower than in model organisms (green algae). Differences in the electron flow saturation curves suggest distinct adaptations to their niche illumination regime. To better understand the distinct strategies in light harvesting optimization, we further recorded the functional antenna size of photosystem II and assessed the components of non-photochemical quenching. We discuss the relation between observed growth rates, photosynthetic performances, and photoprotection strategies with the original cell locations and their life cycle stages to justify ploidy-dependent physiological adaptations to distinct niches in *C. leptoporus*.

Keywords: Photosynthesis, chlorophyll fluorescence, nutrient response, life cycle, coccolithophores.

P 17

THE RESPONSE OF HUMAN GINGIVAL FIBROBLASTS TO METAL IONS ELUTED FROM ORTHODONTIC APPLIANCES

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Human gingival fibroblasts (HGF) are the main components of gingival tissue and play a key role in its maintenance. In this study, HGF are used to test the toxicity of different artificial saliva eluates prepared from orthodontic appliances. Each appliance consists of 2 orthodontic archwires, 20 brackets, 20 ligatures and 4 bands. The elution time was 3, 7, 14 and 28 days.
In all cells treated with experimental eluates, the cells change shape, shrink and curl and tend to form filopodia. In some cases, the formation of tunnelled nanotubes is also observed. Judging by the appearance of the cells under the microscope, the number of dead cells and the size of the plaques, the eluate of 7 and 14 days seems to be the most toxic. It can also be seen that the cells treated with 28-day eluate look more normal, are spindle-shaped and have few filopodia. This correlates with the amount and variety of metal ions (determined by the ICP-MS method) released in a given eluate. The highest amount of total ions released (> 8 mg/l), with the highest amount of nickel (> 4 mg/l), iron (> 3 mg/l) and chromium ions (> 0.6 mg/l), was in the 14-day eluate. The quantity and variety of metal ions released in a particular eluate have different effects on the cells and activate different enzymes of the stress reaction. The superoxide dismutases (SOD1 and SOD2) and other enzymes of the stress response (glutathione and thioredoxin related) determined by WB analyses have been shown to be involved in the oxidative stress response. One of the enzymes, glutathione peroxidase 4, whose expression decreases in response to elution time, leads to an accumulation of lipid peroxides, resulting in ferroptotic cell death. In addition, the 14-day eluate also showed a genotoxic effect and there is a dose-dependent trend of genotoxicity (determined by the comet assay). The stressful influence of the eluted metal ions was also confirmed at the mitochondrial level – all measured parameters (basal and maximal respiration, spare respiratory capacity and total ATP production, determined by seahorse analysis) decreased with increasing amounts of eluted ions.
So the beautiful Hollywood smile is not as harmless as we thought.

P18

QUALITY, QUANTITY AND NEXT GENERATION SEQUENCING ANALYSIS OF CIRCULATING CELL-FREE DNA OBTAINED FROM LIQUID BIOPSY SAMPLES OF PATIENTS WITH COLORECTAL CANCER

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Introduction: Circulating cell-free DNA (ccfDNA) has a resounding diagnostic potential in colorectal cancer (CRC) because it can reflect the accumulation of genetic modifications that cause CRC. The aim was to determine the quality, quantity, and presence of pathologic gene variants in ccfDNA obtained from liquid biopsy samples of patients with CRC using next-generation sequencing (NGS).
Materials and methods: Peripheral blood from 31 CRC patients was collected in CellSave tubes (Menarini Silicon Biosystems), plasma was separated by two-step 10 min centrifugation at 4°C (1900×g, then 16,000×g) and ccfDNA was isolated using the QIAamp® Circulating Nucleic Acid Kit (Qiagen). To verify the presence of a fragment corresponding to ccfDNA (160-170 bp) and the absence of high-molecular weight (HMW) DNA (>1000 bp), the isolates were analysed using the High Sensitivity DNA Kit on Bioanalyzer 2100 (Agilent Technologies). The amounts of isolated ccfDNA *per* mL of plasma were calculated by fluorometric measurement using the Qubit dsDNA HS Assay Kit (Invitrogen) on DS-11 FX (DeNovix). NGS was performed using the Archer LIQUIDPlex™ UNIVERSAL Solid Tumor panel (Invitae) on NextSeq550 (Illumina). Sequencing data analysis was performed using cut-off variant allele frequency (VAF) ≥0.0055. Detected gene variants were classified according to ClinVar.
Results: The amount of isolated ccfDNA ranged from 5.81-34.66 ng *per* mL of plasma. A fragment corresponding to ccfDNA was present in all isolates without detected HMW-DNA contamination. NGS revealed the presence of only one somatic pathogenic variant *BRAF* NM_004333.4:c.1799T>A in two samples (VAF=0.113 and 0.027) which was also reported as a germline variant. The remaining detected variants (N=21) were reported only as germline variants, among which pathogenic variants were present in *TP53* (N=5), *KRAS* (N=2) and *PIK3CA* (N=1) in 7/31 samples.
Conclusion: Isolates of ccfDNA of suitable quality and quantity for NGS were prepared from liquid biopsy samples obtained from CRC patients. Although

multiple variants were detected, including *BRAF* NM_004333.4:c.1799T>A, which was reported to have prognostic value in CRC and is associated with poor outcomes, considering the heterogeneity of ccfDNA, comparison of the obtained NGS results with NGS results from cancer tissue samples is necessary to validate their potential as biomarkers for CRC.

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P19

UNRAVELING SEX-SPECIFIC PATTERNS IN HNSCC: EXPLORING THE CLINICAL SIGNIFICANCE OF SEX HORMONE RECEPTORS DIFFERENTIAL EXPRESSION IN HNSCC

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Head and neck squamous cell carcinoma (HNSCC) encompasses a heterogeneous spectrum of malignancies originating from the squamous cells lining the mucosal surfaces in the head and neck region. Beyond the established risk factors such as tobacco and alcohol consumption, along with infections by high-risk HPV strains, emerging research highlights the endocrine microenvironment as another risk factor. Males exhibit a considerably higher relative risk for HNSCC, up to six times greater than females. This suggests the presence of either male-specific risk factors or protective hormonal and metabolic mechanisms in females. Consequently, we have initiated a comprehensive investigation into the involvement of both nuclear and membrane sex hormone receptors (SHRs) in HNSCC. Using quantitative real-time PCR (qPCR), we analyzed mRNA expression of nuclear and membrane androgen (*AR*, *OXER1*, *CACNA1C*, *SLC39A9*), estrogen (*ESR1*, *ESR2*, *GPER1*, *SCN2A*), and progesterone receptors (*PGR*, *PAQR5*, *PAQR6*, *PAQR7*, *PAQR8*, *PAQR9*, *PGRMC1*, *PGRMC2*) in 93 primary HNSCC tumors, 26 positive lymph nodes and 42 healthy oral mucosa samples. The difference in relative gene expression levels was compared with the patient's age, sex, stage and grade of cancer, HPV status and primary tumor site. Our findings revealed the median age at diagnosis was 64 years. Patients above this age showed higher expression levels of all androgen receptors, membrane estrogen receptors and three membrane progesterone receptors (*PAQR5*, *PAQR6* and *PGRMC1*). Among the cohort, 77.3% were men, while 22.7% were women. In women, there was a notable increase in expression levels of *CACNA1C*, *OXER1*, *GPER1*, *SCN2A*, *PGR*, *PAQR8* and *PAQR9* genes. Additionally, HPV DNA was detected in 18 (15.1%) out of 119 samples. The majority of tissue samples were categorized as stage IV, with a significant difference in *PAQR8* expression in relation to tumor stage. There was a significant decrease in *AR* expression in grade 3 tumors compared to predominant grade 2 tumors. This study represents the first comprehensive investigation into all three types of sex hormone receptors identified in HNSCC, including nuclear and membrane forms. Our findings highlight the significant role

of all sex hormone receptors, especially membrane ones, in crucial cellular processes and their influence on HNSCC progression.

P20

THE EFFECT OF SMALL MOLECULE MIF ENOLASE INHIBITOR TE-91 ON ACTIVATION AND METABOLIC REPROGRAMMING OF MACROPHAGES

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Introduction: Macrophage migration inhibitory factor (MIF) is one of the oldest cytokines discovered. MIF can inhibit the migration of macrophages, induce cell adhesion and phagocytosis and also plays a key role in numerous acute, chronic, and autoimmune inflammatory diseases, such as sepsis, chronic pneumonia, or inflammatory bowel diseases. A protective effect of MIF inhibition has also been described in various inflammatory models *in vivo* and *in vitro*. In this work, we investigated the effect of a small molecule MIF enolase inhibitor TE-91 on macrophage activation and its influence on inflammatory metabolic reprogramming in M1 polarized macrophage cells.

Materials and methods: Raw 264.7 mouse macrophage cells were induced with 10 ng/mL IFN-γ or with 100 ng/mL LPS + 10 ng/mL IFN-γ after a treatment with 20 μM TE-91. Macrophage activation was determined by measuring cellular ROS production via DHR 123 fluorescent dye, nitrite production with Griess-Ilosvay reagent, and TNF-α expression with Ready-Set-Go ELISA kit. Mitochondrial oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured with Seahorse XFp Extracellular Flux Analyser. Using specific mitochondrial respiratory chain inhibitors, key bioenergetic parameters, such as basal respiration, ATP production, proton leakage, maximal respiration, and spare respiratory capacity were determined.

Results: IFN-γ treatment increased ROS, nitrite, and TNF-α production compared to non-treated control cells. Surprisingly, TE-91 further elevated the macrophages' ROS production, however nitrite production and TNF-α expression were diminished significantly compared to IFN-γ treated cells. Moreover, LPS+IFN-γ reduced basal respiration, ATP production, proton leak, maximal respiration, and spare respiratory capacity in macrophages compared to vehicle control cells. In contrast to these, TE-91 significantly improved these parameters except for proton leak and spare respiratory capacity. In addition, LPS+IFN-γ increased basal ECAR, which was inhibited by TE-91 treatment.

Conclusion: Although the pharmacological MIF inhibitor TE-91 increased macrophage ROS production, it reversed inflammatory metabolic reprograming in M1 macrophages. These findings raise the possibility of testing TE-91 in animal *in vivo* models of diverse inflammatory disorders.

Abbreviations: LPS: lipopolysaccharide, IFN-γ: interferon-gamma, ROS: reactive oxygen species, DHR123: dihydrorhodamine 123, TNF-α: tumor necrosis factor alpha

Keywords: MIF, macrophage activation, metabolic reprogramming, mitochondria

P21

BIOAVAILABILITY AND INTESTINAL ABSORPTION OF BIOACTIVE MOLECULES FROM PLANT EXTRACTS AND FOOD BORNE PESTICIDE RESIDUES IN 2D CULTURE OF CaCo2 CELLS

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Caco-2 cellls, as a model of human intestinal epithelium, enable investigation of absorption mechanisms in the digestive tract for various molecules absorbed from food. In this work, we used a 2D culture of CaCo2 cells (CaCoReady, Spain), in in double wells with semipermeable polycarbonate filters. Myrtle extracts were applied to the upper chambers (apical side-A) in concentrations of total polyphenols (TP) of 800 µg TP GAE/mL medium. In this part of the experiment, we wanted to determine which of the 30 analysed polyphenolic plant biomolecules from the extract pass the intestinal barrier, and to what extent the relationship between their chemical structures, glycosylation, interactions during absorption, and basal or apical transport through the intestine influence entry into the human body after consumption. In the same way, in a separate experiment, we evaluated the interactions and transitivity of 30 pesticides (100 ng/mL each individually), which are often detected as residues in food. Biomolecules and pesticides were analyzed by HPLC-LC chromatography. The results indicate that Myrtle and glycosylation of, for example, of the most abundant polyphenols such as quercetin or myricetin, in the CaCo2 model of intestinal absorption, is the best model for demonstrating the turnover of glycosylated polyphenols. Glycosylation has profound effects on increasing bioavailability and stability. This result is significantly important because with glycosylated compounds can be used as food additives, therapeutics and nutraceuticals with enhanced apsorption. Among the pesticides, the cellular model shows selective absorption of only some of the applied pesticides, which indicates selective transport by transport proteins on cell membranes and the need for additional experiments that would determine the

correlation between the structure of the pesticide and the affinity for certain protein transporters and protein channel in the intestine.

P22

METABOLIC SHIFT IN HEPATIC CANCER CELLS CULTURED IN 3D COLLAGEN SCAFFOLD

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Over time, cell culture models have evolved from simple 2D monolayer cultures in Petri dishes to sophisticated 3D platforms, mimicking in vivo conditions. These 3D cultures replicate microenvironmental features of the extracellular matrix, which significantly influence cellular behavior and function. In this work, we investigate the mechanistic basis of cellular responses to physical cues in 3D cell culture. Specifically, we have focused on metabolic pathways mTOR and YAP and their crosstalk in cell cultured in 3D. Using biomaterial-based 3D cell culture model and techniques such as biochemical assays, genetic manipulation and immunodetection, we elucidate the molecular mechanisms underlying mechanotransduction in hepatic cancer cells cultured on 3D collagen scaffolds. Our research aims to deepen our understanding of tumor cell plasticity and signaling pathways, while also potentially informing the development of more predictive cell culture systems, including liver organoid models for drug validation.

P23

IDENTIFICATION AND CHARACTERIZATION OF PROTEINS RELATIVELY RESISTANT TO CARBONYLATION IN *E. coli* AND MAMMALIAN CELL PROTEOMES

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The proposed concept that the common cause of aging and age-related diseases is due to the oxidative damage to the proteins, which leads to irreversible change, i.e. protein carbonylation, which ultimately leads to the loss of protein function. To understand the natural protective mechanisms against protein carbonylation, aim of this study was to identify and biochemically and functionally characterize proteins that are intrinsically more resistant to carbonylation. To that end, both mammalian and bacterial cell lysates were subjected to UV radiation to induce oxidative damage, followed by aminooxy-biotin tagging of the carbonylated proteins and their removal with streptavidin beads. The remaining non-carbonylated proteins were then identified with mass spectrometry and characterized in detail using various bioinformatic approaches. Overall, 599 and 1078 proteins were identified as being naturally resistant to carbonylation (prior to UV-induced oxidative damage), whereas 299 and 418 proteins were identified as remaining resistant to carbonylation upon UV oxidation in bacterial and mammalian proteome, respectively. Functional enrichment identified that carbonylation-resistant proteins are involved in biosynthesis and metabolism of amino acids and nucleotides, heat shock as response to oxidative stress, and ribosome-related processes. Furthermore, they have a lower molecular weight and are more likely to be found in protein complexes. These findings offer insight on the protein-intrinsic characteristics that protect proteins against carbonylation, as well as cellular processes that evolved to be the most resistant to oxidative damage. Protecting proteins against carbonylation is a promising approach to slow down aging and postpone the onset of age-related diseases.

P24

OLIVE LEAF POLYPHENOLS ALTER THE EXCITOTOXICITY OF GLUTAMATE BY MODULATING GLUTAMATE TRANSPORT IN THE RAT BRAIN AFTER INDUCED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

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Glutamate (Glu) is the most important excitatory neurotransmitter of the central nervous system. An increase in extracellular Glu concentration leads to abnormal synaptic signalling and thus to neuronal excitotoxicity and death. The efficiency of Glu transmission depends on the correct function and expression of numerous receptors and transporters located on both neurons and glial cells. Abnormalities in the expression of glutamate receptors, transporters or metabolising enzymes have been observed in animal models of multiple sclerosis (MS) such as experimental autoimmune encephalomyelitis (EAE). In this study, we investigated the effect of olive leaf polyphenols on glutamate excitotoxicity.

Dark Agouti (DA) rats were used to study the effect of olive leaf extract therapy on EAE. The animals were divided into three main groups: 1 - the control group, 2 - the EAE-induced group and 3 - the EAE group, which was continuously treated with olive leaf extract (OLE) intraperitoneally (*i.p.*) for 10 days from the onset of EAE induction. The administered dose of olive leaf extract (OLE) was 1024 mg/kg, while the concentration of oleuropein was 45.96 mg/kg. In addition, group 3 received olive leaf tea (1.5% w/v, *ad libitum*) instead of water from the beginning to the end of the study. Groups 2 and 3 were sacrificed on day 20 after induced EAE.

The clinical course was observed until day 30 after EAE induction. The expression of glutamate receptors and transporters (GLUR1, NMDAR1, NMDAR2, GLUR5, GRID2, GLAST1, GLT1), antioxidant enzymes (SOD1, SOD2), demyelination/remyelination markers (MBP, TREM2, VAMP2), neurodegeneration (NeuN, SIRT1) and activation markers of microglia (IBA1) were determined in the homogenate of the rat brain by immunoblotting. In addition, immunofluorescence staining of GLAST, GLT1, SIRT1 and IBA1 was performed on paraffin-embedded tissue.

Conclusions: Our results show that olive leaf polyphenols are able to attenuate the intensity of EAE-induced symptoms and delay the clinical course of the disease. Moreover, increased expression of the glutamate transporter EAAT2/GLT1 and the antioxidant enzyme SOD2 as well as preserved myelin integrity by MBP and

expression of TREM2, activated microglia (IBA1) and preserved neurons (SIRT1) were found in the EAE+OLE 20d group. Therefore, this study suggests that OLE may have a positive impact on the course of MS treatment.

P25

MONITORING THE CELL CYCLE DYNAMICS OF PROMOTER ACTIVITY WITH NET-SLIC-CAGE REVEALS NOVEL RULES OF CELL CYCLE STAGE-SPECIFIC TRANSCRIPTIONAL REGULATION

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The eukaryotic cell cycle includes coordinated changes in transcriptional regulation punctuated by checkpoints to control its progression. The misregulation of this cellular process in multicellular animals can lead to abnormal development and cancer. Our work aims to understand the genome-wide promoter usage and the dynamics of enhancer activity at single-nucleotide level during cell cycle progression. Cap analysis of gene expression (CAGE) is a methodology that facilitates the identification of promoters and enhancers by quantitative mapping of capped RNA 5'-ends to capture transcription start sites (TSSs) at single-nucleotide resolution. Our work used Super-Low Input Carrier-CAGE (SLIC-CAGE) to profile TSS positions and expression levels from cells isolated from cell cycle stages by flow cytometry, which allowed us to identify sets of promoters with cell-cycle dependent activity. We developed NET-SLIC-CAGE, a novel method that combines Native Elongating Transcript-CAGE (NET-CAGE) and SLIC-CAGE, to obtain the TSS signal of nascent RNA, which increases the time resolution from cell cycle-sorted cells and boosts the transcriptional signal coming from active enhancers. Our integrative analysis of SLIC-CAGE from total RNA, and NET-SLIC-CAGE of nascent RNA, provided the first insights into patterns in RNA stability by comparing total vs nascent RNA levels at different cell cycle stages. We demonstrated and characterised differences in the transcription initiation dinucleotide frequency, along with RNA levels, at specific subsets of promoters in nuclear vs total RNA samples, as well as along the cell cycle. We conclude that NET-SLIC-CAGE is a powerful new methodology that, in combination with total SLIC-CAGE, enables the discovery of the regulatory and structural properties of non-coding sequences involved in cell cycle progression, addressing key gaps in our understanding of the fundamental mechanisms of transcription regulation.

P26

THE ROLE OF NON-CODING RNAS IN THE PROGRESSION OF IDH-MUTANT ASTROCYTOMAS TO GRADE 4 AFTER TREATMENT

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Low grade IDH-mutant (IDHmut) astrocytomas are heterogeneous brain tumors that are associated with a median overall survival of 7-10 years. However, patient survival is significantly worsened upon tumor progression to grade 4. Dysregulated non-coding RNAs (ncRNAs) have been linked to therapy resistance and invasion of the surrounding normal tissue in gliomas. Despite advances in the field, little is known about the role of ncRNAs in the progression of IDHmut astrocytomas.

We used a transcriptomic data-driven approach to uncover how ncRNAs regulate tumor progression at the molecular level. We collected matched tumor samples (before and after progression to grade 4) from six IDHmut astrocytoma patients and measured their genome-wide transcriptome and non-coding RNA expression profiles, respectively. By comparing our data with the The Cancer Genome Atlas (TCGA), consisting of unmatched primary tumors, and Glioma Longitudinal AnalySiS (GLASS), consisting of matched tumors, cohorts, we found that genes shared among all three datasets are related to cell proliferation, DNA repair and ERBB signaling. Genes unique to post-treatment progression are related to synaptic plasticity.

We found 32 differentially expressed (DE) microRNAs upon progression and identified 84 unique DE target genes. Gene set enrichment analysis showed that the target genes were associated with cell proliferation and SUMOylation. Furthermore, we observed 40 dysregulated long non-coding RNAs that can regulate 64 DE target genes enriched in neurodevelopment, cell proliferation and ERBB signaling.

Combined with our ongoing gene regulatory network analysis and modeling, we aim to unravel the molecular mechanisms through which the identified non-coding RNAs exert their function to promote astrocytoma progression after therapy.

P27

NME6 - STED MICROSCOPY VISUALIZATION AND IMPORT TO MITOCHONDRIA

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NME6 is a member of the nucleoside diphosphate kinase (NDPK) family of genes/proteins with crucial roles in controlling the cellular nucleotide homeostasis, membrane remodelling, cell signalling and metastasis suppression. The well characterized NME1-4 family members transfer the terminal phosphate from d(NTP)s to d(NDP)s through a high-energy phospho-histidine intermediate, and are, therefore, catalytically active. To this date the data on other NME proteins, including NME6, were scarce and inconsistent. Our recent comprehensive work clarified and extended the overall knowledge on the human NME6. We found NME6 to be present in the cell in two different isoforms. The recombinant NME6 protein is largely monomeric, and consequently, it does not exhibit the NDPK activity *in vitro*. Immunofluorescence and cell fractioning procedures revealed that NME6 resides in the mitochondrial matrix, probably adjacent to the mitochondrial inner membrane. Overexpression of NME6 negatively influenced oxidative phosphorylation without altering mitochondrial potential, mass or network characteristics. A screen for NME6 protein partners revealed its interaction with RCC1L, a protein involved in mitoribosomal assembly and translation.

Our present work is aimed at detecting the crucial signals within the NME6 sequence which allow it to enter the mitochondrion since NME6 does not possess the canonical mitochondrial targeting sequence. By using GFP fused deletants of NME6 we were able to localize a crucial region in the N-term portion of the protein responsible for the import of NME6 into the mitochondrion. Further, we are using STED (stimulated emission depletion) microscopy to directly see the fine details of the mitochondria and define the NME6 localization visually and in more detail. The goal of our study is to perform dual color STED between NME6 and markers of submitochondrial compartments. Our preliminary data excluded the presence of NME6 at the outer mitochondrial membrane while the analysis of the localization with other mitochondrial compartments is still under construction.

P28

INFUSE vs. OSTEOGROW: BIOLOGICAL ACITIVITY, RELEASE PROFILE AND EARLY CELLULAR RESPONSE

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INTRODUCTION: Infuse, consisting of rhBMP2 on an absorbable collagen sponge (ACS) is the only commercially available osteoinductive device for bone regenearation that showed major drawbacks. A promising alternative is a novel osteoinductive device Osteogrow consisting of rhBMP6 and autologous blood coagulum (ABC), which shows great potential in mitigating the shortcomings associated with ACS. This study aimed to determine biological activity of two osteoindcutive proteins, rhBMP2 and rhBMP6, as well as to determine release profile of mentioned BMP carriers, ACS and ABC. Furthermore, early cellular response of ectopic bone formation induced by aforementioned osteoinductive devices was eavaluated in rat subcutaneous assay at time points 24h and 72h, with determination of of lymphoid, myeloid and mesenchymal cell populations.
METHODS: The bioactivity of rhBMP2 and rhBMP6 was measured in a C2C12-BRE-Luc cell assay treated with a range of concentration from 1 to 400 ng/mL, while rhBMP2 release profile from ABC and ACS was determined by ELISA kit for rhBMP2. In order to determine earlly cellular response to mentioned osteoinductive devices, obtained cell suspensions were labeled with the following rat antibodies: CD3-PE, B220, GR-FTIC, CD11b-APC, CD44-AF647, CD90-APCCy7 and CD140a. Cell suspensions were passed through Attune flow cytometer and obtained dana was analyzed using the FlowJo software.
RESULTS: Cumulative release study showed that rhBMP2 was released from ACS in higher amount (85%) than from the ABC (56%) over 72 hours. Lymphoid populations ($CD3^+$ and $B220^+$), were present in significantly higher numbers in rhBMP6/ABC implants, 24h and 72h after implantation. In the contrary, myeloid population (GR^+CD11b^+), was significantly higher in rhBMP2/ACS implants after 24h and 72h. There was no difference among mesenchymal populations ($CD44^+$), however further sorting showed that adipoprogenitors were mostly present in rhBMP2/ACS implants, while early multipotent progenitors (EMP) were mostly present in rhBMP6/ABC implants after implantation 24h.
CONCLUSION: Novel biocompatible device for bone healing, rhBMP6/ABC showed sustained release profile in comparion to the rhBMP2/ACS. Myeloid populations were significantly higher in rhBMP2/ACS indicating stronger immune response probably due to the xenogenic origin of ACS. Furthermore, adipoprogenitors were mostly present in rhBMP2/ACS implants, while early EMP were mostly present in rhBMP6/ABC implants.

P29

EXPLORING THE MECHANICAL CHARACTERISTICS OF TUMOUR MICROENVIRONMENT IN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths globally, largely due to its ability to metastasize in vital organs such as the liver or lungs. The tumour microenvironment (TME) has a significant influence on cancer invasiveness. For example, CRC often exhibits an unusually stiff matrix compared to healthy tissue. The change in the mechanical properties of the TME can enhance metastasis through mechanisms such as epithelial-mesenchymal transition due to increased mechanical stress. Although the role of mechanical forces in TME is well-recognised, research has been limited by a lack of comprehensive *in vitro* models. In the current study, we utilise CRC cell lines and organoids cultured in two novel hydrogels to establish models suited for studying CRC invasiveness and mechanobiology. We cultured the colorectal cancer cell line HCT116 in two novel customisable hydrogels: a natural ECM-mimicking chemically modified hyaluronic acid (HA-Cx), and a synthetic "Plug-and-Play" protein-carbohydrate crosslinked with SpyCatcher/SpyTag -functionalized recombinant proteins. Additionally, we cultured the patient-derived CRC organoid line CRC_rc3 in the HA-Cx hydrogel. By altering the crosslinking strength of the SpyCatcher/SpyTag -hydrogel we plan to mimic the TME stiffness changes during cancer progression. We have synthesized both the natural HA-Cx and the synthetic SpyCatcher/SpyTag hydrogels and tested their functionality. We have established the CRC cell line HCT116 in both hydrogels, and the PDO line CRC_rc3 in the HA-Cx hydrogel supplemented with Matrigel. Our current findings establish the biocompatibility of the natural HA-Cx and synthetic SpyCatcher/SpyTag hydrogels and indicate their suitability for the study of CRC invasiveness with the cell line HCT116, and in the case of the HA-Cx hydrogel also in the context of patient-derived organoids. In the next steps we will establish the protocols and experimental set up required for the study and assessment of CRC invasiveness with the PDOs in both hydrogels. The long-term goal is to set up a platform to develop personalised therapies for CRC patients utilizing the tailored hydrogel models.

P30

THE MyD88 SIGNALING ACCOMPANIED BY MICROBIOTA CHANGES SUPPORTS URINARY BLADDER CARCINOGENESIS

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Urinary bladder cancer (BC) is the seventh most common type of cancer that causes significant impairment of life quality and carries a high risk of mortality. Infection with *Schistosoma haematobium* can cause BC, uropathogenic *Escherichia coli* is linked to BC, and the urinary microbiota of BC patients differs from that of healthy individuals. Furthermore, intravesical instillation of the bacterium *Bacillus Calmette-Guerin* is the gold-standard treatment for non-muscle invasive BC. Since the importance of the microbiota in the development of BC is evident, investigating the receptors and signaling molecules involved in bacterial recognition in BC is crucial. To address this, we exposed *Tlr4* and *Myd88* knock-out (KO) mice to the bladder carcinogen N-butyl-N-(4-hydroxylbutyl)-nitrosamine (BBN) during two (acute) or twelve weeks (chronic protocol). Urinary bladder pathology and gene expression, as well as the gut microbiota, were studied. Acute exposure to BBN showed no difference in bladder pathology despite differences in mice's ability to recognize and respond to bacteria. However, chronic treatment of *Myd88*^{KO} mice resulted in reduced invasiveness of tumors, while the absence of functional *Tlr4* did not affect BC development or progression. These differences correlate with increased *Faecalibaculum* genus abundance and decreased microbial diversity in *Myd88*^{KO} mice, highlighting the significant role of microbiota composition and MyD88-mediated signaling in bladder carcinogenesis.

P31

BIOSCAVENGING CAPACITY OF BUTYRYLCHOLINESTERASE REDUCES INHIBITION IMPACT OF ORGANOPHOSPHATE COMPOUNDS IN HUMAN BLOOD

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Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) are serine esterases which perform a crucial role in nerve impulse transmission by hydrolysing neurotransmitter acetylcholine (ACh). Although AChE primarily breaks down ACh, BChE can also hydrolyse various drugs and xenobiotics and it has for years been explored as a bioscavenging enzyme. Organophosphorus compounds (OP), like nerve warfare agents and pesticides, are potent inhibitors of AChE and BChE due to the phosphorylation of their catalytic site, and without a prompt treatment poisoning can result in fatal outcome. Administration of exogenous BChE isolated from human plasma could neutralise OP in the bloodstream before they reach target tissues, in combination with a potent oxime antidote that reactivates the inhibited cholinesterases. To investigate the oxime-assisted catalytic scavenging potency of BChE, we chose two *N,N*-aryl imidazolium oximes which showed potent *in vitro* reactivation of VX-, sarin-, cyclosarin-, tabun- and paraoxon-BChE conjugates. Their overall reactivation efficiency was up to 2,400-fold more prominent compared to pyridinium oximes used in standard medical treatment, especially for nerve agent cyclosarin. We tested the scavenging capability of oxime-exogenous BChE pairs in *ex vivo* conditions in samples of human whole blood inhibited with cyclosarin with a ratio of cyclosarin to supplemented BChE being 10:1 and 100:1. The results showed revival of over 60% of cholinesterase blood activity within 20 min using 10 μM oxime concentration. Moreover, the combined application of 10 μM standard pyridinium oxime HI-6 with a selected imidazolium oxime enabled about 90% of cholinesterase reactivation within 10 min. These results are a breakthrough in counteracting research, as this is an improvement of outcome when either oxime is used alone or without applied exogenous BChE. We also performed a docking study to identify important interactions of imidazolium oximes within the BChE active site. Our results provide evidence that imidazolium oximes could act as potent reactivators against a range of OPs, as well as effectively recover blood cholinesterase activity, which offers potential for further development of catalytic bioscavenging systems for OP poisoning.

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P32

EFFECT OF INTERLEUKIN-1 ALPHA ON MEGAKARYOPOIESIS AND PLATELET FORMATION IN VITRO

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In the event of blood vessel injury, specialized blood cells called platelets are crucial in recognizing the site of trauma and initiating clot formation. Platelets are derived from megakaryocytes (MKs) that arise from hematopoetic stem cells in the bone marrow. MKs go through a specific differentiation program, mainly controlled by thrombopoetin (TPO). Physiologically, MKs undergo comprehensive maturation of their nucleus and cytoplasm, resulting in large and polyploid cells that extend cytoplasmic processes termed proplatelets into the bone marrow sinusoids, thus releasing platelets. When the number of platelets in the circulation is low, MKs are able to rapidly release platelets via a rupture mechanism which was found to be mediated mostly by interleukin-1 alpha (IL-1α) *in vivo* (Nishimura *et al.*, *J Cell Biol* 2015). In this study, we were interested in the molecular changes affecting MK maturation under conditions of low platelet count. Therefore, we aimed to investigate the functional effects of IL-1α on megakaryopoiesis by *in vitro* culturing murine BM-derived MKs with TPO or TPO together with IL-1α. We observed that stimulation of BM progenitor cells with IL-1α resulted in approximately 30% higher MK yield compared to those treated solely with TPO on day 3. Furthermore, MKs cultured with IL-1α exhibited significantly larger size and a greater number of nuclear lobes per cell. Flow cytometric analysis of released platelet-like particles (PLPs) has revealed a notably elevated count of CD61+ events subsequent to IL-1α stimulation with no changes in proplatelet formation, which suggests heightened platelet release. Remarkably, MKs cultured with IL-1α demonstrated reduced expression of GPIbα, GPIbβ, GPIX, and β3-integrin, alongside decreased von Willebrand factor (vWF) content, confirmed by Western blot and immunofluorescence. Our preliminary results indicate that IL-1α drives Golgi into dispersed vesicles typical of large, mature cells. Surprisingly, MKs from IL-1α cultures exhibited a substantially increased presence (>50%) of other cell nuclei within their cytoplasm, indicative of emperipolesis, which was confirmed by neutrophil marker (Ly6G) immunofluorescent staining. Taken together, our findings imply that *in vitro* IL-1α stimulation triggers an alternate mechanism of MK maturation which promotes increased cell size, ploidy, and heightened emperipolesis, but lower expression of hemostatic proteins.

P33

PLASTICS IN FOCUS: THE INFLUENCE OF POLYSTYRENE ON THE FITNESS OF FRESHWATER ALGAE

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The widespread use of polystyrene (PS) particles in many commercial products often leads to their presence in aquatic ecosystems, the effects of which are not yet well understood. To investigate the effects of PS particles on the freshwater alga *Chlorella vulgaris*, cells were treated with 40 mg L^{-1} PS, which is considered the upper limit for human exposure to styrene monomers. The accumulation of PS particles in the cells and on the EPS layer around the cells was measured by Py-GC-MS analysis, while visualization of the cells under native conditions and analysis of the cell ultrastructure was performed by TEM. To determine the fitness of the algae after treatment with PS particles, the content of reactive oxygen species (ROS), the level of lipid peroxidation and protein damage were analysed, while the antioxidant potential was investigated by measuring the activity of antioxidant enzymes. The results show that the PS particles are mostly bound to the EPS layer around the algal cells, although internalization of PS in the cells was also observed. No increase in ROS content was observed after treatment with PS particles, which is consistent with an increased activity of antioxidant enzymes, especially peroxidases. On the other hand, PS particles caused significant lipid peroxidation, which is consistent with the observed ultrastructural damage, manifested mainly by plasmolysis. In addition, PS particles caused significant carbonylation of proteins, probably due to protein absorption at the surface of the particles. In conclusion, the EPS layer significantly attenuates the phytotoxic effect of PS particles by keeping the majority of the particles outside the cells, while the internalized PS particles cause protein damage and some degree of oxidative stress, as evidenced by increased activity of peroxidases and reduced integrity of the endomembrane system.

P34

CHOLESTEROL OXIME OLESOXIME ASSESSED AS A POTENTIAL LIGAND OF HUMAN CHOLINESTERASES

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Olesoxime, a cholesterol derivative with an oxime group, possesses the ability to cross the blood-brain barrier, and has demonstrated excellent safety and tolerability properties in clinical research. These characteristics indicate it may serve as a centrally active ligand of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), whose disruption of activity with organophosphate compounds (OP) leads to uncontrolled excitation and potentially life-threatening symptoms. To evaluate olesoxime as a binding ligand and reactivator of human AChE and BChE, we conducted *in vitro* kinetic studies with the active metabolite of insecticide parathion, paraoxon, and the warfare nerve agents sarin, cyclosarin, tabun, and VX. Our results showed that both enzymes possessed a binding affinity for olesoxime in the mid-micromolar range, higher than the antidotes in use (i.e. 2-PAM, HI-6, etc.). While olesoxime showed weak ability to reactivate AChE, cyclosarin-inhibited BChE was reactivated with an overall reactivation rate constant comparable to that of standard oxime HI-6. Moreover, in combination with the oxime 2-PAM, the reactivation maximum increased by 10-30% for cyclosarin- and sarin-inhibited BChE. Molecular modeling revealed productive interactions between olesoxime and BChE, highlighting olesoxime as a potentially BChE-targeted therapeutic. Moreover, it might be added to OP poisoning treatment to increase the efficacy of BChE reactivation, and its cholesterol scaffold could provide a basis for the development of novel oxime antidotes.

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P35

UNIQUE RECOGNITION OF tRNA^{Ile} BY ISOLEUCYL-tRNA SYNTHETASE TYPE 2 IN *Priestia megaterium*

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Aminoacyl-tRNA synthetases (aaRS) play a key role in protein synthesis by covalently linking cognate amino acids and tRNAs. However, some aaRS can mischarge cognate tRNA with non-cognate amino acids structurally resembling the cognate ones. To prevent errors in protein synthesis, these aaRSs evolved the editing domain responsible for the hydrolysis of incorrectly aminoacylated tRNA. Isoleucyl-tRNA synthetase (IleRS) is an enzyme that charges tRNA^{Ile} with isoleucine. In bacteria, two types of IleRS have been found, IleRS1 and IleRS2. They differ in the structure of the C-terminal domain responsible for the tRNA^{Ile} recognition. We have recently solved[1] the first structure of IleRS2 with a fully resolved C-terminal domain. The structure revealed that in IleRS2, like in IleRS1, the C-terminal domain comprises three subdomains (SD1-3). The SD1 and SD2 subdomains in both IleRS types are structurally homologous, except for an SD2-insertion present only in IleRS2. On the contrary, the SD3 subdomain adopts different folds. Here we present a structural model of IleRS2 bound to tRNA^{Ile} at 5.5 Å resolution, which indicates an involvement of SD3 in anticodon recognition and an interaction of the SD2-insertion with the D-loop of tRNA^{Ile}. Model-based kinetic analysis demonstrated that the strictly conserved Arg964 establishes an interaction with the tRNA^{Ile} anticodon base G34. This supports the view that the SD3 subdomains, despite their different folds, are used in both IleRS types to recognize the first anticodon base. Mutation of residues Lys862 and Phe865 from the SD2-insertion results in a 10-fold increase in K_M for tRNA aminoacylation, supporting the existence of the interaction observed in the structural model. Further kinetic analysis showed that Lys862Ala and Phe865Ala mutants, despite having wild-type editing domains, may surprisingly accumulate incorrectly aminoacylated norvalyl-tRNA^{Ile} (Nva-tRNA^{Ile}) in a steady state. Taking into account the large distance between the SD2-insertion and the editing site, where hydrolysis of Nva-tRNA^{Ile} occurs, the most plausible explanation for the observed Nva-tRNA^{Ile} accumulation is that the SD2-insertion holds Nva-tRNA^{Ile} during its translocation from the synthetic to the editing site.

[1]A. Brkic, M. Leibundgut, J. Jablonska, V. Zanki, Z. Car, V. Petrovic-Perokovic, A. Marsavelski, N. Ban, I. Gruic-Sovulj *Nat.Commun.*, 2023, **14**, 5498.

P36

STRUCTURE AND pH STABILITY OF THE MINIMAL PROGENITOR TOXIN COMPLEX B

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Botulinum neurotoxins (BoNTs) are the most dangerous biological toxins known. These toxins are naturally co-expressed with a non-toxic partner protein called non-hemagglutinin (NTNH), forming a tight complex known as the minimal progenitor toxin complex (M-PTC). NTNH plays a protective role, shielding the toxin from harsh environmental conditions. BoNTs, especially M-PTC/A and M-PTC/B, are used to treat muscle spasms and other movement disorders. In this study, we present the structure of BoNT/B-NTNH/B (M-PTC/B) at a 2.7 Å resolution, determined by single-particle cryo-electron microscopy. This high-resolution structure provides a comprehensive understanding of how BoNT/B and NTNH/B interact and stabilize each other, forming a robust complex. Additionally, we investigated the pH stability of the complex by conducting structural analysis to identify residues involved in its pH-sensing mechanism and performing a pH stability assay. The results indicate that the complex begins to dissociate at pH values greater than 6. Given that M-PTC/B is already FDA-approved for therapeutic use, understanding its structure is crucial. This knowledge enhances our understanding of the toxin's mechanism of action and is essential for the development of new therapeutics utilizing the specific properties of the toxin. By leveraging this detailed structural information, it may be possible to create more stable and effective treatments for various neuromuscular conditions, expanding the therapeutic potential of these powerful biological agents.

P37

ALTERNATIVE SPLICING OF *BPM2* AS A MECHANISM FOR ESTABLISHING A FUNCTIONAL DIVERSITY OF MATH-BTB PROTEIN FAMILY IN *Arabidopsis thaliana*

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Protein BPM2 from *Arabidopsis thaliana* is a member of the MATH-BTB protein family. This family is characterized by two functional domains, MATH (Meprin and TRAF Homology) and BTB/POZ (Bric a Brac, Tramtrack, Broad Complex), and some MATH-BTB proteins have an additional BACK domain located C-terminally to BTB. In addition, some of the proteins contain one or more nuclear localization signals (NLS) in their sequences. The MATH-BTB family proteins are involved in numerous developmental processes at the cellular, genetic and epigenetic levels, and most of their known functions require the presence of all three domains in both plants and animals. In addition to its physiological importance, an interesting characteristic of the family is that the number of its members is significantly higher in grasses (Poaceae) compared to dicotyledons. While there are only six MATH-BTB genes (*BPM1-6*) in the genome of *A. thaliana*, some grass genomes contain dozens of MATH-BTB genes. Therefore, the question arises as to how *A. thaliana* and other dicotyledons compensate for the wide range of functions that have been characterized for the MATH-BTB proteins in grasses. The results of this work suggested alternative splicing of primary transcripts as a mechanism for the acquisition of functional diversity of MATH-BTB family proteins in *A. thaliana*. Based on the most comprehensive *A. thaliana* transcriptome data analysis AtRTD3, we identified 16 *BPM2* transcript isoforms encoding nine different BPM2 proteins. The N-terminal part of all BPM2 protein isoforms is identical, whereas their C-terminal parts, containing BTB and BACK domains or NLS, vary significantly, indicating different functional properties of the isoforms. Our previous knowledge of grasses suggests the existence of MATH-BTB proteins with precise spatial and temporal functions in development. Therefore, we analyzed the expression of different *BPM2* isoforms in *A. thaliana* embryos, ovules, germinated seeds, seedlings, leaves, roots and flowers under control and temperature stress conditions. Here, the expression atlas of the different *BPM2* splice variants will be presented and the possible function of alternative *BPM2* splicing during development and stress response will be discussed.

P38

EXPLORING THE INTERACTIONS OF MANGANESE-DEPENDENT TRANSCRIPTION REGULATORS SloR AND ScaR WITH DNA – AN EXPERIMENTAL STUDY

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Transition metal ions are essential for living cells, they serve as centers of enzyme catalysis and structural constituents, and they enable many processes within cellular metabolism. However, their excessive concentration can be toxic. Most common mechanism for regulating metal ion homeostasis in bacteria is via metal sensing transcription factors [1]. Metal ions bind reversibly to these proteins, thus modulating their DNA binding affinity. The main causative agent of dental caries is *Streptococcus mutans* [2]. One of the important factors of colonization of the oral cavity by *S. mutans* is competition with cohabitating species. *Streptococcus gordonii* is a non-cariogenic relative of *S. mutans*, which also inhabits the oral cavity. To maintain the homeostasis of manganese ions (Mn(II)), both of these bacteria employ manganese-dependent transcription regulators, SloR (*S. mutans*, [3]) and ScaR (*S. gordonii*, [4]). Understanding the modes of action of SloR and ScaR, and especially their differences, if any can be identified, might contribute to the understanding of dental caries formation and to the development of novel strategies for preventing and fighting it. Using combination of experimental and computational methods, structural and dynamical properties of SloR and ScaR, and their interactions with Mn(II) ions and DNA were investigated. The purification protocol for SloR and ScaR was established and an experimental study of their binding to DNA was conducted.

P39

DOMAIN SCOUTING AND EXPRESSION OPTIMISATION OF HUMAN PNPLA7 PROTEIN IN *E. coli* USING ALPHAFOLD2 GUIDANCE

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PNPLA7 (*patatin-like phospholipase domain containing protein 7*) is a transmembrane lysophospholipase with a possible role in the phosphatidylcholine catabolism. However, its physiological role is not well understood, and protocols to produce protein suitable for kinetic and structure determination experiments are not available either. The goal of our research was to try to express human PNPLA7 in *Escherichia coli* BL21(DE3) Codon Plus and optimize purification for the enzyme activity assays. Human PNPLA7 is 1342 amino acid long and has one transmembrane domain near the N-terminus, three cyclic nucleotide binding sites (CNB 1-3), and patatin domain with the active site. First, we tried to overexpress full-length PNPLA7 lacking the transmembrane domain in *E. coli* Codon Plus, but this variant of the enzyme overexpressed poorly. After examination of the Alphafold2 model of PNPLA7, we decided to express only its patatin domain, the patatin domain plus additional α/β fold domain in the vicinity of the patatin domain with hitherto unknown function in this enzyme (patatin-AB, or patatin α/β), and CNB 2-3 without the active site. All three constructs are much shorter compared to the full PNPLA7 and lack flexible loops. Our western blot results show that all three constructs express in a much higher yield than PNPLA7 lacking only the transmembrane domain. Next, we performed activity assays on supernatants of cell lysates overexpressing these constructs with p-nitrophenyl valerate as a substrate. Our results show that patatin-AB and patatin domain are enzymatically active in contrast to the CNB 2-3. Interestingly, patatin-AB shows 2-fold higher activity when compared to sole patatin domain. While purification remains a challenge ahead, this brings us a step closer to obtain an active enzyme form for future kinetic studies and deciphering a role of PNPLA7 and its possible interactions.

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P40

VIMENTIN EXPRESSION IN KIDNEY AND CHANGES CAUSED BY CISPLATIN NEPHROTOXICITY IN WISTAR RATS

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Vimentin, intermediate filament protein, indicate cells of mesenchymal origin and whether epithelial-mesenchymal transition (EMT) occurs. Vimentin is normally expressed in mesangial cells in the kidney glomeruli as support to endothelia and in pericytes around capillary network of *vasa recta*. However, we found that boundaries between S3 segments of proximal tubules (PT) do have inserts of few vimentin positive cells that continue as Thin Descending Limb of Henle (TDLH) in inner stripe and papilla, together with a few vimentin intensive interstitial cells and tight layer around endothelia. Similar findings were present in a several species. Cisplatin, one of the oldest chemotherapeutic agents for solid tumours, cause acute nephrotoxicity and in a rat model damage strait S3 segment of the PT. We expected EMT to manifests itself throughout molecular and morphological changes, because epithelial cells of S3 segment de-differentiate, lose polarity and epithelial barrier function. Changes in the expression of vimentin would indicate whether EMT occurs in cisplatin nephrotoxicity. Archival samples were used, gained after a single treatment of males with cisplatin (i.p. at a dose of 5 mg/kg). The most pronounced toxicity effect occurs on the 5th day after treatment, but time course of vimentin distribution was monitored each day. Vimentin was observed with immunofluorescence on tissue sections and its abundance was monitored in the homogenates of kidney tissue zones by western blot analysis. The appearance of redistributed intensive vimentin expression was observed over time, and increase in the presence of proteins in cortex and outer stripe was found on 5th day with decrease in inner stripe and papilla. In conclusion, cisplatin nephrotoxicity showed, instead of EMT, multiplication and migration of vimentin positive cells from interstitium and TDLH in inner stripe and papilla toward damaged S3 segment that started as early as 1st day, leaving endothelial *vasa recta* cells without support. Our results indicate an involvement of mesenchymal cell proliferation, migration and invasion that can explain the changes in kidney function early as 1st day polyuria, pointing to mechanisms of specific kidney tissue repair, source of observed fibrosis and/or possible origin of secondary neoplasm in patients treated with cisplatin.

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P41

ATYPICAL ARGINYL-tRNA SYNTHETASE OF PREDATORY BACTERIA *Herpetosiphon aurantiacus* AND ITS POTENTIAL PROTEIN INTERACTORS

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Aminoacyl-tRNA synthetases (aaRS) are enzymes that charge tRNAs with their cognate amino acids. In the first step, the amino acid is activated at the expense of ATP, and in the second, the aminoacyl moiety is transferred to the tRNA. AaRS are structurally and mechanistically divided into two classes, I and II. In class I, a highly conserved histidyl-x-glycyl-histidine motif (HxGH, x represents a hydrophobic amino acid, usually isoleucine), found in the active site, is essential for ATP binding in the activation step. Certain natural variations of the HxGH motif were shown to provide evolutionary advantage to their host. For instance, the glycyl-isoleucyl-histidyl-histidine (GIHH) variation of some isoleucyl-tRNA synthetase provides them hyper-resistance to natural antibiotic mupirocin. Our recent research has found a duplication of arginyl-tRNA synthetase (ArgRS, class I) in the predatory bacterium *Herpetosiphon aurantiacus*. HaArgRS1 appears as the main enzyme that contains a typical HVGH motif and performs the canonical function, i.e. production of Arg-tRNA^{Arg}. In contrast, HaArgRS2 has an atypical arginyl-valyl-aspartyl-histidine (RVDH) motif instead of HVGH, lacks the canonical function, and is expressed at a lower level than HaArgRS1 under normal growth conditions. To explore whether HaArgRS2 maintains a non-canonical role through the interaction(s) within the *H. aurantiacus* proteome, we performed the pull-down assay using StrepTagII-labeled proteins followed by SDS-PAGE and mass spectrometry for the interactor analysis. Preliminary data suggest the most promising potential interactors are the typical HaArgRS1 itself and a previously uncharacterized protein containing the NACHT domain. Further pull-down assay optimization and *in vitro* characterization of the potential interactors is needed for firm conclusions.

1. Brkic, A. *et al*. Antibiotic hyper-resistance in a class I aminoacyl-tRNA synthetase with altered active site signature motif. *Nat Commun* **14**, 1–12 (2023).

P42

GENOME EDITING OF THE *VvPDS* GENE IN GRAPEVINE (*Vitis vinifera* L.) SOMATIC EMBRYOS VIA BIOLISTICS

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Grapevine cultivation is known for its heavy pesticide use in order to control various pests and diseases. On the other hand, this dependence on chemical protection raises environmental and health concerns and creates a need for inovative and sustainable agricultural practices. Due to climatic and economic pressures, traditional breeding methods for developing improved grapevine cultivars aren't cost-effective nor time-efficient. This is due to a long generation time and a high heterozygosity of grapevine, which substantially prolong breeding programs. Therefore, to improve biotic and abiotic stress tolerance and reduce pesticide dependence of existing grapevine varieties, there is a neccessity to use faster breeding approaches through next generation genetic engineering technologies like genome editing. In this study, as a proof of concept, we wanted to demonstrate targeted mutagenesis by using a single plasmid harboring both, Cas9 nuclease and the gRNA, on grapevine somatic embryos (SE). This *in vitro* system ensures the maintanance of the desired genotype after modification and a relatively easy whole plant regenareation. As an editing target, we chose the *VvPDS* locus of grapevine which represents an excellent marker gene due to its role in carotenoid biosynthesis i.e. its disruption results in readily obsevable leaf bleaching. As a transformation approach, we used biolistics/particle bombardment, which involves shooting DNA-coated microscopic particles into plant tissues at high velocity. Once inside the cell, the CRISPR-Cas9 plasmid can be expressed with or without genomic integration. Following particle bombardment, we successfully regenerated whole plants from grapevine somatic embryos. These plants exhibited sequence alterations in the targeted *VvPDS* region. However, the CRIRSP-Cas9 induced mutations were not homozygous/biallelic, which would have resulted in visible white sectors. Although a grapevine reference genome was used for designing the gRNA target, here we demonstrate the necessity of the initial biallelic genotyping of each targeted genomic region in grapevine. In addition, we also report on vacuum agroinfiltration of young grapevine leaves as a means for preliminary validation of the applied editing vectors.

P43
THE ROLE OF UvrD IN PRESPACER PREPARATION FOR CRISPR-Cas IMMUNITY

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CRISPR-Cas (clustered regularly interspaced short palindromic repeats – CRISPR associated genes) is a prokaryotic adaptive immune system that targets invading mobile genetic elements (MGEs). This system represents the genetic memory of MGEs by integrating a small piece of the invader's DNA, called a protospacer, as new spacer into the CRISPR array of the invaded cell. The process of spacer acquisition is called adaptation and is mediated by a Cas1-Cas2 complex and sometimes by other Cas proteins. The spacers are later transcribed into long pre-crRNA and processed by Cas proteins into short crRNAs (CRISPR-RNA). The CRISPR-Cas immune defence system relies entirely on the crRNA molecules arising from the CRISPR locus. In *Escherichia coli*, the integration of spacers is mediated by the Cas1-Cas2 complex and supported by the host protein RecBCD. RecBCD is required for the generation of single-stranded (ssDNA) fragments from the processing of double-stranded DNA breaks (dsDNA) that occur at broken replication forks through its helicase activity. In this study, we investigated the possibility that another host helicase is involved in prespacer preparation. We found that spacer integration is reduced in the absence of UvrD helicase and increased when UvrD is overexpressed, suggesting that UvrD helicase plays a role in prespacer preparation. UvrD is known to remove RecA filaments, but deletion of *recA* in the *uvrD* mutant did not, whereas deletion of *recF* rescued spacer integration. Since the Cas1-Cas2 complex needs ssDNA substrates for binding, UvrD could be involved in the preparation of these substrates within the dsDNA (ssDNA gaps).

P44

MAPPING OF BARTH SYNDROME PATHOGENIC VARIANTS LINKS TAFAZZIN STRUCTURAL ALTERATIONS AND MONOLYSOCARDIOLIPIN BINDING

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Background/Objectives/Issue/Case: We previously mapped pathogenic mutations of human tafazzin to functional domains and observed that the HX4D and MT1 domains contain significantly more residues that result in Barth Syndrome when mutated. Using molecular dynamics (MD) simulations, we interrogated the effects of mutations to key residues of these domains, on tafazzin's tertiary structure and monolysocardiolipin (MLCL) binding.
Methods/Project: Using AlphaFold and molecular modeling software, we generated 3D models of human tafazzin. We used molecular docking techniques on both an experimentally obtained (crystalized) tafazzin structure from *Yarrowia lipolytica* and the modeled human enzyme. Next, we focused on pathogenic mutations to D75, which borders the HX4D domain, and R94, which resides within the MT1 domain, and characterized structural changes to the enzyme and MLCL binding efficacy *in silico*.
Results: The crystalized tafazzin from *Y. lipolytica* is bound to one molecule of MLCL, which was found to localize into a pocket stabilized by binding interactions with 12 amino acids. Seven of these residues were found to be conserved in human tafazzin, including D75. Human D75 was also shown to be most densely surrounded in 3-D space by residues that can also carry pathogenic mutations. R94 was found to have the largest recorded number of pathogenic mutational variants. Upon introducing D75 mutations *in silico*, MD simulations showed significant structural changes to the tafazzin enzyme. In contrast, the introduction of R94 mutations showed less structural changes. Together, these results suggest potential for distinct molecular mechanism resulting in altered MLCL binding and transacylase activity.
Conclusions/Lessons learned: The analysis of key residues within the HX4D and MT1 domains of Tafazzin, which are important for MLCL biding, provides insights into two potentially distinct molecular mechanisms of Barth syndrome affecting the active site of tafazzin. These results provide a basis for accelerating the characterization of tafazzin's active site and molecular mechanisms of Barth syndrome.

P45

IMPACT OF PROTEIN DELETIONS IN GPI ANCHOR SYNTHESIS PATHWAY ON YEAST SURFACE DISPLAY EFFICIENCY

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The yeast surface display encompasses the yeast's innate ability to enrich its cell wall with mannosylated proteins, thus creating a dynamic and self-sustaining catalyst on the external surface of the yeast. The effectiveness of recombinant protein immobilization relies on the inherent capability of the cell wall to harbor and present these proteins on the exterior surface of the yeast cell. Namely, the yeast cell wall is saturated with numerous native proteins, among which a significant part takes covalently linked GPI-proteins. GPI-linked proteins include over 30 diverse proteins incorporated into the wall via a remnant of glycosylphosphatidylinositol (GPI) anchor at the C-terminal end of the protein. The synthesis of the GPI anchor occurs on a phosphatidylinositol lipid within the endoplasmic reticulum through a series of enzymatic reactions. By selectively eliminating proteins involved in GPI anchor synthesis, the quantity of GPI-linked proteins on the cell surface might be diminished, potentially generating extra space and facilitating the effective binding of recombinant proteins. To achieve this goal, we examined how the deletion of four proteins (Arv1, Gpi7, Gup1, and Cos16) involved in the distinct stages of GPI anchor synthesis would influence the yeast surface display efficiency of Pir2BLA protein. Pir2BLA is a recombinant protein obtained by fusing the gene encoding for Pir2, a native cell wall protein that integrates into the wall through its N-terminal end, with BLA, gene coding for β-lactamase, serving as a reporter enzyme. The efficiency of recombinant Pir2bla protein exposure was monitored by measuring β-lactamase activity and the Western blot of cell wall proteins.

P46

BCHE POLYMORPHISM EFFECT ON THE EFFICIENCY OF 5-(2-AMINO-1-HYDROXYETHYL) PHENOL CARBAMATES AS POTENTIAL NEW DRUGS FOR ADVANCED ALZHEIMER`S DISEASE TREATMENT

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Alzheimer`s disease (AD) is the most prevalent neurodegenerative disease and its treatment is based mainly on increasing the concentration of the neurotransmitter acetylcholine (ACh) by inhibiting the enzymes responsible for its hydrolysis, primarily acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Since several studies demonstrated the importance of BChE in the regulation of ACh concentrations in the late stages of AD, BChE selective inhibition evolved into a promising approach for the treatment of advanced AD. Carbamates have become structurally desirable in drug design due to their good pharmacological properties and are an important structural motif in drugs in use for AD treatment, where they act as cholinesterase inhibitors and display significant positive effects on cognitive symptoms. In our study, we designed and synthesized thirteen 5-(2-amino-1-hydroxyethyl) phenol carbamates with the aim to evaluate them as potential inhibitors of human BChE – usual and atypical variant. It is worth emphasizing that the efficiency of any drug that targets BChE activity might be affected by human BCHE gene polymorphisms with more than 70 isoforms, characterized by different catalytic properties or lower concentration than usual BChE. For example, individuals with atypical BChE (result of point mutation Asp70→Gly) are at risk in case of using anticholinergics and drugs such as muscle relaxants. All of the synthesized compounds displayed a time-dependent inhibition of both BChE variants with inhibition rate constants within 10^3-10^6 M^{-1} min^{-1}, while inhibition of atypical BChE was 30 to 1400 times slower in comparison to usual BChE. The largest difference in inhibition rate was observed for carbamates with piperidine in the carbamoyl and hydroxyaminoethyl chain. Molecular docking has shown that carbamate groups are oriented towards the bottom of the active site of usual BChE, while in the case of atypical BChE, due to its narrow active site gorge the accommodation of this compound into the active site is aggravated and delayed.

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P47

DIPEPTIDYL PEPTIDASE 3 KNOCKOUT IN 293FT CELLS DOWNREGULATES NRF2 IN THE BASAL CONDITIONS

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Nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that governs the expression of more than 200 genes involved in the response to oxidants and electrophiles. In the basal state, NRF2 levels are kept low through its interaction with Kelch-like ECH-associated protein 1 (KEAP1), which binds it in the E3 ligase CUL3-RBX1 complex. In this complex, NRF2 is ubiquitinated and subsequently degraded in the proteasome. The NRF2 monomer is bound to the KEAP1 dimer through higher affinity ETGE and lower affinity DLGx motifs. NRF2 is ubiquitinated only when both motifs are bound to KEAP1 and the complex is in the appropriate conformation for ubiquitination.

The canonical mechanism of NRF2 activation after the exposure of cells to oxidative or electrophilic stress involves the oxidation of KEAP1 reactive cysteine residues. This changes the conformation of the complex and blocks NRF2 ubiquitination. There are also several modes of non-canonical activation of NRF2, including the binding of competitive protein interactors to KEAP1, which leads to the dissociation of the lower affinity DLGx motif from KEAP1.

Dipeptidyl peptidase 3 (DPP3) is one of the KEAP1 interactors that contains an ETGE motif and competes with the NRF2 DLGx motif for binding to KEAP1. In vitro studies have shown that DPP3 overexpression decreases NRF2 ubiquitination and activates the expression of several NRF2-controlled genes. The DPP3-KEAP1 interaction is enhanced under oxidative stress conditions induced by hydrogen peroxide. Overexpression of DPP3 also correlates with increased NRF2 activity and poor prognosis in lung and breast cancer samples. It is assumed that DPP3 might be involved in the activation of NRF2 in cancer, but it might also play a role in regulating the KEAP1-NRF2 response to hydrogen peroxide.

We used the CRISPR-Cas9 approach to produce a DPP3-knockout 293FT cell line and performed RNAseq analysis of KO-DPP3 versus WT 293FT cells. We identified downregulation of NRF2, as well as several NRF2-controlled genes, in DPP3-KO cells. The results were confirmed by western blot, indicating that DPP3 is involved in the control of NRF2 activity in non-tumorigenic cells under basal conditions.

P48

THE IMPACT OF ENDOPLASMIC RETICULUM STRESS ON THE MODULATION OF THE UROKINASE PLASMINOGEN ACTIVATION SYSTEM

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Urokinase plasminogen activator (urokinase, uPA) is a highly specific extracellular protease which activates plasminogen to produce plasmin. Plasmin participates in extracellular matrix remodelling, cell migration and invasion and has many important physiologic roles. The uPA system comprises of urokinase, PAI1, its inhibitor, and uPAR, a membrane urokinase receptor. The aim of the project was to investigate whether endoplasmic reticulum (ER) stress modulates uPA activity. ER stress appears when unfolded proteins accumulate in ER and induce signalling leading to unfolded protein response (UPR). The response includes inhibition of protein translation, increase in the chaperone expression and can lead to autophagy or apoptosis. In two tumour cell lines, ER stress was induced by treatment with specific ER stressor, thapsigargin. In a glioblastoma cell line, an increase in uPA activity was observed, while in a breast cancer cell line, a decrease was detected. Expression of uPA and PAI1 proteins were analysed in cell lysates of control and treated cells. Furthermore, uPA system and UPR gene expression was analysed by qRT-PCR. Both, uPA and PAI1 expression was affected. The chaperone inhibitor decreased ER stress effects on the urokinase activity. ER stress in both cell lines also inhibited cell proliferation. As many chemotherapeutics induce ER stress, the increased expression of ER stress genes could influence the cell survival and modulated uPA system can additionally affect response on chemotherapy.

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P49

ASSOCIATION OF LIVER ENZYME LEVELS WITH COVID-19 SEVERITY

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As the COVID-19 pandemic persists, it's becoming increasingly important to understand the connection between liver function and the severity of the disease. Elevated liver enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), have been reported in COVID-19 patients, but their association with disease severity remains unclear.

The study included 750 positive COVID-19 patients who had been recruited at General Hospital Tešanj. Patients were stratified into three groups based on disease severity (mild, moderate, and severe). Liver enzyme levels were determined using standard IFCC procedures.

Our results revealed that AST levels were significantly higher in patients with a severe clinical outcome (61.56 ± 6.24) compared to those with mild or moderate symptoms (47.53 ± 3.24). Patients with mild symptoms exhibited the lowest AST levels (43.89 ± 3.46). Statistically significant differences were observed between the "mild" and "severe" groups ($p < 0.001$), as well as between the "moderate" and "severe" groups ($p = 0.033$). Additionally, the lowest ALT levels were observed in patients with mild symptoms (46.92 ± 3.79), while the highest levels were observed in patients with a severe clinical outcome (52.89 ± 4.71). However, no statistically significant differences were found among the three groups of patients.

Elevated levels of AST and ALT are associated with increased COVID-19 severity. Monitoring liver enzyme levels may help identify patients at risk of developing severe disease and guide clinical management strategies.

Keywords: COVID-19; liver enzymes; AST, ALT; disease severity

P50

RESOLVING THE MYTH OF GANGLIOSIDE PRESENCE IN MITOCHONDRIA: CHARACTERIZATION OF MITOCHONDRIAL GANGLIOSIDOME

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Gangliosides, complex glycosphingolipids, are dominant glycome determinants in the brain. They play important roles in cell signalling and ion transport across the membrane. Gangliosides are synthesized by stepwise addition of monosaccharide units to their ceramide core catalysed by specific glycosyltransferases. Defects in three genes in the ganglioside biosynthesis pathway have been described in humans, causing congenital disorders which exhibit some classical forms of mitochondrial disease: infantile-onset symptomatic epilepsy syndrome, a complex form of hereditary spastic paraplegia and West syndrome. However, the molecular mechanism leading from the changed ganglioside composition to the documented dysfunction in cellular energy metabolism in these patients remains an enigma. Gangliosides are generally considered practically undetectable anywhere except in the plasma membrane, with some literature reports determining their presence in endoplasmic reticulum (ER), mitochondria-associated ER and nuclear membranes.

The aim of this pilot study was to investigate whether gangliosides can be detected in brain mitochondria, and if so, to provide their detailed structural characterization. For this purpose, we isolated mitochondria from cortical brain tissue of adult C57BL/6 mice by subfractionation using series of centrifugations, and ultracentrifugation in discontinuous Percoll gradients. The purity of mitochondrial preparations was assessed by Western blotting for mitochondrial markers (cytochrome c oxidase and mitochondrial outer membrane translocase TOMM20) and plasma membrane markers (Na^+/K^+-ATPase and plasma membrane Ca^{2+}-ATPase) to rule out any potential contamination. Gangliosides were extracted and purified from mitochondria by established protocols of our group. The isolated gangliosides were analysed by high performance thin layer chromatography (HPTLC) visualised by resorcinol-HCl and dot blot using specific anti-ganglioside antibodies. After we determined that gangliosides are indeed present in mitochondrial membranes, we proceeded to fully structurally characterize them by mass spectrometry (MS) and tandem MS. Analysis was performed through negative ion mode using high resolution hybrid quadrupole time-of-flight mass

spectrometer (QTOF-micro MS), equipped with a nanoelectrospray ionization (nanoESI) source. We detected different ganglioside species in the mitochondrial membrane, including GM1, GD1a, GD1b and GT1b.

This study represents the first report characterizing the mitochondrial gangliosidome and highlights a critical need for exploring gangliosides as potentially highly important factors in both mitochondrial architecture and function.

P51

ENHANCING SENSITIVITY IN ALPELISIB AND FULVESTRANT QUANTITATION BY SWEEPING MEKC-MS/MS

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Breast cancer is the most common cancer in the world presenting a huge burden for healthcare systems. One of the promising ways to improve clinical outcomes is the implementation of therapeutic drug monitoring (TDM). However, analytical methods which are prerequisites for successful implementation of TDM are still scarce. Currently, there are no reported methods for the determination of alpelisib (ALP) and fulvestrant (FUL) suitable for TDM.

Herein, we report the first-ever sweeping micellar electrokinetic chromatography coupled with tandem mass spectrometry (MEKC-MS/MS) method for ALP and FUL. Separation of these drugs was possible due to the use of ammonium perfluorooctanoate (APFO) as a volatile, MS-compatible surfactant. Since these breast cancer drugs are found in very low concentrations in patients' plasma, optimization of the method's sensitivity was needed.

Sheath liquid interface parameters, classical electrospray ionization (ESI) source parameters, and Agilent Jet Stream parameters were tested. Effects of sheath liquid composition (MeOH, EtOH, ACN or i-PrOH, 50-70 % and formic acid 0.05-0.2 %) and sheath liquid flow (3-8 µL/min), drying gas temperature (250-350 °C) and gas flow (7-11 L/min), nebulizer pressure (8-12 psi), capillary voltage (2000-3000 V), sheath gas flow (5-11 L/min), sheath gas temperature (100-250 °C) and nozzle voltage (0-2000 V) were investigated. Varying some of the parameters showed negligible effects, while others showed a remarkable increase in sensitivity. For example, changing the nozzle voltage alone increases the sensitivity 7 times.

Optimal conditions for the sensitivity of the method were 60 % MeOH with 0.05 % formic acid as sheath liquid delivered at 3 µL/min, drying gas at 300 °C and 9 L/min flow rate, 10 psi nebulizer pressure, 2500 V capillary voltage, sheath gas at 250 °C and 5 L/min and 2000 V nozzle voltage. In the end, much better sensitivity was achieved, enabling the detection of these drugs at their plasma concentrations. In further studies, the proposed sweeping MEKC-MS/MS method will be validated for its intended purpose and applied to patients' plasma samples.

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P52

DEVELOPMENT OF A COMPUTATIONAL PIPELINE FOR THE DESIGN OF MONOBODY BINDERS

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Monobodies (Mobs) represent a class of synthetic antibody-like proteins derived from Human fibronectin type III[1]. Mobs offer several advantages over monoclonal antibodies (mAbs), including their monomeric assembly, absence of glycosylation, and lack of disulfide bonds. These features help make Mobs suitable for expression in bacteria, providing an economic benefit over mAbs. This type of protein has been engineered in laboratory settings to have high affinity to oncologic[2,3] and viral targets[4,5], suggesting their potential as future therapeutic agents. However, computational pipelines to design Mob binders remain scarce[6].

Therefore, this work aims to develop a computational platform capable of generating Mob binder sequences with strong affinity for specific protein targets. To that end, we leveraged RFdiffusion[7] to design de novo proteins (fold-conditioned to possess Mob structure) in complex with relevant protein targets. Then, entirely new sequences were designed with ProteinMPNN [8] for the selected poses. The most promising outcomes were determined by filtering through AlphaFold2[9,10] and biophysical metrics.

The protocol was applied against relevant viral therapeutical targets, such as SARS-CoV-2's Region Binding Domain and Stem region. Assessments through AlphaFold2 tests and molecular dynamics simulations indicate that this de novo design protocol generates stable proteins. Some selected sequences were tested in the recently published AlphaFold3[11], and a few have predictions of remarkably similar interfaces as the designs from the end of the pipeline. These promising findings are now under experimental validation using Yeast Display screenings against their respective targets. This work showcases an innovative platform for the design of Mobs which will boost their therapeutic applications.

[1]Koide, A., et al. *Journal of molecular biology* 284.4 (1998):1141-1151.
[2]Schmit, N.E., Neopane, K., & Hantschel, O. *ACS chemical biology* 14.5 (2019):916-924.
[3]Park, S.-H., et al. *PLoS One* 10.7 (2015):e0132976.
[4]Kondo, T., et al. *Science advances* 6.42 (2020):eabd3916.

[5]Kondo, Taishi, et al. *Life Science Alliance* 5.6 (2022).
[6]Eguchi, R.R., et al. *bioRxiv* (2022):2022-12.
[7]Watson, J.L., et al. *Nature* 620.7976 (2023):1089-1100.
[8]Dauparas, J., et al. *Science* 378.6615 (2022):49-56.
[9]Jumper, J., et al. *Nature* 596.7873 (2021):583-589.
[10]Roney, J.P., and Ovchinnikov, S. *Physical Review Letters* 129.23 (2022):238101.
[11]Abramson, J., et al. *Nature* (2024):1-3.

P53

DIVERSITY IN THE BIVALENT AND MULTIVALENT INTERACTIONS OF THE LC8 HUB PROTEIN

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LC8 was originally recognized as a member of the multi-subunit dynein motor complex, but it has been described as a hub protein involved in many different cellular processes. It is able to interact with an extremely high variety of partners (more than 100 verified partners; see LC8 Hub*), however, the core structural basis for the complex formation is common. LC8 homodimerization is necessary for the functionality, because both sub-units participate in the formation of the binding pocket. All known LC8 partners adopt a β-strand upon binding to the LC8 homodimer interface. Additionally, the LC8 dimer is symmetric, therefore two identical binding sites are capable of interacting with two ligands on the opposite sides of the complex. The detailed structure of the LC8 dimer in free form and in complex is extensively studied. These complexes are mainly heterotetrameric structures, in addition to a few hetero-hexamers. The published peptide complexes almost exclusively focus on the segment that makes physical/chemical contact with the binding pocket of LC8. A few studies examined the structural properties of longer protein segments with bivalent binding sites, however, all of these were X-ray crystallography studies without addressing the dynamics of this region.

GKAP is a postsynaptic density scaffold protein contributing to the regulation and modulation of signal transduction through the postsynaptic cell via NMDA receptors and other partners. It is mostly disordered, with high structural flexibility even in the binding regions. GKAP harbors two LC8 binding linear motifs having atypical amino acid composition. Both binding segments lack the consensus TQT anchor region (LC8 Hub*), and have atypical residues also in other positions of the binding region. We have observed small but consistent differences even in the two highly similar binding sites, hinting at a previously undescribed diversity of LC8 interactions.

This prompted us to conduct a detailed computational analysis of residue-residue interactions in available LC8 structures. Our main focus is on the subtle structural changes in sidechain rotamers and interaction types that might influence the stability of the complexes. In addition, special emphasis is given to small structural rearrangements upon LC8 complexation.

P54

HUMAN TRYPSIN-ISOENZYME INHIBITORS FROM FINNISH CYANOBACTERIA

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Proteases play important roles in all living organisms. About one third of the over 500 known human proteases are serine proteases. These include trypsins, which are among the best characterized proteases. Among three different human trypsin-isoproteases, trypsin-3 appears to play an important role in the metastatic spread of cancer. Recently selective targeting of trypsin-3 has been shown to reduce the cancer cell invasion. However, due to high sequence and functional similarity, it is difficult to produce selective trypsin-3 inhibitors. Cyanobacteria produce potent inhibitors of trypsins. Here we tested the selective inhibition of three human trypsin-isoproteases using crude extracts of 505 strains, belonging to 19 different genera of cyanobacteria isolated from Baltic Sea and Finnish lakes. Extracts of 87 strains (17.2%) showed strong inhibition of one or more trypsin-isoproteases. The strains producing these inhibitors belonged especially to *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix* genera. Extracts of 13 strains showed selective inhibition activity. Of these, 3 preferentially inhibited trypsin-2 and -3 and 10 trypsin-1 and -2. Up to now, we have isolated and characterized trypsin inhibitors among aeruginosin family of cyanobacterial peptides (suomilide and varlaxins) and a dipeptide called radiosumin C. The most potent of these inhibited human trypsins with subnanomolar IC_{50} values. Interestingly, these peptides showed different selectivity profiles for inhibiting different human trypsin-isoproteases. Suomilide also inhibited the invasion of aggressive and metastatic PC-3M prostate cancer cells without effecting the cell proliferation. These results show that cyanobacteria are rich source of protease inhibitors with interesting selectivity profiles. The inhibitory peptides we have isolated and characterized may serve as leads for development of selective and potent trypsin inhibitors.

P55

FINE-TUNING THE PRODUCTION OF RECOMBINANT PROTEINS USING tRNA POST-TRANSCRIPTIONAL MODIFICATIONS

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Heterologous protein production is an important source of industrial and medicinal proteins. However, several options for improving the protein yields are yet to be explored. Current research has led to a novel idea of using optimized transfer RNA (tRNA) pools to achieve more efficient transgene expression. Post-transcriptional tRNA modifications (PTMs) are known to be essential for the proper function of the translation machinery. Nevertheless, the impact of tRNA PTMs on protein production still remains to be investigated. Here, we propose a proof-of-concept to assess the effect of tRNA PTMs on heterologous protein production. To this end, we will use a cell-free protein synthesis (CFPS) system based on *Saccharomyces cerevisiae* that allows for easy manipulation of the RNA content. We will isolate tRNA pools from variously stressed yeast cells to create a library of modified tRNAs. Next, we will reconstitute the tRNA content of the expression system to match the needs of the specific reporter construct. We will analyze the translational efficiency using fluorescent/luminescent markers and evaluate tRNA modification dynamics by performing RNA mass spectrometry coupled with oligonucleoside sequencing and tRNA sequencing. The outcome of this research will shed a new light on the role of tRNA PTMs as translational modulators and provide new methodological approaches for enhancing heterologous protein production.

P56

SKIN MICROBIOTA IN SUBJECTS WITH HAND ECZEMA AFTER PROBITECT-C EMOLLIENT PLUS APPLICATION

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Introduction: Hand eczema (HE) is an inflammatory skin condition affecting palms and fingers, characterized by redness, erythema, scaling, fissures and hyperkeratosis of epidermis, as well as symptoms of itch and pain. HE can evolve into a chronic condition with persistent disease resulting in a high social and economic impact for the affected individual.
PROBITECT-C is an innovative emollient plus lotion consisting of a cosmetic base with three active ingredients based on human microbiota, specifically designed for alleviating symptoms of HE. The clinical study assessed the effects of PROBITECT-C during a 6-week treatment period in adult HE patients by evaluating skin microbiota, skin condition and patient reported outcomes.
Materials and methods: The prospective double-blind randomized placebo-controlled clinical study compared PROBITECT-C and a control emollient treatment during 6 weeks of twice daily application on the affected skin. DNA was extracted from skin swabs and microbiota composition was determined following 16S RNA amplicon sequencing (V3-V4 region), QIIME, DADA2 and ALDEx2R processing. Efficacy was evaluated between two time-points (day 0 and week 6) using: HECSI disease index, transepidermal water loss, hydration, erythema, redness, lichenification, pH, scaliness and skin microtexture as well as parameters from two questionnaires (Dermatological Life Quality Index (DLQI) and Numeric Rating Scale (NRS) for average and worst pruritis (itching)).
Results: PROBITECT-C treatment demonstrated a significant increase of *Streptococcaceae*, *Xanthomonadaceae*, *Gemellaceae*, *Methanobacteriaceae* bacterial families, accompanied by a significant reduction in the *Dietziaceae* family, with this trend less pronounced or absent in the control group. Both emollients significantly decreased *Placococcaceae* and *Beijerinckiaceae*, while the control emollient lowered *Moraxellaceae* family. The observed effects were translated into significant clinical improvements: decreased HECSI index and reduced skin redness in the PROBITECT-C group compared to the control.

Participants reported a significant increase in the quality of life and reduced itching during emollient application.
Conclusion: PROBITECT-C, an innovative emollient plus, increased the abundance of microbiota constituents associated with healthy human skin (e.g. *Streptococcaceae*), reducing hand eczema symptoms and increasing quality of life in adult patients with HE.

P57

INTERPLAY BETWEEN ONTOGENY AND REGULATORY EVOLUTION OF OHNOLOGUES FOLLOWING AUTOPOLYPLOIDIZATION IN SALMONIDS

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Whole genome duplication (WGD) has been proposed as a key driver of diversification and the evolution of novel morphology in vertebrates. Morphological evolution is linked to gene expression during embryogenesis; earlier research has found that minimal transcriptomic divergence across orthologues occurs at the phylotypic stage, where the basic vertebrate body plan is established. The ancestor of salmonids underwent a WGD event by autopolyploidization, known as Ss4R, ~100 mya. Atlantic salmon still conserves ~55% of its ohnologues (WGD paralogues) originating from Ss4R as two functional copies. This has established salmonids as a popular WGD model. A lack of high temporal resolution embryonic time course data has, however, limited our understanding of how ohnologue evolutionary divergence is influenced by ontogenetic constraints. To understand how ontogenetic constraints shaped post-Ss4R ohnologue divergence, we generated RNA-seq, ATAC-seq and ChIP-seq datasets spanning 14 stages of embryogenesis and 8 mature and immature tissues in Atlantic salmon and rainbow trout, two salmonids that separated ~20Mya. These datasets were used to measure transcriptomic and regulatory constraints across different stages and tissues between Atlantic salmon ohnologues, as well as between Atlantic salmon and rainbow trout orthologues. This analysis revealed, for the first time, a period of maximum transcriptome similarity between ohnologues during the phylotypic stage. To understand how ontogenetic constraints, affect the epigenetic landscape, we quantified the proportion of accessible regions retained in duplicate post-Ss4R by overlaying enhancer and promoter ATAC-seq peaks with whole genome alignments. This revealed the highest proportion of duplicated open

chromatin regions at the phylotypic stage and in brain. Our results present an overall connection between ohnologue gene expression and regulatory divergence following Ss4R, highlighting previously unexplored interactions between ontogenetic constraints and ohnologue regulatory divergence during post-WGD evolution.

P58

CREATING A MODEL SYSTEM FOR STUDYING DNA-PROTEIN CROSSLINK REPAIR IN HUMAN CELLS USING CRISPR/dCas9 TECHNOLOGY

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DNA-protein crosslinks (DPCs) are highly toxic DNA lesions that originate from endogenous (aldehydes, ROS) and exogenous (chemotherapeutics, UV radiation) agents. They interfere with replication and transcription and lead to genomic instability, consequently causing carcinogenesis and neurodegeneration. DPC induction is used as a mechanism of chemotherapeutic agents like camptothecins, etoposide, and olaparib. CRISPR/Cas9 is a gene-editing tool that uses single-guided RNA (sgRNA) to target and cleave specific DNA sequences, while dCas9 is a modified version used for transcriptional regulation without cutting DNA. A model system for the measurement of DPC repair (DPCR) in human cells has not yet been developed. Therefore, we aim to create a DPC consisting of dCas9 crosslinked to the specific site in the genome which could be used to measure the rate of DPCR using RT-qPCR (reverse transcription quantitative PCR). If dCas9 forms a DPC at the target gene and inhibits its transcription, we can detect silencing with RT-qPCR. Once the dCas9-DPC is repaired, gene silencing will no longer be detectable. We selected eight genes that we decided to target with dCas9 and designed 1-3 sgRNAs for each of them. These sgRNAs were then synthesized and combined with dCas9 in a complex, which was subsequently transfected into HEK293T cells. The silencing of target genes was quantified using RT-qPCR. All cellular DPCs were isolated using the modified RADAR assay, and specific dCas9-DPC was detected using Western blot. Out of all selected target genes, we detected significant silencing of two genes: *TDP2* (tyrosyl-DNA phosphodiesterase 2) and *MATE1* (multidrug and toxin extrusion protein 1). Observed silencing indicates dCas9 presence at the target gene site. We then proceeded with DPC isolation and detection in transfected cells. The obtained results show that dCas9 forms DPCs in human cells with and without specific sgRNA. In conclusion, dCas9 forms DPCs in human cells and, when coupled with specific sgRNA, can effectively silence target genes. These findings suggest that the specific crosslinking of dCas9 could be used as a model for DPCR measurements in human cells and that dCas9 crosslinking could be one of the mechanisms of dCas9 toxicity in human cells.

Key words: DNA repair, DNA-protein crosslinks, CRISPR/dCas9, human cells, model system.

P59

SIRT3 AFFECTS SEX-SPECIFIC DIFFERENCES IN OXIDATIVE AND ANTIOXIDANT PARAMETERS IN KIDNEY AND BRAIN TISSUE IN MICE

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Mitochondrial deacetylase Sirtuin 3 (Sirt3) plays a pivotal role in maintaining cellular redox homeostasis and mitochondrial function. Growing evidence suggests that metabolic and oxidative stress elicit sex-specific responses in various organs. This study aims to elucidate the sex- and organ-specific effects of Sirt3 deficiency in mice, with a particular focus on the kidney and the brain. Using Sirt3 wild-type (WT) and knockout (KO) mouse model, we analyzed the expression levels of Sirt3, PGC-1α, CuZnSOD, MnSOD, and Catalase (Cat) proteins using Western blot analysis. Additionally, we quantified oxidative stress markers, including protein carbonylation and lipid peroxidation, using ELISA and fluorometric assays respectively. CuZnSOD, MnSOD and Cat activities were determined using standard enzymatic assays. Our findings reveal significant sex- and organ-specific variations in response to the loss of Sirt3. In the kidney, Sirt3 KO impacted both sexes differently, with KO females displaying lower CuZnSOD and Cat activity compared to males. In contrast, brain tissue from WT females exhibited higher activities of all three antioxidant enzymes compared to males, which may indicate an inherent sex-specific protective mechanism. Interestingly, brain shows significantly lower expression of Sirt3, CuZnSOD, MnSOD, and Cat, but higher expression of PGC-1α protein. Furthermore, the study underscores the organ-specific responses to Sirt3 deficiency, with kidney showing a different pattern of oxidative damage and enzyme activity compared to brain. These differences suggest distinct regulatory mechanisms of redox homeostasis in these organs, mediated by Sirt3. In conclusion, our research provides comprehensive insights into the complex interplay between Sirt3 function, oxidative stress, and antioxidant defenses in murine kidney and brain. The observed differences between these two organs and the impact of sex together emphasize the importance of considering these factors in the development of therapeutic strategies targeting mitochondrial function and redox homeostasis.

P60

EFFECTS OF BEP CHEMOTHERAPY ON AMINO ACID EXCRETION IN TESTICULAR CANCER PATIENTS

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Bleomycin, etoposide, and platinum (BEP) chemotherapy is the standard treatment for patients with germ cell tumours (GCTs). Most patients have excellent outcomes, however, the cure for patients with advanced disease is less efficient. Tumour cells have elevated metabolic demands for nutrients due to their rapid proliferation, and therefore they increase their uptake of amino acids, structural units of proteins, and other macromolecules. It has been recently discovered that depleting specific amino acids can be lethal to tumour cells and that decreased amino acid availability might contribute to chemotherapy success. Therefore, monitoring parameters like amino acid excretion can provide insight into the effects of chemotherapy. In this study, the impact of combined BEP chemotherapy on excretion of amino acids in testicular cancer patients was investigated. A quantitative profile of 30 amino acids measured in 60 patients, ages 18 to 50, both before and after completing two cycles of chemotherapy was determined by GC-MS using the Phenomenex EZ:faast kit. When compared to pre-chemotherapy values, the Wilcoxon matched pairs test results (with a Bonferroni adjustment of $p<0.0016$) showed a significant increase in the excretion of 26 out of 30 amino acids: alanine (64%), sarcosine (64%), glycine (83%), α-aminobutyric acid (72%), valine (74%), leucine (61%), isoleucine (31%), threonine (192%), serine (56%), proline (117%), aspartic acid (128%), thioproline (121%), aspartic acid (40%), methionine (19%), glutamic acid (93%), phenylalanine (53%), α-aminoadipic acid (28%), α-aminopimelic acid (120%), glutamine (53%), ornithine (42%), glycine-proline (40%), lysine (78%), histidine (56%), tyrosine (93%), tryptophan (63%), and cystine (50%). Increased excretion of amino acids in the urine can further contribute to reduced tumour growth and increase the success of chemotherapy. Understanding tumour-specific amino acid dependencies will provide valuable insights to develop targeted therapies that could reduce the drug's side effects. Supported by the European Union—Next Generation EU (Program Contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006).

P61

N-GLYCANS OF BOTH FC AND FAB REGIONS OF IGG ARE CHANGED IN CHILDREN WITH TYPE 1 DIABETES

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IgG, as the most abundant plasma glycoprotein and a key molecule of the immune system, is the most researched protein from the aspect of N-glycosylation. IgG has an evolutionarily conserved glycosylation site in the Fc domain of the heavy chain, but it is estimated that 15-25% of IgG molecules also contain N-glycans in the variable region of the antigen-binding (Fab) domain. Previous research by our group has shown that changes in overall N-glycosylation of IgG occur at the onset of type 1 diabetes (T1D). The changes were most pronounced in complex, sialylated structures which are presumed to come from glycans on the Fab region.

To test the origin of these differentiating glycans, we used a novel method to investigate Fab and Fc N-glycosylation separately. IgG isolated from human plasma was captured using Fc affinity beads. Bound IgG was treated with an enzyme that cleaves IgG in the hinge region into two fragments (IdeS) with the Fc region staying bound to the beads, whilst the $F(ab)_2$ fragment is released. The two fragments were then analysed separately by enzymatic release of N-glycans using N-glycosydase F, and their subsequent fluorescent labelling with procainamide. Glycans were analysed using normal-phase UPLC. Structural composition of glycans was determined using UPLC-MS.

Applying the method described we analysed 108 samples of children with type 1 diabetes and 98 samples of healthy children. Looking at the structural differences between Fc and Fab glycans we found that most of the glycans of Fab contain sialic acids, whilst the occurrence of sialylated glycans in the Fc region was rare. Also, oligomannose glycans seemed exclusive to the Fab region. Statistical analysis of differences confirmed the findings of our previous study on whole IgG, and showed changes in both Fc and Fab glycans, with changes being more pronounced in the glycans from the Fab fragment. On Fc, significant change was observed on a core fucosylated monogalactosylated glycan with bisecting GlcNAc (FA2[3]BG; adjusted p value 0.0003), while on Fab changes in the two oligomannose glycans (Man5 and Man7, adjusted p values 0.0338) and digalacotsylated sialylated glycans (A2G2S2 and A2G2S1, adjusted p values 0.0035).

P62

DECIPHERING DNA-PROTEIN CROSSLINK REPAIR IN VIVO USING CRISPR-CAS GENE EDITING IN A ZEBRAFISH MODEL

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DNA-protein crosslinks (DPCs) are the second most common DNA lesions caused by byproducts of cellular processes such as aldehydes and reactive oxygen species, as well as helical alterations. DPCs obstruct all DNA transactions and deficient repair of these toxic lesions is associated with the onset of cancer, neurodegeneration and aging. Induction of DPCs is used in medicine to treat many cancers and understanding the repair at the organismal level could provide an impetus for the development of new drugs and combination therapies. In addition, accumulation of DPCs lead to aging and neurological disorders, so it is important to understand the mechanisms behind the crosslink repair. In recent years, we and other groups have gained mechanistic insights into the DPC repair (DPCR) factors. However, the function of repair factors at the organism level is still largely unknown. In my group, we use the zebrafish model and CRISPR-Cas gene editing to study the interplay of DPCR factors and sub-pathways including proteolysis-, nuclease- and tyrosyl-DNA phosphodiesterase-dependent repair at the biochemical and cellular level. I will present our recent discoveries using three new zebrafish strains generated with the CRISPR-Cas system: a catalytic mutant and a C-terminal mutant of the ACRC protease involved in DPCR, as well as a transgenic strain with the inactive DPCR factor, tyrosyl-DNA phosphodiesterase 1 (TDP1). We have found that ACRC is an essential protease in vertebrate development, as a catalytic mutation leads to early embryonic lethality. By injecting ACRC (WT) mRNA constructs into mutant embryos, we were able to grow the transgenic line and perform DPCR analyses. We also show that TDP1 is required for the resolution of topoisomerase 1- and histone-DPCs at the organism level and in human cells, and we characterise a novel TDP1-mediated repair pathway for histone-DPC repair. Our results provide insights into the complex DPCR pathways and their implications for human disease and cancer treatment.

P63

AN INTEGRATED LANDSCAPE OF GANGLIOSIDES, NEUROPLASTIN, AND PLASMA MEMBRANE CA^{2+}-ATPase IN HUMAN GLIOBLASTOMA

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One of the most common types of malignant brain tumors, glioblastoma, accounts for about 65% of all gliomas. They develop fast and penetrate the neighboring brain tissue. Gangliosides, sialylated membrane glycosphingolipids, have long been recognized as important factors in the development and metastatic potential of tumors. Altered ganglioside expression and concentration are well documented in different types of brain tumors, including glioblastoma. However, in healthy tissue, gangliosides perform an array of vital functions, such as modulating the expression, micro-localization, and function of membrane proteins. One of the proteins affected by ganglioside environment is neuroplastin (Np), cell adhesion glycoprotein involved in synaptic plasticity, cognition, neurodegeneration, as well as the control of calcium homeostasis as an auxiliary subunit of plasma membrane Ca^{2+}-ATPase (PMCA). Even though it is heavily influenced by ganglioside composition it was not investigated in connection to brain tumors, despite the fact that it was found to increase tumor invasion in breast cancer with metastatic potential. The aim of this study was to use a comprehensive approach and analyze the expression of Np and PMCA in correlation to the ganglioside composition of human glioblastoma samples compared to control tissue. For this purpose, we performed a pilot study and analyzed the composition of ganglioside by high-performance thin-layer chromatography and immunoblotting, while Np and PMCA expression was determined by Western blotting in glioblastoma and control tissue samples. The preliminary results of ganglioside analysis are in accordance with the literature and show a significantly different ganglioside pattern between glioblastoma and control samples. Protein expression analysis revealed a decreased expression of both distinct Np isoforms and PMCA in glioblastoma in comparison with control tissue. This exploratory study provides valuable insights into molecular features of glioblastoma where alteration of ganglioside composition is coupled to differential expression patterns of selected membrane proteins. Therefore, an approach integrating the analysis of gangliosides and proteins known to be affected by gangliosides is an excellent starting point for

further investigation on a larger number of samples. Since gangliosides have been recognized as potential therapeutic targets for treatment of several tumor types, this study may offer new directions in glioblastoma research.

P64

EVALUATING THE INFLUENCE OF tRNA POST-TRANSCRIPTIONAL MODIFICATIONS ON TRANSLATION

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Post-transcriptional modifications (PTMs) are universally present on all RNA species, being most prevalent in transfer RNA (tRNA). PTMs modulate translation by altering isoacceptor recognition and codon-anticodon pairing, impacting its processivity and fidelity. They are essential for translational adaptation to stress, like nutrient starvation, infection, and other homeostasis-disrupting processes. Despite the wealth of information showcasing the importance of PTMs, there is currently no approach for evaluating how translation can be driven by PTMs. In this study, we aim to systematically evaluate the impact of individual tRNA PTMs on translation and enhance protein synthesis by fine tuning PTM levels. First, we will generate differently modified tRNA pools by exposing *S. cerevisiae* cultures to different stresses, such as salinity, temperature, and oxidizing substances. The pools' PTM levels will be characterized and comparatively analysed using LC-MS and tRNA-seq. To establish the translational importance of individual PTMs, we will perform translational analysis with the generated tRNA pools and *in vitro* transcribed hypomodified tRNAs. Translational analysis will be performed in a yeast cell-free protein synthesis (CFPS) system with dedicated mRNA reporter molecules, devoid of cellular RNA. In the final stage, we will tailor an artificial tRNA pool for the target-of-interest. Optimal PTM distribution along with codon optimization will be implemented in the CFPS system to enhance the production of an industrially relevant protein. These findings will not only identify key regulatory components in the intricate process of translation, but will also establish a novel tool for translational modulation.

P65

UNVEILING THE MOLECULAR DANCE: EXPLORING SloR AND ScaR PROTEIN-DNA INTERACTIONS THROUGH MD SIMULATIONS

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Streptococcus mutans and *Streptococcus gordonii* are oral bacteria that form a plaque biofilm on the tooth surface. *S. mutans* is pathogenic, contributing to the development of dental caries, while *S. gordonii* is typically considered a commensal bacterium [1]. Like other divalent cations, manganese is essential for the survival of streptococci in the host. The scarcity of manganese impairs several streptococcal processes including oxidative stress defense, de novo DNA synthesis, bacterial survival, and virulence. To maintain metal ion homeostasis, *S. mutans* and *S. gordonii* rely on manganese-dependent transcription regulators, SloR (*S. mutans*) and ScaR (*S. gordonii*). SloR and ScaR coordinate the expression of virulence genes with metal ion transport across the cell surface in response to the availability of environmental manganese [2,3]. As part of our investigation into the transcriptional factors SloR and ScaR, we employed molecular dynamic (MD) simulations to explore their interaction with corresponding DNA sequences.

References:

[1] Mattos-Graner RO, Klein MI and Smith DJ. *Curr Oral Health Rep* 1, 70-78 (2014)

[2] Rolerson E. et al. *J Bacteriol* 188, 5033-44 (2006)

[3] Stoll KE. Et al. *Biochemistry* 48, 43, 10308–10320 (2009)

P66

TICAL CONTROL OF COVALENT PROTEIN ASSEMBLY TO INVESTIGATE EARLY CELL ADHESION FORMATION

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Optical control of intracellular proteins allows precise regulation of complex biological systems at high resolution. This control is often achieved through light-regulated protein interactions, triggering changes in protein localization, protein degradation or gene expression. However, most optogenetics tools suffer from inefficient activation in light and spontaneous return to the inactive state. To overcome these limitations, we turned to our previously developed peptide/binder-pair termed SpyTag003/SpyCatcher003. This 'protein superglue' forms an irreversible isopeptide bond at an ultra-fast reaction rate and allows defined assembly of biological building blocks to complex structures. Here, we established a system for the optical control of SpyCatcher003 in mammalian cells. We used site-specific incorporation of an unnatural amino acid at the reactive site of SpyCatcher003, blocking its reactivity until activation by cell-friendly 405 nm light. Initially, we confirmed robust SpyCatcher003 activation upon 405 nm light exposure using *in vitro* methods. We then applied the system to the optical control of focal adhesion protein talin to investigate the early moments of adhesion formation and fibroblast cell attachment. Within seconds of the optical activation of talin, we observed fast lamellipodium extension and a corresponding decrease in the rate of actin retrograde flow. Analysis of cell spreading and polarization kinetics suggested initial cell attachment is a biphasic process. Finally, the irreversible optical activation of talin allowed us to study the timeline of adhesion complex assembly in extended experiments, revealing hierarchy of recruitment for key focal adhesion proteins. The methods presented in here allow precise optical control over covalent protein conjugation in living cells, creating diverse opportunities in cell biology and protein nanoassembly.

P67

IDENTIFICATION OF POTENTIAL NOVEL GLI2 TRANSCRIPTIONAL TARGETS IN PROSTATE CANCER BY PROTEIN ARRAY

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The Hedgehog-GLI pathway is one of the major developmental pathways. In adult organisms, this pathway is predominantly active in stem cells. However, aberrant activation of this pathway in adults is involved in the onset of various cancers, including prostate cancer. GLI transcription factors are fundamental components of the Hedgehog-GLI signaling cascade, mediating its intracellular effects. Previous research has demonstrated that dysregulation of the Hedgehog-GLI pathway is associated with several malignancies, affecting the expression of target genes. Specifically, a study on melanoma cells, using RNA-seq, identified 77 cytokines and chemokines as novel GLI targets. In the present study, we aimed to identify cytokines and chemokines influenced by GLI2 in prostate cancer cells using a protein array.

In this research, spheroids are used as a 3D cell model, as they provide a more accurate representation of tumor characteristics. First, the spheroid cultivation method was optimized by determining the optimal cell number and spheroid formation technique. We utilized the LNCaP prostate cancer cell line, which was cultured and transfected with either an empty plasmid (mock) or a plasmid encoding GLI2 to induce its overexpression. Following transfection, the spheroids were cultured and the supernatant for analysis was collected 48h post-transfection when the spheroids we formed.

The protein array analysis of the supernatant from GLI2-overexpressing spheroids revealed a downregulation of four cytokines compared to mock-transfected spheroids. Contrarily, KLK3 (PSA), an important diagnostic biomarker for prostate cancer, was upregulated in GLI2-transfected cells relative to mock-transfected cells.

These results indicate that the GLI2 transcription factor regulates the expression of KLK3 in LNCaP cells, highlighting its potential role in the development of targeted therapies for prostate cancer.

P68

DISULFIDE LINK IN PLANT CYTOSOLIC AMINOACYL-tRNA SYNTHETASE IS IMPLICATED IN OXIDATIVE STRESS RESPONSE

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In general, cytosolic proteins do not contain disulfide link due to high reducing power of the cytosol. Unexpectedly, the crystal structure of cytosolic seryl-tRNA synthetase (SerRS) from plant *Arabidopsis thaliana* revealed a unique disulfide bond between Cys213 and Cys244. SerRS belongs to a family of aminoacyl-tRNA synthetases, that link amino acid to cognate tRNA, thus providing substrates for translation on the ribosome. Cysteines involved in *Arabidopsis* SerRS disulfide link are evolutionary conserved in all green plants, but not in other organisms, indicating their biological importance in plants. In order to decipher the role of disulfide bond in plant SerRS we have prepared mutant variants and compared their thermal stability, activity and structure with the wild-type protein. We discovered that both cysteines are important for protein stability, but with contrary effects. Crystal structure of C244S variant showed higher rigidity and very extensive network of noncovalent interactions in the vicinity of residues 213 and 244 which correlated with its higher stability. Interestingly, mass spectrometry analysis showed that disulfide link is present in solution only when the wild-type protein is incubated with H_2O_2, while it was observed in SerRS crystal structure despite addition of high concentration of reducing reagent DTT during crystallization. This once more confirmed propensity of Cys213 and Cys244 to form a disulfide bridge. Indeed, we have identified structural features of the protein microenvironment, including vicinity of positively charged Arg, hydrogen bond formation and sulfur–π interaction, which may influence reactivity of these cysteines and promote formation of disulfide link. In the presence of H_2O_2, aminoacylation activity of the wild-type protein was not abolished, while the activity of mutants was severely impaired. Altogether, our data show that evolutionary conserved cysteines in plant SerRS do not form structural or alosteric disulfide, but they may act as a sink for reactive oxygen species thus enabling the protein to function under oxidative stress conditions which can be beneficial considering that translation of stress-related mRNAs is important for the efficient plant cellular stress response.

P69

CfDNA ANALYSIS IN LIQUID BIOPSY OF PATIENTS WITH TESTICULAR GERM CELL TUMOR

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Testicular germ cell tumors are the most common neoplasia in the males aged 18 – 45, with nonseminomas having worse prognosis and the potential to develop chemoresistance. Significant diagnostic challenges persist especially in serum tumor marker negative (STM) patients. In this study we have investigated the application of cellfree DNA (cfDNA) parameters in liquid biopsies for early detection and monitoring orchidectomy success.

For the purpose of the study 41 NSE patients and 114 healthy controls were recruited. The diagnostic potential of cfDNA parameters (quality and fragmentation) as well as cfDNA methylation (RASSF1A, PRSS21, and LINE1) in blood plasma and seminal plasma samples was investigated. The diagnostic potential of investigated cfDNA parameters was analyzed using logistic regression. Pre and postoperative NSE patients' samples were analyzed to assess orchidectomy success.

In blood plasma hypomethylation of LINE1 and hypermethylation of RASSF1A have shown an AUC of 0.89, a sensitivity/specificity of 83%/83% for NSE patients and have detected 79% of STM patients. In blood plasma LINE1cfDNA methylation postoperatively exhibited a return to healthy controls cfDNA methylation levels. In seminal plasma the increase in cfDNA fragmentation and cfDNA hypermethylation of LINE1 and PRSS21 have shown an AUC of 0.83, a sensitivity/specificity of 88%/65% for NSE patients and have detected 85% of STM patients.

Both seminal and blood plasma have shown great promise as liquid biopsies in NSE for the purpose of early detection. LINE1 cfDNA methylation has exhibited potential

as a biomarker of NSE in both blood and seminal plasma as well as the first biomarker of orchidectomy success.

P70

p53 FAMILY PROFILE IN PATIENT-DERIVED MELANOMA CELL LINES RESISTANT TO TARGETED THERAPY

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Malignant melanoma remains the most aggressive and treatment-resistant form of skin cancer with increasing incidence. Most of the patients harbor the BRAF V600E mutation, which enabled the development of MAPK inhibitors that have revolutionized the treatment of melanoma patients. Despite the significant improvements in treatment the majority of patients develop resistance whose mechanisms are still not completely understood. Based on their distinct roles in carcinogenesis, diverse levels of expression, localization and/or mutual inhibitory interactions, we hypothesize that p53 family isoforms might be involved in development of resistance to targeted therapy of melanoma. A respectable number of the BRAF-mutated melanomas bear the mutations of *TP53* and *TP63*, while p73 is never mutated. All p53 family members generate multiple isoforms with diverse functions in tumorigenesis. Shorter isoforms of the p53 family, particularly those lacking transactivation domain, show a dominant-negative effect towards tumor-suppressive family members. Using qPCR and western blot analysis, we determined the p53/p63/p73 expression profiles in a panel of five BRAF-mutated patient-derived melanoma cell lines with different sensitivity to BRAF and/or MEK inhibitors. We have found altered levels of p53 family expression both on gene and protein level correlating with acquired resistance to BRAFi/MEKi and/or BRAFi targeted therapy. Hence, we suggest that uncovering the specific p53 family code could potentially lead to revealing of novel therapeutic targets in melanoma treatment. In addition, modifying expression of the p53 family proteins or targeting their functional pathways linked to MAPK inhibitor resistance could be a potential therapeutic strategy to overcome resistance to MAPK inhibitors in melanoma.

P71

ERK INHIBITORS AS A NOVEL THERAPY IN KIDNEY MITOCHONDRIAL DYSFUNCTION

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Kidney diseases are becoming a public health problem, and the prevalence worldwide is over 10%. Kidneys have one of the highest densities of mitochondria due to the high requirements for energy consumption, so their normal function and homeostasis in the kidneys are extremely important. Mitochondrial dysfunction is important in the pathogenesis of various kidney diseases that lead to an energy crisis and contribute to epithelial atrophy, inflammation, or cell death. A mechanistic understanding of the pathogenesis of kidney disease limits the development of new drugs. Extracellular signal-regulated kinases (ERK) activity is implicated in signaling pathways leading to autophagy/mitophagy, oxidative stress, and apoptosis in the kidney, and their role in renal mitochondrial dysfunction is not fully investigated. Changes in mitochondrial membrane potential play an essential role in MAPK-dependent cell death. Modulation of ERK could play a key role in the development of effective therapy. A model of mitochondrial dysfunction in human proximal tubular (HK-2) and embryonic kidney cells (HEK) and a model of mitochondrial transfer of pERK- and pERK+ mitochondria is established. The role of different drugs that inhibit ERK activation (U0126, SCH772984, Mirdametinib, VX-11e, Ulixertinib) in mitochondrial dysfunction and transfer is examined by monitoring mitochondrial membrane potential, ATP levels, and the expression and localization of proteins involved in mitochondrial oxidative stress, apoptosis, autophagy/mitophagy, and oxidative phosphorylation. Our first results imply that ERK activation is responsible for maintenance of mitochondrial population and control of glycolysis and has a role in the mitochondrial transfer. It is crucial to determine exact role of ERK in mitochondrial dysfunction to understand the pathophysiological role of mitochondrial dysfunction in kidney diseases and to contribute to the development of new drugs and the understanding of signaling pathways.

P72

EXAMINATION OF VIRAL PepH3 PEPTIDE-FUNCTIONALIZED NANOPARTICLES ON A CULTURE MODEL OF THE BLOOD-BRAIN BARRIER

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Nanoparticles (NPs) are promising new tools to increase the transfer of drugs across the blood-brain barrier (BBB) to the CNS. With appropriate ligands, vesicular NPs are suitable for targeted drug delivery across the BBB. The aim of this study was to investigate the PepH3 peptide, isolated from the capsid protein of Dengue virus as a targeting ligand of NPs to elevate the cargo penetration across the BBB.
In our experiments, we prepared PepH3-targeted NPs loaded with Texas-Red labelled bovine serum albumin (TR-BSA) or single-domain antibody (sdAb) against amyloid beta peptide as cargo. The physico-chemical properties of NPs, such as particle size, polydispersity index and surface charge were measured by dynamic light scattering. The encapsulation efficiency was detected by spectrofluorimeter or western blot. The effect of PepH3-targeted NPs on the viability of primary rat brain endothelial cells was monitored by impedance measurement. The cellular uptake and co-localization of NPs cargo with endoplasmic reticulum (ER), Golgi apparatus and lysosomes were visualized by confocal microscope. The cellular internalization, mechanisms of cellular uptake, and the penetration of NPs across the culture model of the BBB was quantified by spectrofluorimeter.
The average diameter of non-targeted and N-PepH3 particles was between 98-207 nm, respectively. NPs have a slightly negative surface charge and a relatively narrow size distribution. The encapsulation efficiencies of non-targeted and PepH3-targeted TR-BSA-loaded NPs were 32 and 24 %, respectively, and for sdAb-loaded NPs they were 93 and 68 %, respectively. PepH3 as a targeted ligand successfully increased cellular internalization of TR-BSA cargo at each time point compared to the non-targeted group. After cellular uptake, TR-BSA colocalized with ER and Golgi and limited amounts of lysosomes. Uptake of the TR-BSA cargo was an energy- and surface charge-dependent process and was partially mediated by endocytosis. The transfer of PepH3-targeted NPs containing sdAb cargo after 24 h of incubation had significantly higher penetration through the BBB model compared to non-targeted NPs.
Our results demonstrated that PepH3 is a good candidate to be used as a peptide for targeted brain delivery of therapeutic biomolecules.

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P73

INTERPLAY OF ISOLEUCINE MISTRANSLATION AND OXIDATIVE STRESS FROM A PROTEOMIC PERSPECTIVE

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Although fidelity of protein biosynthesis is maintained on many levels during replication, transcription and translation, incorporating incorrect amino acids into proteins (mistranslation) occurs at the frequency of 1 in 10 000. Mistranslation usually has negative consequences on the cells, such as protein aggregation, loss of protein function, impaired growth and morphological changes. However, some studies have shown that moderate mistranslation may lead to positive effects, such as adaptation to oxidative stress in the example of *Escherichia coli*. Aminoacyl-tRNA synthetases (aaRSs) play a major role in keeping translation faithful by pairing amino acids to their respective tRNAs. Ten out of 24 aaRSs are equipped with an editing domain that hydrolyzes misaminoacylated tRNAs. This study investigates the effect of isoleucine mistranslation on adaptation to oxidative stress by growing a strain of *E. coli* with an editing-deficient (ED^-) isoleucyl-tRNA synthetase (IleRS) in medium with valine (Val) or norvaline (Nva). Val and Nva, structurally similar amino acids to isoleucine, are recognized as substrates by IleRS ED^- and can be incorporated into proteome instead of isoleucine. The bacteria were exposed to mistranslating conditions overnight and it was shown that exposed bacteria better tolerate subsequent exposure to oxidative stress. In order to correlate adaptation to oxidative stress to isoleucine mistranslation levels prior to oxidative stress, total proteins were isolated and the proteome was analyzed by LC-MS/MS. It was shown that levels of isoleucine mistranslation go up to 30%, depending on the Val or Nva concentrations in the medium. Using quantitative mass spectrometry, differential protein expression in samples with or without Val or Nva has been analyzed by performing a functional gene ontology enrichment analysis in order to identify cellular processes that enable adaptation of bacteria to oxidative stress.

P74

SEX-DEPENDENT MODULATION OF OXIDATIVE AND ANTIOXIDATIVE STATUS IN NOD MICE: POTENTIAL CONTRIBUTORS TO THE DEVELOPMENT OF DIABETES

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The destruction of pancreatic beta cells, leading to insulin deficiency, is characteristic of type 1 diabetes mellitus (T1DM), a complex autoimmune disease. The NOD mouse strain, which is naturally susceptible to diabetes, provides a valuable model to study the intricate relationship between metabolic dysregulation, oxidative stress and mitochondrial dysfunction. Understanding sex differences in susceptibility to developing diabetes and the molecular mechanisms involved is central to tailoring therapeutic interventions. The study investigates how diet-induced metabolic changes affect antioxidant defence, oxidative damage and the expression of important proteins involved in lipid metabolism and mitochondrial function. Our study focusses on investigating the liver, an important metabolic organ, in male and female NOD mice on standard and high-fat diets. We assessed the carbonyl content and lipid peroxidation, indicative of oxidative stress, in liver samples obtained from male and female mice fed with a high-fat diet (HFD) for both 6 weeks and 6 months. Furthermore, we evaluated the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), to assess the effectiveness of antioxidant defense mechanisms in alleviating the impact of nutritional stress. By recognising possible sex-specific characteristics, we aim to elucidate sex-dependent factors that influence the development of diabetes.

P75

ANTICANCER EFFECTS OF BETULINIC ACID ON HCT116 COLORECTAL CANCER CELLS: ROLE OF OXIDATIVE STRESS, AUTOPHAGY, AND SIGNALING PATHWAYS

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Colorectal cancer (CRC) is a disease with high malignancy and mortality rates. Current antitumor therapies for CRC are only partially effective, thus highlighting the need for new therapeutic agents. Betulinic acid (BA) is a pentacyclic triterpenoid extracted from birch with a range of biological properties. Recent studies have shown that BA has significant cytotoxicity against various types of human cancer cells, indicating its potential in cancer treatment. However, the efficacy of BA on human colorectal cancer tumor cells remains unclear. The purpose of our study was to evaluate the anticancer activity of BA in human colorectal cancer HCT116 cells *in vitro* and to investigate the possible mechanisms involved.

BA dose-dependently reduced the viability of HCT116 cells, with an IC_{50} of 4.26 μM after 72 hours of treatment. BA induced oxidative stress in HCT116 cells by increasing the expression of enzymes HO-1, GPX-1, and GPX-4 in a dose-dependent manner. To further investigate the impact on reduced viability of HCT116 cells, BA induced autophagy in cancer cells by increasing the expression of microtubule-associated protein 1A/1B-light chain 3 beta-II (LC3B-II), which was found to have a protective role. We also demonstrated the colocalization of proteins TOMM20 and LC3B associated with autophagy in HCT116 cells.

To elucidate the mechanism of BA's cytotoxicity on HCT116 cells, we studied the activation of key signaling pathways involved in carcinogenesis. BA treatment induced the expression of transcriptional proteins FOXO3a and Sirt6 and decreased the expression of p-AMPK and p-mTOR in HCT116 cells in a dose-dependent manner. BA exhibited an inhibitory effect on MAPK signaling pathways, evidenced by reduced expression of p-ERK1/2, p-JNK1/2, and p-p38 in a dose-dependent manner.

According to these results, cell death induced by betulinic acid can be attributed to a combination of oxidative stress, inhibition of key signaling pathways (ERK, JNK, p38), and metabolic pathways (p-AMPK, p-mTOR), as well as the activation autophagy. These mechanisms collectively contribute to cell death through non-apoptotic pathways, indicating a complex response of tumor cells to BA treatment.

P76

EFFECTS OF SIRTUIN 3 ON METABOLIC AND REDOX HOMEOSTASIS IN MOUSE LIVER DURING AGEING

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Ageing is associated with progressive cellular and physiological deterioration, particularly in the liver, where changes in oxidative stress and antioxidant defence mechanisms occur. Sirtuin 3 (Sirt3), a member of the sirtuin family and the main mitochondrial deacetylase regulating numerous metabolic processes, plays a crucial role in the regulation of mitochondrial function and redox homeostasis. It is also known to be associated with longevity in mammals and humans as an important anti-ageing molecule and regulator of many age-related diseases. In this study, we investigated the effects of a Sirt3 knockout (KO) on age-related changes in the liver of mice, focusing on markers of oxidative damage, antioxidant enzyme activities and metabolic profiles. Survival analysis of mice showed a greater dependence of males on the protective function of Sirt3. This observation motivated us to investigate the interplay of Sirt3 and sex in metabolic regulation during ageing. In liver samples from young (4 months) and aged (14 months) wild-type (WT) and Sirt3 KO mice, we measured carbonyl content and lipid peroxidation as biomarkers of oxidative stress. In addition, we measured the activity of two important antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), to assess the capacity of antioxidant defence mechanisms. Furthermore, we analysed the changes in the metabolic profiles of the liver samples using NMR spectroscopy. Sirt3, as a regulator of metabolism, is an extremely attractive target for pharmaceutical treatments and therapeutic opportunities, especially during ageing when metabolism is disturbed. Therefore, understanding the role of Sirt3 in liver ageing may provide insights into therapeutic interventions to combat age-related liver diseases. In addition, understanding the sex differences will greatly enhance the development of relevant sex-specific treatments for age-related diseases.

P77

SEQUENCE OF ECTOPIC OSTEOGENESIS IN RATS INDUCED BY rhBMP6 DELIVERED IN AUTOLOGOUS BLOOD COAGULUM WITH SYNTHETIC CERAMICS

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Introduction: Bone morphogenetic proteins are potent osteoinductive molecules commonly used in clinics for bone regeneration. We have recently developed Osteogrow-C, a novel autologous bone graft substitute comprised of recombinant human Bone morphogenetic protein 6 (rhBMP6) delivered within autologous blood coagulum (ABC) as a BMP carrier combined with synthetic ceramics as compression resistant matrix. The aim of this study was to investigate the time course of ectopic osteogenesis in rats induced by Osteogrow-C implants containing ceramics in a size range from 500 to 1700 µm.
Material and methods: Osteogrow-C implants were prepared as follow: lyophilized rhBMP6 (20 µg per implant) was dissolved in water and added to freshly withdrawn autologous blood. Blood containing rhBMP6 was mixed with biphasic calcium phosphate ceramic particles (size 500-1700 µm; TCP/HA ratio 80/20) and left to coagulute. Osteogrow-C implants were bilateraly implanted subcutaneously in the axillary regions of Sprague Dawley rats. Animals were killed 7, 14, 21, 35 and 50 days following surgery to observe the sequence of ectopic osteogenesis. Number of implants was 10 per each time point. All extracted implants were scanned on microCT and processed histologically.
Results: MicroCT analyses revealed that rhBMP6 induced extensive bone formation in implants 14 days following subcutaneous Osteogrow-C implantation. Histological analyses revealed that 7 days following surgery ongoing endochondral ossification was present at the peripheral portions of the implants while 14 days after implantation newly formed bone was present throughout the implant between ceramic particles. On day 21 after implantation ectopic osteogenesis induced bv rhBMP6 has reached its final stage and bone was present at the ceramic surfaces and in the pores, At the end of the observation period the structural properties of newly formed bone were similar as on day 21, however, the number of adipocytes was increased and they became the predominant cell population in the bone marrow.
Conclusion: Conclusively, our study elucidates the dynamics of ectopic osteogenesis induced by rhBMP6 in ABC with ceramics. Osteogrow C implants with ceramic particles induced bone in rat ectopic site, demonstrating excellent

osteoinductive properties. Hence, Osteogrow C emerges as a promising novel therapeutic solution for bone regeneration.

P78

TYROSINE PHOSPHORYLATION OF CYTOCHROME *C* MODULATES ITS BINDING TO NUCLEAR SET/TAF-IB

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Post-translational modifications (PTMs) of proteins are ubiquitous processes present in all life kingdoms, involved in the regulation of protein stability, subcellular location, and protein activity.

In this context, cytochrome *c* (C*c*), a key component of the mitochondrial electron transport chain, is subjected to PTMs that downregulate its electron transfer activity in vivo. Furthermore, it is known that modifications such as Cc Tyr48 phosphorylation abrogate its interaction with its cytoplasmic, apoptosis-related targets. Notwithstanding the efforts put in describing the role of C*c* phosphorylation in the cytosolic and mitochondrial context, the function of C*c* phosphorylation in the nucleus remained elusive.

In this work, we delved deeper into the role of the of C*c* phosphorylation on Tyr48 within the nuclear compartment by examining its interaction with SET/TAFIβ nuclear histone chaperone. Through a combined approach of molecular dynamics simulations and biophysical assays, we elucidated that the Tyr48 phosphorylation induces local structural perturbations that lead to a 7-fold drop in binding affinity to the histone chaperone, partially impeding the inhibition of SET/TAFIβ. In line with the cytoprotective role of this PTM in the cytoplasm, these results suggest that C*c* Tyr48 phosphorylation would lead to a poised state which partially allows the chromatin remodelling activity of SET/TAFIβ under chronic, homeostatic levels of genotoxic stress, altogether upgrading the precision level in the tuning of the cell stress response mechanisms.

Our findings reveal, for the first time, that phosphorylation impact the nuclear, stress-responsive functions of C*c* in the nucleus and provide a framework to explore novel aspects of C*c* posttranslational regulation in this compartment.

P79

ANALYSIS AND ENGINEERING OF KIT.METRNL SIGNALLING TO BOOST HEART TISSUE REGENERATION AFTER MYOCARDIAL INFARCTION

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KIT, a receptor tyrosine kinase, is dimerized at the cell surface by the cytokine stem cell factor (SCF) to initiate fundamental signalling pathways, including hematopoiesis, gametogenesis, and melanogenesis. However, recent studies have shown that KIT can also bind meteorin-like protein (METRNL) to trigger heart tissue regeneration [4]. Ischemic heart diseases are the leading cause of mortality and morbidity worldwide. Therefore, revealing the molecular principles underlying this completely new function of KIT might open new therapeutic opportunities for maximizing post-infarction recovery. However, the field is currently plagued by a paucity of mechanistic details of the KIT.METRNL complex assembly. Here, we provide critical new data towards elucidating the structure-function landscape of the novel interaction between KIT and METRNL and a blueprint for how KIT can be activated by two structurally distinct ligands. The gained knowledge is a necessary prerequisite to guide us to rationally engineer METRNL towards improving its interaction with KIT to elicit intensified signalling that can lead to amplified heart tissue regeneration. Collectively, the project will impact basic and applied cardiovascular research and promises to provide new possibilities for alleviating the enormous socioeconomic burden associated with these diseases.

[1] L. K. Ashman, *The International Journal of Biochemistry & Cell Biology*, 1999, 31: 1037–1051.
[2] K. Verstraete, S. N. Savvides, *Nature Reviews Cancer*, 2012, 12: 753–766.
[3] R. Roberts, D. Govender, *Journal of Clinical Pathology*, 2015, 68: 671–674.
[4] M. R. Reboll et al., *Science*, 2022, 376: 1343–1347.
[5] G. A. Roth et al., *The Lancet*, 2018, 392: 1736–1788.
[6] T. Vos et al., *The Lancet*, 2020, 396: 1204–1222.

P80

EXPRESSION PATTERNS OF TIGIT AND LAG-3 IMMUNE REGULATION RECEPTORS ACROSS MOLECULAR SUBTYPES OF MUSCLE-INVASIVE BLADDER CANCER

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BACKGROUND & OBJECTIVE

Muscle-invasive Bladder cancer (MIBC) is an aggressive disease which requires early detection and multimodal treatment. Immune checkpoint inhibitors (ICIs) showed to be very effective. In our research we asses expression of possible ICI targets, TIGIT and LAG-3 in MIBC.

METHODS

63 FFPE samples of MIBC (33 luminal and 30 basal molecular subtypes), were analyzed for tumor histology, muscle invasion, and molecular subtype by immunohistochemistry (gata-3, CK20, CK5/6, p16). Immunohistochemical expression of immune checkpoints, T-cell immunoglobulin with ITIM domain (TIGIT) and lymphocyte activation gene 3 (LAG-3) was assessed as percentage of positive tumor cells and inflammatory cells in the tumoral/peritumor stroma.

RESULTS:

TIGIT showed heterogeneous expression, membranous epithelial expression and cytoplasmic expression in inflammatory cells. Epithelial expression of TIGIT was found in 39 patients, percentage of positive cells ranged from 1% to 50%. In luminal/basal subtype it was found in 18/21 cases. In inflammatory cells it was expressed in all samples, ranging from 1 to 70%. Most of the cases showed more than 5% positivity, in luminal/basal subtype it was found in 31/26 cases. LAG- 3 showed lower proportion of positive cells, epithelial cells were entirely negative. Inflammatory cells showed expression in 39 samples, percentage of positive cells ranged from 1 to 10%. In luminal/basal subtype it was found in 15/24 cases.

CONCLUSION

Our study showed differences in expression of TIGIT and LAG-3, immune regulation receptors across MIBC, without statistical differences in molecular subtypes for TIGIT. TIGIT showed high percentage of positivity in subset of MIBC patients, mostly in inflammatory cells of intra/peritumoral stroma. LAG-3 showed very limited

expression, only in inflammatory cells, with higher expression in basal subtype. Our results can contribute to the design and correlative study of therapeutic response in clinical trials targeting TIGIT and LAG-3.

P81

PROTECTION OF BRAIN ENDOTHELIAL CELLS AS A THERAPEUTIC TARGET IN CENTRAL NERVOUS SYSTEM DISEASES

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Blood–brain barrier (BBB) provides a special compartment necessary for proper functioning of the CNS by actively controlling cellular and molecular trafficking between the blood and the nervous system. The continuous layer of cerebral endothelial cells attached to each other by tight intercellular junctions constitutes the morphological basis of the BBB. The phenotype of the BBB including tight juctions, the lack of fenestrae, specific transendothelial transport systems and efflux pumps regulates the entry of solutes and cells into the brain, and performs metabolic and detoxifying functions to protect the CNS. Emerging evidence supports the crucial role of BBB disruption in the early stages of various brain disorders, and BBB dysfunction has therefore been identified as a potential new therapeutic target.

The degree of BBB disruption is correlated with cognitive dysfunction in neurodegenerative disorders, especially in Alzheimer's disease (AD). Enhancing amiloid beta clearance across the BBB, as well as protection of the BBB from injury are among the new strategies for therapy of AD. In our experiments, pentosan polysulfate (PPS), a polyanionic polysaccharide decreased the toxic effects of amiloid beta peptides in brain endothelial cells and protected the function of the BBB. This new observation may suggest a potential therapeutical application of PPS in AD. Furthermore, the intake of the polyunsaturated fatty acid docosahexaenoic acid (DHA) has been associated with decreased amyloid deposition and reduced risk in AD. Our results proved that DHA may protect not only neurons but also the other elements of the neurovascular unit from toxic effects of amiloid and this effect may be beneficial in AD.

Beside AD, BBB damage is also a major feature in stroke. During and after ischemic stroke, BBB disruption facilitates injury progression and increases the risk of hemorrhage predicting high mortality. In our studies, the histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA/vorinostat) showed a promising therapeutic effect on BBB preservation due to suppression of pro-inflammatory responses and reduced vesicular transcytosis.

Our results demonstrated that protecting BBB functions as a therapeutic target in brain disorders may lead to the development of new approaches for the treatment of CNS diseases.

P82

DECODING ITIH5 FUNCTION IN MELANOMA RESISTANCE TO TARGETED THERAPY

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Melanoma is skin cancer that develops from melanocytes and is one of the most aggressive tumor types due to high metastatic potential. Melanoma is prone to metastasis due to high phenotypic plasticity of melanoma cells that can undergo switch between different transcriptional states. The phenotype switching is a process that includes transition of melanoma cells between slow-cycling/invasive and proliferative states, and involves changes in different signaling pathways. Therefore, phenotype switching contributes to targeted therapy resistance in melanoma. To investigate the molecular mechanisms of resistance to targeted therapy we generated and characterized melanoma cell lines with in vitro acquired resistance to BRAFi targeted therapy. As a consequence of BRAFi-driven phenotype switching, the BRAFi-resistant cell lines exhibited specific features of slow-cycling cells which included morphological features of EMT-like cells, enhanced resistance to chemotherapy, changed levels of cell-cycle regulators, reduced proliferation and migration ability. Additional features included reactivation of MAPK or activation of PI3K/AKT signaling pathways dependent on the cell line. In order to determine the transcriptomic signature associated with the resistance to BRAFi targeted therapy, we performed transcriptomic analysis and observed that only few genes are down-regulated in both cell lines independent of the time of exposure to BRAFi therapy. Among validated genes that had reduced expression in BRAFi-resistant compared to BRAFi-sensitive melanoma cells we identified p53-regulated gene, ITIH5. ITIH5 was known to be involved in the stabilization of extracellular matrix and functions as a tumor suppressor in different tumor entities, including melanoma. Therefore, we next performed functional analysis of ITIH5 to determine its role in melanoma cell features. Although we didn't observe changes in sensitivity to BRAFi in our cell line models, we observed increased expression of the proliferation marker PCNA upon ITIH5 silencing. Furthermore, using an interactive database that clusters cells depending on differentiation, we observed reduced expression of ITIH5 in undifferentiated, highly invasive cells, which points to the potential role of ITIH5 in the plasticity of melanoma cells. Therefore, our further research will be focused on unraveling mechanisms of ITIH5 suppression dependent on BRAFi therapy and the influence of ITIH5 on different characteristics of melanoma cells resistant to BRAFi.

P83

TUMOR PROTEIN p53 (TP53) MUTATIONS IN CROATIAN POPULATION – PRELIMINARY RESULTS

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Tumor protein p53 (TP53) is a crucial protein that functions as a tumor suppressor. It plays a pivotal role in preventing cancer development by regulating cell division and ensuring the stability of the genome. Mutations in the TP53 gene are strongly associated with various hematological malignancies, including leukemia and lymphoma. TP53 mutations are detected in a subset of chronic lymphocytic leukemia (CLL) cases. Patients with TP53-mutated CLL often have a more aggressive clinical course, shorter time to treatment failure, and inferior overall survival compared to those without TP53 mutations. TP53 mutations are observed in a subset of patients with both Hodgkin (HL) and non-Hodgkin Lymphoma (NHL), particularly in aggressive subtypes such as diffuse large B-cell lymphoma (DLBCL). The aim of this study was to determine the incidence and recurrence of TP53 gene mutations in the Croatian population of patients with CLL and NHL. Dubrava University Hospital is the only institution in Croatia that performs testing for mutations in the TP53 gene using Next-generation sequencing (NGS) technology (Illumina, Cambridge Ltd.). Since its implementation in routine laboratory diagnostics, 180 patients diagnosed with CLL and NHL have been tested for TP53 mutations. A total of 42 mutations were found in 29 patients (23 CLL and 6 NHL), of which 41 were classified as pathogenic and one as a variant of uncertain clinical significance (VUS). Missense mutation resulting in nonfunctional p53 has been found in 31 patients. In addition to missense mutations, deletions of one or more bases were observed, of which four deletions resulted in frame shift termination at different codons (31, 33, 46, 79), one in a reading frame shift, while five mutations were observed in introns or in the splice acceptor site of the TP53 gene. Only 3 out of the 42 mutations recurred at least 2 times in different patients: c.376-1 G>A, c.641 A>G, and c.818 G>A. Based on these preliminary data, it can be concluded that TP53 gene mutations occur in 16% of the Croatian population of CLL and NHL patients and that the number of recurrent mutations is very small (7%).

P84

SEED TREATMENT WITH COLD PLASMA IN COMBINATION WITH PLASMA-ACTIVATED WATER AND AEROPONICS CULTIVATION FOR PLANT IMPROVEMENT

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Seed treatment with cold plasma (CP) and plasma-activated water (PAW) are green technologies that stimulate seed germination, plant morphometric parameters, biomass production, and disease resistance by inducing changes in plant biochemical phenotype. These effects depend on multiple intrinsic characteristics of the plant (species, seed dormancy state, age etc.) and variable factors such as CP parameters, cultivation conditions, biotic and biotic stressors, etc. Plant cultivation in soilless systems may substantially change the CP effect.

We have investigated different combinations of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC)), PAW and aeroponics cultivation: 1) the effect of seed treatment with CP and imbibition in PAW on germination of *Lactuca sativa* seeds, morphometric and biochemical traits of seedlings, 2) the effect of seed treatment with CP when plants are cultivated in aeroponics system, and 3) the effect of seed treatment with CP and plant spraying with PAW when plants are cultivated in field conditions (ongoing).

Both seed treatment with CP and PAW increased germination rate and early growth of seedling roots (up to 15-21%) when applied separately, however, a combination of CP pre-treatment and PAW did not enhance the stimulatory effect. The concentrations of photosynthetic pigments and total phenolic compounds (TPC) were from 9 to 28% higher in the leaves of *L. sativa* seedlings from positively affected treatment groups. When lettuce was grown in aeroponics, CP pre-treatment had a trend of increased leaf number and biomass. For comparison, in our study on *Stevia rebaudiana*, the CP effect on biomass strongly depended on CP type: DBD increased (66%) and CC decreased (64%) biomass. CP did not affect natural sweeteners steviol glycosides (SGs) concentration in aeroponics whereas SGs biosynthesis was stimulated and biomass was unaffected when plants were grown in the soil.

We have demonstrated that a short (3, 5 min) seed treatment with two types of CP and PAW has the potential to substantially enrich plant material with valuable secondary metabolites and possibly increase biomass by applying adjusted treatment protocols and choosing soilless or soil conditions for different plants.

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P85

Saccharomyces cerevisiae Mub1, A SUBSTRATE ADAPTOR OF E3 UBIQUITIN LIGASE, LINKS CELL WALL ROBUSTNESS TO THE UBIQUITIN-PROTEASOME SYSTEM

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Yeasts evolved a sophisticated regulatory system to build and maintain their cell wall, the key structure through which they interact with their environment. However, our understanding of how cell wall homeostasis interfaces with basic cellular functions largely remains elusive. In this study, we investigate the role of *Saccharomyces cerevisiae* Mub1, an adaptor protein for the E3 ubiquitin ligase Ubr2, known for its involvement in controlling proteasome genes via the transcription factor Rpn4. We demonstrate that cells lacking Mub1 tolerate standard cell wall stressors better than wild-type strains. This enhanced resilience arises from the activity of several transcription factors, leading to the inhibition of cell wall remodelling – a complex evolutionary program that becomes counterproductive under chronic cell wall stress in the laboratory environment. Our findings suggest that Mub1 regulates not only Rpn4 but a broader array of transcription factors, positioning itself as a critical yet overlooked regulatory node that directly links cell wall integrity to the ubiquitin-proteasome system (UPS).

P86

EARLY SAFETY EVALUATION OF *N*-ALKYL QUATERNARY QUINUCLIDINES AS POTENTIAL DRUG CANDIDATES USING *IN VITRO* TESTS ON HepG2 CELLS

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Quinuclidine derivatives have been recognized as promising candidates for the treatment of cholinergic system-linked disorders due to their structural resemblance to neurotransmitter acetylcholine. Recently, we characterized 14 *N*-alkyl quaternary quinuclidines (QOH-Cn and QNOH-Cn; n = 8 – 16) as reversible inhibitors of human acetylcholinesterase and butyrylcholinesterase. These findings suggest their therapeutic potential in the cholinergic neurotransmission system. Here, we present assessment of the compounds' safety profiles through a battery of *in vitro* cell tests. Human hepatocytes (HepG2) were used to determine *N*-alkyl quaternary quinuclidines influence on cell viability and homeostasis, membrane integrity and oxidative status. Seven out of 14 analogues showed cytotoxic effect (IC_{50} = 7.9 – 550 μM) and the toxicity of the compounds increased with the increase of the alkyl chains (specifically C12, C14 and C16) for both groups, alcohols and oximes. However, only oximes displayed a notable time-dependent toxicity. Although such results point to a type of regulated cell death like apoptosis, further analysis did not reveal a significant activation of specific caspases 3, 8 and 9. Exposure of HepG2 cells to LOAEL concentrations of cytotoxic quinuclidines for 4 h triggered lactate dehydrogenase (LDH) release and decrease in the membrane mitochondrial potential, indicating membrane damage to the cells. In tested time-frame and concentration range, all cytotoxic quinuclidines also induced ROS generation. The specific cell-related effects observed, likely triggered by the free long alkyl chain in quinuclidine derivatives, underscore the importance of efficient early toxicological evaluation in the initial stages of drug development and selection/optimization. Future refinements of *N*-alkyl quaternary quinuclidine structures should not overlook these toxicological assessments. The current findings indicate that bisquaternary quinuclidines with alkyl chains containing 10 or fewer carbon atoms did not exhibit cytotoxic effects. Given their inhibition potential, these compounds can be further investigated as anticholinesterase drugs for cholinergic neurotransmission-linked disorders.

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