

30th SEPTEMBER – 4th OCTOBER 2026
HOTEL KOMPAS, DUBROVNIK

50^{years}
of friendship



HDBMB 2026

UnitingScientists

30th SEPTEMBER – 4th OCTOBER 2026
HOTEL KOMPAS, DUBROVNIK, CROATIA

BOOK OF ABSTRACTS



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HDBMB 2026 MEETING

UNITING SCIENTISTS

BOOK OF ABSTRACTS

International Scientific Congress organized by
the Croatian Society of Biochemistry and Molecular Biology (HDBMB).

September 30 - October 04, 2026
Hotel Kompas, Dubrovnik (Croatia)

Book of Abstracts of the International Scientific Congress organised by the
Croatian Society of Biochemistry and Molecular Biology (HDBMB)
www.hdbmb.hr

HDBMB2026 Congress: Uniting Scientists



CROATIAN SOCIETY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

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Editors

Igor Stuparević, Nino Sinčić, Tea Martinić Cezar, Morana Dulić, Maja Katalinić

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I

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Nino Sinčić

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BOOK OF ABSTRACTS





Dear Colleagues and Friends,

Welcome to Dubrovnik and to the Anniversary Congress of the Croatian Society of Biochemistry and Molecular Biology (HDBMB). This year, we celebrate an important milestone, 50 years of HDBMB, and bring together scientists, educators, students and professionals under the motto “Uniting Scientists”.

Over the coming days, you will enjoy a rich programme of lectures, discussions and poster presentations showcasing the latest advances in biochemistry, molecular biology, biotechnology, biomedicine and related disciplines. Even more importantly, you will have countless opportunities to exchange ideas, establish new collaborations and reconnect with colleagues and friends.

This booklet will help you navigate the scientific programme and social activities. We encourage you to explore broadly, ask questions and engage in discussions. After all, great science often begins with a conversation over a poster, a coffee break or a question that nobody expected.

And while the science takes centre stage, keep an eye on the supporting programme as well. Alongside outstanding lectures and poster sessions, you can look forward to a number of special highlights, including special sessions, a sunset cruise and walk through Dubrovnik’s Old Town, a quiz night, a gala dinner, and perhaps a few pleasant surprises along the way.

Thank you for being part of this special anniversary congress. We wish you an inspiring, productive and memorable stay.

Welcome to HDBMB 2026 and enjoy the congress!

Morana Dulić
Chair of the Scientific Committee

Igor Stuparević
Chair of the Organising Committee

ORGANISERS

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

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CONGRESS SECRETARIAT

info@alpe-adriatours.com
www.hdbmb2026.com

VENUE

Hotel Kompas

Ul. kardinala Stepinca 21, 20000 Dubrovnik, Croatia

CONGRESS PCO

Alpe-Adriatours

Ulica Đure Esterca 15, 48000 Koprivnica, Hrvatska

SUPPORTED BY

FEBS - Federation of European Biochemical Societies

Institute for Medical Research and Occupational Health, Zagreb

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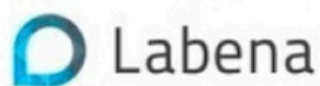




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INFORMATION

VENUE

The HDBMB2026 Congress "Uniting Scientists" will take place at the **Hotel Kompas** Dubrovnik, Croatia.

Hotel address: Ul. kardinala Stepinca 21, 20000, Dubrovnik

Hotel contact number: 020 299 000

The hotel reception is in the main lobby. **Check-in is from 14:00 and check-out by 12:00.** Participants arrange check-in and check-out directly at the reception.

The Conference Centre is immediately to the left of the hotel lobby. The Congress **Registration Desk** is in the conference foyer and stays open daily during the Congress programme. The foyer also hosts the **industry exhibition and the coffee breaks**, and all conference rooms open onto it. **Lunches** and other meals included in the congress programme will be served in a designated area of the hotel restaurant.

REGISTRATION

Registration will take place at the Registration desk in the Meeting lobby of the Hotel Kompas from 14:00 on Wednesday, 30 September.

The registration fee for participants includes admission to lectures and exhibitions, the Book of Abstracts, congress materials, lunches, refreshments during the congress, and admission to all social events, including the congress dinner and excursion. Registration for accompanying persons includes all social events, including the congress dinner and excursion. The certificate of attendance will be provided online after the congress.

LANGUAGE

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

PLENARY ROOM

Lectures and oral presentations will be held in the plenary Meridian Hall and can be followed live. The plenary Meridian Hall will accommodate the lecturers, session chairs, and up to 200 participants. Audience interaction will be possible via microphones in the congress hall.

LECTURES

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

POSTER SESSIONS

Poster presentations will take place in the designated area of the Congress centre Meridian Hall.

All posters should be mounted by 08:30 on Thursday, 01 October, according to the poster list in the Book of Abstracts, and removed before the end of the conference. **All posters must remain displayed on the poster panels throughout the congress.**

All presenters of the posters should stand by their posters during the poster sessions as defined in the Congress Programme.

Poster panels and mounting materials will be available on site. All posters will be evaluated by the Committee. The **FEBS Open Bio Poster Award**, **BioFactors Poster Award**, and **MEDiLAB Poster Award** will be presented to the best poster presenters at the closing ceremony.

SOCIAL EVENTS

All official social events listed in the congress programme are included in the registration fee. Additional hotel charges may apply for any services or goods outside the official contract with the congress organiser. If you have any questions regarding services or goods included in the registration fee, please contact the organisers.

EXCURSION AND CONGRESS DINNER

The congress excursion and congress dinner are included in the registration fee for all registered participants.

Excursion

Friday, 2 October | 16:20-21:00 (22:00)

Galleon Tirena Sunset Cruise & Dubrovnik Old Town Walk

Participants should gather in the lobby of Hotel Kompas Dubrovnik immediately after the Special Session ends. The buses will depart at 16:20 sharp. Participants who arrive late will not be able to join the organised excursion. The buses will take participants to board the Galleon Tirena. A selection of snacks and a welcome drink will be served on board. Additional drinks may be purchased individually. After returning to the Old City Port, participants will continue with a guided walk through Dubrovnik's Old Town. There will also be some free time. Return buses to Hotel Kompas Dubrovnik will depart from Pile Gate at 21:00 and 22:00.

Gala Dinner with Entertainment

Saturday, 3 October | 20:00–24:00

Hotel Dubrovnik Palace – Elafiti Restaurant

The Gala Dinner will take place at the Elafiti Restaurant, Hotel Dubrovnik Palace. Hotel Dubrovnik Palace is approximately a 15-minute walk from Hotel Kompas, and therefore no organised transportation will be provided. Following dinner, the evening will continue with a DJ programme at the Orlando Night Club within Hotel Dubrovnik Palace. The drinks must be paid for individually, so we recommend bringing cash and/or a credit card.

NOTES

Please note that all lunches are included in the congress registration fee. In addition, the Welcome Reception and its accompanying dinner on the first evening, as well as the Gala Dinner, are included in the registration. Other dinners are not included and are at participants' own expense.

PROGRAMME

SEP 30

WEDNESDAY

14:00 – 17:00

Registration

Meeting lobby

Meridian Hall

17:00 – 18:00

Opening ceremony

50 years of HDBMB

18:00 – 18:50

Plenary lecture 1

Chair: Vedrana Filić Mileta

Ana Traven (Melbourne, Australia)

Silent killers: how human fungal pathogens manipulate host immunity

19:00 – 21:00

Welcome reception

Hotel Kompas Dubrovnik

Meridian Hall

09:00 – 10:20 Session 1

Chairs: Morana Dulić and Ivana Ivančić Baće

09:00 – 09:25 Marta Popović (Zagreb, Croatia)

The repair of DNA-protein crosslinks: Developing new disease models and identifying molecular causes of human diseases

09:25 – 09:50 Mirta Boban (Zagreb, Croatia)

Mechanisms of protein quality control under acute metabolic stress

09:50 – 10:05 Ita Gruić-Sovulj (Zagreb, Croatia)

Speed-driven evolution of tRNA^{Ala} recognition by isoleucyl-tRNA synthetase

10:05 – 10:20 Damir Baranašić (Zagreb, Croatia)

Asymmetric regulatory evolution of parental subgenomes across embryonic development in allotetraploid common carp

10:20 – 11:30 Poster session 1 · Exhibition · Refreshment

(Odd poster numbers)

Session 2

11:30 – 13:05

Chairs: Mihaela Matovina and Maja Herak Bosnar

11:30 – 11:55 Marko Šestan (Rijeka, Croatia)

Teach your immune system new tricks (sit, stay and attack on cue)

- 11:55 – 12:20 **Vjekoslav Tomaić (Zagreb, Croatia)**
High-risk HPV E6 oncoproteins interact with MAML1 to maintain their protein stability and modulate notch signaling to delay keratinocyte differentiation
- 12:20 – 12:35 **Antonija Braut (Rijeka, Croatia)**
HSV-1 relocalizes ADAR1 and exploits it to inhibit stress granule formation
- 12:35 – 12:50 **Marija Šimić (Zagreb, Croatia)**
Revisiting redundancy: Unique roles of individual Rac1 GTPases in *Dictyostelium discoideum*
- 12:50 – 13:05 **Igor Weber (Zagreb, Croatia)**
Intracellular Rock and Roll: How the small GTPase Rac1 oscillates in the cell membrane
- 13:05 – 15:00 **Lunch break**
- 15:00 – 16:20 **Session 3**
Chairs: Nino Sinčić and Damjan Franjević
- 15:00 – 15:25 **Nevenka Meštrović (Zagreb, Croatia)**
Centromere: At the crossroads of function and evolution
- 15:25 – 15:50 **Dunja Leljak Levanić (Zagreb, Croatia)**
Beyond Proteasomal Degradation: BPM1 shapes epigenetic regulation of E3 ubiquitin ligase genes in *Arabidopsis* embryos
- 15:50 – 16:05 **Martina Šeruga Musić (Zagreb, Croatia)**
'*Candidatus Phytoplasma solani*' effectors SAP11-like and SAP54-like alter the transcriptomic profile in *Arabidopsis thaliana* transgenic lines

OCT 01

THURSDAY

- 16:05 – 16:20 **Lucija Markulin, Labena (Zagreb, Croatia)**
eDNA for Species Detection: Applications and advances in ddPCR technology
- 16:20 – 17:20 **Poster session 2 · Exhibition · Refreshment**
(Even poster numbers)
- 17:20 – 19:00 **Education Session**
Chairs: Marko Žarak and Ferhan G. Saĝin
- 17:20 – 18:10 **Plenary lecture 2 (FEBS Education lecture)**

Cormac Taylor (Dublin, Ireland)
Pride in progress: LGBTQIA+ Scientists in STEM
- 18:10 – 18:35 **Knut Teigen (Bergen, Norway)**
Beyond supervision: Mentorship across Snowsports and academic careers
- 18:35 – 19:00 **Ly Villo (Tallinn, Estonia)**
Safe to learn, sustainable to teach?
- 19:00 – 21:00 **Free time**
- 21:00 – 23:00 **Quiz night (Meridian Hall)**

Meridian Hall

09:00 – 09:50

Plenary lecture 3

Chair: Antonija Jurak Begonja

Iva Tolić (Zagreb, Croatia)

Mitotic vulnerabilities in cancer

09:50 – 10:45

Session 4

Chairs: Antonija Jurak Begonja and Vedrana Filić Mileta

09:50 – 10:15

Siniša Volarević (Rijeka, Croatia)

Ribosomal Protein Haploinsufficiency Cooperates with *TP53* Loss to Rewire Translation, Metabolism, and Promote Tumorigenesis

10:15 – 10:30

Ivana Novak Nakir (Split, Croatia)

Signaling networks governing receptor-mediated mitophagy in erythropoiesis

10:30 – 10:45

Sara Čabrijan (Rijeka, Croatia)

Phosphatidylinositol-4-kinase alpha mediates platelet aggregation through integrin activation

10:45 – 11:45

Poster session 3 · Exhibition · Refreshment

(Sponsored by Gorea Plus)

(Odd poster numbers)

11:45 – 13:20

Session 5

Chairs: Maja Katalinić and Igor Stuparević

11:45 – 12:10

Zrinka Kovarik (Zagreb, Croatia)

Acetylcholinesterase as a target of xenobiotics and therapeutic agents: An enzyme kinetic perspective

- 12:10 – 12:35 **Miguel Castanho (Lisbon, Portugal)**
Resisting the resistant: the struggle for a new generation of antibacterial biofilm drugs.
- 12:35 – 12:50 **Marko Močibob (Zagreb, Croatia)**
Dual response of *Bacillus thuringiensis* to antibiotics targeting aminoacyl-tRNA synthetases
- 12:50 – 13:05 **Mladen Tzvetkov (Greifswald, Germany)**
Chasing the mechanistic basis of SLC22 transporters: the YER/W motif is essential for the basic transport mechanism
- 13:05 – 13:20 **Antonio Zandona (Zagreb, Croatia)**
Targeting oxidative stress at the blood–brain barrier with nicotinamide derivatives
2025 HDBMB Young Scientists Award
- 13:20 – 15:00 **Lunch break**
- 15:00 – 16:20 **Special session**
European Research Council (ERC) – Funding opportunities and advice for future applicants
- 16:20 – 21:00 **Excursion – Galeon Tirena Sunset Cruise & Old Town Walk**
(Meeting lobby)

Meridian Hall

09:00 – 09:50

Plenary lecture 4

Chair: Jasenka Wagner Kostadinović

Ronald L. Schnaar (Baltimore, USA)

Immune regulation by human glycans and glycan binding proteins

09:50 – 10:30

Session 6

Chairs: Jasenka Wagner Kostadinović and Olga Gornik Kljaić

09:50 – 10:15

Barbara Viljetić (Osijek, Croatia)

Age- and region-dependent responses to cuprizone-induced demyelination in the mouse brain

10:15 – 10:30

Božana Blažević (Zagreb, Croatia)

Organelle screening by automated microscopy reveals numerous alterations upon cholesterol accumulation in *NPC1/2*-knockout cells

10:30 – 11:30

Poster session 4 · Exhibition · Refreshment

(Even poster numbers)

11:30 – 13:05

Session 7

Chairs: Dubravka Švob Štrac and Antonio Zandona

11:30 – 11:55

Ivana Munitić (Rijeka, Croatia)

OPTN variants in neurodegenerative diseases: Insights from experimental models and disease-specific variant analyses

- 11:55 – 12:10 **Ena Šimunić (Zagreb, Croatia)**
Sirtuin 3 drives sex-specific hepatic metabolic adaptation to Western diet-induced stress
- 12:10 – 12:25 **Andrea Gelemanović (Split, Croatia)**
Multi-omics integration for decoding inflammaging as a shared driver of age-related multimorbidity
- 12:25 – 12:40 **Marko Žarak, Astra Zeneca (Zagreb, Croatia)**
From biomarkers to targeted therapy: Precision molecular diagnostics in oncology
- 12:40 – 12:55 **Katarina Trajković (Zagreb, Croatia)**
Contact-dependent, non-cell-autonomous control of stress-induced cell fate in heterogeneous cell populations
- 12:55 – 15:00 **Lunch break**
- 15:00 – 16:50 **Session 8**
Chairs: Marta Popović and Jasmina Rokov Plavec
- 15:00 – 15:25 **Janoš Terzić (Split, Croatia)**
Contribution of microbiota and inflammation to urinary bladder cancer biology
- 15:25 – 15:40 **Neda Slade (Zagreb, Croatia)**
Characterization of melanoma cells resistant to MAPK inhibitors in the context of p53 family
- 15:40 – 15:55 **Nikolina Stojanović (Zagreb, Croatia)**
Regulation of reticular adhesions by KANK2 and talin2 in two melanoma cell lines

OCT 03

SATURDAY

- 15:55 – 16:10 **Maja Herak Bosnar (Zagreb, Croatia)**
Mitochondrial NME6: A new look at an old protein family
- 16:10 – 16:35 **Karoly Mirnics (Omaha, USA)**
Sterol pathway disruption in pregnancy: a link to autism
- 16:35 – 16:55 **Special Session**
Dubrovnik's gastronomy note presented by Ana Bitanga
- 16:55 – 17:30 **Exhibition · Refreshment**
- 17:30 – 18:30 **Special Session**
Quo Vadis HDBMB?
- 20:00 – 24:00 **Gala dinner with entertainment**
(Hotel Palace Dubrovnik, Elafiti Restaurant)

OCT 04

SUNDAY

Meridian Hall

09:00 – 09:50

Plenary lecture 5

Chair: Maja Katalinić

Ivan Đikić (Frankfurt, Germany)

Guardians of cellular homeostasis – Ubiquitin and autophagy networks

09:50 – 10:20

Closing ceremony

POSTER LIST

P1

**NGS-IDENTIFIED COMPLEX *SERPING1* ALLELE WITH CIS CONFIGURATION
CONFIRMED BY SANGER SEQUENCING IN PEDIATRIC HEREDITARY ANGIOEDEMA**

Irena Abramović, Petra Anić, Ivana Rako

P2

**SIGNATURES IN CIS-REGULATORY REGIONS ASSOCIATED WITH MULTICELLULAR
DEVELOPMENT IN SOCIAL AMOEBA *D. discoideum***

Katarina Kajan, Bojan Žunar, Jonathan R. Chubb, Boris Lenhard, Damir Baranašić,
Vlatka Antolović

P3

**ANTITUMOR ACTIVITY AGAINST MELANOMA OF ROYAL JELLY AND AF4-
ENRICHED VESICULAR FRACTION**

Vanja Baričević, Ena Kramarić, Anna Placci, Ignacija Vlašić, Mario Kurtjak, Stefano
Giordani, Valentina Marassi, Mladenka Malenica

P4

**FLUORESCENCE LIFETIME IMAGING UNCOVERS COMPARTMENT-SPECIFIC
MEMBRANE TENSION ALTERATIONS UPON CHOLESTEROL ACCUMULATION IN
NPC1-DEFICIENT MICROGLIA AND ASTROCYTES**

Ana-Marija Bogner, Lea Vidatić, Vincent Mercier, Dimitri Moreau, Sabina Tahirović,
Silva Katušić

P5

**C3 GLYCOPROTEIN AS A BIOMARKER OF EARLY METABOLIC AND INFLAMMATORY
CHANGES IN WOMEN**

Valentina Borko, Maksym Shmatkov, Dinko Šoić, Antonio Starčević, Olga Gornik

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THE UHRF1–SPRTN AXIS SAFEGUARDS GENOME AND EPIGENOME INTEGRITY

Maria Bošković, Mateo Glumac, Vincent Cahais, Felicia Chung Fei Lei, Koraljka
Husnjak, Thorsten Mosler, Borna Markušić, Cyrille Cuenin, Rita Khoueiry, Zdenko
Herceg, Ivan Đikić, Janoš Terzić

P7

**ANALYTICAL AND CLINICAL VERIFICATION OF DIGITAL PCR FOR *MYD88*
MUTATIONS IN cfDNA ACROSS BREAST CANCER SUBTYPES AND STAGES**

Andro Brzović, Marko Žarak, Sandra Šupraha Goreta

P8

BIOAVAILABILITY OF CREATINE SUPPLEMENTS: A META-RESEARCH ON METHODOLOGIES USED IN RANDOMIZED CONTROLLED TRIALS

Viljemka Bučević Popović, Antonela Sovulj, Sanda Raić, Ivana Pezelj, Malgorzata M Bala, Livia Puljak

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ERK SIGNALING IN CELLULAR RESPONSES TO MITOCHONDRIAL DYSFUNCTION

Ana-Marija Budeš, Antonijo Grčić, Roberta Ogrizović, Robert Domitrović, Iva Suman

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CHARACTERIZATION OF MEGAKARYOCYTES AND PLATELETS FROM OCRL KO MICE

Ana Bura, Leopoldo Staiano, Maria Antonietta De Matteis, Antonija Jurak Begonja

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COMPUTATIONAL AND EXPERIMENTAL INVESTIGATION OF INTERACTIONS BETWEEN MANNOSYLATED DESMURAMYL PEPTIDES AND MANNOSE RECEPTORS

Željka Car, Katarina Leko, Marija Paurević, Vesna Petrović Peroković, Josip Požar, Rosana Ribić

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FROM CHROMOSOMES TO CLINICAL FINDINGS: BIOLOGY IN CLINICAL CYTOGENETICS

Antonija Crnečki, Antonija Piškor, Lucija Belužić, Tea Mejovšek Herceg, Sara Drk Kiralj, Julijana Marinac, Janja Postružin, Manuela Žugaj, Matea Katić

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EARLY METAZOAN ORIGINS OF BRMS1: STRUCTURE, FUNCTION AND EVOLUTION

Antea Talajić, Kristina Dominko, Bastien Proust, Nikolina Škrobot Vidaček, Matija Harcet, Helena Četković

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GALECTIN-3: A POTENTIAL MISSING LINK IN BOTULINUM TOXIN ANALGESIA

Jerka Dumić, Mihael Grdunac, Lidija Bach-Rojecky

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LATENT AUTOIMMUNE DIABETES IN ADULTS AND TYPE 1 DIABETES SHARE A COMMON GLYCOSYLATION SIGNATURE OF IgG AND COMPLEMENT COMPONENT 3 (C3)

Marko Dužević, Branimir Plavša, Domagoj Kifer, Edit Pape Medvidović, Marijana Vučić Lovrenčić, Olga Gornik

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TARGETING GLIOBLASTOMA VIA NOVEL PHENYL DERIVED ACRYLONITRILES

Katja Ester, Antonija Mamić, Marijana Hranjec

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GENE EXPRESSION CHANGES INDUCED BY A REPLICATION DEFICIENT HUMAN ADENOVIRUS TYPE 26 VECTOR IN THE CONTEXT OF USING $\alpha v\beta 3$ INTEGRIN AS A RECEPTOR

Roberto Galov, Mario Stojanović, Dragomira Majhen

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ENGINEERING A PROBIOTIC YEAST FOR SWEET PROTEIN PRODUCTION: A STEP TOWARD FUNCTIONAL SWEETENERS

Romana Ivković, Bojan Žunar, Renata Teparić, Igor Stuparević

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PROFILING AND FUNCTIONAL CHARACTERISATION OF p53 FAMILY ISOFORMS IN PRIMARY MELANOMA CELL LINES RESISTANT TO COMBINATION TARGETED THERAPY

Janja Josić, Anđela Horvat, Christine Supina Pavić, Ignacija Vlašić, Neda Slade

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ROLE OF ENDOSOMAL COMPARTMENTS ACIDIFICATION DURING MCMV INFECTION IN FIBROBLASTS

Ljerka Karleuša Mujkić, Tamara Gulić, Hana Mahmutefendić Lučin, Pero Lučin

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A HIGH-THROUGHPUT AFFINITY-FREE GLYCOPROTEOMIC WORKFLOW ENABLES SIMULTANEOUS PROFILING OF FIBRINOGEN AND IgG N-GLYCOSYLATION IN HUMAN PLASMA

Toma Keser, Dinko Šoić, Olga Gornik

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OVERCOMING THE LIMITS OF STANDARD ANTIDOTES: BREAKTHROUGHS IN ORGANOPHOSPHATE NERVE AGENT SCAVENGING

Dora Kolić, Ines Primožič, Zrinka Kovarik, Nikolina Maček Hrvat

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DEVELOPMENT OF ANTIBACTERIAL COMPOSITE POLYMER SURFACES BY INCORPORATION OF LASER-SYNTHEZIZED NANOPARTICLES

Laura Nuić, Luka Perković, Rafaela Radičić, Nikša Krstulović, Lucija Krce

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HOW SERUM TYPE INFLUENCES CELLULAR HEALTH AND CELL CULTURE EXPERIMENTAL OUTCOMES

Matea Kurtović Kodžoman, Maja Katalinić, Tea Žugec

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COMPLEX EXPOSURE PATTERNS OF PHTHALATES: THE ROLE OF LIFESTYLE AND INDOOR ENVIRONMENT IN ADULT MEN

Lucija Kutleša, Andreja Jurič, Blanka Tariba Lovaković

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IN SEARCH OF NEW TREATMENTS AGAINST *Helicobacter pylori*: CHARACTERIZATION OF ADENYLOSUCCINATE LYASE

Ivana Radetić, Ivo Crnolatac, Ivana Leščić Ašler

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REWIRING NAÏVE CRISPR ADAPTATION IN *Escherichia coli*

Tomislav Mamić, Mirta Žagar, Ivana Ivančić Baće

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STRUCTURAL MODIFICATION OF NICOTINAMIDES AND ITS IMPACT ON THE MODULATION OF KEY SIGNAL-TRANSDUCTION RECEPTORS INVOLVED IN SYNAPTIC NEUROTRANSMISSION

Lucija Marcelić, Antonio Zandona, Katja Vuković, Valentina Bušić, Dajana Gašo-Sokač, Alejandra Mendez, Carlos Villalobos, Lucía Nuñez, Maja Katalinić

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MITOPHAGY IN PLACENTAL HEALTH AND DISEASE: INTEGRATIVE MOLECULAR PROFILING AND EXPERIMENTAL MODELS OF TROPHOBLAST DIFFERENTIATION

Mija Marinković, Ana Rožić, Tina Domjanović, Stipe Dumančić, Petar Đolonga, Antonela Matijaš Hrgović, Pio Labor, Kristijan Čačija

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ALLIANCE4LIFE PROJECT VIRTUAL RESEARCH CENTER: FOSTERING RESEARCH EXCELLENCE THROUGH THE VIRTUAL DEPARTMENT OF METABOLISM AND ENDOCRINOLOGY OF SKELETAL MUSCLE

Tomaž Marš, Katarina Miš, Klemen Dolinar, Blaž Kociper, Anja Srpčič, Ksenja Babič, Chiedozie Kenneth Ugwoke, Denis Korady, Nejc Umek, Sergej Pirkmajer

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IN VITRO NON-INNervATED AND INNervATED MODELS FOR STUDYING SKELETAL MUSCLE DEVELOPMENT, REGENERATION AND CONTRACTION-RELATED METABOLIC ADAPTATION

Tomaž Marš, Katarina Miš, Klemen Dolinar, Blaž Kociper, Anja Srpčič, Ksenja Babič, Sergej Pirkmajer

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THE PROTEASE ACTIVITY OF ACRC/GCNA IS ESSENTIAL DURING EARLY VERTEBRATE DEVELOPMENT FOR REPAIRING DNA-PROTEIN CROSSLINKS.

Cecile Otten, Marin Kutnjak, Christine Supina-Pavić, Marija Pranjić, Ivan Antičević, Marta Popović

PLENARY LECTURE ABSTRACTS

PL1

SILENT KILLERS: HOW HUMAN FUNGAL PATHOGENS MANIPULATE HOST IMMUNITY

Ana Traven

Biomedicine Discovery Institute, Monash University Department of Biochemistry and Molecular Biology and the Infection Program, Melbourne, Australia

The talk will discuss recent advances in our understanding of the battle between immune cells and microbial pathogens, with a focus on metabolic interactions, competition for essential nutrients and the immune response pathways that are shaped by these interactions. We study *Candida albicans*, a yeast commensal of the human gut and mucosal surfaces, as well as *Candida auris*, an environmental yeast that displays substantial resistance to antifungal drugs. Both species cause deadly infections in immunocompromised individuals, and are classified as “critical priority” human fungal pathogens by the WHO. Understanding how pathogens and immune cells respond to metabolic challenges during infection could reveal mechanisms that maintain physiological homeostasis, ultimately underpinning the development of improved therapeutic strategies that could complement existing efforts in antifungal drug discovery.

PL2

PRIDE IN PROGRESS: LGBTQIA+ SCIENTISTS IN STEM

Cormac Taylor

University College Dublin School of Medicine, Dublin, Ireland

Equality, Diversity and Inclusion (EDI) initiatives in Science, Technology, Engineering, and Mathematics (STEM) have come under attack in recent years in an organised, well funded and agenda-driven movement. This attack is damaging science. In this lecture, I will discuss the importance of supporting EDI not only to ensure equal opportunity and fairness but also as a key strength in performing high quality science. LGBTQIA+, and other minority scientists are underrepresented in STEM and are being lost due to the challenges of being accepted and allowed to live their authentic lives. I will discuss challenges facing LGBTQIA+ and other minority scientists and offer solutions to this issue including addressing the importance of visibility, recognising intersectionality and celebrating role models of the past and present.

PL3

MITOTIC VULNERABILITIES IN CANCER

Iva M. Tolić

Ruđer Bošković Institute, Zagreb, Croatia

Accurate chromosome segregation during mitosis is crucial for maintaining genomic stability. Errors in this mechanical process lead to aneuploidy, a hallmark of cancer and severe developmental disorders. While the mitotic spindle has classically been viewed as a structure governed primarily by pulling forces on chromosomes, our work over the past decade has revealed a highly dynamic, three-dimensional mechanochemical network where pushing, pulling, and rotational forces coexist to drive chromosome alignment and segregation. In this talk, I will highlight how my laboratory integrates cutting-edge imaging modalities, laser ablation, optogenetic control, and theoretical modeling to uncover the biophysical principles and scaling laws governing the mitotic spindle. First, I will discuss the discovery of bridging fibers, a previously unknown class of microtubules that promote chromosome alignment at the spindle midplane. To explain how chromosomes become aligned, we proposed a Flux-Driven Centering Model. Guided by this framework, we attenuated microtubule poleward flux, which rescued chromosome alignment and reduced segregation errors in cancer cell lines. In our latest study, we discovered a universal power-law scaling of mitotic spindles across eukaryotes, driven by mechanical chromosome crowding. This crowding dictates the spatial constraints of the spindle, providing insight into the evolution of open mitosis and mitotic cell rounding, as well as polyploidy tolerance in tumors. Together, these biophysical discoveries highlight crucial mechanical and structural vulnerabilities within the mitotic apparatus, offering new mechanistic approaches to disrupt cancer cell division.

PL4

IMMUNE REGULATION BY HUMAN GLYCANS AND GLYCAN BINDING PROTEINS

Ronald L. Schnaar

Johns Hopkins University School of Medicine Department of Physiology, Pharmacology & Therapeutics, Baltimore, Maryland, United States of America

Human glycans - glycoproteins, glycolipids and proteoglycans - are prominent at cell surfaces and in the extracellular milieu. Their varied sugar moieties are complex but tractable, and they shape cell behaviors throughout the body. Regulating the immune system is among their major functions, from initiating and moderating inflammation to directing antibody-dependent cytotoxicity and immune tolerance. Glycan regulatory effects are initiated, in part, by their binding to glycan binding proteins, also called lectins. Evolution generates polypeptide “carbohydrate-recognition domains” on lectins responsible for binding to glycans, sometimes with exquisite sugar structural specificity. Other protein domains on lectins translate glycan binding into changes in cell behavior required for a healthy immune system. Among immune regulatory lectins are members of the 14-member human Siglec (sialic acid binding immunoglobulin-type lectin) family. Most Siglecs are expressed on overlapping subsets of immune cells, and most are immune inhibitory when engaged by their glycan ligands. The discovery of the Siglec family raises challenges and opportunities, including identification of high-affinity glycan structures that engage Siglecs and exploring these and structure-based mimetics as immune inhibitory therapeutics. Our results reveal that a highly restricted set of glycans in each tissue are biosynthetically modified to carry Siglec-binding glycans, and that their expression is regulated in inflammatory diseases.

PL5

GUARDIANS OF CELLULAR HOMEOSTASIS - UBIQUITIN AND AUTOPHAGY NETWORKS

Ivan Đikić

Goethe University Institute of Biochemistry II and Max Planck Institute for Biophysics, Frankfurt/Main, Germany

Cellular homeostasis relies on highly coordinated quality-control systems that monitor protein integrity, organelle function, and genome stability. Among these, ubiquitin-dependent signaling and selective autophagy form an integrated network that safeguards cells against proteotoxic, metabolic, and genotoxic stress. Beyond their classical roles in protein degradation, ubiquitin pathways orchestrate diverse processes including endocytosis, translation, innate immunity, and DNA repair, thereby shaping cellular adaptation and survival. In this lecture, I will discuss how ubiquitination and autophagy cooperate to maintain cellular integrity under conditions of stress. I will present recent insights into how defects in RNA splicing, caused by deficiency of the snRNP component USP39, trigger proteotoxic stress responses that engage selective autophagy pathways. I will also highlight mechanisms by which loss of the metalloprotease SPRTN, a key factor resolving DNA-protein crosslinks, couples genome instability to ubiquitin-driven quality control systems. Finally, I will discuss how mechanistic insights into ubiquitin networks are enabling new therapeutic strategies. Through the PROXIDRUGS initiative, we are developing proximity-inducing modalities, including PROTACs and molecular glues, that harness ubiquitin-dependent degradation to target disease-relevant proteins, nucleic acids, and even organelles. Together, these studies illustrate how ubiquitin and autophagy networks function as central guardians of cellular homeostasis and how their manipulation opens new avenues for therapeutic intervention.

INVITED LECTURE ABSTRACTS

L1

THE REPAIR OF DNA-PROTEIN CROSSLINKS: DEVELOPING NEW DISEASE MODELS AND IDENTIFYING MOLECULAR CAUSES OF HUMAN DISEASES

Marta Popović, Cecile Otten, Marin Kutnjak, Ivan Antičević, Marija Pranjić, Leona Verasto

Ruđer Bošković Institute, Zagreb, Croatia

DNA-protein crosslinks (DPCs) are the second most common DNA lesions, obstructing replication and transcription. 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 repair at the organismal level could drive the development of new drugs and combination therapies. Additionally, the accumulation of DPCs leads to aging and neurological disorders, making it important to understand the mechanisms behind crosslink repair. In recent years, we and other groups have gained mechanistic insights into DPC repair (DPCR) factors. However, the function of repair factors at the organismal level remains 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 levels. I will present our recent discoveries using four new zebrafish strains generated with the CRISPR-Cas system: a catalytic mutant and a C-terminal mutant of the ACRC protease involved in DPCR, and transgenic strains with inactive DPCR factors, tyrosyl-DNA phosphodiesterases 1 and 2 (TDP1 and TDP2). We found that ACRC is an essential protease in vertebrate development, as a catalytic mutation leads to early embryonic lethality caused by the accumulation of specific DPCs, including histones, topoisomerases, and Parp1. We also show that in early development, the SPRTN protease cannot compensate for ACRC function. In addition, unpublished data about the DPCR pathway in TDP1- and TDP2-deficient zebrafish will be presented. Our results provide insights into the complex DPCR pathways and their implications for human disease and cancer treatment.

L2

MECHANISMS OF PROTEIN QUALITY CONTROL UNDER ACUTE METABOLIC STRESS

Mihaela Pravica¹, Dina Franić¹, Matko Bazdan¹, Antonio Bedalov², Mirta Boban¹

¹*University of Zagreb, School of Medicine Croatian, Institute for Brain Research, Zagreb, Croatia* ²*Fred Hutchinson Cancer Center, Seattle, United States of America*

Acute metabolic stress poses a major challenge to protein quality control, yet how cells maintain proteostasis under these conditions remains unclear. In budding yeast, abrupt glucose depletion rapidly lowers ATP levels and promotes the formation of chaperone-containing inclusions, raising the possibility that ATP-dependent degradation of misfolded proteins is compromised. Here, we show that selective degradation of misfolded proteins remains active during acute glucose starvation despite reduced ATP levels. Using model misfolded substrates in *Saccharomyces cerevisiae*, we find that misfolded proteins are efficiently degraded during both early and prolonged phases of glucose depletion. This process requires the proteasome and its functional 19S regulatory particle, indicating that ATP-dependent proteasomal activity persists during metabolic stress. In addition, nucleus-vacuole junctions (NVJs) support efficient degradation during prolonged starvation, pointing to a role for organelle contact sites in sustaining proteostasis under energy limitation. These findings show that cells preserve proteasome-mediated proteostasis during acute glucose starvation, while NVJs help sustain degradation capacity when metabolic resources become scarce.

L3

TEACH YOUR IMMUNE SYSTEM NEW TRICKS (SIT, STAY AND ATTACK ON CUE)

Marko Šestan

University of Rijeka, Faculty of Medicine, Department of Histology and Embryology, Rijeka, Croatia

Conditioning the immune response provides a powerful framework for understanding how associative cues can shape host defense. In this paradigm, a neutral sensory stimulus is repeatedly paired with an immunomodulatory event, allowing the organism to later reproduce elements of the immune reaction when exposed to the cue alone. These learned immune adjustments reveal that the nervous and immune systems form a tightly integrated predictive network capable of storing and retrieving immunological states. By dissecting the mechanisms underlying this phenomenon—from autonomic signaling to neuroimmune circuits—we demonstrated that conditioned responses can be harnessed to enhance immune function.

L4

HIGH-RISK HPV E6 ONCOPROTEINS INTERACT WITH MAML1 TO MAINTAIN THEIR PROTEIN STABILITY AND MODULATE NOTCH SIGNALING TO DELAY KERATINOCYTE DIFFERENTIATION

Vjekoslav Tomać

Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

α - and β -human papillomaviruses (HPVs), two of the most extensively studied HPV genera due to their impact on human health, exhibit distinct biological properties. High-risk α -HPVs are strongly associated with a broad range of malignancies, whereas β -HPVs primarily act as cofactors in cutaneous carcinogenesis. Despite these differences, both genera encode the E6 oncoprotein, which is essential for viral infection and contributes to cellular transformation. E6 oncoproteins contain an LXXLL-binding motif that mediates critical protein–protein interactions, allowing α -E6 to form a complex with E6AP and β -E6 to interact with MAML1. Although these interactions were once considered a defining distinction between α - and β -HPVs, we demonstrate that high-risk α -E6 oncoproteins also bind MAML1, revealing a previously unrecognized and functionally significant overlap between the two groups. This interaction stabilizes E6 and likely alters MAML1 function, contributing to the enhanced proliferative capacity of HPV-transformed cells. Furthermore, high-risk α -E6 expression delays keratinocyte differentiation, a process essential for the viral life cycle, and is accompanied by altered expression of Notch-responsive genes, consistent with reduced Notch signalling. Notably, these effects mirror those induced by β -E6, suggesting that α - and β -E6 proteins converge on the Notch pathway. Together, our findings highlight a shared strategy among HPV types to disrupt differentiation programs and promote a cellular environment favorable for viral persistence.

L5

CENTROMERE: AT THE CROSSROADS OF FUNCTION AND EVOLUTION

Nevenka Meštrović

Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Cell division, encompassing mitosis and meiosis, is fundamental to growth, development, regeneration and reproduction across all living organisms. Mitosis involves the straightforward division of somatic cells, whereas meiosis is a more complex process that produces haploid gametes essential for sexual reproduction. Both processes ensure the accurate transmission of genetic material – chromosomes – to daughter cells and subsequent generations. A critical component in this process is the centromere, a specialised chromosomal locus defined by the centromeric protein CenH3. Paradoxically, despite its vital role, studies across diverse organisms reveal that centromeres undergo remarkably rapid evolution at both the DNA (genetic) and protein (epigenetic) levels. Beyond their essential role in cell biology and evolution, centromeres also have intriguing implications for medicine. Notably, centromeric regions are disrupted in up to 40–60% of certain tumours, underscoring their fragility and potential involvement in tumorigenesis. Our research on mitotic and meiotic asexual nematodes provides new insights into centromere evolution and function. We found that the conservation of genetic and epigenetic centromere components in exclusively mitotic organisms results from centromere evolution occurring outside the conflict zone of meiosis. Furthermore, our comparative genomic analyses among different species highlight how reproductive strategies influence centromere organisation and evolutionary trajectories, offering new perspectives on their fundamental biological roles. This research advances our understanding of centromeric determinants, their evolution and epigenetics.

L6

BEYOND PROTEASOMAL DEGRADATION: BPM1 SHAPES EPIGENETIC REGULATION OF E3 UBIQUITIN LIGASE GENES IN *Arabidopsis* EMBRYOS

Dunja Leljak-Levanić¹, Mateja Jagić¹, Tamara Vuk¹, Lucija Markulin¹, Nenad Malenica¹, Snježana Mihaljević², Sandra Vitko¹, Željka Vidaković-Cifrek¹, Nataša Bauer¹

¹University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia ²Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

MATH-BTB proteins are best known as substrate-specific adaptors of CUL3-dependent E3 ubiquitin ligase complexes that mediate proteasomal degradation. Recent evidence that the *Arabidopsis* MATH-BTB protein BPM1 interacts with the RNA-directed DNA methylation (RdDM)-associated proteins DMS3 and RDM1 suggests an additional epigenetic role. Chromatin immunoprecipitation analyses identified shared genomic targets of BPM1 and DMS3, including *FBW2* and *RKP*, two genes encoding distinct E3 ubiquitin ligases. *FBW2* and *RKP* are associated with post-transcriptional gene silencing and cell cycle regulation, respectively, and both loci contain transposable element-derived sequences in their promoter regions enriched in RdDM-related methylation marks. To further examine the regulatory relevance of these shared BPM1 and DMS3 targets, DNA methylation and gene expression analyses were performed in early and cotyledonary somatic embryos, as well as in cotyledonary zygotic embryos, using *Arabidopsis* lines overexpressing BPM1 or DMS3, a line with reduced BPM expression, and the RdDM-deficient line *dms3-1*. Increased methylation within distal promoter regions of *FBW2* and *RKP* was positively associated with gene expression, particularly in cotyledonary zygotic embryos, with a stronger effect observed for *FBW2*. Together, these findings reveal a previously unrecognized CUL3-independent role of BPM1 in epigenetic regulation and suggest that BPM1 contributes to the fine-tuning of gene expression during embryogenesis through RdDM-dependent mechanisms. They further indicate that BPM1 may coordinate ubiquitin-mediated regulatory pathways both directly, through proteasomal degradation, and indirectly, through epigenetic control of genes encoding other classes of E3 ubiquitin ligases.

L7

BEYOND SUPERVISION: MENTORSHIP ACROSS SNOWSPORTS AND ACADEMIC CAREERS

Knut Teigen

University of Bergen, Department of Biomedicine, Bergen, Norway

Drawing on his experience as both a professor at the University of Bergen and Head of Education within Snowsports Norway, Knut Teigen explores the parallels between mentoring in higher education and the development of ski professionals. In both settings, individuals are expected to meet demanding standards under pressure, yet no two people follow the same path to excellence. This talk examines a central challenge of mentorship: how to help different individuals achieve the same high standard while recognizing that they learn, develop, and perform in different ways. Effective mentors are able to adapt their approach without lowering expectations. Drawing on lessons from coaching ski professionals preparing for international certification assessments, Knut argues that one of the most important qualities of a mentor is curiosity. While the standard may be the same for everyone, the path to reaching it rarely is. The best mentors resist relying on a single formula for success and instead remain curious about the individual, adapting their approach while maintaining high expectations.

L8

SAFE TO LEARN, SUSTAINABLE TO TEACH?

Ly Villo

Tallinn University of Technology Department of Chemistry and Biotechnology, Tallinn, Estonia

In my biochemistry teaching, I increasingly encounter students whose learning is affected by mental-health difficulties or individual needs. How should lecturers respond when they want to help but lack training, clear guidance and appropriate support? I will describe how our university is developing a dedicated service to advise on students' needs and possible accommodations. Psychological safety, however, is relevant to all students. How can we make it possible to ask questions, make mistakes and learn collaboratively while maintaining academic standards? And what about us – the educators? We face increasing workloads and burnout. To whom can lecturers turn for help? Can we build a learning culture that supports students without exhausting the people who teach them? This presentation shares experiences and invites discussion without claiming to have all the answers.

L9

RIBOSOMAL PROTEIN HAPLOINSUFFICIENCY COOPERATES WITH *TP53* LOSS TO REWIRE TRANSLATION, METABOLISM, AND PROMOTE TUMORIGENESIS

Siniša Volarević

University of Rijeka, Faculty of Medicine, Molecular Medicine and Biotechnology, Rijeka, Croatia

Ribosome biogenesis is fundamental for cell proliferation, and haploinsufficiency of ribosomal protein (RP) genes typically impairs growth. Paradoxically, heterozygous RP mutations are frequently observed in cancer, often in conjunction with *TP53* alterations, suggesting functional cooperation between these lesions in tumor development. However, the mechanistic basis of this interaction remains incompletely understood. To address this, we generated mouse embryonic fibroblasts with inducible heterozygous deletion of *Rps6* and/or *Trp53*. *Rps6* haploinsufficiency activated the RPL5/RPL11–p53 nucleolar stress checkpoint, resulting in p53-dependent cell cycle arrest and reduced proliferation. In contrast, concomitant loss of *Trp53* abrogated this checkpoint and restored proliferative capacity. Unexpectedly, *Trp53*-deficient, *Rps6*-haploinsufficient cells exhibited enhanced proliferation relative to *Trp53* loss alone, accompanied by robust activation of mTORC1, MYC, and E2F transcriptional programs. Mechanistically, this signaling rewiring increased cap-dependent translation of *Rps6* mRNA, partially compensating for impaired ribosome biogenesis. However, this adaptive response came at a cost, inducing metabolic dysregulation and proteotoxic stress. Together, these alterations created a permissive state for malignant transformation that exceeded the oncogenic effects of *Trp53* loss alone. Our findings define a mechanistic framework in which RP haploinsufficiency cooperates with *Trp53* loss to reprogram translational control and metabolic homeostasis, thereby promoting tumorigenesis. Importantly, this reveals a potential therapeutic vulnerability in cancers harboring concurrent defects in ribosome biogenesis and p53 signaling.

L10

ACETYLCHOLINESTERASE AS A TARGET OF XENOBIOTICS AND THERAPEUTIC AGENTS: AN ENZYME KINETIC PERSPECTIVE

Zrinka Kovarik

Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are essential enzymes in cholinergic neurotransmission and detoxification. AChE rapidly hydrolyzes acetylcholine at cholinergic synapses, while BChE acts as an endogenous bioscavenger by hydrolyzing a broad range of xenobiotics and anticholinesterase agents. Organophosphorus (OP) pesticides and nerve agents irreversibly inhibit both enzymes through phosphorylation of the catalytic serine residue. Inhibition of AChE results in acetylcholine accumulation, causing overstimulation of cholinergic receptors and potentially life-threatening cholinergic crisis. Although BChE inhibition has fewer direct physiological consequences, its detoxification capacity makes it an important biomarker of exposure and a promising therapeutic target. Despite decades of research, OP poisoning remains difficult to treat because currently available oximes show limited efficacy, particularly against aged AChE and within the central nervous system (CNS). Recent advances in medicinal chemistry have led to the identification of highly efficient oxime reactivators with improved kinetics toward phosphorylated cholinesterases. In particular, click chemistry-derived oxime libraries have yielded potent BChE reactivators, supporting the development of pseudo-catalytic bioscavengers as a novel therapeutic strategy against nerve agent intoxication. In parallel, the design of CNS-active oximes capable of crossing the blood–brain barrier through favorable oxime–oximate equilibria represents a promising approach for restoring cholinesterase activity in the brain. This presentation will highlight recent progress in understanding the structural and mechanistic basis of cholinesterase inhibition and reactivation. Advances in enzyme structural biology, computational modeling, and site-directed mutagenesis have enabled the rational design of more effective antidotes by identifying amino acid residues critical for oxime-mediated reactivation. Furthermore, recent *in vitro* kinetic studies, supported by efficacy demonstrated in mouse and macaque models, have identified next-generation oxime reactivators that outperform currently used antidotes. Together, these developments provide new opportunities for designing catalytic bioscavengers and more effective therapies for OP poisoning.

L11

RESISTING THE RESISTANT: THE STRUGGLE FOR A NEW GENERATION OF ANTIBACTERIAL BIOFILM DRUGS

Miguel Castanho^{1,2}

¹Gulbenkian Institute for Molecular Medicine, Lisboa, Portugal Miguel Castanho Lab, Lisbon, Portugal ²Faculdade de Medicina da Universidade de Lisboa Institute of Biochemistry, Lisbon, Portugal

Antimicrobial peptides (AMP) have been intensively studied in artificial lipid vesicles and planktonic bacteria. A step forward in the cellular biophysics of AMP is the study of peptide diffusion in biofilms, which consist in bacterial colonies living in a gel-like environment formed by an extracellular matrix of polysaccharides, nucleic acids, and proteins formed by the bacteria themselves. This extracellular matrix protects physically and chemically the bacterial colony from deleterious agents, including antibiotics. Because biofilms account for more than 80% of all hospital-related infections in humans, it is urgent to address biofilm penetration in modern antibiotic development strategies. Peptides are relatively big compared to most conventional antibiotics but they are also flexible, which makes diffusion in gels plausible for this class of molecules. The biofilm matrix diffusion-membrane lysis coupling has been characterized by us using biophysical techniques capable of reporting peptide diffusion through a *S. aureus* biofilms, killing bacteria along the way. Confocal microscopy reveals a diffusion coefficient is consistent with a crowded medium, and a combination of Atomic Force Microscopy and other imaging techniques reports the impact of AMP on the density and roughness of the biofilm. Importantly, bacteria are killed but the biofilm is not dispersed.

L12

AGE- AND REGION-DEPENDENT RESPONSES TO CUPRIZONE-INDUCED DEMYELINATION IN THE MOUSE BRAIN

Barbara Vilječić¹, Robert Rončević^{2,3}, Viktoria Čurila⁴, Ana Eldić⁵, Vedrana Ivić⁴, Željko Debeljak⁶, Marija Heffer⁴

¹J. J. Strossmayer University of Osijek, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Osijek, Croatia ²University Hospital Center Osijek, Department for Diagnostic and Interventional Radiology, Osijek, Croatia

³J. J. Strossmayer University of Osijek, Faculty of Medicine, Department of Anatomy and Neuroscience, Osijek, Croatia ⁴J. J. Strossmayer University of Osijek, Faculty of Medicine, Department of Medical Biology and Genetics, Osijek, Croatia ⁵J. J. Strossmayer University of Osijek, Department of Chemistry, Osijek, Croatia ⁶University Hospital Center Osijek, Clinical Department for Laboratory Diagnostics, Osijek, Croatia

Cuprizone-induced demyelination is widely used to study myelin damage and subsequent remyelination, yet it remains unclear whether motor performance, myelin-associated protein expression and lipid-domain organization return toward physiological profiles during the post-cuprizone period. We aimed to assess age- and region-dependent post-cuprizone remodeling in the mouse brain, with emphasis on the demyelination-remyelination interval, using an integrated behavioral, immunohistochemical and lipidomic approach. Male C57BL/6 mice aged 3 and 6 months were assigned to control or cuprizone-fed groups (0.2% cuprizone for 20 days) and analyzed 20 days after cuprizone withdrawal. Motor function was assessed using footprint analysis, rotarod and activity cage tests. Brain tissue was analyzed immunohistochemically for MAG, MBP and complex gangliosides (GM1, GD1a, GD1b and GT1b). Lipid profiles were mapped by MALDI-TOF imaging mass spectrometry (IMS) in negative ion mode (m/z 600– 1000) in the corpus callosum, anterior commissure, striatum and motor cortex. IMS data were reprocessed to include only m/z features with FDR-adjusted statistical significance and biologically relevant changes in signal intensity. Post-withdrawal behavioral testing indicated largely transient, age- and test-dependent motor alterations: younger mice showed acute changes in spontaneous activity, whereas mature mice displayed more pronounced deficits in rotarod performance. Myelin-marker analysis revealed regionally variable alterations, particularly in commissural white matter, with MAG and MBP showing different sensitivities to treatment and age. Ganglioside staining suggested selective remodeling of GM1- and GD1a-enriched membrane microdomains rather than uniform disruption of complex gangliosides. Reprocessed IMS data revealed region-specific changes in phospholipid- and sulfatide-related m/z features, most evident in white matter tracts and more extensive in 3-month-old mice, while mature animals showed fewer but distinct alterations. These results suggest that, 20 days after cuprizone withdrawal, motor performance may partly return toward control levels while myelin-associated molecular profiles remain altered. Combined with MAG/MBP and ganglioside analyses, IMS showed distinct age- and region- dependent molecular patterns across analyzed brain regions, highlighting commissural white matter as a particularly sensitive site of post-cuprizone change.

L13

OPTN VARIANTS IN NEURODEGENERATIVE DISEASES: INSIGHTS FROM EXPERIMENTAL MODELS AND DISEASE-SPECIFIC VARIANT ANALYSES

Josip Peradinović¹, Ema Jalšić¹, Toni Franjkić¹, Raffaello Cimbri¹, Nikolina Mohović¹, Nikolina Kovačević¹, Dario Brizić¹, Andrea Markovinović², Fabiola De Marchi³, Ahmad Al Kleifat⁴, Lucia Corrado⁵, Gordana Apic⁶, Robert B. Russell⁷, Ivana Munitić¹

¹University of Rijeka, Faculty of Biotechnology and Drug Development, Laboratory of Molecular Immunology, Rijeka, Croatia ²University of Rijeka, Faculty of Medicine, Department of Anatomy, Rijeka, Croatia ³Maggiore della Carità Hospital, University of Piemonte Orientale Department of Neurology, Novara, Italy ⁴King's College London Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, London, United Kingdom of Great Britain and Northern Ireland ⁵University of Piemonte Orientale Department of Health Sciences, Novara, Italy ⁶Cell Networks GmbH Metisox, Heidelberg, Germany ⁷Heidelberg University BioQuant, Heidelberg, Germany

Mutations in the *OPTN* gene are associated with several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and glaucoma. *OPTN* encodes optineurin, a multifunctional adaptor protein involved in inflammatory signalling, autophagy, intracellular trafficking, and several other cellular processes, yet its contribution to disease remains incompletely understood. Both loss-of-function (LoF) and gain-of-function mechanisms have been proposed. Here, we investigated the functional consequences of *OPTN* LoF using mouse and human experimental models and performed computational analyses of disease-associated *OPTN* variants. Our C-terminal truncation mouse model (*Optn470T*), which mimics ALS-associated protein insufficiency, did not develop overt ALS/FTD-like pathology, even during advanced aging or following systemic inflammatory challenge. Nevertheless, primary *Optn470T* microglia and macrophages exhibited impaired TBK1-mediated IFN- β responses, with no major alterations in NF- κ B signalling or innate immune functions, findings that were recapitulated following *OPTN* silencing in human monocyte-derived cells. To further investigate the role of *OPTN* insufficiency in ALS/FTD pathogenesis, *Optn470T* mice were crossed with transgenic *TDP-43G348C* mice expressing a human ALS-associated variant. Compared with *TDP-43-G348C* mice, double-mutant animals displayed improved disease outcomes during aging and following inflammatory challenge, indicating that *OPTN* insufficiency was protective in this setting. In parallel, computational analyses of *OPTN* variants reported in neurodegenerative diseases identified disease-specific variant distributions and distinct patterns of predicted pathogenic variants across ALS, FTD, and glaucoma. Together, these findings showed that *OPTN* LoF alone was insufficient to cause overt neurodegeneration but altered TBK1-mediated inflammatory signalling and influenced disease phenotypes in the presence of additional pathogenic factors. Disease-specific *OPTN* variant distributions further supported distinct molecular mechanisms underlying ALS/FTD and glaucoma, providing new insights into *OPTN*-associated neurodegenerative diseases.

L14

CONTRIBUTION OF MICROBIOTA AND INFLAMMATION TO URINARY BLADDER CANCER BIOLOGY

Janoš Terzić

University of Split School of Medicine, Split, Croatia

Bladder cancer (BC) is the fourth most common malignancy in men, with a three- to fourfold higher incidence than in women. In addition to sex and age, cigarette smoking represents the major environmental risk factor for BC. Using the N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)-induced mouse model of bladder carcinogenesis, we recently demonstrated that depletion of the gut microbiota prevents tumor development by blocking the microbial activation of the procarcinogen BBN. Specifically, gut bacteria convert BBN into the carcinogenic metabolite N-butyl-N-(3-carboxypropyl)nitrosamine (BCPN), identifying microbiota-mediated procarcinogen activation as a previously unrecognized mechanism of bladder cancer initiation. As nitrosamines are also abundant constituents of cigarette smoke, these findings suggest that the gut microbiome may modulate the carcinogenic effects of smoking through microbial metabolism of environmental carcinogens. Consistent with this concept, analysis of human stool samples revealed substantial interindividual variability in microbial nitrosamine-converting capacity, indicating that differences in gut microbiota composition may contribute to individual susceptibility to bladder cancer. Beyond tumor initiation, microbiota-dependent signaling through MyD88 and the pro-inflammatory IL-6/Stat3 axis promotes tumor growth and invasiveness. Collectively, these findings establish the gut microbiota as a regulator of bladder carcinogenesis, driving tumor initiation through metabolic activation of procarcinogens and facilitating disease progression via inflammatory signaling pathways.

L15

STEROL PATHWAY DISRUPTION IN PREGNANCY: A LINK TO AUTISM

Karoly Mirnics, Zeljka Korade

University of Nebraska Medical Center Munroe-Meyer Institute, Omaha, United States of America

Cholesterol plays a fundamental role in normal embryonic development, and disturbances in its biosynthetic pathway may result from either pathogenic genetic variants or exposure to certain prescription medications. This study examined whether maternal use of fifteen sterol biosynthesis inhibiting medications (SBIMs) during pregnancy was associated with an increased risk of autism spectrum disorder (ASD) in offspring. Using the Epic Cosmos database, we analyzed linked maternal and child health records for births occurring between 2014 and 2023, with follow-up extending through December 2025. The study population consisted of 6,135,213 children with corresponding maternal health records. Associations between prenatal exposure to aripiprazole, atorvastatin, bupropion, buspirone, fluoxetine, haloperidol, metoprolol, nebivolol, pravastatin, propranolol, rosuvastatin, sertraline, simvastatin, and trazodone and subsequent ASD diagnosis were assessed using Cox proportional hazards models. Maternal exposure to one or more SBIMs during pregnancy was associated with a 1.47-fold increased risk of ASD (95% CI, 1.45–1.49) after adjustment for potential confounding factors. Furthermore, each additional SBIM prescribed concurrently was associated with a 1.33-fold increase in ASD risk (95% CI, 1.32–1.34), with the risk rising to 2.33-fold when four or more SBIMs were prescribed simultaneously. Across the ten-year study period, 234,971 children (3.8%) received an ASD diagnosis. Among these cases, 35,152 (15.0%) had mothers who were prescribed at least one SBIM during pregnancy. The prevalence of SBIM use among pregnant women also increased substantially over the study period, rising from 4.6% in 2014 to 16.8% in 2023. These findings suggest that exposure to SBIMs during pregnancy may pose risks to fetal neurodevelopment. Given that these medications account for more than 400 million prescriptions annually in the United States, their potential effects should be carefully considered when prescribing them to pregnant patients.

SHORT ORAL PRESENTATION ABSTRACTS

SL1

SPEED-DRIVEN EVOLUTION OF tRNA^{Ala} RECOGNITION BY ISOLEUCYL-tRNA SYNTHETASE

Petra Modrušan¹, Alojzije Brkić¹, Andre Lecona Buttelli², Marc Leibundgut³, Igor Živković¹, Nenad Ban³, Liam Longo^{2,4}, Ita Gruić-Sovulj¹

¹*University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia*

²*Earth-Life Science Institute, Institute of Science Tokyo, Tokyo, Japan* ³*Institute of Molecular Biology and Biophysics, ETH Zürich Department of Biology, Zürich, Switzerland* ⁴*Blue Marble Space Institute of Science, Seattle, United States of America*

Aminoacyl-tRNA synthetases define the genetic code by covalently pairing amino acids to their cognate tRNAs. Amino acid binding occurs within a dedicated active site pocket at the catalytic domain, while recognition of a large tRNA substrate additionally requires the distal domain(s) for contacting tRNA identity elements. Here, we explore the case of isoleucyl-tRNA synthetase (IleRS), which exhibits variation in the architecture of its C-terminal tRNA^{Ala}-recognition domains. Using kinetic, structural, and bioinformatic approaches, we demonstrated that the architecture, likely reflecting an IleRS ancestral state, is conserved across IleRS enzymes in all domains of life. However, variations arise from the dynamic loss of a subdomain in some bacterial and archaeal lineages that binds the tRNA D-loop. We experimentally reconstructed this loss, showing that IleRS variants that lack interactions with the D-loop remain functional both in vitro and in vivo and may even exhibit faster aminoacylation. Our data indicate that this subdomain originally emerged as a binding module mediating tRNA indirect recognition but may have later co-opted for specific base recognition. Additional remodelling involved replacing the ancestral anticodon-recognition domain with a Zn-treble-clef domain, a transition that apparently arose under selective pressure to increase aminoacylation speed in bacteria, at the cost of some stringency in anticodon recognition. Overall, our results support the view that the tRNA-recognition domains of IleRS have been repeatedly reshaped under selection for increased translational speed.

SL2

ASYMMETRIC REGULATORY EVOLUTION OF PARENTAL SUBGENOMES ACROSS EMBRYONIC DEVELOPMENT IN ALLOTETRAPLOID COMMON CARP

Alba Madrero Pardo¹, Ada Jimenez-Gonzalez^{3,6}, Yavor Hadzhiev⁴, Bojan Žunar⁴, Boris Lenhard^{2,5}, Ferenc Mueller³, Damir Baranašić^{1,2,5}

¹Ruđer Bošković Institute, Division of Computational and Data Science, Zagreb, Croatia

²MRC Laboratory of Medical Sciences, London, United Kingdom of Great Britain and Northern Ireland

³University of Birmingham, Department of Cancer and Genomics Sciences, Birmingham, United Kingdom of Great Britain and Northern Ireland

⁴University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Biochemistry, Zagreb, Croatia

⁵Imperial College London Institute of Clinical Sciences, London, United Kingdom of Great Britain and Northern Ireland

⁶University of Sheffield, Sheffield Institute for Translational Neuroscience (SITraN), Sheffield, United Kingdom of Great Britain and Northern Ireland

Whole-genome duplication is a recurrent force in vertebrate evolution, and its aftermath raises a fundamental question: how does the embryonic genome resolve the resulting regulatory redundancy? Common carp (*Cyprinus carpio*), a species of major aquacultural importance, is an allotetraploid that arose ~12.4 million years ago through hybridisation between two diploid cyprinid ancestors, with two distinguishable subgenomes (A and B) coexisting within a single nucleus. To map the regulatory consequences of this duplication, we generated a multi-omic atlas of carp embryonic development spanning 12 developmental stages, integrating RNA-seq and CAGE for transcriptome and base-pair resolution promoter activity, ATAC-seq for chromatin accessibility, and CUT&Tag of H3K4me3, H3K27ac, and H3K27me3 to map active promoters, active enhancers, and repressed regions. From these data, we found over 250,000 regulatory elements genome-wide and functionally classified them for each stage and subgenome. This allowed us to assess chromatin landscapes and capture the regulatory potential of the genome. Our analysis revealed three main findings. First, we tested the developmental hourglass model, the proposal that mid-embryonic (phylotypic) stages are the most evolutionarily constrained, between subgenomes: regulatory divergence between A and B narrows toward the phylotypic stage, extending the principle of mid-developmental constraint to the parental halves of a single hybrid genome. Second, subgenome B dominates the regulatory landscape, contributing disproportionately to active promoters and enhancers across embryogenesis. Third, comparison with the diploid outgroup zebrafish reveals that subgenome B retains the majority of ancestral cis-regulatory elements, while subgenome A is under purifying selection – a directional resolution of regulatory redundancy in which B preserves the ancestral programme while A is reduced to its most essential elements and lineage-specific innovations. Together, these results establish carp as a powerful system for studying regulatory evolution after whole-genome duplication.

SL3

HSV-1 RELOCALIZES ADAR1 AND EXPLOITS IT TO INHIBIT STRESS GRANULE FORMATION

Antonija Braut, Igor Jurak

University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia

Human cells harbor a variety of versatile innate immune sensors such as Toll-like receptors (TLRs), RIG-I like receptors (RLRs), etc. which recognize pathogen-derived molecules and mount life-saving responses, including stress granule formation, translational shutdown, and apoptosis. To prevent excessive activation under homeostatic conditions, these sensors are tightly regulated by cellular proteins, but they can also be targeted by pathogens to evade immune detection. Although the exact mechanisms of immune evasion by herpes simplex virus 1 (HSV-1) remain incompletely understood, our previous work identified ADAR1, an immunomodulatory RNA-editing protein, as a potential proviral factor, as its depletion markedly impairs HSV-1 replication. Moreover, we showed that a key signaling pathway involves PKR, a cytoplasmic sensor of viral dsRNA. ADAR1 is a versatile protein that exists in two isoforms, the constitutively and abundantly expressed p110 and the interferon-inducible p150. To further investigate the subcellular distribution and function of ADAR1 during the course of HSV-1 infection with a focus on PKR activation and its downstream effects such as stress granule formation we analyzed ADAR1-depleted RPE1 cells using fluorescence microscopy. Our results reveal extensive changes in the localization of both ADAR1 isoforms during HSV-1 infection. Early in infection, ADAR1 p110 colocalizes with nucleolar markers (nucleolin, fibrillarin, UBF, B23) but this is markedly reduced or lost later in infection, most probably due to nucleolar disruption. Later stages of infection are characterized by p110 relocalization to the nuclear periphery, likely making way for the growing viral replication compartments (VRCs). In contrast, the p150 isoform aggregates in the nucleus at later stages, possibly aiding viral replication. In addition, we observed stress granule formation in infected ADAR1-depleted cells, which coincides with the activation of PKR and reduced viral replication. Together, these findings suggest that HSV-1 may exploit multiple mechanisms, involving both host and viral factors, to suppress stress granule formation and facilitate efficient replication.

SL4

REVISITING REDUNDANCY: UNIQUE ROLES OF INDIVIDUAL Rac1 GTPases IN *Dictyostelium discoideum*

Marija Šimić¹, Maja Marinović¹, Mihaela Matovina², Igor Weber¹, Vedrana Filić¹

¹Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia ²Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia

The small GTPase Rac1 is one of the best-characterised Rho family members and is a key regulator of the actin cytoskeleton. Human Rac1 regulates many actin-related processes, including cell motility, adhesion, division, macropinocytosis, and phagocytosis. The protist *Dictyostelium discoideum*, a highly motile amoeba, encodes three orthologues of human Rac1 named Rac1A, Rac1B, and Rac1C. These three GTPases share over 90% amino acid sequence identity with each other and over 80% with human Rac1, as well as identical effector domains. Given this high degree of sequence identity, functional redundancy among them has long been assumed in the literature. To test this assumption and investigate the roles of individual Rac1 proteins, we generated all single, double, and triple *rac1* knockout mutants. We analysed growth of mutant cells in suspension, which relies on efficient macropinocytosis and cytokinesis, and growth on solid media with bacteria as a food source, which depends on phagocytosis, cytokinesis, cell-substrate adhesion, and cell motility. We found that cells lacking Rac1A – *rac1A*-, *rac1A*-/1B-, and *rac1A*-/1B-/1C- – have reduced growth rates in suspension compared to wild-type cells, and this phenotype can be explained by a significant cytokinesis defect exhibited by these cells when grown in suspension. Moreover, all *rac1*- mutants, except *rac1C*-, exhibit slower growth on bacterial lawns. Further analysis showed that all mutants lacking *rac1A* have a cell-substrate adhesion defect. It remains to characterize the efficiency of macroendocytosis to fully elucidate the growth defects of mutant cells. We then examined whether the loss of one *rac1* gene triggers compensatory upregulation of the remaining paralogues. The qPCR results showed strikingly elevated expression of *rac1B* and/or *rac1C* upon loss of the *rac1A* gene, thus complicating interpretation of the mutant phenotypes. These results indicate that Rac1A, Rac1B, and Rac1C, like human Rac1, regulate actin-dependent cellular processes and that, in addition to redundant functions, individual *Dictyostelium* Rac1 GTPases also display unique roles. ACKNOWLEDGMENT: This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-2024-05-6331.

SL5

INTRACELLULAR ROCK AND ROLL: HOW THE SMALL GTPase Rac1 OSCILLATES IN THE CELL MEMBRANE

Igor Weber¹, Vedrana Filić¹, Nenad Pavin², Marko Šoštar¹

¹Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia ²University of Zagreb, Faculty of Science, Department of Physics, Zagreb, Croatia

Periodic motions are common in biology and occur at the levels of ecosystems, organisms, organs, cells, and intracellular proteins. Proteins that regulate cell polarization are especially prone to cyclic spatial and temporal fluctuations. Rho family GTPases are small signaling proteins that play a central role in regulating cell polarity and motility by controlling actin cytoskeleton remodeling. Intracellular oscillations of Rho family GTPases have been observed in many species and cell types, including yeast, plant, and mammalian cells. We show that a protein from this group, Rac1, displays oscillating and polarized patterns in the plasma membrane of free-living amoeboid cells of the protist *Dictyostelium discoideum*. The active form of Rac1, Rac1-GTP, can repeatedly move from one side of the cell to the other (rocking motion) or move along the cell membrane (rolling motion), with a typical period of 2 to 5 minutes. To simulate the observed dynamics, we developed a mass-conserving reaction-diffusion model based on the circulation of Rac1 between the membrane and the cytoplasm, its activation by guanine nucleotide exchange factors (GEFs), deactivation by GTPase-activating proteins (GAPs), and interaction with the IQGAP-related protein DGAP1. Our theoretical model accurately reproduces the experimentally observed dynamic patterns of active Rac1 and DGAP1, including the counterintuitive anti-correlation between their peak cortical intensities. In particular, the model simulates the detailed features of the observed traveling and standing waves, including their periods, amplitudes, and shapes. The model shows how cell size, protein amounts, and reaction kinetics of the interacting proteins determine whether Rac1 remains homogeneously distributed in the membrane, polarizes, or oscillates. It also shows how Rac1 dynamics depend on negative feedback mechanisms mediated by its negative regulators. While GAP primarily influences the temporal aspects of pattern formation, DGAP1 influences its spatial aspects. **ACKNOWLEDGMENT** This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-2024-05-6331.

SL6

'*Candidatus Phytoplasma solani*' EFFECTORS SAP11-LIKE AND SAP54-LIKE ALTER THE TRANSCRIPTOMIC PROFILE IN *Arabidopsis thaliana* TRANSGENIC LINES

Dino Davosir, Iva Marković, Martina Šeruga Musić

University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

In pathogenic bacteria, effector genes encoding small proteins that affect host development and physiology are frequently found. Phytoplasmas (genus '*Candidatus Phytoplasma*') encompass a group of uncultivable insect-transmitted bacteria affecting a wide range of plant species, including economically important crops. As endocellular pathogens, phytoplasmas secrete their effectors directly into the host cell, enabling them modulation of developmental and metabolic processes as well as the host immune responses. In our previous studies, we identified putative effector genes by sequencing and comparative analyses of several '*Ca. P. solani*' genomes. Subsequent studies on generated transgenic arabidopsis (*Arabidopsis thaliana*) lines overexpressing effectors SAP11-like and SAP54-like revealed significantly altered plant growth and development, as well as the interactions of effectors with plant transcription factors. In the scope of this study, we aimed to further identify SAP11-like and SAP54-like effectors' host targets and their impact on a transcriptomic level. Tissue from 15-day-old transgenic seedlings overexpressing SAP11-like and SAP54-like genes, as well as wild-type, was used for the analyses. Two independent lines for each effector were used. After isolation of total RNA, quality check, library preparation with polyA enrichment and RNA sequencing were performed (Novogene Europe). The results of bioinformatic analyses have shown considerable changes on the transcriptomic level in all transgenic arabidopsis lines, when compared to the wild-type. Differential gene analysis has revealed a high number of upregulated and downregulated genes indicating that normal metabolic and physiological processes are disturbed by expression of both SAP11-like and SAP54-like effectors in the early stage of development. Functional KEGG enrichment analysis has shown that photosynthesis-related genes are the most downregulated ones, together with those involved in glucosinolate biosynthesis and plant hormone transduction pathways. On the other hand, significant upregulation was found in phenylpropanoid biosynthesis genes, starch and sucrose metabolism and genes involved in plant-pathogen interaction pathways. However, differences in transcriptomic profiles were observed between SAP11-like and SAP54-like transgenic lines. Our results offer the first insight into the global changes of plant transcriptome in response to '*Ca. P. solani*' effectors. Further studies will aim to reveal new effector targets and elucidate mechanisms by which phytoplasmas disturb plant physiology and defense responses.

SL7

eDNA FOR SPECIES DETECTION: APPLICATIONS AND ADVANCES IN ddPCR TECHNOLOGY

Lucija Markulin

Labena d.o.o., eDNA Labs, Zagreb, Croatia

Environmental DNA (eDNA) provides a sensitive and non-invasive approach to biodiversity assessment and environmental monitoring. Conventional biological monitoring can be time-consuming and often relies on specialized taxonomic expertise, creating a need for more efficient and scalable methods. DNA recovered directly from environmental samples can be analyzed using targeted approaches, such as qPCR and droplet digital PCR (ddPCR), or community-level approaches based on next-generation sequencing (NGS). At eDNA Labs, we have developed and applied eDNA workflows across freshwater, marine, and aquaculture environments, using both targeted ddPCR and community-level NGS approaches to address different monitoring needs. In the Sava River, eDNA was used for fish monitoring through species-specific ddPCR assays, complemented by NGS-based analysis of fish communities. In marine aquaculture, eDNA metabarcoding was applied to monitor changes in algal communities associated with feeding and nutrient input. ddPCR-based eDNA monitoring was also used for non-invasive detection of fish pathogens directly from water samples, showing its potential for early detection before clinical signs appear. Targeted eDNA assays have also been developed for monitoring invasive and endangered species across freshwater and marine ecosystems. ddPCR is particularly well suited for eDNA analysis, where target DNA is often present at low concentrations and within complex environmental samples. By partitioning samples into thousands of individual droplets, it enables sensitive detection and absolute quantification without the need for standard curves. Recent advances in digital PCR, including the Bio-Rad QX700™ series, expand these capabilities with seven-color multiplexing, higher throughput, and streamlined workflows. These developments offer new opportunities for simultaneous detection of multiple targets and for expanding eDNA applications to broader environmental monitoring programs.

SL8

SIGNALING NETWORKS GOVERNING RECEPTOR-MEDIATED MITOPHAGY IN ERYTHROPOIESIS

Tina Domjanović¹, Ana Rožić¹, Ivan Blažević², Jörn Dengjel³, Mija Marinković⁴, Ivana Novak¹

¹*University of Split, School of Medicine, Laboratory for Cancer Research, Split, Croatia*

²*University Hospital of Split, Department of Pediatric Disease, Split, Croatia* ³*University of Fribourg Department of Biology, Fribourg, Switzerland* ⁴*University of Split, Faculty of Science, Split, Croatia*

Mitophagy is a selective form of autophagy that maintains cellular homeostasis by eliminating damaged or superfluous mitochondria. Defects in this process are increasingly linked to a wide spectrum of human diseases, underscoring its physiological importance. While the PINK1/Parkin pathway has been extensively characterized, receptor-mediated mitophagy has emerged as a critical mechanism in developmental and differentiation contexts, like erythropoiesis and retinal maturation. Here, we show that receptor-mediated mitophagy is finely tuned by post-translational modifications, notably phosphorylation and ubiquitination which modulate receptor activity and mitochondrial turnover. We focus on the upstream regulatory networks controlling BNIP3L/NIX-driven mitophagy, aiming to elucidate the kinase–phosphatase circuits that govern its activity and enable efficient mitochondrial clearance during erythroid maturation. By dissecting these regulatory pathways, we seek to define how precise modulation of receptor-mediated mitophagy supports mitochondrial clearance and drives terminal erythroid differentiation. We will present our most recent findings which advance our understanding of mitophagy regulation on development context and may reveal novel therapeutic targets for diseases associated with mitochondrial dysfunction and defective erythropoiesis.

SL9

PHOSPHATIDYLINOSITOL-4-KINASE ALPHA MEDIATES PLATELET AGGREGATION THROUGH INTEGRIN ACTIVATION

Sara Čabrijan¹, Ana Bura¹, Steven G. Thomas^{2,3}, Steve P. Watson^{2,3}, Natalie S. Poulter^{2,3}, Antonija Jurak Begonja¹

¹*University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia*

²*University of Birmingham, Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, Birmingham, United Kingdom of Great Britain and Northern Ireland*

³*Universities of Birmingham and Nottingham Centre of Membrane Proteins and Receptors (COMPARE), The Midlands, United Kingdom of Great Britain and Northern Ireland*

Phosphatidylinositol-4-kinase alpha (PI4KA) is an enzyme that, upon cell activation, transiently associates with the plasma membrane (PM), where it generates phosphatidylinositol 4-monophosphate (PI4P), a major lipid precursor of phosphatidylinositol-(4,5)-bisphosphate (PIP2). PIP2 is a well-studied signaling molecule shown to be crucial for cytoskeletal reorganization and activation, but the independent roles of its precursor PI4P and the PI4KA enzyme are yet to be elucidated. A recent study reported altered platelet counts and cytoskeletal defects in B-cells in carriers of PI4KA mutations (Saetini et al., 2025 J Clin Immunol). Therefore, by utilizing a pharmacological approach, we aimed to determine the role of PI4KA in human platelets (hPLTs). We analyzed CRP-activated hPLTs for aggregation (lumi-aggregometry), size of aggregates (light microscopy), integrin $\alpha\text{IIb}\beta\text{3}$ activation and P-selectin exposure (flow cytometry), as well as calcium mobilization (kinetic fluorescence). In addition, we examined adhesion and cytoskeletal reorganization (F-actin polymerization and microtubular coil reorganization) together with the levels of PI4P/PIP2 at the PM in collagen-spread platelets, by immunofluorescence. PI4KA inhibition followed by CRP activation led to a significant decline in platelet aggregation, as well as a reduced size of aggregates. Activation studies revealed a significant decrease in integrin $\alpha\text{IIb}\beta\text{3}$ activation, with no effect on secretion of α -granules (P-selectin). This was paralleled by a decrease in Ca^{2+} mobilization, which could be associated with the exhaustion of the PIP2 pool. At later stages of spreading on collagen, PI4KA inhibition resulted in a decrease in both PI4P and PIP2 at the PM of hPLTs. In addition, the inhibition significantly decreased lamellipodia formation, increased the percentage of cells with F-actin spikes and retained the microtubular coil, thus markedly impairing cytoskeletal organization during platelet spreading. The results demonstrate a critical role of PI4KA in integrin activation, which is essential for platelet aggregation and may aid in the development of new antithrombotic agents.

SL10

DUAL RESPONSE OF *Bacillus thuringiensis* TO ANTIBIOTICS TARGETING AMINOACYL tRNA SYNTHETASES

Marko Močibob, Ita Gruić-Sovulj

University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Aminoacyl-tRNA synthetases (aaRS) are essential enzymes for ribosomal translation, as they charge tRNA with corresponding amino acids. In each organism (or cellular compartment) there should be a single aaRS specific for each of twenty standard amino acids, but this is more an exception than a general rule. A notable example is bacterium *Bacillus thuringiensis*, where ten additional aaRS exist: two isoforms of IleRS, ThrRS, TrpRS, HisRS, LysRS, MetRS, ArgRS, ProRS and three TyrRS instances. For the closely related bacterium *Prestia megaterium*, we have shown that additional IleRS confers resistance to the antibiotic mupirocin, a natural product targeting bacterial IleRS. To test if *B. thuringiensis* is a superbug resistant to multiple antibiotics targeting aaRS, we treated bacteria with mupirocin, halofuginone, borrelidin and indolmycin, the inhibitors of IleRS, ProRS, ThrRS and TrpRS, respectively. We found that *B. thuringiensis* is indeed more resistant to borrelidin and indolmycin than *B. subtilis*, which lacks duplicated ThrRS and TrpRS, but the resistance to borrelidin and indolmycin is modest compared to high mupirocin resistance of both *P. megaterium* and *B. thuringiensis*. Proteomic analysis revealed that in all four cases, expression of cryptic, divergent aaRS genes in *B. thuringiensis* was increased upon antibiotic treatment. Surprisingly, antibiotic treatment in all four cases upregulated also genes involved in amino acid biosynthesis, and revealed additional, previously unrecognized biosynthetic gene operons with novel regulatory mechanisms. Proteomic analysis painted more complex picture than anticipated, suggesting that modulation of amino acid synthesis may contribute to resistance to antibiotics targeting aaRSs.

SL11

CHASING THE MECHANISTIC BASIS OF SLC22 TRANSPORTERS: THE YER/W MOTIF IS ESSENTIAL FOR THE BASIC TRANSPORT MECHANISM

Mladen Tzvetkov, Sarah Römer, Marleen Meyer-Tönnies

Institute of Pharmacology, C_DAT Felix-Hausdorf-Str. 3, Greifswald, Germany

Transporters of the SLC22 family handle as substrates an exceptionally broad spectrum of structurally diverse organic molecules. Among the most polyspecific members are the organic cation transporters OCT1, OCT2, and OCT3, as well as the organic anion transporters OAT1, OAT2, and OAT3. Their broad substrate specificity and high expression in pharmacokinetically relevant organs, including liver, kidney, and intestine, make them key determinants of drug disposition and important targets of pharmacological research. Using human OCT1 as a model transporter, we investigated the mechanistic role of amino acids predicted from recent cryo-EM structures to constrain the substrate binding pocket and regulate conformational transitions. Functional analyses of Y361, E386, and R439 identified the YER motif as a central element of OCT1 transport. The negative charge of E386 and the positive charge of R439 were essential for transport of all tested substrates, whereas charge reversal did not restore activity, indicating that these residues are not merely involved in electrostatic pairing but also in additional transport-relevant interactions. In contrast, Y361 showed pronounced substrate-specific effects. Molecular dynamics simulations and mutagenesis suggested that aromatic or lipophilic substrates can partially compensate for the loss of Y361, while tryptophan at this position most efficiently restored transport, supporting a role of Y361 in hydrogen-bond-mediated interaction with E386 and R439. In parallel, we analyzed W217, W354, and I446 as additional residues shaping the intracellular side of the binding pocket. W354 was indispensable for OCT1 function across 27 structurally diverse substrates, with only W354Y partially preserving activity. Functional data and simulations indicate that W354 stabilizes the outward-open conformation through interaction with N453 in transmembrane helix 10 and thereby supports the conformational switch required for transport. W217, in contrast, showed substrate-dependent effects and functionally interacted with F244, suggesting a compensatory mechanism contributing to substrate specificity. Together, our data identify the YER/W network as a key mechanistic element of OCT1. While E386, R439, and W354 are essential for the basic transport cycle, Y361 and W217 fine-tune substrate-specific transport, providing a mechanistic explanation for the remarkable polyspecificity of SLC22 transporters.

SL12

TARGETING OXIDATIVE STRESS AT THE BLOOD–BRAIN BARRIER WITH NICOTINAMIDE DERIVATIVES

Antonio Zandona

Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

Oxidative stress is a major contributor to blood–brain barrier (BBB) dysfunction and is increasingly recognized as an early event in the development of neuroinflammatory and neurodegenerative disorders. Nicotinamides are closely linked to cellular energy metabolism, redox regulation, and antioxidant defense, making their synthetic derivatives attractive candidates for protecting the BBB against reactive oxygen species (ROS)-mediated injury. In this study, we evaluated a series of synthetic nicotinamide derivatives as potential barrier-protective agents in *in vitro* BBB models exposed to tert-butyl hydroperoxide-induced oxidative stress. All tested derivatives were non-toxic and attenuated oxidative stress in brain endothelial cells, but NA-4OCH₃ showed particularly strong protective activity. It attenuated ROS production and restored key antioxidant and mitochondrial parameters, including glutathione activity, mitochondrial membrane potential, and mitochondrial and cytosolic superoxide dismutase activity. Importantly, it also preserved BBB integrity, as demonstrated by increased transendothelial electrical resistance, elevated claudin-5 levels, and reduced permeability of barrier markers. To further assess translational relevance, the protective effects were compared in human pluripotent stem cell-derived brain endothelial cells and primary rat brain endothelial cells. Although both models showed oxidative stress-induced barrier impairment, NA-4OCH₃'s efficacy depended strongly on species and cellular origin, with human cells displaying more pronounced recovery. These findings identify NA-4OCH₃ as a promising lead candidate for further structural optimization and highlight the importance of model selection when evaluating BBB- targeted antioxidant strategies.

SL13

ORGANELLE SCREENING BY AUTOMATED MICROSCOPY REVEALS NUMEROUS ALTERATIONS UPON CHOLESTEROL ACCUMULATION IN *NPC1/2*-KNOCKOUT CELLS

Božana Blažević¹, Lea Vidatić¹, Ana-Marija Bogner¹, Stefania Vossio², Vincent Mercier², Dimitri Moreau², Silva Katušić¹

¹*Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia* ²*University of Geneva, ACCESS Geneva, Geneva, Switzerland*

Niemann-Pick type C disease (NPC) is a rare, monogenic, neurodegenerative disease and lysosomal storage disorder. It is caused by mutations in *NPC1* or *NPC2* genes resulting in synthesis of dysfunctional NPC1 or NPC2 proteins. These proteins are ubiquitously expressed late endosomes/lysosomes (LE/Lys) cholesterol trafficking proteins that mediate transport of cholesterol and other lipids from LE/LYs to other cellular compartments. Although cholesterol accumulation in LE/Lys and lysosomal dysfunction is key pathological feature of NPC disease, in this work we tested the hypothesis that, due to disrupted lipid homeostasis and transport, other organelles could also be affected in the course of the disease. To investigate this, we performed an automated confocal microscopy screening and quantitative analysis of immunofluorescently labelled HeLa *NPC1/2*-knock-out (KO) vs. wild type (WT) cells. HeLa cells were stained with a panel of over 20 markers to profile organelle changes within the endolysosomal and recycling pathway, endoplasmic reticulum (ER), Golgi apparatus, mitochondria and cytoskeleton. Imaging was performed using an ImageXpress Micro Confocal High-Content Imaging System and several parameters, including signal intensities and morphological descriptors (size, shape, cellular localization, etc.) were quantified. Comprehensive organelle profiling in *NPC1/2*-KO cells revealed widespread structural and functional alterations across all organelles analyzed. Endolysosomal analysis revealed enlarged but fewer vesicular structures in *NPC1/2*-KO cells, indicating trafficking defects within this pathway. Recycling endosome markers were decreased in KO cells, consistent with disrupted endosome dynamics and autophagy. Lipid droplets were reduced in both number and size, while mitochondria appeared elongated. ER/Golgi profiling showed alterations in transport-related markers and cytoskeletal analysis pointed to disrupted vesicle trafficking. These findings demonstrate widespread organelle dysfunction beyond the endolysosomal pathway in NPC disease and suggest that NPC1 and NPC2 may play additional roles in organelle biogenesis. Future studies are needed to elucidate how these subcellular changes contribute to NPC neuropathology, and to validate the observed alterations in neuronal and glial cells, in which they may underlie neurodegeneration and neuroinflammation - the two main characteristics of NPC disease. This work was supported by the Croatian Science Foundation under the project number IP- 2022-10-7325 (to S.K.).

SL14

SIRTUIN 3 DRIVES SEX-SPECIFIC HEPATIC METABOLIC ADAPTATION TO WESTERN DIET-INDUCED STRESS

Ena Šimunić¹, Kate Šešelja¹, Iva I. Podgorski¹, Robert Belužić¹, Marija Pinterić¹, Marijana Popović Hadžija¹, Mirna Halasz¹, Morana Jaganjac¹, Tobias Madl³, Hansjörg Habisch², Xinyu Wang², Tihomir Balog¹, Sandra Sobočanec¹

¹Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia ²Medical University of Graz, Division of Medicinal Chemistry, Graz, Austria ³BioTechMed Graz BioTechMed Graz, Graz, Austria

Obesity is a major global public health challenge driven in large part by sustained consumption of a Western diet, which imposes chronic nutrient overload and disrupts systemic metabolic homeostasis, with profound consequences for liver function. Hepatocytes, as central metabolic hubs, and particularly their mitochondria, must accommodate this excess nutrient flux, leading to metabolic stress and adaptive remodeling. Sirtuin 3 (Sirt3), a mitochondrial NAD⁺-dependent deacetylase, regulates fatty acid oxidation, oxidative phosphorylation and antioxidant defenses, and is therefore critical for maintaining metabolic flexibility. This study investigated sex-specific metabolic responses to WD-induced stress, focusing on Sirt3, using both *in vivo* and *in vitro* approaches in liver tissue and primary murine hepatocytes. Eight-week-old wild-type (WT) and *Sirt3* knockout (KO) mice were fed WD for four weeks. Plasma biochemical parameters, mitochondrial respiration and selected hepatic protein expression were analysed. To model WD-induced stress *in vitro*, primary hepatocytes isolated from male and female WT and KO mice were exposed to a lipid mixture under high-glucose conditions for 6 hours. Lipid accumulation was assessed using Oil Red O staining. Reactive oxygen species (ROS) levels and relevant protein expression were analyzed. Metabolic alterations in hepatocytes and liver tissue were further characterized using LC-MS- and NMR-based metabolomics. Metabolomic profiling of primary hepatocytes revealed depletion of PUFA- containing lysophospholipids in KO cells, suggesting altered membrane composition and remodeling. WD treatment reduced levels of sterol lipids, consistent with changes in membrane properties under metabolic stress. Furthermore, WD treatment increased lipid accumulation in all groups, with the most pronounced effect in KO female hepatocytes. Notably, KO female hepatocytes exhibited elevated mitochondrial ROS compared to WT counterparts. *In vivo*, plasma biochemical parameters indicated altered hepatic metabolism in KO females while reduced expression of PGC-1 α in the liver further supported sex-specific metabolic adaptation to nutrient stress. Despite decreased expression of this key mitochondrial regulator, mitochondrial respiration remained unchanged, indicating preserved mitochondrial function. Collectively, these findings identify Sirt3 as sex-specific regulator of hepatic metabolic flexibility both *in vivo* and *in vitro*, under WD-induced stress, highlighting differential adaptive mechanisms between males and females.

SL15

MULTI-OMICS INTEGRATION FOR DECODING INFLAMMAGING AS A SHARED DRIVER OF AGE-RELATED MULTIMORBIDITY

Andrea Gelemanović¹, Paula Štancl², Maja Kuzman³, Nikola Kotur⁴, Biljana Stanković⁴

¹*Mediterranean Institute for Life Sciences, University of Split Bioinformatics Group, Split, Croatia* ²*University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Bioinformatics Group, Zagreb, Croatia* ³*Kuzman Consulting d.o.o., Zagreb, Croatia* ⁴*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade Group for Molecular Biomedicine, Department of Human Molecular Genetics and Genomics, Belgrade, Serbia*

Population aging and the increasing prevalence of noncommunicable diseases (NCDs) represent one of the greatest public health and socioeconomic challenges of modern society. Cardiovascular, metabolic, neurodegenerative, and malignant diseases account for the majority of global mortality and frequently co- occur in elderly populations, suggesting the existence of shared molecular mechanisms underlying disease initiation and progression. Among these mechanisms, inflammaging, i.e. chronic low-grade inflammation, has emerged as a central hallmark of biological aging, driven by complex interactions between epigenetic remodeling, immune dysregulation, microbiome alterations, and environmental exposures. Despite major advances in high-throughput technologies, the molecular networks linking inflammaging to multimorbidity remain insufficiently understood, while the lack of standardized multi-omics integration strategies limits reproducibility and clinical translation. Here we represent INFLAMomx, a novel pan-European COST Action, aiming to establish a multidisciplinary research network focused on investigating inflammaging as a shared driver of age-related diseases through advanced multi-omics integration approaches. By combining genomics, epigenomics, transcriptomics, proteomics, metabolomics, and microbiomics within systems biology and AI-driven analytical frameworks, the Action seeks to identify shared molecular signatures, biological pathways, and biomarkers associated with healthy aging and chronic disease susceptibility. A major objective of INFLAMomx is the harmonization and standardization of bioinformatic workflows for reproducible multi-omics research, including the development of best-practice guidelines, shared analytical pipelines, and centralized repositories of relevant datasets and protocols. In addition, the Action promotes interdisciplinary collaboration among molecular biologists, biochemists, bioinformaticians, clinicians, computational scientists, industry stakeholders, and policymakers, while supporting training, mobility, and knowledge exchange for early-career researchers across Europe. By shifting the current paradigm from a disease-centric toward a health-centric and prevention-oriented approach, INFLAMomx aims to accelerate translation of multi-omics discoveries into clinical applications and contribute to innovative strategies for preventing age-related multimorbidity and reducing the growing healthcare burden associated with aging populations.

SL16

FROM BIOMARKERS TO TARGETED THERAPY: PRECISION MOLECULAR DIAGNOSTICS IN ONCOLOGY

Marko Žarak^{1,2}

¹*Dubrava University Hospital Clinical Department of Laboratory Diagnostics, Department of Molecular Diagnostics and Genetics, Zagreb, Croatia* ²*University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia*

Precision molecular diagnostics supports personalized oncology by linking tumor biology to clinical decisions. Its clinical value depends on aligning the clinical question with the specimen, assay, timing and interpretation. This lecture examines four complementary molecular questions: which gene is altered, whether a repair pathway is deficient, what changes during disease evolution, and which findings within a broader genomic profile are clinically relevant. Homologous recombination repair (HRR) gene testing, including *BRCA1* and *BRCA2*, and homologous recombination deficiency (HRD) assessment illustrate the distinction between a genetic alteration, a functional state and an accumulated genomic consequence. In ovarian cancer, genomic instability testing extends molecular stratification beyond individual variants, while requiring interpretation within the validated assay and clinical setting. Genomic scars reflect a history of deficient repair and do not directly measure current repair function. Detection of *ESR1* mutations in circulating tumor DNA in estrogen receptor-positive, HER2-negative advanced breast cancer illustrates the importance of sampling time and longitudinal assessment of tumor evolution. Molecular detection, clinical validity and demonstrated clinical utility must be distinguished when evaluating emerging testing strategies. Tissue and plasma provide complementary information, but a negative plasma result may be uninformative when tumor-derived DNA is insufficient. Examples from lung and prostate cancer highlight limited tissue availability, spatial and temporal heterogeneity, and the selection of focused panels or comprehensive genomic profiling (CGP). Coordinated testing can help preserve tissue and reduce delays associated with sequential investigations. Test selection should consider relevant alteration classes, analytical performance, turnaround time and the clinical purpose of broader profiling. Clinical actionability, including tumor-agnostic applications, must be evaluated for the specific alteration, disease context and strength of evidence. Tumor-only findings may warrant germline assessment but cannot establish constitutional status. A negative tumor result does not exclude inherited cancer predisposition. Quality assurance, transparent reporting of limitations and multidisciplinary interpretation are essential to translate molecular findings into clinically useful answers. The laboratory report should communicate the significance of each relevant finding, unresolved questions and the need for additional assessment within the clinical decision window. Sponsorship statement: This lecture is part of an AstraZeneca-sponsored symposium and has been commissioned and funded by AstraZeneca.

SL17

CONTACT-DEPENDENT, NON-CELL-AUTONOMOUS CONTROL OF STRESS-INDUCED CELL FATE IN HETEROGENEOUS CELL POPULATIONS

Jelena Budimir², Nikola Pavlović², Andrea Gelemanović², Vanda Juranić Lisnić³, Svetlana Sperling⁴, Milena Ninković⁴, Miroslav Radman², Katarina Trajković¹

¹*Ruđer Bosković Institute, Division of Molecular Medicine, Zagreb, Croatia* ²*University of Split Mediterranean Institute for Life Sciences, Split, Croatia* ³*University of Rijeka, Center for Proteomic, Rijeka, Croatia* ⁴*University Medical Center Göttingen, Department of Neurosurgery, Göttingen, Germany*

Cellular responses to damage are typically studied in homogeneous cultures, yet tissues are inherently heterogeneous, with injured cells coexisting alongside intact neighbors. How this cellular context shapes damage outcomes remains insufficiently understood. Here, using mammalian fibroblast models, we investigated how intact neighboring cells influence the fate of severely stressed cells within mixed populations. We show that intact cells selectively promote the clearance and apoptotic progression of highly damaged cells, while having no effects at lower stress levels that predominantly induce senescence. This context-dependent effect is accompanied by pronounced changes in nuclear apoptotic phenotypes and a reduction in the retention of damaged cells within the population, consistent with accelerated elimination. Transcriptomic profiling reveals that damaged cells in the presence of intact neighbors undergo markedly attenuated transcriptional reprogramming, including reduced activation of oxidative stress responses, antioxidant pathways, and repair-associated processes, and adopt expression profiles more similar to intact cells. Mechanistically, this regulation requires direct cell–cell contact and is not mediated by diffusible factors, gap junctions, or tunneling nanotubes. Instead, integrative analysis of gene expression with ligand–receptor interaction networks demonstrates extensive remodeling of intercellular communication, affecting pathways related to cytokines, chemokines, growth factors, adhesion, and innate immune signaling. These findings support a model in which direct contact with intact neighbors suppresses stress-adaptive programs while promoting apoptotic resolution of severely damaged cells. Collectively, this work identifies a contact-dependent, non-cell-autonomous mechanism that links cellular fitness to fate decisions within heterogeneous populations, providing a conceptual framework for how multicellular systems coordinate the recognition and elimination of compromised cells. This principle may represent a general mode of tissue-level quality control with implications for aging, regeneration, and disease contexts.

SL18

CHARACTERIZATION OF MELANOMA CELLS RESISTANT TO MAPK INHIBITORS IN THE CONTEXT OF p53 FAMILY

Janja Josić¹, Anđela Horvat¹, Ignacija Vlašić¹, Christine Supina Pavić¹, Ana Tadijan^{1,2}, Tihana Marcuš¹, Ivan Milas³, Jean-Christophe Bourdon⁴, Neda Slade¹

¹*Ruđer Bošković Institute, Division of Molecular Medicine, Laboratory for Protein Interactions, Zagreb, Croatia* ²*Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia* ³*Sestre Milosrdnice University Hospital Center Clinics for Tumors, Zagreb, Croatia* ⁴*University of Dundee School of Medicine, Dundee, United Kingdom of Great Britain and Northern Ireland*

The identification of *BRAF* as a driver mutation in melanoma enabled the development of targeted therapies, revolutionizing patient treatment. Alongside immunotherapy, patients with BRAFV600E mutation are commonly treated with combined BRAF and MEK inhibitor (BRAFi/MEKi) therapy, whereas patients with *NRAS* Q61 mutations, an alternative activating alteration within the MAPK pathway, are primarily treated with immunotherapy or MEK inhibition. Despite significant clinical benefits, most patients eventually develop therapeutic resistance, and the underlying molecular mechanisms remain incompletely understood. In addition to MAPK pathway reactivation or activation of the PI3K/AKT signaling pathway, increasing evidence suggests that dysregulation of the p53 pathway contributes to acquired resistance in both *BRAF*- and *NRAS*-mutated melanoma. The aim of this study was to investigate the involvement of p53 family members in the development of resistance to BRAF/MEK-targeted therapy in metastatic melanoma. Using primary *BRAF*- or *NRAS*-mutated melanoma cell lines differing in their sensitivity to BRAFi/MEKi or MEKi, respectively, we analyzed p53 family expression. Gene expression was assessed by RT-qPCR, while protein levels, subcellular localization, and protein-protein interactions were examined by Western blot analysis, immunofluorescence and co-immunoprecipitation. Distinct expression patterns of p53 family isoforms were observed and correlated with acquired resistance to MAPK inhibitors at both the mRNA and protein levels across melanoma models. Co-immunoprecipitation analyses revealed interactions between p53 α (FLp53) and several short p53/p73 isoforms, suggesting their potential dominant-negative effect on FLp53 activity. Immunostaining demonstrated altered subcellular localization of p53 isoforms in BRAFi/MEKi-resistant cell lines compared with sensitive cell lines. Ongoing studies involving depletion of potentially inhibitory isoforms are expected to further clarify their contribution to the development of therapeutic resistance. These findings demonstrate that melanoma exhibits imbalanced p53 family isoform expression, which may contribute to the development of resistance to targeted therapy. Future studies will focus on the functional relevance of altered expression, subcellular localization, and interactions among p53 family isoforms in therapy resistance. A better understanding of the functional roles of these isoforms may uncover novel therapeutic targets and support the development of new strategies to overcome MAPK inhibitor resistance in melanoma.

SL19

REGULATION OF RETICULAR ADHESIONS BY KANK2 AND TALIN2 IN TWO MELANOMA CELL LINES

Anja Rac¹, Marija Lončarić¹, Nikolina Stojanović¹, Mahak Fatima², Mirna Rešetar¹, Dalibor Hršak³, Jonathan D. Humphries⁴, Martin J. Humphries², Andreja Ambriović-Ristov¹

¹*Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia* ²*Faculty of Biology, Medicine & Health, University of Manchester Manchester Cell-Matrix Centre, Manchester, United Kingdom of Great Britain and Northern Ireland* ³*Ruđer Bošković Institute, Division of Electronics, Zagreb, Croatia* ⁴*Manchester Metropolitan University Department of Life Science, Manchester, United Kingdom of Great Britain and Northern Ireland*

Integrins bind extracellular matrix proteins and, upon clustering, form multimolecular integrin adhesion complexes (IACs) that connect to and regulate the cell cytoskeleton, influencing various aspects of cell behaviour. Alongside well-characterized focal adhesions (FAs) and fibrillar adhesions (FBs), a new class of IACs, reticular adhesions (RAs), have been identified. RAs, formed by integrin $\alpha\text{V}\beta 5$, lack actin association and classical FAs markers and their physiological role in cells remains poorly understood. Previously, we showed that two melanoma cell lines, MDA-MB-435S and RPMI-7951, preferentially use integrin $\alpha\text{V}\beta 5$ (which can form FAs or RAs) for adhesion under long-term culture conditions. Here we investigate the composition of RAs in these two melanoma cell lines. We treated the cells with the actin polymerisation inhibitor cytochalasin D to disrupt FAs and enable isolation and mass spectrometry-based analysis of RAs components. Western blotting, immunofluorescence microscopy and proximity ligation assays (PLA) were performed to assess protein expression and localization within RAs. The effects of talin2 and KN motif and ankyrin repeat domains protein 2 (KANK2) knockdown on RA composition were examined in both melanoma cell lines. Our results identified known RA-associated proteins, including the Adaptor protein 2 (AP2) complex, disabled homolog 2 (DAB2), Numb and talin2 in both lines, along with a new protein, KANK2. PLA following actin disruption confirmed the proximity of KANK2 and talin2 in RAs. Knockdown experiments demonstrated that although both talin2 and KANK2 are located in RAs, neither is essential for RA formation. Talin2 knockdown resulted in reduced abundance of RA components in both cell lines. In MDA-MB-435S cells, KANK2 knockdown produced a similar effect. However, in RPMI-7951 cells, KANK2 knockdown had no significant effect on RA components abundance, reflecting the differential localization and role of KANK2 in this cell line. In conclusion, we provide a comprehensive analysis of RAs in two melanoma cell lines and identify KANK2 as a novel RA-associated protein. The differential effects of KANK2 knockdown on the abundance of RAs underscores its distinct role in $\alpha\text{V}\beta 5$ -mediated adhesions, FAs and RAs, across the two cell lines. These findings emphasize the importance of adhesion crosstalk in regulating integrin $\alpha\text{V}\beta 5$ -mediated cell adhesions.

MITOCHONDRIAL NME6: A NEW LOOK AT AN OLD PROTEIN FAMILY

Maja Herak Bosnar¹, Bastien Proust², Isabel Garcia-Saez³, Serena Luis⁴, Carlo Petosa³, Uwe Schlattner⁵

¹*Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia* ²*Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia* ³*Institut de Biologie Structurale (IBS) Epigenetics and Molecular Pathways (EPIGEN) Group, Grenoble, France* ⁴*Université Grenoble Alpes UFR de Pharmacie, Grenoble, France* ⁵*Université Grenoble Alpes INSERM U1055, Grenoble, France*

Eukaryotic NME proteins (also known as nucleoside diphosphate kinases, NDPKs, or Nm23) maintain cellular nucleotide balance and, having arisen early in evolution, have diversified into multiple biochemical and biological roles across cellular compartments. Interest in this family increased after NME1 was identified as a founder of a novel group of genes/proteins - metastasis suppressors. The human NME family consists of ten proteins divided into two groups: Group I members share high sequence homology and display NDPK activity as homo or heterohexamers, whereas Group II proteins show low sequence homology and often have no or questionable kinase activity. This study aims to define the structure, biophysical properties, function, and interaction network of the mitochondrial Group II member NME6. Using gel filtration, glutaraldehyde crosslinking, western blotting, and NDPK assay we show that NME6 predominantly exists as a monomer and lacks detectable NDPK activity *in vitro*. Mining and comparing large proteomic datasets of the mitochondrial matrix identified RCC1L (RCC1-like G exchanging factor-like protein) as a high confidence NME6 interactor. This direct interaction was confirmed by PLA, immunoprecipitation, and, more recently, by analytical size exclusion chromatography of the NME6–RCC1L complex in the presence of GDP. NanoDSF (nano-Differential scanning fluorimetry) further demonstrated that nucleotide binding increases the thermal stability of the complex, but not of NME6 alone, indicating more favorable nucleotide engagement within the complex and suggesting a preference for pyrimidine nucleotides. Complex–nucleotide models were generated using protein–ligand prediction tools, such as AlphaFold3. X ray diffraction data collected at the ESRF (Grenoble) enabled determination of a low resolution three dimensional structure of the NME6–RCC1L complex, providing a structural framework for understanding NME6 function in mitochondria though further improvement is needed. In conclusion, novel investigations show that NME6 interacts with a non-NME protein, specifically RCC1L, rather than forming a canonical NME hexamer, and that it may achieve its NDPK activity through this interaction, challenging the old paradigm of obligatorily hexameric NDP kinases. This could also apply to other Group II NME proteins, which remains to be determined.

POSTER ABSTRACT

P1

NGS-IDENTIFIED COMPLEX *SERPING1* ALLELE WITH CIS CONFIGURATION CONFIRMED BY SANGER SEQUENCING IN PEDIATRIC HEREDITARY ANGIOEDEMA

Irena Abramović, Petra Anić, Ivana Rako

*University Hospital Centre Zagreb, Clinical Department of Laboratory Diagnostics,
Zagreb, Croatia*

Hereditary angioedema type I/II (HAE) is an autosomal dominant disorder caused by pathogenic variants in the *SERPING1* gene encoding C1 inhibitor, a serine protease inhibitor regulating the complement and contact activation pathways. Loss-of-function variants lead to uncontrolled bradykinin release and recurrent episodes of subcutaneous and submucosal edema. We report a pediatric patient with clinical symptoms consistent with HAE, in whom molecular genetic testing using a next-generation sequencing (NGS) panel for monogenic diseases (Illumina Custom Enrichment Panel, MiSeq platform) identified three heterozygous variants in *SERPING1* (NM_000062.3) clustering within a 6-nucleotide window in exon 4: a nonsense variant c.437T>A (p.Leu146Ter) and two frameshift variants c.436_437insC (p.Leu146SerfsTer111) and c.440_441del (p.Val147GlyfsTer109). All three were classified as likely pathogenic according to ACMG criteria and were absent from population databases (gnomAD). The high read depth and base-level resolution of NGS were essential for distinguishing the individual nature of each variant – a substitution and two indels in close proximity – a discrimination that would not be achievable by Sanger sequencing alone, where overlapping signals in such a densely altered region preclude reliable identification of co-occurring insertions and deletions. Bioinformatic analysis using the Emedgene platform (Illumina) demonstrated that the three variants act in concert as a single complex allele, producing a net frameshift described at the protein level as p.(Leu146SerfsTer5), predicting either nonsense-mediated mRNA decay or a severely truncated, non-functional C1 inhibitor. Sanger sequencing was subsequently performed on the proband's father, who carries the same three variants, serving a specific and complementary role: confirmation of cis configuration on a single chromosome, which formally excludes compound heterozygosity and establishes autosomal dominant inheritance with 50% recurrence risk. This case illustrates the diagnostic superiority of NGS for characterizing complex, tightly clustered alleles in rare disease settings, while demonstrating that Sanger sequencing retains an indispensable role as a targeted tool for parental segregation analysis and phase determination.

P2

SIGNATURES IN CIS-REGULATORY REGIONS ASSOCIATED WITH MULTICELLULAR DEVELOPMENT IN SOCIAL AMOEBA *D. discoideum*

Katarina Kajan¹, Bojan Žunar², Jonathan R. Chubb³, Boris Lenhard⁴, Damir Baranašić¹, Vlatka Antolović¹

¹Ruđer Bošković Institute, Division of Electronics, Zagreb, Croatia ²University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Biochemistry, Zagreb, Croatia ³University College London Laboratory for Molecular Cell Biology, London, United Kingdom of Great Britain and Northern Ireland ⁴Imperial College London MRC Laboratory of Medical Sciences, London, United Kingdom of Great Britain and Northern Ireland

We are focused on investigating the cis-regulatory signatures of the transition from unicellularity to multicellularity using the social amoeba *Dictyostelium discoideum*. *Dictyostelium* alternates between a solitary unicellular state and a multicellular developmental program triggered by nutrient deprivation, during which approximately 100,000 cells aggregate to form a multicellular organism that differentiates into future spore and stalk cells. Its dual life cycle, combined with a fully sequenced genome and strong experimental tractability, makes it an ideal model for dissecting fundamental principles of tissue self-organization, cell–cell communication, and developmental patterning. However, despite the whole genome being sequenced in 2005, regulatory regions in *Dictyostelium* remain poorly annotated, limiting our understanding of how gene regulatory networks are reorganized during the transition to multicellularity. To address this, we combined existing genomic and transcriptomic resources with a Cap Analysis of Gene Expression (CAGE) experiment to generate a genome-wide annotation of untranslated regions (UTRs) and promoter landscapes. Analysis of the CAGE dataset provided base-pair resolution of transcription start sites (TSSs) and revealed promoter features consistent with previous assessment, including TATA-box enrichment approximately 30 bp upstream of TSS and poly(T) tracts immediately preceding the TSS, highlighting the biophysical properties of the chromatin necessary for transcription initiation. Combining CAGE results with transcriptomic read-coordinate analyses across developmental stages, we identified distinct differences in motif occurrence and sequence architecture between cis-regulatory regions associated with unicellular and multicellular gene expression programs, suggesting stage-specific regulatory logic underlying developmental transitions.

ANTITUMOR ACTIVITY AGAINST MELANOMA OF ROYAL JELLY AND AF4-ENRICHED VESICULAR FRACTION

Vanja Baričević¹, Ena Kramarić², Anna Placci^{3,4,5}, Ignacija Vlašić⁶, Mario Kurtjak⁷, Stefano Giordani^{3,4,5}, Valentina Marassi^{3,4,5}, Mladenka Malenica¹

¹University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia ²University of Rijeka, Faculty of Medicine, Department of Basic and Clinical Pharmacology and Toxicology, Rijeka, Croatia ³University of Bologna Department of Chemistry 'G. Ciamician', Bologna, Italy ⁴University of Bologna academic spinoff byFlow s.r.l, Bologna, Italy ⁵Biostructures and Biosystems National Institute (INBB) Italian interuniversity consortium, Rome, Italy ⁶Ruđer Bošković Institute, Department of Molecular Medicine, Laboratory for Protein Dynamics, Zagreb, Croatia ⁷Jožef Stefan Institute Advanced Materials Department, Ljubljana, Slovenia

Royal jelly (RJ), a complex natural bee product, represents a promising source of membrane-enclosed vesicle-like nanoparticles (RJVs) with potential biological activity. Although recent studies have associated RJVs with antibacterial, anti-inflammatory, and regenerative effects, their systematic physicochemical characterization and biological evaluation remain limited. The aim of this study was to isolate and characterize RJV nanoparticles using multidetection asymmetric flow field-flow fractionation (AF4) and to compare the antiproliferative activity of native RJ and an AF4-enriched vesicular fraction in melanoma cell lines. AF4 coupled with UV detection and multi-angle light scattering enabled gentle separation of RJ while preserving the native properties of RJVs, allowing their physicochemical characterization. Based on the AF4 fractionation profile, radius of gyration, and UV absorbance, the RJV displayed characteristics consistent with extracellular vesicles and showed minimal protein contamination. Additionally, transmission electron microscopy confirmed the presence of membrane-enclosed nanoparticles. The antitumor activity of native RJ and RJVs was evaluated using the XTT cell viability assay in primary (WM983A) and metastatic (WM983B) human melanoma cell lines. RJ reduced metabolic activity predominantly in metastatic WM983B cells at low concentrations (0.1–5 mg mL⁻¹), whereas the AF4-enriched RJVs did not exhibit significant antiproliferative activity within the tested concentration range (5–25% v/v). These findings demonstrate that AF4 provides an effective and gentle approach for isolating structurally preserved RJVs suitable for downstream physicochemical characterization. Although the isolated RJVs did not reproduce the antiproliferative activity of native RJ under the tested experimental conditions, further characterization of all AF4 fractions is needed to identify the components responsible for the observed biological activity of royal jelly.

P4

FLUORESCENCE LIFETIME IMAGING UNCOVERS COMPARTMENT-SPECIFIC MEMBRANE TENSION ALTERATIONS UPON CHOLESTEROL ACCUMULATION IN NPC1-DEFICIENT MICROGLIA AND ASTROCYTES

Ana-Marija Bogner¹, Lea Vidatić¹, Vincent Mercier², Dimitri Moreau², Sabina Tahirović³, Silva Katušić¹

¹Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia ²University of Geneva ACCESS Geneva, Geneva, Switzerland ³German Center for Neurodegenerative Diseases (DZNE) e.V. Munich Juvenile Neurodegeneration Group, Munich, Germany

Niemann-Pick Type C (NPC) is a rare lysosomal storage disorder caused by mutations in *NPC1* (95% of cases) or *NPC2* genes, impairing cholesterol egress from late endosomes/lysosomes (LE/LYS) and driving neurodegeneration and neuroinflammation. Cholesterol accumulation within the lysosomal membrane causes extreme lipid packing and stiffening, disrupting endolysosomal trafficking. Recent studies have established a connection between membrane tension and intraluminal vesicle (ILV) formation via ESCRT-III as well as MVB accumulation in *NPC1*-deficient microglia due to failed ILV biogenesis. Here, we employed mechanosensitive Flipper-TR and Lyso Flipper-TR probes, which intercalate into the plasma membrane (PM) and LE/LYS membranes, respectively, to characterize compartment-specific lipid packing via fluorescence lifetime imaging microscopy (FLIM). Tightly packed, high-cholesterol membranes planarize the probe's dithienothiophene fluorophore, increasing fluorescence lifetime (τ ↑), while loosely packed membranes yield shorter lifetimes (τ ↓). To validate the approach, we first examined *NPC1/NPC2*-knockout HeLa cells, which displayed elevated LE/LYS lifetime and decreased PM lifetime relative to wild-type (wt) cells showing a compartment-specific tension signatures. We next isolated primary microglia and astrocytes from P5-7 BALB/cNtr-Npc1m1N/J mice using MACS technology. FLIM imaging confirmed a compartment-specific tension signatures in *NPC1*^{-/-} glia: shorter fluorescence lifetime of the PM flipper probe paired with elevated lifetime of Lyso Flipper relative to wt. Cholesterol depletion by methyl- β -cyclodextrin (M β CD) revealed that M β CD increased Lyso Flipper lifetimes in both wt and *NPC1*^{-/-} glia, while PM Flipper probe showed different effect of M β CD-treatment: increased PM Flipper lifetime in *NPC1*^{-/-} astrocytes and decreased lifetime in wt astrocytes. Although M β CD is known to rescue cholesterol accumulation in LE/Lys upon *NPC1*-loss, our findings reveal that it does not restore the NPC membrane tension signatures at both LE/Lys and the PM to that as in wt, suggesting that different lipid profiles may be the underlying driver of this effect. These findings establish FLIM-based lipid packing analysis as a tool to uncover compartment-specific membrane tension dysregulation in *NPC1*-deficient glia, linking cholesterol dyshomeostasis to membrane biophysical changes and to altered endolysosomal trafficking, a key feature of NPC and related neurodegenerative disorders. This work was supported by the Croatian Science Foundation under the project numbers HRZZ- IP-2022-10-7325 (to S.K.), DOK-NPOO- 2023-10-6286 and MOBDOK-2023-10-8823.

P5

C3 GLYCOPROTEIN AS A BIOMARKER OF EARLY METABOLIC AND INFLAMMATORY CHANGES IN WOMEN

Valentina Borko¹, Maksym Shmatkov², Dinko Šoić¹, Antonio Starčević², Olga Gornik¹

¹*University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia* ²*University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Biochemical Engineering, Zagreb, Croatia*

Changes in glycan structures associated with pathological states have led to the growing recognition of glycans as potential disease biomarkers. Based on previous evidence linking oligomannose glycans with metabolic diseases such as diabetes, we investigated the potential of complement component C3 glycosylation as a biomarker of early-stage metabolic disorder in a cohort of 300 women of reproductive age. Relative abundances of C3 glycopeptides were quantified from plasma samples using a high-throughput bottom-up glycoproteomic LC-MS approach. Glycopeptide profiles were used as input features in a classification model for metabolic risk assessment. Participants with CRP ≥ 1 mg/L and HOMA-IR > 1.5 were classified as a “higher risk” class to enable detection of early glycan alterations associated with metabolic and inflammatory dysregulation. The model was trained to predict metabolic-inflammatory changes in participants based on the C3 glycan signature. The dataset (300 participants) was split into 75% training and 25% independent test set, with repeated random splits and cross-validation to ensure robust model performance. A subset of C3 glycopeptides was identified as most informative, with Asn63- N2H5 and Asn63-N2H6 emerging as key contributors to group separation. Model predictions were compared to clinical thresholds to define true positive (TP), true negative (TN), and false positive (FP) groups. Positive individuals met threshold values for HOMA-IR and CRP, while negatives had lower values. FP comprised individuals who did not meet these thresholds but exhibited an altered glycan profile. Comparative analysis revealed that FP individuals occupy an intermediate position between TN and TP groups, not only at the glycomic level but also across biochemical and anthropometric parameters. Specifically, FP individuals showed increased body mass index, waist and hip circumference, and fasting insulin levels compared to TN, yet lower values compared to TP, indicating a gradual progression of metabolic dysfunction. Although the model shows good discriminative performance (ROC AUC = 0.79), a subset of individuals is consistently classified as false positives. Rather than representing model error, these individuals exhibit intermediate biochemical and anthropometric profiles, suggesting a latent stage of metabolic dysfunction.

THE UHRF1–SPRTN AXIS SAFEGUARDS GENOME AND EPIGENOME INTEGRITY

Maria Bošković^{1,2}, Mateo Glumac², Vincent Cahais³, Felicia Chung Fei Lei^{4,3}, Koraljka Husnjak², Thorsten Mosler², Borna Markušić², Cyrille Cuenin^{3,4}, Rita Khoueiry³, Zdenko Herceg³, Ivan Đikić², Janoš Terzić¹

¹*University of Split, School of Medicine Laboratory for Cancer Research, Split, Croatia*

²*Goethe University Frankfurt Institute of Biochemistry II, Faculty of Medicine, Frankfurt, Germany*

³*International Agency for Research on Cancer Epigenomics and Mechanisms Branch, Lyon, France*

⁴*Sunway University, Jalan University, Bandar Sunway Department of Medical Sciences, School of Medical and Life Sciences, Subang Jaya, Malaysia*

Genomic instability in Ruijs–Aalfs progeroid syndrome stems from defective repair of DNA-protein crosslinks (DPCs), lesions that obstruct replication and transcription. The metalloprotease SPRTN is a central mediator of DPC repair, but the regulatory network controlling its activity remains incompletely understood. Using cellular and mouse models, we identify UHRF1 as an E3 ubiquitin ligase that governs SPRTN chromatin localization and is essential for efficient DPC repair. In *Sprtn*-deficient mice, persistent DPC accumulation is associated with reduced UHRF1 protein levels. Because UHRF1 coordinates DNA methylation maintenance, its depletion in the liver causes global DNA hypomethylation with focal hypermethylation, resulting in widespread changes in gene expression. Our work establishes UHRF1 as a critical regulator of SPRTN-dependent DPC repair and demonstrates how disruption of this axis destabilizes both genome and epigenome integrity, thereby linking defective DPC repair to chromatin disorganization and accelerated ageing.

ANALYTICAL AND CLINICAL VERIFICATION OF DIGITAL PCR FOR MYD88 MUTATIONS IN cfDNA ACROSS BREAST CANCER SUBTYPES AND STAGES

Andro Brzović¹, Marko Žarak², Sandra Šupraha Goreta¹

¹*University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia* ²*Dubrava University Hospital, University of Zagreb, Faculty of Pharmacy and Biochemistry, University Department of Laboratory Diagnostics, Zagreb, Croatia*

Breast cancer is a heterogeneous disease characterized by diverse molecular alterations. While *MYD88* mutations are well established in other malignancies, particularly in B-cell lymphomas, where they affect signaling pathways regulating cell proliferation, survival, and immune response, their role in breast cancer remains unclear. This study aimed to analytically and clinically verify the application of digital polymerase chain reaction (dPCR) for detecting the *MYD88* L265P mutation in genomic DNA (gDNA) and circulating (tumor) cell-free DNA (cfDNA) from patients with breast cancer across different stages and molecular subtypes. A total of 31 female patients (median age: 49 years; Luminal B: 65%, Luminal A: 16%, triple-negative: 13%, HER2-positive: 6%; metastatic: 61%) were included. Peripheral blood and plasma samples were collected for gDNA and cfDNA purification. DNA concentration and purity were assessed using spectrophotometric (NanoDrop One, Thermo Scientific, USA) and fluorimetric (Qubit, Thermo Scientific, USA) methods, while cfDNA fragment size was measured by electrophoresis (TapeStation 4120, Agilent Technologies, USA). Digital PCR assay (NioE, Stilla Technologies, France) targeting the *MYD88* L265P was verified by assessing repeatability, intermediate precision, within-laboratory precision, limit of detection (LoD), master-mix solution stability, and comparison with Sanger sequencing. The LoD was 0.156 ng/μL, confirming high analytical sensitivity. Precision analysis showed variability, with coefficients of variation reaching up to 26%. Agreement with Sanger sequencing was moderate (Cohen's kappa = 0.503), reflecting the higher sensitivity of dPCR for low-frequency variants detection. Stability testing demonstrated that master-mix solutions remained stable at -20 °C for at least five days. No *MYD88* L265P mutations were detected in either gDNA or cfDNA samples, suggesting that this mutation is not characteristic of breast cancer in the studied cohort. Overall, dPCR proved to be a sensitive and robust method for mutation detection in liquid biopsy samples. However, further standardization and optimization are required prior to final clinical implementation. These findings also suggest that *MYD88* mutations do not represent clinically relevant biomarkers in breast cancer.

BIOAVAILABILITY OF CREATINE SUPPLEMENTS: A META-RESEARCH ON METHODOLOGIES USED IN RANDOMIZED CONTROLLED TRIALS

Viljemka Bučević Popović¹, Antonela Sovulj², Sanda Raić³, Ivana Pezelj⁴, Malgorzata M Bala⁵, Livia Puljak⁶

¹*University of Split, Faculty of Science, Department of Chemistry, Split, Croatia* ²*University of Split, Faculty of Science, Department of Biology, Split, Croatia* ³*University of Split Mediterranean Institute for Life Sciences, Split, Croatia* ⁴*University of Split, Faculty of Science, Doctoral Study of Biophysics, Split, Croatia* ⁵*Jagiellonian University Medical College Chair of Epidemiology and Preventive Medicine, Department of Hygiene and Dietetics, Krakow, Poland* ⁶*School of Medicine, Catholic University of Croatia Center for Evidence-Based Medicine, Department of Nursing, Zagreb, Croatia*

Creatine is a naturally occurring amino acid derivative with an important role in energy metabolism. In living cells, it is mostly present as phosphocreatine and serves to rapidly regenerate ATP from ADP and inorganic phosphate ("P_i"). Therefore, the main creatine stores are found in tissues with high energy needs, such as skeletal muscle, cardiac muscle, and the brain. Daily creatine requirements (2-4 g) are met through endogenous synthesis from amino acid precursors or through dietary meat and fish. More recently, creatine supplements have emerged as alternative sources of creatine to enhance athletic performance. The benefits of supplementation have also been explored in various human diseases and age-related health conditions. The most commonly used and investigated creatine supplement is, by far, creatine monohydrate ("CrM"). However, research indicates that while "CrM" is considered the "gold standard" for creatine supplementation, it may have limitations regarding its bioavailability. This has led to the development of novel creatine formulations with purportedly improved absorption and delivery to target tissues (e.g., polyethylene glycosylated forms, magnesium creatine chelate, lipid multi-particulate formulated "CrM"). To evaluate how thoroughly randomized controlled trials (RCTs) address the issue of creatine bioavailability, we conducted a meta-research study searching MEDLINE and Embase from inception to March 2024. The analysis included 357 publications from 343 individual creatine RCTs. We extracted data on trial characteristics, types of creatine formulations, mentions of bioavailability, and comparisons between different creatine products. Creatine monohydrate was used in 80.2% of the included trials, though 15.5% failed to specify the exact form used. Bioavailability was explicitly mentioned in only 11.1% of reports, usually confined to the discussion section. Only five trials compared multiple creatine formulations, with just three discussing bioavailability. Furthermore, these comparisons were often compromised by non-equimolar dosing regimens. In conclusion, available RCTs on oral creatine supplementation frequently lack detailed formulation descriptions and rarely investigate bioavailability. Our findings corroborate previous conclusions that marketing claims regarding the purportedly enhanced bioavailability of new creatine formulations are not adequately supported by evidence from clinical trials. Future trials should improve reporting, incorporate bioavailability-related outcomes, and utilize appropriate dosing regimens when comparing alternative creatine formulations.

ERK SIGNALING IN CELLULAR RESPONSES TO MITOCHONDRIAL DYSFUNCTION

Ana-Marija Budeš, Antonijo Grčić, Roberta Ogrizović, Robert Domitrović, Iva Suman

Faculty of Medicine in Rijeka Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia

Mitochondrial dysfunction plays a central role in the progression of various diseases by disrupting cellular energy balance. While mitochondria are key regulators of cellular stress responses, the effects of mitochondrial impairment on signaling pathway activation remain incompletely understood. Extracellular signal-regulated kinases (ERK) activity is implicated in regulating autophagy and mitophagy, oxidative stress, and apoptosis. However, their precise role in mitochondrial dysfunction is not fully characterized. This study aims to examine ERK pathway activation during mitochondrial impairment and clarify its contribution to cell survival and the regulation of stress-induced cellular damage. We investigated the effects of mitochondrial uncouplers—FCCP, CCCP, chloroquine (CQ), and oligomycin (OMY)—on ERK signaling in HEK293 (human embryonic kidney) and HK2 (human proximal tubule) cells. These cell lines differ in metabolic profile: glycolysis-predominant versus high oxidative phosphorylation (OXPHOS). Mitochondrial membrane potential, oxidative stress, apoptosis, and autophagy/mitophagy markers were analyzed using Western blot, immunofluorescence, and metabolic assays. ERK involvement was further examined using specific inhibitors (U0126 and Mirdametinib). Mitochondrial stress induced pronounced, cell-type-specific alterations in ERK signaling and mitochondrial integrity. FCCP and CQ elicited the strongest effects on membrane potential, oxidative stress, apoptotic markers, and autophagy/mitophagy proteins. ERK inhibition partially restored mitochondrial function and modulated glycolytic compensation. This also reduced apoptotic signaling activation, highlighting ERK's central role in maintaining mitochondrial function under stress. ERK pathways critically orchestrate mitochondrial function and cellular stress responses in kidney cells. These findings provide a foundation for ERK-targeted therapeutic strategies in diseases driven by mitochondrial dysfunction.

P10

CHARACTERIZATION OF MEGAKARYOCYTES AND PLATELETS FROM OCRL KO MICE

Ana Bura¹, Leopoldo Staiano², Maria Antonietta De Matteis², Antonija Jurak Begonja¹

¹University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia

²University of Naples Federico II Theleton, Institute for Genetics and Medicine, Naples, Italy

The oculocerebrorenal syndrome of Lowe (LS) is a rare X-linked disorder characterized by a triad of symptoms affecting the brain, the eyes, and the kidneys. LS carriers suffer from central hypothyroidism, mental retardation, congenital cataracts, glaucoma, low molecular weight proteinuria, and aminoaciduria. It is caused by mutations in the *OCRL* gene, which encodes the OCRL protein, a phosphatase that dephosphorylates phosphatidylinositol-4,5-bisphosphate [PI(4,5)P₂], producing phosphatidylinositol-4-monophosphate (PI4P). Several studies and case reports have shown that some LS carriers suffer from haemorrhagic events during surgery, occasionally present with low platelet counts, and more often with abnormal platelet function. Platelets are the smallest blood cells that circulate in the blood in their resting state. Upon detection of the vessel wall injury, they activate, aggregate, and form a clot to stop the bleeding. In our previous study, we analysed the effect of the pharmacological inhibition of OCRL on human platelet spreading (Bura et al., BJH, 2022). We show that OCRL-inhibited platelets do not fully activate due to impairment in the reorganization of actin stress fibers and microtubules, along with the decreased phosphorylation of the myosin light chain. Here, we characterized platelets and megakaryocytes from OCRL KO mice. Immunohistochemical analysis of the bone marrow and spleen showed that OCRL KO mice have similar megakaryocyte numbers to the controls. In addition, immunocytochemical analysis showed that the bone marrow from OCRL KO mice, upon thrombopoietin stimulation, produces similar numbers of megakaryocytes. Furthermore, Western blot analysis showed that OCRL KO megakaryocytes had normal levels of megakaryocyte-specific markers, such as von Willebrand factor, Gplb α , and Gplb β . Taken together, these data suggest that megakaryopoiesis is not affected in OCRL KO mice. The morphology of resting platelets, as shown by immunocytochemistry and electron microscopy, was also unaltered in OCRL KO platelets. Interestingly, spreading of OCRL KO platelets showed that they do not fully spread, as shown by the increased number of platelets displaying filopodia and decreased number of platelets displaying lamellipodia. These results confirm the observed phenotype upon the pharmacological inhibition of OCRL in human platelets and show that the OCRL KO mouse model has impaired platelet spreading similar to LS carriers.

P11

COMPUTATIONAL AND EXPERIMENTAL INVESTIGATION OF INTERACTIONS BETWEEN MANNOSYLATED DESMURAMYL PEPTIDES AND MANNOSE RECEPTORS

Željka Car¹, Katarina Leko¹, Marija Paurević², Vesna Petrović Peroković¹, Josip Požar¹, Rosana Ribić³

¹University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

²Josip Juraj Strossmayer University of Osijek, Department of Chemistry, Osijek, Croatia

³University North University Center Varaždin, Varaždin, Croatia

Muramyl dipeptide (MDP, N-acetylmuramyl-L-alanyl-D-isoglutamine) is the smallest peptidoglycan fragment retaining immunostimulatory activity. Removal of the muramyl moiety and attachment of carbohydrate and/or lipophilic substituents to the desmuramyl peptide core (DMP, L-alanyl-D-isoglutamine) yields analogues with markedly improved pharmacological profiles, and our previous work has shown that mannosylated adamantane-containing DMPs exhibit pronounced immunostimulatory activity *in vivo*.^{1,2} Although mannosylation clearly potentiates the immune response, the molecular basis of this effect, and in particular the contribution of binding to mannose-recognising receptors, has not been clarified. The aim of the present work was to characterise the interaction of representative mannosylated desmuramyl peptides (DMPs) with mannose-binding proteins using complementary computational and experimental approaches. Two protein targets representing mannose-recognising C-type lectin receptors were employed: concanavalin A (ConA), a plant lectin that selectively recognises α -D-mannose through a $\text{Ca}^{2+}/\text{Mn}^{2+}$ -dependent carbohydrate-binding site,³ and the macrophage mannose receptor (MR, CD206), whose carbohydrate recognition domain 4 (CRD4) mediates Ca^{2+} -dependent binding to terminal mannose, fucose, and N-acetylglucosamine residues on glycoconjugates.⁴ Molecular docking studies were performed to determine the preferred binding modes of mannosylated DMPs within the carbohydrate-recognition sites of ConA and MR-CRD4, in order to identify key protein–ligand interactions, and assess complex stability. In parallel, isothermal titration calorimetry was used to determine the binding stoichiometry, affinity, and thermodynamic parameters of selected mannosylated DMP–ConA interactions. The immunological activity of the compounds was evaluated using RAW-Blue™ cells derived from murine RAW 264.7 macrophages, where activation of PRR-mediated NF- κ B/AP-1 signalling induces secretion of the SEAP reporter enzyme quantified with QUANTI-Blue medium. Combined analysis of the obtained data provides, for the first time, a coherent molecular and thermodynamic insight into the recognition of mannosylated desmuramyl peptides by mannose receptors and establishes a rational basis for the design of more potent and selective immunomodulators targeting C-type lectin receptors. Acknowledgements This work has been fully supported by Croatian Science Foundation under the project IP-2025-02-5568.

P12

FROM CHROMOSOMES TO CLINICAL FINDINGS: BIOLOGY IN CLINICAL CYTOGENETICS

Antonija Crnečki, Antonija Piškor, Lucija Belužić, Tea Mejovšek Herceg, Sara Drk Kiralj, Julijana Marinac, Janja Postružin, Manuela Žugaj, Matea Katić

Poliklinika Breyer, Department of Molecular Diagnostics and Genetics, Zagreb, Croatia

Karyotyping remains a valuable diagnostic method in clinical cytogenetics and a valuable diagnostic tool alongside biochemical and molecular diagnostic methods. This is especially evident in reproductive medicine, where the detection of numerical and structural chromosomal abnormalities can provide valuable diagnostic information. The aim of this study was to present the experience of a cytogenetic laboratory in routine healthcare diagnostics and to highlight the role of biological sciences and laboratory expertise from sample processing and chromosome analysis to interpretation and reporting. We retrospectively analysed 252 patients who underwent peripheral blood karyotyping between April 2023 and December 2024. Patient age and sex, indication for testing, karyotype results and turnaround time were evaluated. Cytogenetic analysis included peripheral blood lymphocyte culture, preparation of metaphase chromosomes, microscopic analysis and karyogram preparation. The cohort included 137 (54.4%) male and 115 (45.6%) female patients, with a mean age of 35.5 years. Recurrent pregnancy loss was the most common indication for testing (105/252; 41.7%), followed by infertility (53/252; 21.0%), assisted reproductive procedures (44/252; 17.5%) and abnormal semen analysis (17/252; 6.7%). A normal 46,XX or 46,XY karyotype was reported in 228 (90.5%) patients, while 24 (9.5%) showed findings requiring additional cytogenetic interpretation. These included numerical and structural chromosomal changes and mosaic karyotypes, such as sex chromosome aneuploidies, trisomy 21, translocations and inversions. Nine of the 24 findings occurred in patients referred for recurrent pregnancy loss, while eight were identified in patients undergoing evaluation for infertility or assisted reproduction. Mean turnaround time was 11.5 calendar days, with a median of 11 days. Our experience demonstrates the clinical value of conventional cytogenetics, especially in reproductive medicine, where chromosome analysis may reveal clinically relevant findings that contribute to patient management and genetic counselling. Karyotyping represents a clear example of biology applied in healthcare, combining cell biology, laboratory expertise, morphological assessment and understanding of chromosome pathology. The reliability and clinical value of cytogenetic findings depend on the coordinated work of the entire laboratory team and integration of cytogenetics with biochemistry and molecular biology, highlighting the importance of a multidisciplinary approach to patient care.

P13

EARLY METAZOAN ORIGINS OF BRMS1: STRUCTURE, FUNCTION AND EVOLUTION

Antea Talajić, Kristina Dominko, Bastien Proust, Nikolina Škrobot Vidaček, Matija Harcet, Helena Četković

Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Early-branching metazoans such as sponges (Porifera) provide valuable insight into the emergence of complex gene regulatory systems and cancer-related mechanisms in early animal evolution. Breast cancer metastasis suppressor 1 (BRMS1) is a core component of the Sin3–HDAC chromatin-remodeling complex and a key regulator of migration, invasion, and metastatic colonization in human cancers. Despite its biomedical relevance, the evolutionary history of the BRMS1 protein family and the conservation of its cellular functions across Metazoa remain poorly understood. We performed a comprehensive phylogenetic and structural analysis of BRMS1, BRMS1-like, and SDS3 proteins across fungi, protists, and metazoans. To investigate functional conservation, we cloned and expressed BRMS1 from the freshwater sponge *Eunapius subterraneus*, along with human BRMS1 and BRMS1-like, in mammalian cell lines. Subcellular localization, protein–protein interactions, and cancer-related cellular processes were examined using confocal microscopy, co-immunoprecipitation, and assays for proliferation, colony formation, and migration. Phylogenetic analyses revealed that BRMS1 and BRMS1-like originated from a duplication of an ancestral BRMS1 gene early in vertebrate evolution, whereas SDS3 represents a more ancient paralog. EsuBRMS1 shares conserved domains with human BRMS1 and displays a comparable predicted 3D architecture. In human cells, EsuBRMS1 colocalized with human BRMS1 and BRMS1-like predominantly in the nucleus and partially in the cytoplasm. Co-immunoprecipitation demonstrated that sponge and human BRMS1 proteins coexist within the same complexes. Functionally, overexpression of EsuBRMS1, HsaBRMS1, or HsaBRMS1-like significantly reduced proliferation, colony formation, and migration of MDA-MB-231 breast cancer cells ($p < 0.05$). Our findings demonstrate that key structural features, intracellular localization patterns, protein interaction capacities, and cancer-related functions of BRMS1 are deeply conserved across Metazoa. The ability of a sponge BRMS1 homolog to integrate into human protein complexes and suppress cancer cell migration and proliferation suggests that fundamental BRMS1 activities arose early in animal evolution, predating the emergence of true tissues and tumorigenic processes.

P14

GALECTIN-3: A POTENTIAL MISSING LINK IN BOTULINUM TOXIN ANALGESIA

Jerka Dumić, Mihael Grdunac, Lidija Bach-Rojecky

University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

Chronic pain is closely associated with central sensitisation processes in which spinal microglia play a key role. Galectin-3 (Gal-3), a β -galactoside-binding lectin, released from injured sensory neurons, activates microglia through the TLR4 receptor, promoting the release of pro-inflammatory cytokines and enhances excitatory transmission in the spinal dorsal horn, thereby contributing to the development of neuropathic pain. At the same time, increasing evidence suggests that botulinum toxin type A (BoNT-A), beyond its established neuromuscular effects, exerts significant antinociceptive properties in various chronic pain conditions. BoNT-A is proposed to modulate microglial activation by promoting a shift from the proinflammatory M1 to the anti-inflammatory M2 phenotype; however, the role of Gal-3 in this process remains unclear. Further investigation of the interplay between Gal-3 signalling and BoNT-A-induced analgesia may help elucidate the mechanisms underlying the antinociceptive effects of BoNT-A and improve understanding of its role in chronic pain modulation. Funded by the European Union (NextGenerationEU) under the National Recovery and Resilience Plan 2021 –2026 (NRRP), through the UNIZG Faculty of Pharmacy and Biochemistry institutional project NPO025-FBF-10.

P15

LATENT AUTOIMMUNE DIABETES IN ADULTS AND TYPE 1 DIABETES SHARE A COMMON GLYCOSYLATION SIGNATURE OF IgG AND COMPLEMENT COMPONENT 3 (C3)

Marko Dužević¹, Branimir Plavša¹, Domagoj Kifer², Edit Pape Medvidović³, Marijana Vučić Lovrenčić⁴, Olga Gornik¹

¹University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia ²University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biophysics, Zagreb, Croatia ³Merkur University Hospital Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Zagreb, Croatia ⁴Merkur University Hospital Department of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia

Type 1 diabetes (T1D) and latent autoimmune diabetes in adults (LADA) are autoimmune diabetes subtypes caused by T-cell mediated attack on the insulin-producing beta-cells. Although they share key pathological mechanisms, there is still ongoing debate as to whether these conditions should be considered the same disease, since LADA is distinguished by slower progression and greater clinical heterogeneity. Previously, our group discovered significant changes in N-glycosylation of immunoglobulin G (IgG) and complement component 3 (C3) related to T1D. To investigate whether LADA and T1D share these glycosylation changes, we analyzed IgG and C3 N-glycosylation from plasma samples of 287 adults with either T1D (n=200) or LADA (n=87). IgG was isolated from 100 µl of plasma on a monolithic protein G plate, followed by N-glycans release with N-glycosidase F, fluorescent labeling, and profiling by HILICUPLC. This resulted in 24 chromatographic peaks containing different glycan structures. For C3 analysis, 10 µl of plasma was subjected to enrichment using a Concanavalin A lectin matrix. Isolated C3 fraction was digested with Glu-C, glycopeptides were purified by SPE and analyzed by LC-MS. Nine glycopeptides were quantified from two C3 glycosylation sites. Results showed no significant differences in IgG and C3 N-glycosylation profiles between LADA and T1D. Glycosylation of these proteins was not related to disease duration or the number of positive autoantibodies. Interestingly, further stratification of patients by sex revealed differences in specific IgG glycan traits between LADA and T1D in women (lower sialylation and galactosylation), which were not observed in men and may be associated with the higher prevalence of autoimmune diseases in women. These findings suggest that previously reported T1D related glycosylation changes are associated with either (pancreatic) autoimmunity or general disruption in glucose metabolism, and do not appear to be specific to any particular subtype of adult autoimmune diabetes.

P16

TARGETING GLIOBLASTOMA VIA NOVEL PHENYL DERIVED ACRYLONITRILES

Katja Ester¹, Antonija Mamić², Marijana Hranjec²

¹*Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia* ²*University of Zagreb, Faculty of Chemical Engineering and Technology, Department of Organic Chemistry, Zagreb, Croatia*

Glioblastoma (GBM) is the most aggressive brain tumor in adults. GBM is highly malignant and resistant to chemotherapy, which leads to poor patient outcome with median survival rate at 12-18 month. Novel therapy that would improve patient outcome is urgently needed. Acrylonitrile derivatives represent an important class of compounds in medicinal chemistry due to their ability to interact with biological targets, and novel research data indicate their potential to target GBM. Here we present design, synthesis and antiproliferative study of 15 novel differently substituted phenyl acrylonitriles. MTT screening using glioblastoma (U251, U87) and lung carcinoma (A549) cell lines revealed selectivity toward glioblastoma cells. We identified 5 compounds, bromo- and nitro-substituted acrylonitriles, demonstrating antiproliferative activity in submicromolar range. Further mechanistic study involving Cell Cycle and Reactive Oxygen Species-induction analysis pointed toward different cellular targets in cells depending on the type and position of the substituent. Novel phenyl derived acrylonitriles represent promising scaffolds for further optimization and identification of novel targets for GMB therapy.

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GENE EXPRESSION CHANGES INDUCED BY A REPLICATION DEFICIENT HUMAN ADENOVIRUS TYPE 26 VECTOR IN THE CONTEXT OF USING $\alpha\text{v}\beta 3$ INTEGRIN AS A RECEPTOR

Roberto Galov, Mario Stojanović, Dragomira Majhen

Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Human adenoviruses (HAdVs) are non-enveloped double stranded DNA viruses spanning more than 100 types classified into subgroups A-G. Extensive knowledge of their molecular biology as well as development of many methods for their manipulation resulted in HAdVs becoming attractive candidates for vector design and they are currently widely utilised as vectors for gene transfer and vaccination. Low seroprevalence adenoviral species, like human adenovirus type 26, are of increasing interest. Recently two HAdV-D26-based vaccines were approved for use in humans for preventive purposes: a vaccine against Ebola and a vaccine against COVID-19. Our previous data provide insight into the interesting nature of the HAdV-D26 infection pathway suggesting that depending on the receptor status this virus can enter the cell in a different manner which can be independent of dynamin-2, clathrin and/or caveolin-1. We also reported that HAdV-D26-induced IL-6 gene expression is $\alpha\text{v}\beta 3$ integrin dependent and NF- κ B mediated. However, data on how HAdV-D26 uses cell surface receptors for forward signalling to activate cell growth, intracellular transport or innate immune response are still insufficient. Therefore, in this study we analysed host cell transcription profile in response to HAdV-D26 infection in the context of using $\alpha\text{v}\beta 3$ integrin as a receptor. We did RNA-seq analysis in A549 and A549-E6 cells overexpressing $\alpha\text{v}\beta 3$ integrin at 1h and 3h post-infection with HAdV-D26. Data were collected from three independent experiments and analysed using tools available on BMKCloud platform. Gene list functional annotation and clustering was done using DAVID database. Mock-infected cells were used to account for the differences between the two cell lines and to isolate infection-specific gene expression changes. We found that 86 and 84 named genes showed differential expression between the A549 and A549-E6 cells at 1h and 3h post-infection with HAdV-D26, respectively. A large number of these genes are connected with extracellular space activity, such as: *FRP1*, *STC2*, *SCUBE3*, *BMPER*, *SMOC1*, *CTSD*, *LOXL4*, *SPARC*, *IL12A*, and *IL24*. Several genes connected with innate immune response and cytokine activity were also identified, namely: *EREG*, *HK1*, *CXCL1*, *IL12A*, and *IL24*, which provide a baseline for future studies of virus-host interactions in adenovirus research.

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ENGINEERING A PROBIOTIC YEAST FOR SWEET PROTEIN PRODUCTION: A STEP TOWARD FUNCTIONAL SWEETENERS

Romana Ivković, Bojan Žunar, Renata Teparić, Igor Stuparević

University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory of Biochemistry, Zagreb, Croatia

Excessive sugar consumption is associated with major health risks, increasing the demand for safe, low-calorie alternatives. Natural sweet-tasting proteins are promising substitutes due to their high sweetness potency and non-caloric properties; however, their low abundance in native sources necessitates the development of efficient microbial production systems. Although previous studies have demonstrated the feasibility of producing such proteins in *Saccharomyces cerevisiae*, the use of probiotic hosts remains largely unexplored. In this study, we evaluated the probiotic yeast *Saccharomyces boulardii* as a novel host for the production of sweet-tasting proteins. Two industrially relevant proteins, thaumatin and brazzein, were successfully expressed in *S. boulardii*. Protein production and secretion into the surrounding medium were confirmed by Western blot analysis, although secretion efficiency remained limited. To our knowledge, this is the first report of heterologous expression of sweet-tasting proteins in this probiotic strain. These results demonstrate the feasibility of using *S. boulardii* as a production platform for natural sweet proteins and provide a basis for further optimization of expression and secretion systems. As a probiotic strain, *S. boulardii* could ultimately enable the development of whole-cell sweeteners with added functional benefits; however, achieving efficient surface display will be essential, and this study represents an initial step towards that goal.

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PROFILING AND FUNCTIONAL CHARACTERISATION OF p53 FAMILY ISOFORMS IN PRIMARY MELANOMA CELL LINES RESISTANT TO COMBINATION TARGETED THERAPY

Janja Josić, Anđela Horvat, Christine Supina Pavić, Ignacija Vlašić, Neda Slade

Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Metastatic melanoma is the most aggressive form of skin cancer and accounts for over 80% of cutaneous cancer-related deaths. A high proportion of melanomas harbour activating mutations in the *BRAF* gene, and patients with *BRAF* V600E mutations are treated with BRAF and MEK inhibitors (BRAFi/MEKi). Although these therapies initially show clinical efficacy, resistance frequently develops due to molecular alterations, including changes in alternative splicing and expression of p53 family isoforms, which regulate tumour suppressor activity and may contribute to resistance mechanisms. Primary melanoma cell lines were established from metastatic melanoma patients treated with combined BRAFi/MEKi therapy. In addition, a paired model of therapy-sensitive and therapy-resistant cell lines derived from the same patient was generated to investigate mechanisms of acquired resistance. Expression of p53 family isoforms (p53, p63, p73) was analysed at the mRNA and protein levels using quantitative PCR and Western blotting. MITF expression was assessed by qPCR and compared with non-melanoma cancer cell lines. Immunofluorescence analysis was performed to evaluate subcellular localisation of selected isoforms and associated cellular markers, while functional studies involving isoform silencing and analysis of proliferation- and cell cycle-related markers are ongoing. Melanoma sublines displayed distinct morphological phenotypes corresponding to melanocytic, mesenchymal, and mixed populations. Differential expression of p53 family isoforms was detected at both mRNA and protein levels across melanoma models. Preliminary data from the paired sensitive-resistant system indicate distinct isoform expression patterns and altered subcellular localisation between therapy-sensitive and resistant cells, suggesting dynamic regulation of p53 family members during acquisition of resistance. These findings indicate that p53 family isoforms may contribute to resistance to combined BRAFi/MEKi therapy in melanoma and highlight their potential as biomarkers or therapeutic targets. Funding: This project is funded by the Croatian Science Foundation (DOK-2023-10 and IP-2022-10-1375).

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ROLE OF ENDOSOMAL COMPARTMENTS ACIDIFICATION DURING MCMV INFECTION IN FIBROBLASTS

Ljerka Karleuša Mujkić, Tamara Gulić, Hana Mahmutefendić Lučin, Pero Lučin

Faculty of Medicine Department of Physiology, Immunology and Pathophysiology, Rijeka, Croatia

The nature of the endosomal compartments is determined by the presence of specific membrane proteins, as well as by their different luminal pH. Generally speaking, the more mature compartment is, its pH is lower. The acidification itself is controlled by v-ATPase (v-Vacuolar-type H⁺-ATPases), an ubiquitous transmembrane protein. Murine cytomegalovirus (MCMV) uses endosomal system for its replication, and it should be expected that the changes in the properties of the endosomal system (i.e. luminal pH) would impact MCMV infection cycle. We investigated the impact of the endosomal acidification on establishing MCMV infection, as well as on the production of infectious viral progeny. By using reversible chemical inhibitor Concanamycin A (ConA), we inhibited function of v-ATPase before and up to the infection, during the entire infection, and at specific timepoints in the MCMV infection life cycle. We checked for expression of key MCMV proteins, specific changes in cellular morphology as well as MCMV progeny production using qPCR, plaque assay and immunofluorescent methods. We showed that endosomal acidification appears to be crucial for MCMV infection lifecycle, since there had been very reduced numbers of viral progeny after the ConA treatment throughout the entire infection period, showed both by qPCR and plaque assay. Even though the expression of early MCMV proteins is established, we noticed a shift in infection timeline, and retention of viral progeny in the cell nucleus, or in the cytoplasm after the ConA treatment up until infection with MCMV. If the inhibition occurred later in the infection, VAC is formed, but the viral progeny is kept both in larger clusters in the cytoplasm, or in the nucleus. Our results show that acidification of endosomal compartments seems to be crucial both for establishing and maintaining MCMV infection, and for functional viral progeny production in fibroblast, with yet unclear mechanisms that need to be elucidated.

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**A HIGH-THROUGHPUT AFFINITY-FREE GLYCOPROTEOMIC WORKFLOW ENABLES
SIMULTANEOUS PROFILING OF FIBRINOGEN AND IgG N-GLYCOSYLATION IN
HUMAN PLASMA**

Toma Keser, Dinko Šoić, Olga Gornik

*University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of
Biochemistry and Molecular Biology, Zagreb, Croatia*

Fibrinogen is a major plasma glycoprotein with central roles in blood coagulation and inflammatory processes. In contrast, immunoglobulin G (IgG) is the most extensively studied plasma glycoprotein, with well-established alterations in glycosylation associated with numerous diseases and inflammatory conditions. Changes in fibrinogen glycosylation have also been implicated in a range of pathological conditions; however, its site-specific N-glycosylation profile remains insufficiently characterized in clinical settings due to analytical challenges. Previously, we developed a high-throughput LC-MS workflow for site-specific analysis of fibrinogen N-glycosylation based on a cost-effective ethanol precipitation enrichment strategy. While robust methods for subclass-specific IgG glycosylation analysis are well established, they typically require affinity chromatography-based IgG isolation prior to proteolytic digestion, C18 clean-up, and LC-MS analysis, which limits throughput and increases sample preparation complexity. In this study, we present a novel, streamlined approach that enables the simultaneous enrichment and analysis of fibrinogen and IgG directly from plasma, eliminating the need for affinity chromatography. The method integrates ethanol-based protein precipitation with optimized sample preparation and LC-MS analysis, enabling simultaneous site-specific profiling of fibrinogen glycopeptides and subclass-specific profiling of IgG glycopeptides within a single analytical workflow. This approach significantly reduces sample handling time and cost while maintaining analytical performance in terms of sensitivity, reproducibility, and glycopeptide coverage. The developed workflow represents an efficient tool for high-throughput glycoproteomic studies and has strong potential for application in large clinical cohorts, enabling the discovery of glycosylation-based biomarkers and improving our understanding of protein-specific glycosylation changes in disease.

OVERCOMING THE LIMITS OF STANDARD ANTIDOTES: BREAKTHROUGHS IN ORGANOPHOSPHATE NERVE AGENT SCAVENGING

Dora Kolić¹, Ines Primožič², Zrinka Kovarik^{1,2}, Nikolina Maček Hrvat¹

¹*Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia* ²*University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia*

Organophosphorus (OP) warfare nerve agents pose a lethal threat by inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), triggering excessive stimulation of cholinergic receptors and leading to respiratory arrest and long-term nerve damage in severe poisoning cases. Current standard-of-care oxime antidotes, which recover activity of inhibited cholinesterases (ChE), lack universal efficacy against the various OPs and show moderate reactivation potency against the understudied A-series agents. Additionally, they struggle to reactivate BChE, which compromises its effectiveness as a competent OP bioscavenger by limiting its ability to remove the OP from the bloodstream and from vulnerable AChE- rich tissues. Therefore, we explored alternatives to the standard-of-care oximes and assessed the *ex vivo* reactivation potency of previously *in vitro* identified potent bispyridinium and N,N'-dibenzyl imidazolium oxime reactivators in human whole blood (hWB) inhibited with G- and A-series agents. When hWB was supplemented with A-230, bispyridinium bis-oxime MMB-4 reactivated about 60 % of total blood ChE activity within 5 hours at the tested K_i AChE concentration, whereas the standard-of-care monoxime HI-6 achieved only 30 % reactivation. Increasing the concentrations of both oximes (up to 1 mM) resulted in a maximal recovery of 80 % of total activity. The remaining 20 % of inhibited activity likely reflects unreactivated BChE, as both oximes were poor *in vitro* reactivators of A-230-inhibited BChE. Two imidazolium oximes identified as effective BChE reactivators restored over 60 % of blood ChE activity. However, the recovery was achieved after 24 hours, due to the slow dephosphylation rate of A-230-conjugate. In contrast, when hWB was inhibited by G-agent cyclosarin, reactivation with 10 μ M of a lead imidazolium oxime was rapid, restoring over 60 % of ChE activity within 20 minutes. Notably, a synergistic effect was observed when combining the standard oxime HI-6 with an imidazolium oxime, achieving nearly 100 % reactivation within just 10 minutes. Although the performance of tested oximes is improved over standard-of-care oximes, refinement is necessary to maximize efficiency in the treatment of OP poisoning. This research was supported by the Croatian Science Foundation (HrZZ IP-2022-10-6685), and the European Union – NextGenerationEU (BioMolTox).

DEVELOPMENT OF ANTIBACTERIAL COMPOSITE POLYMER SURFACES BY INCORPORATION OF LASER-SYNTHEZIZED NANOPARTICLES

Laura Nuić², Luka Perković¹, Rafaela Radičić^{4,3}, Nikša Krstulović³, Lucija Krce¹

¹*University of Split, Faculty of Science, Department of Physics, Split, Croatia* ²*University of Split, Center of Excellence for Science and Technology, Split, Croatia* ³*Institute of Physics CALT, Zagreb, Croatia* ⁴*Jožef Stefan Institute, Department of Surface Engineering, Ljubljana, Slovenia*

Antimicrobial resistance and microbial contamination of surfaces remain major challenges in healthcare, food safety, and packaging applications. Consequently, there is a growing need for safe-by-design antibacterial materials that suppress bacterial growth without promoting resistance development or introducing potentially harmful chemicals into the environment. Polymer surfaces represent an attractive platform for such applications due to their widespread use and ease of modification. In this work, we present two complementary approaches for the fabrication of antibacterial composite polymer surfaces based on the incorporation of laser-synthesized nanomaterials into the polymer surface. Nanoparticles were produced by pulsed laser ablation in water, providing a surfactant-free and environmentally friendly route to nanomaterial synthesis. The resulting nanomaterials were incorporated into polymer surfaces either by atmospheric pressure plasma jet treatment or by thermal annealing, enabling the formation of functional near-surface composite layers with tailored physicochemical properties. The influence of the fabrication strategy on surface morphology, chemical composition, wettability, and antibacterial performance was investigated using various characterization techniques. The results demonstrate that both plasma-assisted and thermally induced nanomaterial incorporation significantly modify surface properties and generate pronounced antibacterial activity. These findings support the further development of safe-by-design antibacterial composite polymer surfaces suitable for food packaging and related applications.

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HOW SERUM TYPE INFLUENCES CELLULAR HEALTH AND CELL CULTURE EXPERIMENTAL OUTCOMES

Matea Kurtović Kodžoman¹, Maja Katalinić¹, Tea Žugec²

¹*Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia* ²*Faculty of Food Technology and Biotechnology, Laboratory for Biology and Microbial Genetics, Zagreb, Croatia*

Human 2D and 3D cell culture models are of a great importance in medical and pharmaceutical research, especially when investigating cellular proliferation, metabolism, toxicity and drug response. However, this implies growing and maintaining cells under strictly controlled laboratory conditions. In the period between 1940s and 1950s it has become clear that one of the most important of all cell media supplements is serum, which is rich in hormones, proteins, growth factors and attachment factors that promote cell proliferation, differentiation and survival. However, the origin and percentage of serum in the medium has a strong influence on the cellular health and activation of various cellular processes. More recently, concerns over inconsistency, batch variations, as well as animal welfare have led to development of serum-free media and semi-defined serum substitutes. In this study, we investigated the effects of different types of serum and one serum substitute (standard FBS, Xtra FBS, human serum and Ultrosor G) on cell viability, metabolic activity, colony formation and ability of forming spheroids. In addition, metabolic activity and viability was examined in different concentrations for all mentioned serums (2%, 5%, 10% and 15%). Microscopic images show differences in the size and shape of the spheroids, but also in the rate at which aggregates are formed, depending on the type of serum added to the cell growth medium. Metabolic activity assay which strongly correlates with cell number, cell proliferation rate and overall cellular health showed significant differences between serum types, especially during longer exposure periods. Colony forming was also affected by different serum types, but, interestingly, the serum that showed the biggest increase in cell viability and metabolic activity assay was not the same that resulted in bigger colony formation. The results demonstrate that the choice of serum significantly affects cell culture outcomes, influencing spheroid morphology, aggregation dynamics, metabolic activity, and colony-forming ability. In addition, our results suggest that different serum types may selectively impact distinct aspects of cellular behavior, emphasizing the importance of careful serum selection depending on the specific goals of the experiment. Acknowledgment: This work was funded by the European Union – Next Generation EU (BioMolTox, Class: 643-02/23-01/00016, Reg. No. 533-03-23-0006 and Croatian Science Foundation project IP-2025-02-7665

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COMPLEX EXPOSURE PATTERNS OF PHTHALATES: THE ROLE OF LIFESTYLE AND INDOOR ENVIRONMENT IN ADULT MEN

Lucija Kutleša, Andreja Jurič, Blanka Tariba Lovaković

Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

Phthalates are ubiquitous environmental contaminants widely used as plasticizers and consumer product additives, and increasing evidence suggests their adverse effects on endocrine function and reproductive health. Urinary concentrations of 12 phthalate metabolites were analysed in 52 healthy Croatian men of reproductive age (22 – 46 years) in order to assess exposure patterns and potential associations with lifestyle and indoor environmental factors. Among the analysed metabolites, mono-octyl phthalate (MnOP) and monoisononyl phthalate (MiNP) were below the limit of quantification (LOQ) in all urine samples. The highest measured concentrations were observed for monoethyl phthalate (MEP; range <LOQ–224.0 ng/mL) and monoisobutyl phthalate (MiBP; range <LOQ–125.6 ng/mL), indicating widespread exposure to low-molecular-weight phthalates. Participants completed a questionnaire regarding dietary habits, use of plastic-related consumer products, and indoor environmental exposures. Non-parametric statistical analyses revealed several associations between urinary metabolite concentrations and self-reported lifestyle factors. Participants who reported minor home refurbishments within the previous year had significantly higher urinary MiBP concentrations compared to those who had not undertaken such activities. In contrast, individuals who had renovated their homes within the past five years showed significantly lower monobutyl phthalate (MBP) concentrations. Positive correlations were also observed between mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) concentrations and weekly meat consumption, supporting the role of diet as a potential exposure pathway. Significant inverse associations were identified between MBP concentrations and the frequency of paper cup use, while additional correlations involving plastic cup use and personal care products suggested complex exposure patterns linked to everyday behaviours. Following Benjamini–Hochberg correction for multiple testing, only the association between MBP concentrations and paper cup use remained statistically significant. To our knowledge, this study represents the first report on urinary phthalate metabolite levels in the Croatian population. The findings highlight the contribution of lifestyle habits and indoor environmental factors to phthalate exposure and emphasize the importance of biomonitoring studies in evaluating population-level exposure to endocrine-disrupting chemicals. The study is funded by the Croatian Science Foundation (grant HRZZ-IP-2024-05- 8815, PlastRepRisk) and European Union – Next Generation EU (Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006, BioMolTox).

**IN SEARCH OF NEW TREATMENTS AGAINST *Helicobacter pylori*:
CHARACTERIZATION OF ADENYLOSUCCINATE LYASE**

Ivana Radetić³, Ivo Crnolatac², Ivana Leščić Ašler¹

¹Ruđer Bošković Institute, Division of Physical Chemistry, Zagreb, Croatia ²Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia

³University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Helicobacter pylori is a gram-negative bacterium that colonizes human stomach. Since its discovery by Marshall and Warren in 1984, it was proven to participate in development of several serious diseases, including peptic ulcer, gastric cancer, and gastric mucosa associated lymphoid tissue lymphoma. As a result, *H. pylori* is considered a significant threat to human health and has been classified as a class I human carcinogen. It is estimated that around 1/2 of world population is infected with *H. pylori*, fortunately mostly without symptoms. The currently recommended treatment of *H. pylori* infections includes simultaneous administration of multiple drugs that target different molecular pathways. However, these therapies are often ineffective, and the number of *H. pylori* strains that are resistant to used antibiotics is on the rise. Given these issues, it is necessary to find alternative molecular mechanisms for drug development to effectively eradicate *H. pylori*. The enzymes participating in synthesis of purines present possible targets for new antibacterial drugs, and this is especially emphasized in *H. pylori* which lacks several enzymes of de novo purine synthesis and relies solely on the purine salvage pathway. This pathway includes 9 enzymes, of which only 2 from this bacterium are experimentally characterized: adenylosuccinate synthetase (AdSS) and purine nucleoside phosphorylase (PNP). Our goal is to investigate enzymes of *H. pylori* purine salvage pathway in more detail, their properties, potential protein-protein interactions and possible use as drug targets for eradicating this pathogen. Here we present our results on purification and characterization of adenylosuccinate lyase (AdSL), the enzyme that catalyzes the conversion of adenylosuccinate (product of AdSS-catalyzed reaction) to AMP and fumarate. The AdSL enzyme was overexpressed in *E. coli* and purified via immobilized metal affinity chromatography and size-exclusion chromatography. Its kinetic constants were determined by measuring initial reaction velocities across a range of adenylosuccinate concentrations. Effect of various conditions (buffer, pH, ionic strength) on AdSL stability was assessed using the differential scanning fluorimetry assay. Additionally, CD spectroscopy measurements and protein crystallization screens were undertaken, in order to facilitate structural characterization of the AdSL enzyme.

Tomislav Mamić, Mirta Žagar, Ivana Ivančić Baće

University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

CRISPR-Cas is a prokaryotic adaptive immune system that protects against mobile genetic elements (MGEs) through a crRNA-mediated interference mechanism. Immunity is established by the integration of short DNA fragments (spacers), derived from invading MGEs, into specific chromosomal loci called CRISPRs. The acquisition of new spacers is essential for CRISPR immunity, and this step is called “CRISPR adaptation”. CRISPR adaptation can be divided into two types based on the mechanism of spacer acquisition: naïve and primed adaptation. Both processes require the Cas1–Cas2 complex, which is responsible for spacer capture and integration into the CRISPR array. *In E. coli*, *cas1* and *cas2* are the last and the least expressed genes within the seven-gene operon (*casABCDE12*). Primed adaptation is much more efficient than naïve adaptation and is therefore easier to study. It is well observed when *cas1* and *cas2* are expressed as part of a long polycistronic mRNA from an inducible chromosomal pBAD promoter. In contrast, naïve adaptation is typically observed only upon overexpression of *cas1* and *cas2* from plasmids. However, plasmids are often unstable in many mutant strains and can be lost over time, complicating data interpretation. To address this limitation, we constructed a strain in which a T7 promoter was inserted upstream of the chromosomal *cas1* and *cas2* genes at their native locus – replacing previously used pBAD which proved inefficient. Here, we present new data on strain construction, strain verification, as well as the effects of T7-driven chromosomal overexpression of *cas1* and *cas2* on naïve CRISPR adaptation.

STRUCTURAL MODIFICATION OF NICOTINAMIDES AND ITS IMPACT ON THE MODULATION OF KEY SIGNAL-TRANSDUCTION RECEPTORS INVOLVED IN SYNAPTIC NEUROTRANSMISSION

Lucija Marčelić¹, Antonio Zandona¹, Katja Vuković¹, Valentina Bušić², Dajana Gašo-Sokač², Alejandra Mendez³, Carlos Villalobos³, Lucía Nuñez³, Maja Katalinić¹

¹*Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia* ²*Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology, Department of Applied Chemistry and Ecology, Osijek, Croatia* ³*Institute of Biomedicine and Molecular Genetics of Valladolid Pathophysiology of Intracellular Calcium group, Valladolid, Spain*

Nicotinamides, derivatives of vitamin B3, are known as essential molecules for the body function as critical precursors for NADH and NADPH, participating in major redox reactions, cellular homeostasis, metabolism, as well as neurotransmission. In this study, we investigated the effects of 22 synthetic nicotinamide derivatives (NAs) to determine how structural changes influence their effects at the cellular level and the level of receptors associated with acetylcholine (ACh), serotonin (5-HT) and N-methyl-D-aspartate (NMDA) neurotransmission. These receptors under investigation play roles in various conditions and diseases such as addiction, depression, ADHD, cognitive impairments associated with psychotic disorder or neurodegenerative disorder like Alzheimer's disease. Modulating the activity of these receptors may help mitigate symptoms linked to these disorders and enhance cognitive and emotional health. All nicotinamide derivatives investigated here share a common core derived from a nicotinamide-based scaffold, while differing in the substituents and their position on the structure. We assessed the compounds inhibition of nicotinic acetylcholine receptors (nAChR), serotonin receptors (5-HTR) in human neuronal cell line (SH-SY5Y) and NMDA receptors in primary cerebellum rat cells, as well as their cytotoxicity and mitotoxicity. Our data show that structural modifications strongly influence receptor activity. The most promising compound from the series was the para-phenyl-substituted nicotinamide (NA-Ph), and its corresponding isonicotinamide analogue (INA-Ph) having activity in micromolar concentration. Both compounds exhibited selectivity for nAChR modulation. In contrast, the compound with the highest potency for 5-HTR modulation was the simplest nicotinamide in the series with no substituent (NA-H). Regarding the impact on NMDA receptors, only NA-Ph showed stronger inhibitory activity while three additional compounds from the series, tested for comparison, did not exhibit similar activity. This compound exhibited an IC_{50} around 100 μ M, whereas the remaining three compounds displayed substantially higher IC_{50} values, indicating lower potency. The majority of the compounds didn't show any cytotoxicity or mitotoxicity on selected cell lines up to 500 μ M, although a few induced notable toxicity. Together, these findings highlight several NAs and INAs as promising leads for the development of selective inhibitors within the neurotransmitter receptor system. Acknowledgment: This work was supported by the Croatian Science Foundation grants (IP-2025-02-7665 to M.K and DOK-NPOO-2023-10-4267 to L.M) and spanish AEI grant PID2024-159238OB-I00.

MITOPHAGY IN PLACENTAL HEALTH AND DISEASE: INTEGRATIVE MOLECULAR PROFILING AND EXPERIMENTAL MODELS OF TROPHOBLAST DIFFERENTIATION

Mija Marinković¹, Ana Rožić², Tina Domjanović², Stipe Dumančić⁴, Petar Đolonga⁴, Antonela Matijaš Hrgović¹, Pio Labor¹, Kristijan Čačija¹

¹University of Split, Faculty of Science Department of Physics, Department of Biology, Split, Croatia ²University of Split, School of Medicine Immunology and Medical Genetics, Split, Croatia ³University Hospital Split, Institute for Pathology, Split, Croatia ⁴University Hospital Center, Split Department of Obstetrics and Gynecology, Split, Croatia

Pregnancy complications such as preeclampsia and preterm birth remain leading causes of maternal and neonatal morbidity and mortality worldwide, largely due to the lack of early biomarkers and targeted therapeutic strategies. Increasing evidence points to mitochondrial dysfunction as a central driver of placental insufficiency, yet the underlying quality-control mechanisms remain insufficiently understood. Mitophagy, the selective removal of damaged mitochondria, has emerged as a critical regulator of cellular homeostasis, but its role in human placental biology—particularly receptor-mediated pathways—remains largely unexplored. This study addresses this critical knowledge gap through an integrative and translational research approach combining human samples, high-throughput molecular profiling, and experimental models. A well-defined biobank of placental tissues from healthy, preeclamptic, and preterm pregnancies was established, enabling comprehensive multi-level analyses. Initial findings reveal distinct alterations in mitophagy-related gene and protein expression, supported by transcriptomic data indicating broader dysregulation of mitochondrial quality-control pathways in pathological placentas. To bridge descriptive and mechanistic insights, a robust in vitro model of trophoblast differentiation was developed using forskolin-induced syncytialization of BeWo cells, providing a controlled platform for functional studies of mitochondrial regulation. Importantly, this work is being extended into in vivo systems through the development of a mitophagy-specific knockout mouse model combined with a preeclampsia-like (L-NAME) model, enabling longitudinal and mechanistic investigation of disease progression. By integrating clinical material with advanced molecular and experimental approaches, this research positions mitophagy as a key regulatory axis in placental function. The project has strong potential to identify novel biomarkers for early detection of pregnancy complications and to uncover new therapeutic targets, ultimately contributing to improved maternal and fetal health outcomes.

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ALLIANCE4LIFE PROJECT VIRTUAL RESEARCH CENTER: FOSTERING RESEARCH EXCELLENCE THROUGH THE VIRTUAL DEPARTMENT OF METABOLISM AND ENDOCRINOLOGY OF SKELETAL MUSCLE

Tomaž Marš, Katarina Miš, Klemen Dolinar, Blaž Kociper, Anja Srpčič, Ksenja Babič, Chiedozie Kenneth Ugwoke, Denis Korady, Nejc Umek, Sergej Pirkmajer

University of Ljubljana, Faculty of Medicine Alliance4Life Virtual Research Center, Department of Metabolism and Endocrinology of Skeletal Muscle, Ljubljana, Slovenia

Alliance4Life Action Project. Alliance4Life is a consortium of leading life science and health research institutions from Central and Eastern Europe dedicated to strengthening research and innovation excellence, promoting institutional development, and enhancing international competitiveness. The A4L_BRIDGE project builds on the achievements of the Alliance by addressing key challenges related to scientific culture, research careers, innovation capacity, and science policy. Through targeted actions, knowledge sharing, and collaborative initiatives, the project supports institutional reforms and contributes to the development of a more integrated and competitive European Research Area. Virtual Research Center within Alliance4Life. To facilitate scientific collaboration across institutions and disciplines, Alliance4Life has established a Virtual Research Center composed of thematic virtual departments. These departments serve as platforms for networking, knowledge exchange, training, and the development of joint research activities. Covering diverse biomedical fields, including neurobiology, cardiovascular research, cancer biology, medical microbiology, and metabolic health, the Virtual Research Center promotes webinars, journal clubs, workshops, and collaborative funding initiatives, thereby strengthening connections among researchers throughout the consortium. Virtual Department of Metabolism and Endocrinology of Skeletal Muscle. The Virtual Department of Metabolism and Endocrinology of Skeletal Muscle focuses on understanding skeletal muscle as a central regulator of whole-body metabolism, a target of endocrine regulation, and a source of signaling molecules such as myokines. Research activities investigate the effects of obesity, diabetes, aging, and physical inactivity on skeletal muscle function and metabolic health. The department promotes interdisciplinary and international collaborations while integrating advanced research approaches, including multi-omics technologies and language model-based methodologies. As part of its activities, the department organized the mini-conference “Integrative (Patho)physiology of Skeletal Muscle and Metabolic Disorders: From Cellular Mechanisms to Interventions” in January 2026, providing a forum for presenting recent findings and establishing new collaborative opportunities. Funding: This work was supported by the Horizon Europe project A4L_BRIDGE (Grant Agreement No. 101136453).

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IN VITRO NON-INNervATED AND INNervATED MODELS FOR STUDYING SKELETAL MUSCLE DEVELOPMENT, REGENERATION AND CONTRACTION-RELATED METABOLIC ADAPTATION

Tomaž Marš^{1,2}, Katarina Miš^{1,2}, Klemen Dolinar^{1,2}, Blaž Kociper^{1,2}, Anja Srpčič^{1,2}, Ksenja Babič^{1,2}, Sergej Pirkmajer^{1,2}

¹*University of Ljubljana, Faculty of Medicine Alliance4Life Virtual Research Center, Department of Metabolism and Endocrinology of Skeletal Muscle, Ljubljana, Slovenia*

²*University of Ljubljana, Faculty of Medicine Institute of Pathophysiology, Ljubljana, Slovenia*

Skeletal muscle is a highly adaptable tissue that contributes not only to locomotion but also to whole-body metabolic regulation and inter-organ communication. Muscle contractions induce profound structural, metabolic, and endocrine adaptations, including the production and secretion of myokines that influence numerous physiological processes. While these adaptations have been extensively characterized *in vivo*, the molecular mechanisms linking muscle contractions to metabolic and endocrine responses remain incompletely understood. The development of physiologically relevant *in vitro* models is therefore essential for advancing research in skeletal muscle biology. Traditional *in vitro* models based on primary human skeletal muscle cells or myogenic cell lines have provided valuable insights into myogenesis, muscle differentiation, and regeneration. However, human myotubes cultured under standard conditions do not contract spontaneously and therefore only partially reproduce the functional state of skeletal muscle *in vivo*. Consequently, these non-innervated models have limitations when studying contraction-dependent processes, including activity-induced metabolic adaptations and endocrine signaling. To overcome these limitations, innervated neuromuscular co-culture systems have been developed. Classical models employing human skeletal muscle cells co-cultured with embryonic rat spinal cord explants enable the formation of functional neuromuscular junctions and spontaneous neurogenic contractions. These systems have proven useful for studying neuromuscular junction development, motor neuron–muscle communication, and mechanisms involved in neuromuscular disorders. More recently, human stem cell- derived motor neuron co-cultures have emerged as promising non-animal alternatives that preserve essential features of neuromuscular signaling while addressing ethical concerns associated with animal- derived tissues. This report summarizes the advantages and limitations of non-innervated and innervated skeletal muscle models and discusses their applications in studying muscle development, regeneration, neuromuscular interactions, ion transport regulation, endocrine activity, and contraction-induced metabolic adaptation. We propose that advanced human neuromuscular co-culture systems represent an important next step toward understanding the mechanisms by which motor neuron-driven contractions regulate skeletal muscle physiology in health, ageing, and disease, while simultaneously contributing to the reduction of animal use in biomedical research.

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Ccw12-BASED SURFACE DISPLAY ENABLES EFFICIENT GLUCOSYL GLYCEROL PRODUCTION IN YEAST

Tea Martinić Cezar, Antonia Paić, Bojan Žunar, Renata Teparić

University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Chemistry and Biochemistry, Laboratory of Biochemistry, Zagreb, Croatia

Saccharomyces cerevisiae is widely used microorganism for the production of recombinant proteins and valuable biotechnological goods. However, not all enzymatic reactions can be performed inside yeast cells due to incompatible conditions, requiring alternative strategies. One such example is the synthesis of glucosyl glycerol (GG) using sucrose phosphorylase, which requires phosphate-free, low-water, and high- glycerol conditions. Sucrose phosphorylase catalyzes the one-step synthesis of glucosyl glycerol from sucrose and glycerol, producing fructose and glucose as by-products. This process currently relies on enzyme purification and chemical immobilization, followed by additional purification steps to remove by-products, which increases both cost and processing time. To overcome this obstacle, we developed an alternative approach for glucosyl glycerol synthesis based on yeast surface display. In this approach, yeast cells immobilize the enzyme directly on their cell surface using native cell wall proteins, simplifying enzyme production and preparation. At the same time, yeast cells metabolize glucose and fructose by-products, reducing the need for downstream purification. To achieve this, we first designed a bipartite anchoring system based on the highly abundant cell wall protein Ccw12, enabling covalent C-terminal attachment of the recombinant enzyme to the yeast surface. The gene encoding sucrose phosphorylase from *Leuconostoc mesenteroides* was fused to Ccw12-based anchor and expressed under the control of the phosphate-inducible *PHO5* promoter, ensuring secretion and immobilization of the enzyme on the cell surface. Localization of the enzyme was confirmed by western blot analysis, while activity and kinetic parameters were determined using the UPLC method. During a five-day reaction, the engineered system produced up to 37 g L⁻¹ of glucosyl glycerol, with no detectable glucose or fructose remaining in the reaction mixture. The immobilized enzyme also remained stable after 10 days of storage at 4 °C. This work presents the first eukaryotic microbial cell factory for glucosyl glycerol production and highlights the potential of surface display for bioprocesses that cannot be performed inside the cell.

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THE ROLE OF SALL4 IN PERINATAL TESTIS: AN *IN VITRO* siRNA-BASED APPROACH

Dajana Krsnik², Ena Vilagoš^{3,4}, Ana Planinić^{1,3}, Nino Sinčić^{1,3}, Krševan Medić^{1,3}, Ana Katušić Bojanac^{1,3}

¹University of Zagreb, School of Medicine, Department of Medical Biology, Zagreb, Croatia ²Forensic Science Centre "Ivan Vučetić" Forensic Science Centre "Ivan Vučetić", Zagreb, Croatia ³University of Zagreb, School of Medicine Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia ⁴University of Zagreb, School of Medicine, Department of Anatomy and Clinical Anatomy, Zagreb, Croatia

Continuous spermatogenesis is essential for maintaining male fertility. The foundation of this process is the balance between the pluripotency and self-renewal of germ cells, with one of the responsible molecular components being the SALL4 protein (Spalt-like transcription factor 4; Sal-like protein 4). Sall4 is mainly expressed during fetal testicular development. It plays a key role in the migration of primordial germ cells and the regulation of stem potential. Based on recent knockout studies, it is believed that in the postnatal period, Sall4 promotes the specification of spermatogonial stem cells, but the underlying molecular mechanisms remain insufficiently characterized. The objective of this study was to investigate Sall4 gene function in spermatogonial development through siRNA silencing of its expression during the perinatal period *in vitro* using rat testicular tissue culture. To examine the impact of Sall4 silencing on perinatal spermatogonial development, a novel *in vitro* culture system for testicular tissue was established and combined with an optimized siRNA delivery protocol. Testes were isolated from rat fetuses at gestational day 20.5, decapsulated, and cultured at the air–liquid interface under optimized conditions with respect to tissue size, culture duration, and medium composition. siRNA-mediated knockdown of *Sall4* was performed on one-eighth fragments of testes, with appropriate biological and methodological controls included. After seven days of culture, *Sall4* gene expression levels were assessed. Global transcriptomic changes induced by Sall4 silencing were analyzed using bulk RNA sequencing. Selected differentially expressed genes were validated by qPCR. siRNA-mediated silencing resulted in a statistically significant reduction of *Sall4* expression in the siSALL4-treated group compared to the control and to methodological control groups. Transcriptomic analysis revealed alterations in gene expression profiles following *Sall4* knockdown, particularly affecting genes involved in spermatogonial development and testicular differentiation. Genes predominantly expressed in Sertoli cells were downregulated. In addition, changes were observed in the expression of cell cycle–associated genes. These results provide new insights into the role of *Sall4* in perinatal testicular development. Beyond its function in germ cells, *Sall4* appears to influence Sertoli cell–associated gene expression and cell cycle regulation, indicating a broader role in coordinating germ cell–somatic cell interactions during early testis development.

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SPECIES-SPECIFIC DIFFERENCES IN OCT TRANSPORT: DIFFERENCES BETWEEN MOUSE AND RAT Oct1

Marleen Meyer-Tönnies¹, Ole Jensen², Mladen Tzvetkov¹

¹*Institute of Pharmacology, C_DAT, University Medicine Greifswald Pharmacology, Greifswald, Germany* ²*Institute of Clinical Pharmacology, University Medical Center Göttingen Clinical Pharmacology, Göttingen, Germany*

The organic cation transporter OCT1 (SLC22A1) is an uptake transporter that is strongly expressed in the sinusoidal membrane of hepatocytes. There, it mediates the uptake of weakly basic or cationic compounds and may represent the first step in hepatic metabolism or clearance of its substrates. One of the most characteristic features of OCT1 is its polyspecificity. OCT1 binds and translocates a great variety of structurally diverse compounds, among them therapeutically relevant drugs such as metformin, sumatriptan, or fenoterol as well as endogenous compounds such as thiamine (vitamin B1), choline, or serotonin. Despite available cryo-EM structures, the precise transport mechanism and the amino acids involved in polyspecific substrate binding of OCT1 are still not fully understood. Species differences between the human and rodent OCT1 orthologs are well known, regarding both tissue expression and transport kinetics. Mouse and rat Oct1 show high similarities. They are both expressed in liver and in kidney and they share 97% of their amino acid sequence. However, preliminary data indicated differences in transport kinetics for several substrates. The aim of this study was to identify differences in OCT1 function between mouse and rat Oct1 and to identify the amino acids responsible for the observed differences. The results should help to better understand the mechanism of polyspecificity of OCT1. To this end, we used HEK293 cells stably transfected to overexpress mouse and rat Oct1 and compared the transport kinetics of a panel of known OCT1 substrates. As a next step, we exchanged the different amino acids using site-directed mutagenesis and analyzed the effect on substrate uptake, first, in single concentration uptake experiments using transient transfection, then, using concentration-dependent uptake experiments. We observed in part strong differences in both maximal transport capacity ($v_{\max}$) and transport affinity (K_M) between mouse and rat Oct1, which were strongly substrate-specific. The strongest differences were observed for endogenous compounds such as choline and isobutyryl-L-carnitine. Preliminary results pointed to substrate-specific effects of single amino acids mutations. In conclusion, we identified substrate-specific differences in transport kinetics between mouse and rat Oct1. The exact molecular mechanisms remain to be further analyzed.

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THE IMPACT OF PRENATAL EXPOSURE TO α -CYPERMETHRIN ON DNA METHYLATION PATTERNS IN PUBERTAL RAT OVARY

Anja Katić², Lorena Kulaš³, Dajana Krsnik⁴, Marina Miletić^{1,5}, Ana Katušić Bojanac^{1,5}

¹ University of Zagreb, School of Medicine, Department of Medical Biology, Zagreb, Croatia ²Institute for Medical Research and Occupational Health Division of Toxicology, Zagreb, Croatia ³Clinical Hospital Centre Zagreb Women's Diseases and Obstetrics Clinic, Zagreb, Croatia ⁴Forensic Science Centre "Ivan Vučetić" Forensic Science Centre "Ivan Vučetić", Zagreb, Croatia ⁵Centre of Excellence for Reproductive and Regenerative Medicine Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia

Keywords: DNA methylation, α -cypermethrin, ovary, rat. DNA methylation is the first identified and one of the most extensively studied epigenetic mechanisms, playing a crucial role in genomic imprinting and sex-specific gene expression during development. Proper establishment of methylation patterns at imprinted genes is essential for normal gonadal function and reproductive competence. Recent animal studies suggest that the synthetic pyrethroid insecticide and endocrine disruptor α -cypermethrin may alter the offspring epigenome. This study investigated the effects of in utero exposure to α -cypermethrin on DNA methylation patterns in ovarian tissue of female offspring. Pregnant rats have received repeated oral doses of α -cypermethrin (1, 10, or 19 mg/kg) starting on gestational day 6. Female F1 offspring were raised until puberty, after which ovaries were collected for DNA isolation and bisulfite pyrosequencing analysis. LINE-1 retrotransposon sequences were used as markers of global DNA methylation, while differentially methylated regions of the imprinted genes *Igf2r* and *Mest* were also examined. No statistically significant differences in LINE-1 methylation levels were observed between control and treated groups. Similarly, methylation analysis of *Igf2r* and *Mest* showed no significant differences, although a slight decrease in methylation at specific CpG sites within *Igf2r* was detected. In conclusion, prenatal exposure to α -cypermethrin did not significantly affect global DNA methylation or imprinting-associated methylation patterns in ovarian tissue under the tested conditions. However, further studies employing genome-wide epigenetic profiling may provide deeper insight into the potential epigenetic effects of α -cypermethrin on genomic imprinting.

EVALUATION OF OMETHOATE AS A CHOLINESTERASE INHIBITOR

Petar Nakić, Tena Čadež, Goran Šinko, Nikolina Maček Hrvat

Institute for Medical Research and Occupational Health, Department of Toxicology, Zagreb, Croatia

Omethoate (O, O-dimethyl S-methylcarbamoylmethyl phosphorothioate) is a potent synthetic insecticide and acaricide. Chemically, as an organothiophosphate, it belongs to the broader class of organophosphorus compounds (OPs) known for inhibiting the enzyme acetylcholinesterase (AChE) essential for terminating nerve impulses by hydrolyzing neurotransmitter acetylcholine at synapses. OPs also affect non-essential enzyme butyrylcholinesterase (BChE). This mechanism makes it toxic to insects but can also be hazardous to humans and animals if misused or overexposed. Understanding OPs kinetic profile and effect on the cellular level is vital for risk assessment. Therefore, in this work, we investigated omethoate by determining the inhibition rate constant (k_i) of AChE and BChE, and assessing cytotoxic potential across three neuronal cell lines: mouse neuroblastoma (Neuro-2a), mouse motor neuron-like (NSC-34), and human neuroblastoma (SH-SY5Y). Further, to provide structural insights into the selectivity and binding affinity of omethoate, molecular modeling was utilized to evaluate the specific non-covalent interactions within the enzyme active site. Kinetic analysis revealed that omethoate is approximately five-fold more potent AChE inhibitor compared to BChE. To determine the neurotoxic threshold, cell lines were exposed to omethoate concentrations ranging from 0.01 μ M to 1 mM over 24 h. The results showed that omethoate does not induce significant cytotoxic effects in mouse or human neural cells within the tested range. Overall, these results indicate that omethoate primarily poses a cholinergic risk through irreversible AChE inhibition rather than through direct acute neurotoxicity at the cellular level. This study underscores the absolute necessity of integrating kinetic, structural, and cellular data to comprehensively define the toxicological profile of organophosphate pesticides, which, despite being mostly banned or restricted, still present a great risk to human health. This research was supported by the Croatian Science Foundation (IP-2022-10-6685 and DOK 2025-02-7746).

P37**ANTIBODY VALIDATION AND OPTIMIZATION FOR FLOW CYTOMETRIC
DETECTION OF $\gamma\delta$ T LYMPHOCYTE SUBPOPULATIONS IN PERIPHERAL BLOOD**

Rebeka Monika Oklopčić¹, Martina Mihalj^{2,4}, Vera Plužarić^{1,4}, Maja Tolušić Levak^{3,4}, Marija Šola⁴, Stana Tokić^{1,4}

¹ Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Department of Laboratory Medicine and Pharmacy, Osijek, Croatia ²Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Department of Dermatovenereology, Osijek, Croatia ³ Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Department of Histology and Embryology, Osijek, Croatia ⁴University Hospital Center Osijek, Department of Dermatology and Venereology, Osijek, Croatia

$\gamma\delta$ T lymphocytes represent an unconventional T cell population comprising 1–10% of peripheral blood T cells. These cells, capable of rapid effector function and MHC-independent antigen recognition, hold key roles in bridging innate and adaptive immunity. Accurate and reliable identification of $\gamma\delta$ T cell subpopulations by flow cytometry, particularly V δ 1+, V δ 2+ and V δ 1-V δ 2- subsets, remains technically challenging due to variable antibody performance, fluorochrome selection and signal intensity. Therefore, the aim of this methodological study was to optimize and validate antibody panels for precise detection of peripheral $\gamma\delta$ T cell subsets. Peripheral blood mononuclear cells were isolated from human blood samples and stained with different combinations of monoclonal antibodies targeting CD3, pan- $\gamma\delta$ TCR, TCRV δ 1 and TCRV δ 2 markers. Pan- $\gamma\delta$ TCR and TCRV δ 2 antibodies from two different manufacturers were examined across a range of titrations and fluorochrome conjugates on the BD FACS Canto II cytometer in two independent laboratories. Single stain titration experiments demonstrated differences in signal intensity, background staining and clone-dependent binding efficiency. BioLegend PE-Cy7 pan- $\gamma\delta$ TCR (clone B1) and PerCP/Cy5.5 TCRV δ 2 (clone B6) showed acceptable performance at recommended dilutions but exhibited increased background and reduced separation at higher dilutions. In contrast, Miltenyi Biotec antibodies, particularly PerCP-Vio700 pan- $\gamma\delta$ TCR (clone REA591/11F2) and PE TCRV δ 2 (clone 123R3) consistently produced the cleanest signal, superior resolution and minimal spectral spillover across all tested dilutions, maintaining signal quality while reducing reagent consumption. Full stain panel comparisons further confirmed this pattern. Panels incorporating Miltenyi PerCP-Vio700 pan- $\gamma\delta$ TCR and PE TCRV δ 2 yielded the most stable and reproducible gating, while combinations using BioLegend antibodies showed greater variability and occasional loss of population separation. Overall, our validation demonstrates that Miltenyi Biotec $\gamma\delta$ T cell antibodies, particularly PerCP-Vio700 pan- $\gamma\delta$ TCR and PE TCRV δ 2, provide the most reliable, reproducible and high resolution detection of $\gamma\delta$ T cell subsets, making them the preferred choice for downstream sorting and transcriptomic experiments. These findings highlight the importance of systematic antibody validation in flow cytometric analyses of rare immune cell populations.

Nrf2-INDEPENDENT ROLE OF KEAP1 IN ACTIN CYTOSKELETON REGULATION

Marina Oskomić¹, Nikolina Stojanović², Andreja Ambriović-Ristov², Jonathan D. Humphries³, Mihaela Matovina¹

¹*Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Laboratory for Protein Biochemistry and Molecular Modelling, Zagreb, Croatia* ²*Ruđer Bošković Institute, Division of Molecular Biology, Laboratory for Cell Biology and Signalling, Zagreb, Croatia* ³ *Manchester Metropolitan University, Department of Life Sciences, Manchester, United Kingdom*

Keap1 (Kelch-like ECH-associated protein 1) is best known as the principal repressor of the antioxidant transcription factor Nrf2, but its broader cellular roles remain to be elucidated. The Keap1 Kelch (DGR) domain shares homology with the actin-bundling domain in *Drosophila* Kelch, suggesting a conserved capacity for actin interaction. To investigate these functions, siRNA-mediated knockdown (KD) of Keap1 was performed in HeLa cells, resulting in nearly complete disassembly of actin stress fibers, redistribution of α -actinin 1 into a cortical pattern, and a significant decrease in focal adhesion (FA) number and size, assessed by vinculin and talin1 and 2 immunofluorescence microscopy. Despite overall unchanged expression of integrin α V β 5, its signal shifted from the peripheral sites of stress fibers (FAs) into more centralized locations, resembling reticular adhesion structures (RA). Biochemical isolation of integrin adhesion complexes (IACs) confirmed reduction of vinculin, talin1, talin2, and α -actinin 1 and revealed for the first time that endogenous Keap1 is present in IACs. Despite adhesion reorganization, the global F/G-actin ratio remained unchanged, ruling out Keap1 as a regulator of actin polymerization and supporting a role as an actin-bundling and adhesion-stabilizing protein. To determine whether these effects are Nrf2-independent, parallel experiments were conducted in A549 lung cancer cells with constitutively active Nrf2 and a homozygous loss-of-function KEAP1 mutation. Keap1 KD in A549 cells induced stress fiber disassembly and reduced FA number but increased FA size. By contrast, Nrf2 KD did not affect stress fiber organization but increased FA size, indicating that Keap1 and Nrf2 act via distinct mechanisms at adhesion complexes and supporting a Nrf2-independent cytoskeletal role of Keap1. Ongoing mass spectrometry analysis of isolated IACs will clarify changes in adhesion complex protein composition upon Keap1 KD. Functionally, Keap1 KD enhanced both individual and collective migration in HeLa cells without changing cell cycle distribution, whereas in A549 cells migration was diminished, demonstrating cell-type-specific effects. These results identify Keap1 as a novel regulator of actin cytoskeleton organization and focal adhesion dynamics, acting at least in part independently of Nrf2, with important consequences for the migratory behavior of cancer cells.

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ENGINEERING ROBUST YEAST SURFACE DISPLAY SYSTEM FOR HIGH-SALINITY ENVIRONMENTS

Antonia Paić, Tea Martinić Cezar, Renata Teparić, Bojan Žunar

University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Chemistry and Biochemistry, Laboratory for Biochemistry, Zagreb, Croatia

Surface display is a powerful bioengineering strategy that enables the covalent binding of proteins to the outer surface of microbial cells, thereby converting it into a catalytically active platform. This approach is particularly attractive for the immobilization of enzymes, allowing whole-cell systems to function as stable and reusable biocatalysts. In fungal systems, surface display has been predominantly established in the model yeast *Saccharomyces cerevisiae*; however, its limited tolerance to harsh environments constrains its application in marine bioprocesses and saline wastewater treatment. To overcome these limitations, we developed a surface display platform in the halotolerant yeast *Debaryomyces hansenii*, an extremophilic organism capable of growth in environments containing up to 4 M NaCl. To enable efficient genetic manipulation and protein expression in this non-conventional host, we engineered a novel selection marker, constructed expression vectors based on strong constitutive promoters, and characterized promoters responsive to varying salinity conditions. Using this expanded molecular toolkit, we generated *D. hansenii* strains capable of covalently anchoring multiple biotechnologically relevant enzymes to the cell wall. This work represents the first successful transformation of the *D. hansenii* cell wall into an engineered, catalytically active interface, highlighting its potential as a robust platform for biocatalysis under high-salinity conditions and opening new opportunities for industrial and environmental applications.

MACHINE LEARNING–AUGMENTED BIOELECTRONIC SENSING: PLATFORM VALIDATION AND THE PATH TOWARD LUNG CANCER DETECTION

Ana Malen Pascua^{1,2}, Esteban Piccinini^{1,3}, Joaquín Difortí^{1,2,3}, Juan Allegretto¹

¹*Danube Private University Laboratory for Life Sciences and Technology (LiST), Department of Medicine, Faculty of Medicine and Dentistry, Krems 3500, Austria*

²*GISENS BIOTECH Department of Bioelectronics, Berkeley, CA 94704, California, United States of America* ³*National University of La Plata (UNLP) Institute of Theoretical and Applied Physical Chemistry Research (INIFTA) – Department of Chemistry – Faculty of Exact Sciences, 1900, La Plata, Argentina*

Respiratory diseases, and particularly lung cancer, remain major global health challenges, highlighting the need for sensitive, non-invasive, and accessible approaches for early diagnosis. Exhaled breath analysis has emerged as a promising strategy for lung cancer screening through the detection of disease-associated volatile organic compounds (VOCs), offering the potential for label-free and patient-friendly point-of-care diagnostics. This work focuses on the development of an intelligent data science framework to enhance metal-organic framework field-effect transistor (MOF-FET) sensors for the detection and quantification of lung cancer-related VOCs. Semiconductor MOFs will be employed as sensing materials due to their porous structures and suitable electrical properties, which facilitate the adsorption and transduction of target VOCs. Experimental measurements will generate MOF-FET electrical response datasets under controlled conditions, establishing relationships between sensor signals and analyte concentrations. A Random Forest regression model will then be developed, trained, and validated to capture potentially complex and non-linear relationships between sensor responses and VOC concentrations. Model performance will be assessed using appropriate validation strategies and predictive metrics, with optimization aimed at improving quantitative accuracy and robustness. Following validation, the developed data-driven approach will be applied to measurements obtained under physiologically relevant conditions to evaluate its ability to account for increased sample complexity and variability. The integration of machine learning with MOF-FET sensing is expected to improve the interpretation, quantitative analysis, and predictive capability of the sensor platform, supporting reliable and selective VOC detection. This work will contribute to the development of intelligent, non-invasive, and potentially portable sensing technologies for exhaled-breath analysis, with the longer-term objective of enabling early lung cancer detection and strengthening the transition of MOF-based biosensing technologies toward point-of-care applications.

NONINVASIVE, DIGITAL, AND PORTABLE TESTING FOR SMART MONITORING OF CHRONIC KIDNEY DISEASE (CKD)

Esteban Piccinini¹, Joaquin Diforti^{1,2,5}, Martin Mamberti^{3,4}, Silvina Maltas^{3,4}

¹National University of La Plata (UNLP) – CONICET Department of Chemistry – Faculty of Exact Sciences. Institute of Theoretical and Applied Physical Chemistry Research (INIFTA), La Plata, Argentina ²GISENS BIOTECH Department of Bioelectronics, Berkeley, United States of America ³Hospital Interzonal General de Agudos (HIGA) General San Martín - Department of Nephrology Department of Nephrology, La Plata, Argentina ⁴Hospital Interzonal General de Agudos (HIGA) General San Martín - Central Biochemistry Laboratory Central Biochemistry Laboratory, La Plata, Argentina ⁵Danube Private University Laboratory for Life Sciences and Technology, Department of Medicine, Faculty of Medicine and Dentistry, Krems, Austria

Current dialysis monitoring relies on venous blood sampling, limiting frequent and home-based assessment. We evaluated whether capillary blood sampling combined with a portable lab-on-a-chip test could accurately assess urea, potassium, and dialysis adequacy in haemodialysis (HD) and peritoneal dialysis (PD) patients. In this work, we report the results of the Noninvasive Testing of End-stage Kidney Disease (NiTED) clinical study, which evaluated the use of capillary blood as a minimally invasive sample for measuring urea and potassium and assessing dialysis adequacy in end-stage chronic kidney disease. In hemodialysis patients, capillary blood urea and potassium levels were measured before and after dialysis sessions in a paired design, allowing estimation of single-pool Kt/V (spKt/V). In peritoneal dialysis patients, urea and potassium were measured in capillary blood, peritoneal dialysate, and urine, which may enable calculation of total weekly Kt/V. Capillary blood measurements were performed using a digital lab-on-a-chip test (G-LOC, KF4+ test) designed for home use. Measurements obtained with the digital test were compared with reference measurements obtained from venous blood using a standard laboratory autoanalyzer to determine their analytical agreement and clinical applicability. Capillary blood measurements showed excellent agreement with venous blood for pre-dialysis urea and potassium. Urea concentrations measured after HD reflected the expected physiological urea rebound and, after correction, enabled accurate estimation of spKt/V. The portable test achieved accuracies of 96.4% for urea and 97.8% for potassium. In PD, capillary blood and peritoneal dialysate measurements showed no significant differences from the reference laboratory method. Patients strongly preferred capillary over venous sampling because of lower pain, greater convenience, and the feasibility of self-testing.

SERUM MALONDIALDEHYDE IS A STRONG PREDICTOR OF MORTALITY IN ACUTE HEART FAILURE PATIENTS

Marija Pinterić^{2,1}, Iva Klobučar³, Margarete Lechleitner¹, Lidija Hofmann⁴, Matias Trbušić^{3,5}, Gudrun Pregartner⁶, Andrea Berghold⁶, Tobias Madl^{7,8}, Vesna Degoricija⁹, Saša Frank^{1,8}

¹Medical University of Graz Gottfried Schatz Research Centre, Division of Molecular Biology and Biochemistry, Graz, Austria ²Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia ³Sisters of Charity University Hospital Centre Department of Cardiology, Zagreb, Croatia ⁴FH JOANNEUM University of Applied Sciences Institute of Biomedical Science, Department of Health Studies, Graz, Austria ⁵University of Zagreb, School of Medicine Internal medicine, Zagreb, Croatia ⁶Medical University of Graz Institute for Medical Informatics, Statistics and Documentation, Graz, Austria ⁷Medical University of Graz Otto Loewi Research Centre, Division of Medicinal Chemistry, Graz, Austria ⁸BioTechMed-Graz BioTechMed-Graz, Graz, Austria ⁹Sisters of Charity University Hospital Centre Department of Medicine, Zagreb, Croatia

Acute heart failure (AHF) is a syndrome defined as the new onset or worsening of symptoms and signs of heart failure, mostly related to systemic congestion. AHF is associated with high short- and long-term mortality, and early identification of patients at highest risk remains challenging despite the use of established biomarkers and clinical risk scores. Oxidative stress is an important pathophysiological pathway in the development and progression of AHF; however, it has been insufficiently investigated as a prognostic target. This study evaluates the prognostic value of specific serum oxidative stress biomarkers for predicting short- and long-term mortality in hospitalized AHF patients, determining whether they enhance risk stratification beyond current established tools. Admission serum levels of malondialdehyde (MDA), advanced oxidation protein products (AOPPs), catalase, and superoxide dismutase (SOD) were measured in a well-characterized cohort of 315 hospitalized AHF patients. Univariable and multivariable logistic regression analyses were conducted to determine the associations of measured oxidative stress markers with in-hospital, 6-month, and 12-month mortality. Prognostic performance was compared with N-terminal pro-brain natriuretic peptide (NT-proBNP) and established risk scores (ADHERE, GWTG-HF, OPTIMIZE-HF) using receiver operating characteristics curves, the continuous net reclassification index (cNRI) and decision curve analysis. Our results show that serum MDA, catalase, and SOD levels were significantly elevated in non-survivors across all evaluated clinical time points. Following rigorous adjustment for confounding clinical variables in multivariable models, MDA emerged as the only oxidative stress biomarker independently associated with mortality in AHF patients. MDA showed robust prognostic performance, with improvements in AUC and cNRI as well as increased net benefit, when added to NT-proBNP or clinical scores. Survival analysis confirmed lower survival in patients with MDA ≥ 1.45 nmol/L (the median in our cohort). Conversely, AOPPs, catalase, and SOD exhibited limited long-term prognostic utility. In conclusion, this study shows that serum MDA level serves as a potent, independent predictor of short- and long-term mortality in AHF, significantly improving risk classification when combined with existing prognostic biomarkers and clinical scores.

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SEMINAL PLASMA METALS, ACCESSORY GLAND BIOCHEMICAL MARKERS AND CASA-DERIVED SPERM KINEMATIC PARAMETERS IN MEN WITH SUSPECTED INFERTILITY

Alica Pizent¹, Tanja Živković Semren¹, Blanka Tariba Lovaković², Kristijan Peroš³

¹*Institute for Medical Research and Occupational Health, Division of Occupational and Environmental Health, Zagreb, Croatia* ²*Institute for Medical Research and Occupational Health Division of Toxicology, Zagreb, Croatia* ³*Clinical Hospital Merkur University Clinic Vuk Vrhovac, Zagreb, Croatia*

Seminal plasma contains trace elements and biochemical markers originating from male accessory reproductive glands that may contribute to sperm motility and function. However, their relationships with computer-assisted sperm analysis (CASA)-derived sperm kinematic parameters remain incompletely characterized. This cross-sectional study included 113 men attending a reproductive medicine centre due to suspected infertility. Seminal plasma concentrations of zinc (SpZn; median 120,375 µg/L), copper (SpCu; median 81.40 µg/L), and selenium (SpSe; median 40.70 µg/L) were determined alongside biochemical markers of accessory gland secretory function including alpha-glucosidase (ACPH, epididymis), citric acid (CITRA, prostate), fructose (FRUCT, seminal vesicles), and lactate dehydrogenase- X (LDHx, spermatogenic cells). Serum reproductive hormones (FSH, LH, testosterone, estradiol, prolactin, and TSH) were also assessed. CASA kinematic profiling included VAP, VSL, VCL, ALH, BCF, STR, LIN, ELO, and ARE. Spearman rank correlations with false discovery rate (FDR) correction ($p < 0.05$) were used to examine associations, while linear regression identified independent predictors of kinematic parameters. SpZn was strongly associated with ACPH ($r = 0.744$, $p < 0.001$) and CITRA ($r = 0.631$, $p < 0.001$), suggesting that seminal zinc reflects overall accessory gland secretory capacity rather than a single glandular source. SpCu was a significant positive predictor of sperm straightness (STR) in simple linear regression ($\beta = 0.048$, $p = 0.038$), with borderline significance after adjustment for reproductive hormones ($p = 0.064$). FRUCT was negatively correlated with lateral head displacement (ALH; $r = -0.323$, $p = 0.003$), indicating an inverse association between seminal vesicle secretory activity and flagellar oscillation amplitude. LDHx correlated negatively with serum FSH ($r = -0.293$, $p = 0.004$), while SpSe was inversely associated with estradiol ($r = -0.232$, $p = 0.048$). These findings suggest differential associations between seminal plasma constituents and specific CASA kinematic parameters, with SpCu emerging as a predictor of sperm movement linearity and FRUCT as an indicator of flagellar kinematics. Supported by European Union – Next Generation EU (Reg. no. 533-03-23-0006), HumEnHealth and BioMolTox, and performed using the facilities and equipment funded within the European Regional Development Fund project KK.01.1.1.02.0007.

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C3 N-GLYCOPEPTIDES ARE CORRELATED WITH METABOLIC AND INFLAMMATORY MARKERS IN PREGNANCY

Branimir Plavša¹, Marko Dužević¹, Jelena Omazić², Jasenka Wagner³, Olga Gornik¹

¹University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia ²"Dr. Juraj Njavro" National Memorial Hospital, Department of Laboratory Diagnostic, Vukovar, Croatia ³Josip Juraj Strossmayer University, Faculty of Medicine Department of Medical Biology and Genetics, Osijek, Croatia

Gestational diabetes mellitus (GDM) is defined as diabetes diagnosed in second or third trimester and is characterized by an increase in glucose production and decreased peripheral insulin sensitivity combined with its insufficiently elevated production. Previous research has shown that N-glycosylation of total plasma proteins as well as specific immune-associated proteins, such as IgG, AGP and C3, is associated with different diabetes subtypes. To further increase understanding of GDM, and its relation to other diabetes subtypes, we analyzed C3 N-glycosylation in a cohort of 152 pregnant women, 78 with GDM and 74 without GDM as controls. Blood plasma samples were taken in the third trimester. Site-specific N-glycan profiles of C3 were obtained by enriching C3 using lectin affinity chromatography, followed by Glu-C proteolysis, after which the resulting (glyco)peptide mixture was separated by UPLC-MS. Nine oligomannose C3 N-glycopeptides from two N-glycosylation sites (Asn963 and Asn917) were quantified. After data processing and batch correction, linear models were used to examine associations between disease status and different metabolic and inflammatory markers, including glucose, HbA1c, insulin, c-peptide, blood lipids, CRP, IL-6, IL-10 and TNF-alpha. To account for multiple hypothesis testing all p-values were adjusted using the Benjamini-Hochberg method. Significant associations were observed between C3 N-glycosylation and triglycerides, insulin, CRP and HDL. Man8 and Man9Glc on the Asn917 site were associated with triglycerides ($p = 0.01$, $p = 0.005$ respectively), and insulin levels ($p = 0.1$ for both glycopeptides). Man5 on the Asn63 site was associated with CRP levels ($p = 0.04$), while Man6 and Man7 from the same site were associated with HDL ($p = 0.04$ for both glycopeptides). These results are consistent with our previous research on IgG and total plasma protein N-glycosylation in GDM, in which no direct association between glycans and disease status was found; however, associations with metabolic and inflammatory markers were demonstrated. This, along with our previous studies in the general population as well as in young women, which also found the same C3 associations as this current research, indicates a high potential of C3 glycopeptides as biomarkers of metabolic and inflammatory status.

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INTERPLAY OF SPRTN AND p97 IN RESOLVING PROTEIN ADDUCTS

Marija Pranjić, Cecile Otten, Marin Kutnjak, Leona Verasto, Marta Popović

Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia

DNA-protein crosslinks (DPCs) are highly toxic lesions (occurring at frequency of ~6000 per cell per day) that obstruct replication and transcription and must be efficiently resolved to maintain genome stability. The zinc metalloprotease SPRTN plays a central role in replication-coupled DPC repair. AAA+ ATPase p97/VCP is a key component of ubiquitin-proteasome system and is involved in a numerous processes by extracting K48-polyubiquitinated substrates from various cellular compartments. It is known that p97 participates in DNA damage response through chromatin-associated degradation and regulation of double-strand break repair, however its role in DPC repair and its regulation by adaptor proteins remains less well understood. In particular, the functional importance of the SPRTN-p97 interaction in DPC resolution remains poorly characterized. Here, we investigate how disruption of the SPRTN-p97 interaction affects DPC formation and repair using complementary cellular and *in vivo* models. In human HEK293T cells, we employ mutants in the conserved SHP motif of SPRTN, which mediates binding to p97, and assess their impact on DPC formation. Furthermore, we use a zebrafish embryo model (6 hours post fertilization), in which endogenous SPRTN is silenced and subsequently rescued by injection of either wild- type or SHP motif-mutated SPRTN mRNA. Our results will contribute to a deeper understanding of DPC repair pathways and may, in the future, support the development of anticancer strategies targeting the SPRTN-p97 interaction interface.

Roberta Stevanović, Marko Močibob, Valentina Ević, Jasmina Rokov-Plavec

University of Zagreb, Faculty of Science, Division of Biochemistry, Department of Chemistry, Zagreb, Croatia

Aminoacyl-tRNA synthetases (aaRSs) establish the biochemical basis of the genetic code by creating aminoacylated tRNAs which enable the ribosome to translate the language of nucleotides to the language of amino acids. Here, noncanonical role of two aaRSs in oxidative stress is presented. Surprisingly, a unique disulfide bond was identified in the crystal structure of *Arabidopsis thaliana* cytosolic seryl-tRNA synthetase (SerRS) involving cysteines evolutionarily conserved in all green plants. Cysteines Cys213 and Cys244 showed opposing effects on the thermal stability of plant SerRS. Structural features of the protein microenvironment were identified which influence the reactivity of Cys213 and Cys244 and promote the formation of the disulfide link in the oxidizing conditions. The activity of the wild-type protein showed resistance to oxidation with H_2O_2 , while activities of the cysteine-to-serine mutants were impaired. The disulfide link may act as a sink for reactive oxygen species and enable the protein to function under oxidative stress conditions which can be beneficial for an efficient plant stress response. Although the incorporation of incorrect amino acids into proteins is associated with negative consequences, in some cases cells can benefit from this process. A strain of *Escherichia coli* expressing isoleucyl-tRNA synthetase with a non-functional error-repair domain was grown under mistranslating conditions which enabled adaptation of bacteria to oxidative stress. In order to identify cellular mechanisms involved in this unexpected adaptation, differential protein expression was analyzed in mistranslating and wild type strain using quantitative mass spectrometry. Mistranslation at isoleucine positions caused increased expression of chaperones, proteases, proteins involved in peptide uptake and arginine biosynthesis, while expression of proteins involved in the biosynthesis of enterobactin, a siderophore that enables iron transport, was decreased. Increased expression of the RecA protein and decreased expression of the LexA repressor were observed, suggesting that the SOS response is activated. Considering that the human immune system fights pathogenic bacteria via reactive oxygen species, understanding the impact of mistranslation on bacteria and their response to oxidative stress may reveal new mechanism of bacterial evasion of our immune system.

FUNDAMENTAL TRANSCRIPTOMIC AND METABOLOMIC DIFFERENCES BETWEEN KALE (*Brassica oleracea* var. *acephala*) AND ITS WILD RELATIVE (*Brassica incana*)

Kristina Majsec¹, Zrinka Karačić¹, Nikola Major², Tvrtko K. Kovačević², Iva Orehovec¹, Nenad Jasprica³, Smiljana Goreta Ban², Branka Salopek Sondi¹

¹Ruđer Bošković Institute, Department for Molecular Biology, Zagreb, Croatia ²Institute for Agriculture and Tourism, Poreč, Croatia ³University of Dubrovnik, Institute for Marine and Coastal Research, Dubrovnik, Croatia

Brassicaceae encompass many economically important species (cabbage, broccoli, bok choy, kale etc.) that are grown worldwide and have traditionally been consumed in the human diet. The health benefits of Brassica vegetables for human population are mainly attributed to their abundance in “healthy phytochemicals” (glucosinolates, carotenoids, phenols), which has led to their recognition as functional foods. In the last couple of years, kale has gained significant popularity, not only due to health benefits for humans but also because of its remarkable tolerance to abiotic and biotic stresses, especially amid extreme climate change events. Brassica wild relatives, which are morphologically similar to leafy kale, are wild cabbages (*B. incana*, *B. insularis*, *B. macrocarpa*, *B. montana*, *B. rupestris*, etc.). They inhabit marine cliffs and sandy, barren rocks, and are able to survive in extremely unfavorable environmental conditions of increased salinity, high temperature, and drought. Herein, we aim to identify fundamental, genetically determined differences in metabolomic and transcriptomic profiles of kale (*B. oleracea* var. *acephala*) and its close wild relative, wild cabbage (*B. incana*), when grown in the same control conditions. Several accessions of kale and wild cabbage were selected and examined. The underlying differences in metabolomic and transcriptomic profiles of kale and wild cabbage accessions were discussed. Wild cabbage accessions are enriched in upregulated genes involved in glucosinolate biosynthesis when compared to cultivated kale. On metabolomic level, wild cabbage is particularly rich in aliphatic, long chain glucosinolates (4C and 5C). This comparative research between kale and its close wild relative *B. incana* was conducted to generate a dataset that will serve as foundational knowledge for further research focused on wild cabbage. *B. incana* can become a plant model for elucidating tolerance mechanisms against abiotic and biotic stress in Brassicas and can be seen as a potential gene pool for breeding programs.

HELMINTH-DERIVED ANTIMICROBIAL PEPTIDES: ROLE OF THE UNSTRUCTURED DISULFIDE-BRIDGED C-TERMINUS

Iva Stojan¹, Anamarija Budimir¹, Korina Primorac², Andrea Caporale³, Lucija Krce¹, Marija Raguž⁴, Sabrina Pacor⁵, Alessandro Tossi⁵, Tomislav Rončević², Larisa Zoranić¹

¹University of Split, Faculty of Science, Department of Physics, Split, Croatia ²University of Split, Faculty of Science, Department of Biology, Split, Croatia ³CNR Institute of Crystallography, Trieste, Italy ⁴University of Split, School of Medicine Department of Medical Physics and Biophysics, Split, Croatia ⁵University of Trieste Department of Life Sciences, Trieste, Italy

In light of rapidly increasing prevalence of multidrug-resistant microbial strains over recent decades, antimicrobial peptides (AMPs) have emerged as a promising alternative to conventional therapies. These small yet structurally diverse molecules are ubiquitous in nature and exhibit strong antimicrobial as well as immunomodulatory properties. A key advantage of AMPs lies in their rapid and multifaceted mechanisms of action, which are associated with a comparatively low incidence of microbial resistance. Elucidating the structural determinants of AMP activity is therefore essential for their potential clinical use. In this study, structural and functional characteristics of helminth-derived AMPs, namely mesco-2 and mesco-2A, were examined. Mesco-2, derived from the flatworm *Mesocostoides corti*, has an N-terminal α -helix kinked around a palindromic GRGIGRG sequence and an unstructured C terminus containing a disulfide-bridged CLGRC motif, whose function remains poorly understood. Its analogue, mesco-2A, lacks the disulfide bridge within CLGRC loop. Molecular modelling indicated that disulfide bridge within C terminus influences both the flexibility and oligomerization of mesco-2. Both peptides exhibited similar strong antibacterial activity against Gram-positive and -negative strains coupled with low cytotoxicity. Differences were observed in growth kinetics of *E. coli* and *S. aureus* at sub-bactericidal concentrations. Flow cytometry and fluorescence microscopy supported a membrane-associated mode of action for both peptides, with mesco-2A displaying a slower effect. Atomic force microscopy further indicated distinct modes of membrane interaction, while circular dichroism in anionic liposomes revealed differences in peptides' secondary structures. Microscale thermophoresis (MST) confirmed variations in liposome binding behavior indicating that mesco-2A likely interacts as monomers, whereas mesco-2 forms higher-order assemblies, consistent with molecular modelling results. Additionally, a novel MST protocol was developed, employing fluorescently labelled live bacterial cell membranes as targets and AMPs as ligands. This approach enabled quantification of membrane-binding interactions between AMPs and intact bacteria, yielding binding affinities in the low micromolar range for native membrane bilayers. Overall, these findings suggest that the unstructured C-terminal tail is a crucial modulatory element in mesco-2, governing both conformational adaptability and collective membrane interaction, representing a tuneable element for peptide engineering to modify the speed, strength, and cooperativity of antimicrobial action.

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MOLECULAR PROCESSES SHAPING THE *Saccharomyces cerevisiae* CELL WALL AND ENABLING PROTEIN DISPLAY

Igor Stuparević, Romana Ivković, Antonia Paić, Tea Martinić Cezar, Bojan Žunar, Renata Teparić

University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Biochemistry, Zagreb, Croatia

The *Saccharomyces cerevisiae* cell wall is a dynamic, multi-layered extracellular structure essential for maintaining cellular integrity and mediating environmental interactions. This structure consists of an internal β -1,3-glucan and chitin network that provides mechanical strength, and an outer layer of mannoproteins covalently or non-covalently bound to the polysaccharide matrix. Our research identifies a sophisticated network of molecular processes that shape this wall, ranging from post-translational proteolytic maturation to pervasive non-coding RNA regulation. A critical mechanism in cell wall remodelling involves the proteolytic processing of structural proteins. We demonstrate that the glycoside hydrolase Scw4 undergoes sequential cleavage by the trans-Golgi protease Kex2 and cell surface yapsins. This maturation not only activates the enzyme but also acts as a regulatory switch, significantly reducing the protein's potential for covalent attachment to glucan, thereby facilitating structural flexibility. Furthermore, the stability of the cell wall is governed by the nuclear RNA exosome. Inactivation of exosome subunits (Rrp6, Dis3) leads to osmoremedial cell lysis at high temperatures due to defects in protein glycosylation, specifically through the down-regulation of the mannosylation precursor enzyme Psa1 caused by the accumulation of non-coding transcripts. The cell wall architecture is further refined by long non-coding RNAs (lncRNAs), which control the expression of approximately 44% of cell wall-related genes through transcriptional interference and chromatin remodelling. For example, at the *PHO5* locus, antisense transcription facilitates the recruitment of the Rpd3 histone deacetylase, leading to a more repressive chromatin state that decreases the association of the RSC remodelling complex and consequently delays promoter opening during gene induction. Understanding these native immobilisation and regulatory mechanisms provides a robust foundation for Yeast Surface Display (YSD). By leveraging specialised anchors such as the Pir family (alkali-labile ester linkages) or Ccw12 (GPI anchors), we have developed quantitative reporter systems using β -lactamase to measure display capacity and enzymatic activity in the cell's native environment. These insights transform the yeast cell wall into a programmable, living catalytic material suitable for diverse biotechnological and industrial applications. Current research is focused on utilising the probiotic strain *Saccharomyces boulardii* to express and display sweet proteins on the cell surface. This strategy not only circumvents the need for costly protein purification but also allows for the direct application of yeast cells in functional food formulations, combining high-intensity natural sweetness with probiotic benefits.

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MODULATION OF ERK SIGNALING FACILITATES FUNCTIONAL MITOCHONDRIAL TRANSFER

Iva Suman, Antonijo Grčić, Ana-Marija Budeš, Lidija Šimić, Roberta Ogrizović, Robert Domitrović

University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia

Mitochondria are essential for cellular metabolism, primarily driving aerobic respiration and ATP synthesis. Their dysfunction is implicated in numerous pathologies, including cancer, metabolic disorders, and degenerative diseases. In recent years, intercellular mitochondrial transfer has emerged as a promising mechanism for restoring energy homeostasis and rescuing compromised cells. While the extracellular signal-regulated kinase (ERK) pathway is known to regulate cytoskeletal dynamics and localize in mitochondrial membranes, its specific role in the process of mitochondrial transfer remains unexplored. This study investigates whether ERK activation status influences the efficacy and functional outcome of mitochondrial transfer. We established *in vitro* mitochondrial transfer models using HEK293, HK-2, TH-1, and HeLa cells. To assess the functional relevance of ERK modulation, we evaluated transfer success under conditions of altered ERK activity by monitoring several key metabolic and functional parameters. These included glucose consumption and lactate production to assess metabolic shifts toward glycolysis or oxidative phosphorylation, JC-1 dye staining to determine the mitochondrial membrane potential ($\Delta\Psi_m$) of transferred mitochondria—where a red-to-green fluorescence shift indicates loss of functional integrity—and expression profiling of multiple proteins involved in mitochondrial dynamics, stress response, and signaling, specifically HSP-60, TOM20, p-ERK, ERK, TFAM, VDAC3, p-PDH, PDH, p-AMPK, Bcl-2, and Bax. Our results indicate that ERK-dependent mechanisms govern both the efficiency of mitochondrial transfer and the functional integrity of donated organelles. Understanding this pathway not only advances fundamental knowledge of intercellular communication but also holds critical translational value for developing safe and effective mitochondrial transfer-based therapies for diseases linked to mitochondrial dysfunction, including cancer and other bioenergetic disorders.

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SATELLITE DNAs AND TRANSPOSABLE ELEMENTS IN *Ostreidae*: ORGANIZATION, EVOLUTION, AND INTERPLAY

Monika Tunjić Cvitanić¹, Patrik Majcen¹, Matej Kristan Mirković¹, Daniel García-Souto^{2,3}, Juan Pasantes², Miroslav Plohl¹, Eva Šatović Vukšić¹

¹*Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia* ²*Universidade de Vigo Centro de Investigación Mariña, Vigo, Spain* ³*Universidade de Santiago de Compostela Centre for Research in Molecular Medicine and Chronic Diseases, Santiago de Compostela, Spain*

Segments of the genome enriched in repetitive DNA sequences, including satellite DNAs (satDNAs) and transposable elements (TEs), are often referred to as the “dark matter” of the genome. Research on all aspects of satDNAs and TEs is rapidly expanding, as they are increasingly recognized as important structural and functional components of the genome, as well as key drivers of genome evolution. Using a combination of experimental and bioinformatic approaches, we show that oysters represent useful model organisms for studying repetitive DNA. We characterized the satellitomes of six species from the *Ostreidae* family, revealing a high number of satDNAs per genome (33 – 61) and several unusual features of their organization. Oysters exhibit highly interspersed patterns of satDNA distribution throughout the genome, which deviate from conventional models in which satDNAs typically form long arrays that predominantly constitute heterochromatic chromosomal compartments. Furthermore, a substantial proportion of satDNAs were found to be associated with, or derived from, TEs. Notably, the most abundant satDNAs exhibit simultaneous coexistence of multiple organizational states (standalone arrays, Helitron-associated arrays, and intermediate forms), representing a novel level of structural plasticity. This feature appears to be evolutionarily conserved within the family. Helitron-related satDNAs dominate both in chromosomal occupancy and monomer abundance, suggesting that these TEs play a major role in the propagation and genome-wide dispersal of satDNAs. In addition, we demonstrate that satDNAs and TEs in oysters are tightly interconnected, forming networks and complex loci shaped by various genomic rearrangements. We propose that even highly fragmented and degraded TE remnants within heterochromatic “TE graveyards” may serve as a source of novel composite satDNA monomers, effectively transforming these genomic regions into DNA “recycling sites”.

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COMPARISON OF BLOOD CULTURE AND NOVEL PCR - BASED PATHOGEN DETECTION IN SUSPECTED SEPSIS

Lidija Šimić^{1,2}, Iva Marinović¹, Mia Krapić¹, Dunja Vrbanić, Mate Lerga^{1,3}, Martina Pavletić^{1,3}

¹Clinical Hospital Center Rijeka Emergency Department, Rijeka, Croatia ²University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia ³University of Rijeka, Faculty of Medicine, Department of Anesthesiology, Reanimatology, Emergency and Intensive Care Medicine, Rijeka, Croatia

Introduction: Sepsis is a life-threatening condition associated with high morbidity and mortality, and its early recognition in the emergency department (ED) is critical for timely initiation of appropriate therapy. However, the long turnaround time and suboptimal sensitivity of blood cultures, the current gold standard, limit early clinical decision-making and support the use of faster diagnostic approaches such as polymerase chain reaction (PCR). The aim was to compare conventional microbiology and PCR in patients with suspected sepsis, focusing on time to pathogen detection and positivity rate. **Methods:** This study was conducted as part of a prospective study in the ED of a tertiary hospital during 2025-2026. Adult patients (N=91) with suspected sepsis were included, with simultaneous blood sampling for blood cultures and multiplex PCR testing. Primary outcomes were time to pathogen detection and positivity rate, while secondary outcomes included the diagnostic performance of PCR compared with blood cultures and concordance between methods. **Results:** PCR significantly reduced time to pathogen detection (median 9 vs. 123.7 h, $p<0.001$), with a minimum turnaround time of 4.5 h. Blood cultures were positive in 34 patients, while PCR detected pathogens in 28 patients, with 22 overlapping cases. PCR identified pathogens in 6 culture-negative patients further supported by additional microbiological and clinical findings. Sensitivity and specificity of PCR were 65% and 90%, respectively. Although limited by panel coverage, PCR identified antimicrobial resistance genes in 71.4% of cases where target genes were present. **Conclusions:** PCR provides substantially faster pathogen detection and may improve detection rates in suspected sepsis. Despite moderate sensitivity, its high specificity supports its use as a rule-in tool in the emergency department, facilitating earlier recognition and clinical decision-making. These findings support the complementary use of PCR and blood cultures to enhance overall diagnostic accuracy. **Keywords:** Blood culture; Diagnostic accuracy; Emergency department; PCR; Pathogen detection; Sepsis;

EVALUATION OF NOVEL PYRIDINIUM OXIMES USING AN *IN SILICO* ANALYSIS

Goran Šinko¹, Kamil Musilek², David Malinak², Maja Katalinić¹, Dora Kolić¹, Petra Tuksar¹

¹*Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia* ²*University of Hradec Králové Faculty of Science, Hradec Králové, Czechia*

Molecular docking and in silico analysis were used to analyse a group of new class of pyridinium oximes for their reactivation potential, CNS (central nervous system) activity and BBB (blood–brain barrier) permeability. The studied compounds differ in molecular mass and structure of the linker connecting two pyridinium rings. A molecular docking study indicated key interactions between the tested novel pyridinium oximes and residues of the lipophilic active site of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Additionally, a comparison of interaction energies obtained for pyridinium oximes and the known anticholinesterases was performed. Moreover, ADME (absorption, distribution, metabolism and excretion) properties in combination with the physicochemical properties (molecular weight, lipophilicity coefficient, number of hydrogen bond donor and acceptor atoms, number of rotational bonds and polar surface area) of the tested pyridinium oximes were compared to values for CNS-active drugs to estimate the BBB (blood- brain barrier) permeability, since standard oximes used in OP poisoning therapy do not cross the BBB. Our results can be applied in the design of new oximes with improved reactivation efficacy and broad activity toward various different OP compounds. This work was funded by project IPCZ-2025-04-6205.

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DETERMINANTS OF MITOCHONDRIAL IMPORT AND INTRAMITOCHONDRIAL POSITIONING OF NME6 RESOLVED BY STED MICROSCOPY

Maja Šutić², Bastien Proust¹, Lea Vrbančić², Nikolina Škrobot Vidaček¹, Valentina Štimac^{1,2}, Lovro Gudlin¹, Iva Marija Tolić¹, Maja Herak Bosnar²

¹*Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia* ²*Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia*

NME6 is a member of the nucleoside diphosphate kinase (NDPK/NME) family, whose proteins contribute to nucleotide homeostasis, membrane dynamics, intracellular signaling, and suppression of metastatic progression. In contrast to the well-characterized, catalytically active NME1–4 proteins, the function of NME6 remains poorly defined. Our previous work showed that recombinant NME6 is predominantly monomeric, lacks measurable NDPK activity under standard *in vitro* conditions, and localizes to mitochondria. Its overexpression reduced oxidative phosphorylation, and RCC1L was identified as a potential interacting partner, suggesting a role in mitochondrial ribosome biogenesis or gene expression. In this study, we used stimulated emission depletion (STED) microscopy to visualize NME6 with enhanced spatial resolution and to clarify its submitochondrial localization and import determinants. Expression constructs were generated using pSNAPf and pHTC-HaloTag vectors. Wild-type NME6-186 was analyzed in live cells by dual-color STED microscopy together with markers of defined mitochondrial subcompartments. SNAP-tagged NME6 was labeled with SiR-647, and Halo-tagged markers were visualized using JF549. Localization was assessed relative to outer membrane, inner membrane, matrix markers, and RCC1L. To identify sequence elements required for mitochondrial targeting, three additional NME6 variants were generated: an N-terminal deletion lacking residues 1–7, a multiple arginine-to-alanine substitution mutant (R58A, R71A, R96A, R103A, R106A, R108A), and a combined construct carrying both modifications. These variants were designed to test the contribution of the N-terminal region and internal basic residues to mitochondrial import. STED microscopy provided substantially improved resolution of mitochondrial structures and protein distribution compared with confocal imaging. The analyses excluded NME6 localization at the outer mitochondrial membrane and revealed partial colocalization with inner membrane and matrix-associated markers, consistent with previous findings. Ongoing experiments aim to refine its precise submitochondrial position. Comparison of wild-type and mutant constructs is expected to determine whether the N-terminal segment, internal arginine residues, or both are required for efficient mitochondrial targeting. Overall, these results demonstrate the value of STED microscopy for high-precision analysis of submitochondrial protein localization and provide new insight into the targeting mechanisms and potential mitochondrial functions of NME6.

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MULTIMODAL BENEFICIAL EFFECTS OF DHEA(S) IN THE MOUSE MODEL OF ALZHEIMER'S DISEASE

Dubravka Švob Štrac¹, Ana Knezović², Csenge Solyomvari³, Gordana Nedić Erjavec¹, Szidonia Farkas³, Matea Nikolac Perković¹, Jelena Osmanović Barilar², Barbara Čupić¹, Tina Ćurković¹, Dora Zelena³

¹Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia ²University of Zagreb, School of Medicine, Department of Pharmacology, Zagreb, Croatia ³School of Medicine, University of Pecs Institute of Physiology, Pecs, Hungary

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia, affecting approximately 50 million individuals worldwide. AD is pathologically defined by the accumulation of extracellular amyloid- β (A β) plaques and intracellular hyperphosphorylated tau neurofibrillary tangles, which lead to synaptic dysfunction, neuronal loss, cognitive decline and behavioral disturbances. In addition to these classical hallmarks, AD is increasingly recognized as a multifactorial disorder involving several interconnected pathogenic mechanisms, including cholinergic dysfunction and neuroinflammation. Despite substantial advances in understanding its pathology, current therapies primarily provide symptomatic relief and fail to halt or reverse disease progression, emphasizing the need for novel therapeutic approaches. Endogenous neurosteroids, including dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), have been shown to promote neuronal survival, plasticity, and synaptic function; however, their levels are reduced in patients with AD. To investigate the neuroprotective effects of DHEA(S), we employed a pharmacologically induced mouse model of AD using male C57BL/6 mice that received intracerebroventricular (icv) injection of A β peptide. The effects of DHEA(S) (10 mg/kg) administered intraperitoneally (ip) to mice were assessed for up to one month. Behavioral analysis revealed that A β -injected mice exhibited impaired memory performance compared to control group, as assessed by the passive avoidance test and the novel object recognition test. In contrast, administration of DHEA(S) had positive effect on memory. The levels of selected neuropathological markers in the mouse hippocampus homogenates and brain slices were determined post-mortem. A β exposure induced neurotoxic effects on the cholinergic system and triggered time-dependent activation of microglia and astrocytes. Treatment with DHEA(S) preserved cholinergic fiber density and modulated the inflammatory responses of glia cells. Furthermore, the results suggested that the effects of DHEA(S) are mediated, at least in part, through PI3K/Akt signaling pathway, a key regulator of cell survival and neuroplasticity. These findings demonstrate the multimodal neuroprotective effects of DHEA(S), supporting their potential as promising therapeutic candidates for AD. Nevertheless, further studies are required to better characterize the multiple effects of DHEA(S), elucidate the precise molecular mechanisms underlying these actions and evaluate their translational potential.

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EXCHANGE OF MITOCHONDRIA BETWEEN YOUNG AND SENESCENT FIBROBLASTS RESHAPES CELLULAR PHENOTYPE

Ana Tadijan¹, Martina Deželjin¹, Barbara Nikolić², Ivica Rubelj¹

¹*Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia* ²*University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia*

Cellular senescence contributes to tissue ageing not only through cell-autonomous changes, but also through communication with neighboring cells, primarily mediated by the senescence-associated secretory phenotype (SASP). In this study, we propose intercellular mitochondrial transfer as a potential additional mechanism contributing to the spread of senescence. We investigated interactions between young and aged human dermal fibroblasts, with a particular focus on mitochondrial exchange and its possible role in intercellular signalling during ageing. Replicative ageing models of human dermal fibroblasts were established and characterized by tracking population doubling, senescence-associated β -galactosidase activity, cell morphology, and mitochondrial content. Aged fibroblast cultures exhibited an increased proportion of senescent cells, enlarged and more granular morphology, and elevated mitochondrial signal compared with younger counterparts. To investigate intercellular mitochondrial transfer, young and aged fibroblasts were labelled using two complementary approaches: chemical labelling with mitochondrial dyes and stable lentiviral expression of fluorescently labelled mitochondria, and analysed using fluorescence microscopy and flow cytometry. Microscopy revealed the presence of mitochondria within intercellular extensions and in recipient cells, consistent with transfer between cells. Expectedly, in co-culture of young and aged fibroblasts, aged cells displayed a shift towards a more youthful mitochondrial phenotype, whereas young cells showed features associated with an aged mitochondrial phenotype. The contribution of contact-dependent mechanisms was assessed using actin-disrupting agents and co-culture inserts to distinguish direct cell–cell interactions from soluble factor-mediated communication. Notably, our results also indicate that mitochondrial dyes can exhibit signal leakage, which may confound the interpretation of transfer events, underscoring the importance of complementary approaches. In conclusion, our results suggest that mitochondrial exchange may represent an additional layer of intercellular communication contributing to the spread of ageing-associated phenotypes in fibroblast populations.

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IMPACT OF SUBCELLULAR ENZYME LOCALIZATION ON CAROTENOID BIOSYNTHESIS IN *S. cerevisiae*

Renata Teparić, Antonia Paić, Kristijan Vuraić, Romana Ivković, Tea Martinić Cezar, Igor Stuparević, Bojan Žunar

University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory of Biochemistry, Zagreb, Croatia

Carotenoids are pigmented compounds valued for their antioxidant properties and are used as food colourants and dietary supplements. In *S. cerevisiae*, the native MVA pathway provides precursors that are condensed into farnesyl pyrophosphate (FPP) and subsequently geranylgeranyl pyrophosphate (GGPP). These precursors, when combined with heterologous *crt* genes, can be channelled toward carotenoid biosynthesis. Phytoene synthase (*crtB*) converts GGPP into phytoene, which phytoene desaturase (*crtI*) then converts to lycopene, to be subsequently cyclised into β -carotene by *crtY*. Ketolation by *crtW* yields canthaxanthin and hydroxylation by *crtZ* yields zeaxanthin, while the combined activity of both enzymes is required to produce astaxanthin. To enhance carotenoid production, yeast strains have been engineered to increase metabolic flux through the MVA pathway and heterologous genes enabling transformed strains to channel FPP toward subsequent carotenoid production were introduced. However, accumulation of intermediates such as FPP can be cytotoxic. A key strategy to address this is subcellular compartmentalisation, where enzymes are targeted to specific organelles to improve efficiency, reduce toxicity, and promote substrate channeling. This study explored three strategies: targeting enzymes to the endoplasmic reticulum (ER), Zera-bodies, and lipid droplets (LDs). Targeting to the ER was achieved by adding an N-terminal Kar2-derived signal peptide and a C-terminal HDEL ER retention motif. Zera is a synthetic peptide containing an ER signal peptide and a proline-rich region that induces the formation of self-assembling ER-derived vesicles, which can encapsulate fused enzymes and create artificial microcompartments retained in the ER. For targeting to LDs, fusion tags derived from the LD-associated protein oleosin were used. Yeast transformants carrying different combinations of the engineered plasmids exhibited varying efficiencies in carotenoid biosynthesis. The results indicate that adding the Zera sequence to *crtYB*, *crtW*, and *crtZ* did not significantly alter carotenoid levels compared to the control. However, incorporating the Zera sequence into *crtI* abolished pigment production. Targeting enzymes to LDs resulted in negligible carotenoid production. In contrast, targeting *crtW* and *crtZ* to the ER yielded up to a 35% increase in total carotenoid accumulation, highlighting ER localisation as the most effective strategy.

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POTENT PYRIDINIUM OXIMES AS REACTIVATORS OF NERVE AGENT- AND PESTICIDE-INHIBITED ACETYLCHOLINESTERASE

Petra Tuksar¹, Maja Katalinić¹, David Malinak², Kamil Musilek², Zrinka Kovarik^{1,3}, Goran Šinko¹

¹Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia ²University of Hradec Kralove, Faculty of Science, Department of Chemistry, Hradec Kralove, Czechia ³University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Organophosphorus compounds, including nerve agents and pesticides, inhibit acetylcholinesterase (AChE) through phosphorylation of the catalytic serine residue, resulting in accumulation of acetylcholine and disruption of cholinergic neurotransmission, ultimately leading to a life-threatening cholinergic crisis. Oxime-based compounds act as antidotes that restore AChE activity by nucleophilic attack on the phosphorylated enzyme. However, currently available oximes show variable efficacy depending on the specific organophosphate, and no universal reactivator has yet been identified. In this study, seven newly synthesized pyridinium oximes were evaluated for their ability to reactivate AChE inhibited by nerve agents sarin, cyclosarin, tabun, VX, and the pesticide fenamiphos. At 100 μM , two compounds showed high reactivation potency, restoring more than 90% of enzyme activity within 60 minutes for all tested inhibitors except fenamiphos and tabun. Both compounds achieved complete reactivation of VX-inhibited AChE within 10 minutes, with one oxime exhibiting a catalytic efficiency of approximately $90,000 \text{ M}^{-1} \text{ min}^{-1}$, clearly outperforming standard oximes HI-6 and obidoxime. Consistent with these findings, further characterization revealed that several compounds exhibit micromolar dissociation constants for AChE, indicating favorable interactions within the enzyme active site. The cytotoxicity of the compounds was evaluated in HepG2 (hepatocytes) and SH-SY5Y (neuronal) cells over a concentration range up to 400 μM within 24 hours. Overall, the oximes exhibited low cytotoxicity across the tested range. However, the two most potent reactivators showed moderate cytotoxicity in SH-SY5Y cells at concentrations above 200 μM . To complement the experimental findings, computational studies were performed on this novel class of pyridinium oximes, focusing on their predicted ability to cross the blood–brain barrier and exert central nervous system activity. Based on the obtained results, two oximes were identified as promising antidotes for sarin-, cyclosarin-, and VX-inhibited AChE, supporting their further development as therapeutics for organophosphate-induced neurotoxicity and highlighting the importance of future evaluation against additional organophosphorus pesticides.

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LINK BETWEEN DNA-PROTEIN CROSSLINKS AND NEURODEGENERATION: Tdp1-DEFICIENT ZEBRAFISH MODEL

Leona Verasto, Ivan Antičević, Cecile Otten, Marija Pranjić, Marta Popović

Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia

Interactions between DNA and proteins are dynamic and essential for replication, transcription, and DNA repair. Because of various endogenous and exogenous causes, proteins covalently and irreversibly bind to chromosomal DNA, forming DNA-protein crosslinks (DPCs). If DPCs are not properly repaired, they accumulate in cells, causing genome instability and cytotoxicity, which is associated with cancer, neurodegeneration, and aging. There are multiple mechanisms of DPC repair depending on DPC type. Here, we focus on tyrosyl-DNA phosphodiesterase 1 (TDP1), an enzyme involved in the processing of topoisomerase 1 (TOP1)-DPCs. Under physiological conditions, TOP1 relieves torsional stress by introducing single-stranded breaks (SSBs) during DNA replication and transcription. If TOP1 encounters DNA helical alterations, is in the vicinity of elevated aldehyde concentrations due to metabolic processes or after exposure to chemotherapeutics such as camptothecin, it becomes irreversibly bound to DNA and forms TOP1-DPCs. TDP1 hydrolyzes the bond between the tyrosine residue of TOP1 and the DNA 3'-phosphate, generating the 3'-OH end of DNA. However, it cannot remove the whole protein crosslink before upstream processing by proteases. A point mutation in TDP1 causes human syndrome, SCAN1 (spinocerebellar ataxia with axonal neuropathy 1). This autosomal recessive neurodegenerative disease is characterized by a slowly progressive ataxia and cerebellar atrophy with distal sensorimotor axonal neuropathy. Our aim is to determine molecular cause behind the neurodegeneration caused by TDP1 deficiency. To this end, we created TDP1-deficient zebrafish strain in which we analyse TOP1-DPC formation in neurons using the modified RADAR assay and immunostaining, DNA breaks and apoptosis. Neurodegenerative phenotypes in adult and old zebrafish are assessed in respect to swimming patterns, memory and learning ability using the ZANTIKS system.

P60

IDENTIFICATION OF NOVEL TARGET GENES ASSOCIATED WITH ACQUIRED RESISTANCE OF PRIMARY MELANOMA CELLS TO TARGETED THERAPY

Ignacija Vlašić¹, Anđela Horvat¹, Ana Tadijan², Christine Supina Pavić¹, Janja Josić¹, Neda Slade¹

¹*Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia* ²*Ruđer Bošković Institute Division of Molecular Biology, Zagreb, Croatia*

Melanoma is aggressive skin tumor prone to metastasis with frequent mutations in components of MAPK signaling pathway. Due to fast response, BRAF-mutated melanoma was initially treated with monotherapy targeting BRAF kinase (BRAFi), while lately therapy includes combination of inhibitors that target both BRAF and MEK kinases (BRAFi/MEKi). Although initially response to targeted therapy is promising, disease relapses due to acquired resistance to targeted therapy. It has been shown that acquisition of tumor cell resistance to treatment can be associated with altered expression levels of microRNAs (miRNAs). Nevertheless, the association of specific miRNA expression profile and acquired melanoma resistance to BRAFi or BRAFi/MEKi therapy has not been thoroughly investigated. Therefore, we performed comprehensive analysis of miRNA expression using NGS technology on primary melanoma cells that differ in sensitivity to targeted therapy. Differentially expressed miRNAs were determined via sRNAde toolbox between treatment-sensitive primary melanoma cells and melanoma cells with acquired resistance to BRAFi or BRAFi/MEKi targeted therapy. After identifying miRNAs and validating their altered expression using RT-qPCR, we checked which predicted target genes are regulated via selected miRNA using several tools such as TargetScan, mirDIP, miRDB and miRTarBase. After selection of target mRNA, their differential expression between primary cell lines was confirmed by RT-qPCR. We identified 700 miRNAs by miRNA-Seq, from which 58 were shown to be differentially expressed. We have chosen to validate the miRNAs whose expression between sensitive and resistant cells differs at least 5 times. Interestingly, we observed reduced expression of hsa-miR-96-5p in BRAFi and BRAFi/MEKi resistant cells compared to treatment-sensitive cells. Next, we checked which predicted target genes are regulated via differentially expressed miRNAs and identified *FOXO3* as hsa-miRNA-96-5p regulated gene. We validated differential expression of *FOXO3* and observed its increased expression in targeted therapy resistant cells compared to treatment-sensitive cells. Our results show that primary melanoma cells resistant to BRAFi or BRAFi/MEKi have higher expression of *FOXO3* gene, so our further research will be focused on unraveling molecular mechanisms of *FOXO3*-dependent acquisition of resistance to BRAFi or BRAFi/MEKi in melanoma cells.

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NME6 IN MITOCHONDRIA: KEY REGIONS FOR IMPORT AND THE INTERACTION NETWORK

Lea Vrbančić^{1,2}, Bastien Proust^{1,3}, Maja Jazvinščak Jembrek¹, Maja Šutić¹, Maja Herak Bosnar¹

¹Ruđer Bošković Institute, Laboratory for Protein Dynamics, Division of Molecular Medicine, Zagreb, Croatia ²University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia ³Ruđer Bošković Institute, Laboratory for Molecular Genetics, Division of Molecular Biology, Zagreb, Croatia

Nucleoside diphosphate kinases (NDPKs or NME proteins) are evolutionarily conserved proteins involved in essential cellular processes, including proliferation, differentiation, and nucleotide homeostasis. They are divided into two groups, with Group II members being more diverse and less characterized. NME6, a Group II member, localizes specifically to the mitochondrial matrix, likely in proximity to the inner membrane. Although it lacks a canonical mitochondrial targeting signal (MTS), NME6 is efficiently imported into mitochondria. To identify the key regions responsible for this import, we generated a series of GFP-tagged deletants and mutantants and analyzed their localization by confocal microscopy. Our results show that the N-terminal region and specific arginine residues are critical for mitochondrial internalization, suggesting that NME6 uses noncanonical signals for mitochondrial import. Given its mitochondrial localization, identifying the proteins that interact with NME6 is important for understanding its biological function. Our previous studies demonstrated the interaction of NME6 with the GTPase RCC1L, which has also been confirmed by two independent groups, indicating a possible role for NME6 in mitochondrial RNA maturation, ribosome assembly, and/or translation. In addition, the interaction between NME6 and RCC1L was shown to be essential for its enzymatic activity, distinguishing NME6 from other NME proteins. To further explore the function of NME6, we performed interactome analysis using co-immunoprecipitation (co-IP) followed by mass spectrometry (MS). This analysis revealed numerous potential NME6 interactors, providing a basis for better understanding its specific role in mitochondria. The most relevant MS hits, corresponding to mitochondrial proteins detected in NME6 co-immunoprecipitates, will be further validated by co-IP and Western blot analyses in NME6-overexpressing and wild-type cells. Altogether, these findings further characterize NME6 as a mitochondrial protein and provide new insight into the key regions involved in its import and its interaction network.

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3D SPHEROID ARCHITECTURE DRIVES SASP-LINKED SURVIVAL ADAPTATIONS IN OVARIAN CANCER

Nikolina Vučemilo Paripović, Tea Dragičević, Dora Raos, Maja Sabol

Ruđer Bošković Institute, Division of Molecular Medicine, Laboratory for Hereditary Cancer, Zagreb, Croatia

Ovarian cancer remains most lethal gynecological malignancies, with a five-year survival rate below 40 %. A hallmark of its dissemination is the detachment of tumor cells from the primary lesion into the peritoneal cavity, where they survive and spread as multicellular aggregates. This process is strongly supported by the tumor microenvironment, which includes fibroblasts, immune cells and mesothelial cells that collectively establish a pro-inflammatory state. Because of this anchorage-independent mode of metastasis, three-dimensional spheroid cultures—and their co-cultures with stromal and immune components—represent a highly relevant *in vitro* model for studying metastatic behavior and drug resistance in ovarian cancer. In this study, we employed monoculture and co-culture systems of ovarian cancer cell lines (OVCAR-8, OVSAHO and SKOV-3) and HOF fibroblasts to generate three-dimensional spheroids, in parallel with conventional two-dimensional adherent cultures used as a comparative reference. Spheroids were formed in ULA plates for 72 hours for two purposes: (1) RNA isolation and transcriptomic profiling, and (2) fixation and cryosectioning for immunostaining. Tumor monoculture spheroids were used for RNA-seq, whereas both mono- and co-culture spheroids were processed for cryosections. Transcriptomic profiling revealed a distinct signature of up-regulated genes in spheroids compared to 2D cultures, including *TNF*, *IL-8*, *CXCL10* and *MMP9*, coupled with downregulation of proliferation and protein synthesis associated markers. This transcriptional pattern is characteristic of cellular senescence, specifically the Senescence-Associated Secretory Phenotype (SASP), indicating that cells within spheroids are not dead but have stopped the cell cycle and shifted into a pro-inflammatory state. qPCR analysis confirmed the upregulation of SASP-associated chemokines in the 3D model, including IL-8, a key mediator of ovarian cancer aggressiveness. Importantly, immunostaining of cryosectioned spheroids demonstrated robust protein-level expression of SASP markers IL-8 and VEGFA, whereas these signals were absent in 2D cultures. This highlights the unique inflammatory and pro-survival milieu induced by 3D cultivation. Collectively, these findings indicate that OC spheroids downregulate genes for cell growth and division, while strongly upregulating matrix-remodeling enzymes (MMP9), survival factors (STC1) and drug protective molecule (SERPINA1). This phenotype is clinically relevant, as dormant yet metabolically active tumor cells may persist in the peritoneal cavity and resume proliferation under favorable conditions.

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COMPARATIVE EVALUATION OF ENDOTHELIAL CELL MODELS FOR *IN VITRO* BLOOD- BRAIN BARRIER FORMATION

Katja Vuković¹, Anikó Szeckó^{2,3}, Maja Katalinić¹, Mária A. Deli², Szilvia Veszelka², Antonio Zandona¹

¹*Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia* ²*HUN-REN Biological Research Centre Institute of Biophysics, Szeged, Hungary*
³*Doctoral School of Biology, University of Szeged, Szeged, Hungary*

Reliable *in vitro* blood-brain barrier (BBB) models are powerful tools for investigating cerebrovascular disease and for facilitating drug development to the brain. Human brain endothelial cell lines provide a convenient, well-characterized long-term resource for experiments and are widely used in BBB research. However, these cell types may not fully mimic primary cell behaviour, which is essential for studying BBB physiology. In this study, we compared three commonly used endothelial BBB models: human pluripotent stem cell-derived endothelial cells (hEC), primary rat brain endothelial cells (RBEC), and the immortalized human brain endothelial cell line HBEC-5i. Barrier development and integrity were assessed using impedance based real-time monitoring, transendothelial electrical resistance measurement (TEER), permeability assays and immunostaining of claudin-5 (CLDN5) tight junction protein. The three models showed distinct barrier properties. RBEC and hEC developed endothelial barriers, although with different functional profiles. RBEC cells showed lower impedance values than hEC but formed the tightest barrier integrity. This was reflected by the highest TEER values, the lowest permeability to paracellular and transcellular markers, also an increased and well-preserved CLDN5 tight junction organization. hEC demonstrated a better impedance value than primary model, although they had reduced BBB integrity compared to RBEC. Finally, HBEC-5i displayed the weakest barrier phenotype, characterised by poor impedance, reduced TEER, increased permeability to markers, and disrupted CLDN5 junction confirmed by immunostaining. To further address this, we investigated a differentiation method, however further research is needed to appropriate junctional maturation and to acquire a complex BBB phenotype of HBEC-5i cell line. Overall, both RBEC and hEC exhibited BBB-like characteristics, whereas HBEC-5i showed clearly impaired barrier formation. These findings confirm that endothelial cell model selection strongly affects *in vitro* BBB development and the reliability of permeability-related research outcomes. Acknowledgment: This work was funded by the European Union – Next Generation EU (BioMolTox, Class: 643-02/23-01/00016, Reg. No. 533-03-23-0006).

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WHOLE-CELL YEAST-BASED MODULAR BIOSENSOR FOR ESTROGEN AND ESTROGEN LIKE COMPOUND DETECTION

Kristijan Vuraić, Antonia Paić, Renata Teparić, Bojan Žunar

University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Biochemistry, Zagreb, Croatia

Estrogen is crucial to animal and human health, being an essential hormone. However, the prevalence of xenoestrogen and estrogen-like compounds is on the rise since they are byproducts of various industries. Some of them enter the organism and mimic hormones, acting as endocrine disruptors. Since most analytical methods lack the sensitivity to measure their bioavailability, it is of interest to develop whole-cell xenoestrogen biosensors. Previously developed human or animal biosensors have native estrogen receptors, which makes them unreliable measuring tools. Therefore, a more convenient solution is to construct a whole-cell *Saccharomyces cerevisiae* biosensor. For this purpose, we developed a modular estrogen receptor consisting of the DNA-binding domain, a human ligand-binding domain (LBD), and a transactivation domain. The ratiometric two-reporter system, engineered into the 2 μ plasmid, uses constitutive RFP and inducible GFP expression to account for variable plasmid copy number. Based on this concept, we successfully created two biosensors, carrying the LBDs of the human estrogen receptor α (ER α) and estrogen receptor β (ER β) that could detect and report various concentrations of estrogen. After validating our results, we tested the responses of our ER α and ER β biosensors to various xenoestrogens, including bisphenol A (BPA). To further demonstrate our modular concept, we interchanged transactivation domains and incorporated the human androgen LBD into the receptor. Currently, we are optimising the sensitivity of our biosensors. Our work establishes a framework for future development and implementation of robust biosensors in various fields, including environmental monitoring.

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THE CONTRIBUTION OF PROGESTERONE RECEPTOR GENE VARIANTS TO SPONTANEOUS PRETERM BIRTH: INTERPLAY OF GENETIC AND ENVIRONMENTAL FACTORS

Jasenska Wagner¹, Mirta Kadivnik^{1,3}, Kristina Kralik¹, Siniša Šijanović^{1,3}

¹J. J. Strossmayer University of Osijek, Faculty of Medicine, Department of Medical Biology and Genetics, Osijek, Croatia ²J. J. Strossmayer University of Osijek, Faculty of Medicine, Department of Obstetrics and Gynecology, Osijek, Croatia ³University Hospital Center Osijek Clinic of Obstetrics and Gynecology, Osijek, Croatia

Preterm birth (PTB) is a leading cause of neonatal morbidity and mortality worldwide. Many sociodemographic, dietary, medical, environmental, and pregnancy-related risk factors have been shown to increase the risk of spontaneous preterm birth (sPTB). This case-control study was conducted at the Clinic of Gynecology and Obstetrics of the Clinical Hospital Center in Osijek and the Department of Medical Biology and Genetics at the Faculty of Medicine in Osijek, Croatia. It investigated six single-nucleotide polymorphisms (SNPs) in the maternal and foetal progesterone receptor (*PGR*) gene and their association with spontaneous PTB, considering environmental and clinical risk factors. Our study included two groups of pregnant women (term and preterm, 292 in total) and two groups of newborns (term and preterm, 292 in total), and analysed 6 single-nucleotide polymorphisms (SNPs) of *PGR* (rs1042838, rs1042839, rs10895068, rs4754732, rs653752, and rs1942836) in relation to maternal age, foetal gender, and pregnancy complications such as vaginal bleeding. Venous blood samples were taken from pregnant women and blood from the umbilical cord of the newborns once after obtaining informed consent. SNP genotype analysis was performed using TaqMan-based fluorescent probes (Taq-Man SNP Genotyping Assays) on the ABI PRISM 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). Results showed that *PGR* SNPs rs1942836 (OR 0.38, CI 95% 0.15–0.98, $p = 0.03$), rs4754732 (OR 2.4, CI 95% 1.01–5.57, $p = 0.04$), and rs653752 (OR 2.27, CI 95% 1.19–4.34, $p = 0.02$) were associated with PTB when considered in the context of clinical factors (mothers vaginal bleeding during pregnancy, foetal gender and mothers age subsequently), highlighting gene-environment interactions. Our findings suggest that genetic variants of the progesterone receptor gene (*PGR*), particularly rs1942836, rs4754732, and rs653752, may correlate with maternal age, fetal gender, and pregnancy complications such as vaginal bleeding to modulate the risk of spontaneous preterm birth. Their interaction with clinical and environmental factors highlights the multifactorial nature of preterm birth.

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RecG-MEDIATED REPLICATION FORK PROCESSING SHAPES NAÏVE CRISPR ADAPTATION IN *E. coli*

Mirta Žagar, Tomislav Mamić, Damjan Mitić, Ivana Ivančić-Baće

University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

The CRISPR-Cas adaptive immune system defends many bacteria and most archaea against invading mobile genetic elements (MGEs) by memorizing, recognizing, and destroying them. Immune memory is acquired by integrating small fragments of MGEs (spacers) into specific loci called CRISPR, which consist of a series of repeats separated by spacers. This step is called naïve adaptation, and its effectiveness depends on the integration of DNA from an MGE into the CRISPR locus. The Cas1-Cas2 heterohexameric integration complex is essential for adaptation but also requires support from host enzymes. Previous work has shown that stalling and breaking of the DNA replication fork, followed by repair of DNA ends by RecBCD, generate ssDNA fragments that are captured, annealed, trimmed, and integrated into the CRISPR locus by the Cas1-Cas2 complex. Interestingly, RecBCD helicase activity was more important than its nuclease activity, suggesting that any host exonuclease can assist in DNA trimming. To test for roles of other host helicases we generated a mutant strain lacking RecG, a key helicase in replication fork regression and processing. We also cloned this gene to study how its overexpression affects naïve adaptation. We found that overexpression of RecG inhibited naïve adaptation, while in *recG* mutant cells naïve adaptation was highly increased. We then deepened our analysis using MiSeq sequencing of acquired spacers. Since RecG interacts with PriA to help reinitiate stalled replication and with RecA to repair broken replication forks, we will present our findings on how these interactions and different stages of fork rescue and recombination contribute to the generation of pre-spacer DNA fragments.

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LIQUID BIOPSY DETECTION OF ESR1 MUTATIONS IN ENDOCRINE-RESISTANT METASTATIC BREAST CANCER: FIRST RESULTS OF ROUTINE CLINICAL IMPLEMENTATION IN CROATIA

Marko Žarak^{1,2}, Marina Tomičević¹

¹Dubrava University Hospital Clinical Department of Laboratory Diagnostics, Department of Molecular Diagnostics and Genetics, Zagreb, Croatia ²University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

Estrogen receptor 1 (*ESR1*) mutations in the ligand-binding domain may emerge under selective pressure from endocrine therapy and represent an important mechanism of acquired resistance in estrogen receptor-positive, human epidermal growth factor receptor 2-negative (ER+/HER2-) metastatic breast cancer (mBC). Liquid biopsy enables minimally invasive assessment of the current molecular profile of metastatic disease and may detect resistant subclones not represented by a single tissue biopsy. We report initial results of routine *ESR1* mutation testing in Croatia. Between October 2025 and July 2026, 18 women with ER+/HER2- mBC were tested for the first time at disease progression during or after endocrine therapy. Median age was 65 years (range 45-89). Peripheral blood (9 mL) was collected into K₂EDTA tubes. Plasma was prepared by routine two-step centrifugation: initial low-speed separation from blood cells and a second centrifugation to remove residual cellular debris and microparticles. Circulating cell-free DNA (cfDNA) was isolated from 5 mL of plasma using the QIAamp Circulating Nucleic Acid Kit and QIAvac 24Plus system (QIAGEN, Germany). Samples containing at least 1 ng/μL cfDNA were analysed by digital polymerase chain reaction (dPCR) on the Nio E platform using a Crystal dPCR assay covering 17 *ESR1* mutations (Stilla, France). Results were expressed as variant allele frequency (VAF) and mutant copies/μL. A laboratory-defined reporting threshold of 1 mutant copy/μL was applied, with lower signals classified as background noise. All analyses were valid and interpretable. *ESR1* mutations were detected in 2/18 patients (11.1%), both harbouring p.(D538G). In one patient (59 years old), isolated p.(D538G) was detected at 1.36 copies/μL and 0.50% VAF during progression on a CDK4/6 inhibitor combined with an aromatase inhibitor; the result supported replacement of the endocrine partner with fulvestrant. The second patient (53 years old) showed a polyclonal profile comprising five exon 8 mutations, with dominant p.(D538G) at 29.60 copies/μL and 6.34% VAF and four additional alterations affecting residues L536 and Y537 at lower levels during hepatic progression on fulvestrant. Liquid biopsy using plasma-based dPCR enabled detection of both low-level and polyclonal *ESR1* alterations, demonstrating its feasibility for identifying acquired endocrine resistance, capturing tumour heterogeneity and supporting treatment sequencing in mBC.

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FUNCTIONAL DIVERGENCE OF AN ARGINYL-tRNA SYNTHETASE PARALOG IN PREDATORY *Herpetosiphon* BACTERIA

Igor Živković¹, Ana Madunić¹, Petar Vrvilo¹, Valentina Vranješ¹, Marko Močibob¹, Guillaume Hoffmann², Andrés Palencia², Ita Gruic-Sovulj¹

¹University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

²Université Grenoble Alpes, Inserm Institute for Advanced Biosciences, Grenoble, France

Aminoacyl-tRNA synthetases (aaRSs) are grouped into two evolutionarily distinct classes, I and II, both of which use ATP to couple amino acids to their cognate tRNAs for ribosomal protein synthesis. Class I enzymes are defined by the presence of a universally conserved HXGH motif (X is any hydrophobic amino acid), which plays a critical role in ATP binding at the transition state. Naturally occurring deviations from the HXGH motif are extremely rare; yet, when observed, they can bring some unique features. Puzzled by the discovery of two arginyl-tRNA synthetases (ArgRSs), a canonical enzyme with the HVGH motif and its paralog with the RVDH motif, in predatory bacteria of the genus *Herpetosiphon*, we used various biochemical and biophysical approaches to explore their cellular roles. We established that, while canonical ArgRS-HVGH is active in aminoacylation, ArgRS-RVDH fails to bind ATP and is consequently inactive. Moreover, ArgRS-RVDH remained inactive under all tested conditions and with different ATP analogues. Introducing the canonical HVGH motif into ArgRS-RVDH by mutagenesis did not restore activity, indicating that other factors are also responsible for the loss of activity. However, proteomic analyses confirmed that this noncanonical paralogue is expressed in cells at approximately 1000-fold lower levels than the canonical enzyme. To identify potential interactors that may define the function of ArgRS-RVDH, we performed pull-down experiments in combination with AlphaFold3 predictions. Several potential interaction partners identified suggest that ArgRS-RVDH has a regulatory function.

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MAPPING THE CELL WALL PHENOME OF NATURAL *Saccharomyces cerevisiae* ISOLATES AS A PROXY FOR INDUSTRIAL ROBUSTNESS

Bojan Žunar, Antonia Paić, Romana Ivković, Kristijan Vuraić, Tea Martinić Cezar, Igor Stuparević, Renata Teparić

University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Chemistry and Biochemistry, Zagreb, Croatia

The yeast *Saccharomyces cerevisiae* is a common synthetic biology chassis, yet most engineering efforts rely on a handful of laboratory strains optimised for benchtop performance rather than industrial robustness. This mismatch often undermines scale-up to real-world bioprocesses. By contrast, the large pool of phenotypically diverse natural and domesticated *S. cerevisiae* isolates, shaped by centuries of traditional fermentations, remains largely overlooked. To identify the most robust isolates as candidate chassis, we use the cell wall as a proxy for whole-cell robustness, as the cell wall is both the protective barrier shielding the cell from environmental insults and the primary interface through which the cell senses and responds to its surroundings. Thus, we assembled a panel of dozens of genetically and phenotypically divergent natural *S. cerevisiae* isolates and profiled their cell wall composition at single-cell resolution. Mannans, β -glucans, and chitin were fluorescently labelled and quantified by high-throughput flow cytometry, complemented by confocal microscopy to resolve their spatial distribution. This approach revealed a striking range of cell wall phenotypes across the natural diversity space and identified several outlier strains whose composition deviates markedly from canonical laboratory references. These outliers will serve as candidates for next-generation industrial chassis and as a unique resource for dissecting how natural variation shapes cell wall architecture, surface display efficiency, and stress tolerance.

NEUROCHEMICAL BIOMARKERS (AChE AND MAO) IN THE EUROPEAN BROWN BEAR: INFLUENCE OF SEASON AND BIOLOGICAL FACTORS

Suzana Žunec¹, Maja Lazarus², Agnieszka Sergiel³, Đuro Huber⁴

¹*Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia* ²*Institute for Medical Research and Occupational Health, Division of Occupational and Environmental Health, Zagreb, Croatia* ³*Institute of Nature Conservation of Polish Academy of Sciences Department of Wildlife Conservation, Kraków, Poland* ⁴*University of Zagreb Faculty of Veterinary Medicine Department of Biology, Zagreb, Croatia*

Human activity contributes to the accumulation of inorganic and organic pollutants in the environment, posing increasing risks to ecosystem and human health. The One Health concept recognizes the interconnectedness of environmental, animal, and human health, highlighting the need for integrated monitoring approaches. Long-lived mammals with physiological similarities to humans are particularly valuable as sentinel species for assessing the impact of environmental contamination. Previous studies in mammals indicate that monoamine oxidase (MAO) activity is strongly influenced by developmental stage and aging, with species- and tissue-specific patterns reported in rodents and domestic animals. Seasonal modulation of monoaminergic systems has also been reported in primates. In addition, age, sex, as well as ecological variables, including season, has been shown to affect acetylcholinesterase (AChE) activity in wildlife species. Together, these findings suggest that neurochemical biomarkers are sensitive to both intrinsic and extrinsic influences. To improve monitoring in large terrestrial mammals, this study evaluated the effects of biological factors (sex, age, and body condition) and season on AChE and MAO activity in the brain of the brown bear (*Ursus arctos*). Samples of the medulla oblongata were collected from 56 free-living bears hunted in Croatia between 2022 and 2025 in accordance with the Brown Bear Management Plan. Field data included body mass, body length, sex, date of sampling, and location. AChE activity was determined spectrophotometrically using 1 mM acetylthiocholine as substrate with selective inhibition of butyrylcholinesterase, while MAO activity was measured using a luminescence-based assay. Values ranged from 0.38 to 14.85 $\mu\text{mol}/\text{min}/\text{g}$ wet tissue for AChE and from 4.11 to 21.47 $\text{nmol}/\text{min}/\text{g}$ wet tissue for total MAO. Multiple regression analysis indicated that sex significantly influenced AChE activity ($b = 0.316$; $p < 0.001$) after controlling for age, season, and body condition index, although the model explained a relatively small proportion of the variability (28%). The same factors failed to show significant effects on MAO activity. These results represent the first data on brain AChE and MAO activity in brown bears and provide base for further ecotoxicological studies addressing pollutant effects in wild mammals. Acknowledgement: European Union – NextGenerationEU (project BioMolTox and HumEnHealth; Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006), the National Science Centre in Poland (project no. 2020/04/X/NZ4/01327), European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant (project no. PAN.BFB.S.BDN.617.022.2021) and the Polish Ministry of Education and Science (PASIFIC call 1). A part of research was performed using the facilities and equipment funded within the European Regional Development Fund (project KK.01.1.1.02.0007).

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THE PROTEASE ACTIVITY OF ACRC/GCNA IS ESSENTIAL DURING EARLY VERTEBRATE DEVELOPMENT FOR REPAIRING DNA-PROTEIN CROSSLINKS.

Cecile Otten¹, Marin Kutnjak¹, Christine Supina-Pavić¹, Marija Pranjić¹, Ivan Antičević^{1,2}, Marta Popović¹

¹*Rudjer Bošković Institute Division for Marine and Environmental Research, Zagreb, Croatia,*

²*Nanyang Technological University Lee Kong Chian School of Medicine (LKCMedicine), Singapore, Singapore*

DNA-protein crosslinks (DPCs) are specific DNA lesions defined by the irreversible covalent crosslink between a protein and DNA. As a result of their size, unrepaired DPCs block all DNA transactions including replication and transcription, which has severe consequences, ranging from DNA double strand breaks and genomic instability to cell death. At the organismal level, it is associated with cancer, aging and neurodegeneration, based on the studies of very rare genetic diseases associated with defective DNA-Protein Crosslink Repair (DPCR). Various aspects of DPCR have been recently uncovered and mostly studied in vitro, but the mechanisms of DPCR at the organismal level are largely unknown. Proteases play a central role in DPCR, as they remove proteinaceous part of the DPCs, while the peptide remnant crosslinked to DNA is subsequently removed by other repair factors. Here we characterized the in vivo role of the putative protease ACRC/GCNA (ACidic Repeat Containing/Germ Cell Nuclear Antigen) in DPCR by creating zebrafish mutants with inactive *Acrc* using CRISPR/Cas. Maternal zygotic *acrc* mutants are early embryonic lethal; rescue experiments, in which mRNAs encoding various mutated and truncated forms of *Acrc* or other proteases implicated in DPCR were injected into the *acrc* mutants, showed that *Acrc* has a unique role in DPCR via its protease activity. Analysis of DPC levels and composition in rescued embryos using the RADAR assay further identified Histone H3, Topoisomerases 1 and 2, Dnmt1, Parp1, Polr3a and Mcm2 as putative DPC substrates of *Acrc* in vivo. Taken together, our results demonstrate the vital role of the *Acrc* protease in DPC repair in zebrafish during early embryonic development.

LIST OF PARTICIPANTS

A

Abramović Denis, Crux d.o.o.

Abramović Irena, University Hospital Centre Zagreb, Clinical Department of Laboratory Diagnostics, Zagreb, Croatia

Antolović Vlatka, Ruđer Bošković Institute, Division of Electronics, Zagreb, Croatia

B

Bakoš Damir, Kefo d.o.o.

Balog Tihomir, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Baranašić Damir, ¹Ruđer Bošković Institute, Division of Computational and Data Science, Zagreb, Croatia ²MRC Laboratory of Medical Sciences, London, United Kingdom of Great Britain and Northern Ireland ³Imperial College London Institute of Clinical Sciences, London, United Kingdom of Great Britain and Northern Ireland

Baričević Vanja, University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia

Benjak Lana, Biovit d.o.o.

Blažević Božana, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Boban Mirta, University of Zagreb, School of Medicine Croatian, Institute for Brain Research, Zagreb, Croatia

Bogner Ana-Marija, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Borko Valentina, University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

Bošković Maria, ¹University of Split, School of Medicine Laboratory for Cancer Research, Split, Croatia ²Goethe University Frankfurt Institute of Biochemistry II, Faculty of Medicine, Frankfurt, Germany

Braut Antonija, University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia

Brzović Andro, University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

Bučević Popović Viljemka, University of Split, Faculty of Science, Department of Chemistry, Split, Croatia

Budeš Ana-Marija, Faculty of Medicine in Rijeka Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia

Bujas Vesna, Altium d.o.o.

Bura Ana, University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia

Burić Branimir, Biosistemi d.o.o.

C

Car Željka, University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Castanho Miguel, ¹Gulbenkian Institute for Molecular Medicine, Lisboa, Portugal Miguel Castanho Lab, Lisbon, Portugal ²Faculdade de Medicina da Universidade de Lisboa Institute of Biochemistry, Lisbon, Portugal

Crnečki Antonija, Poliklinika Breyer, Department of Molecular Diagnostics and Genetics, Zagreb, Croatia

Č

Čabrijan Sara, University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia

Ć

Ćetković Helena, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

D

Dulić Morana, University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Dumić Jerka, University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

Dužević Marko, University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

Đ

Đikić Ivan, Goethe University Institute of Biochemistry II and Max Planck Institute for Biophysics, Frankfurt/Main, Germany

E

Erdelja Jelena, Mikro+polo d.o.o.

Ester Katja, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

F

Filić Mileta Vedrana, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Franjević Damjan, University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

G

Galov Roberto, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Gelemanović Andrea, Mediterranean Institute for Life Sciences, University of Split, Bioinformatics Group, Split, Croatia

Gornik Kljaić Olga, University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

Grabovac Danijela, VWR International

Gruić Sovulj Ita, University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

H

Herak Bosnar Maja, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Horvat Trupković Doroteja, Biovit d.o.o.

I

Ivančić Baće Ivana, University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

Ivković Romana, University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory of Biochemistry, Zagreb, Croatia

J

Josić Janja, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Jurak Begonja Antonija, University of Rijeka, Faculty of Biotechnology and Drug Development, Rijeka, Croatia

K

Karleuša Mujkić Ljerka, Faculty of Medicine Department of Physiology, Immunology and Pathophysiology, Rijeka, Croatia

Katalinić Maja, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
Keser Toma, University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia
Kolić Dora, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
Kovarik Zrinka, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
Krce Lucija, University of Split, Faculty of Science, Department of Physics, Split, Croatia
Kurtović Kodžoman Matea, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
Kutleša Lucija, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

L

Leljak-Levanić Dunja, University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia
Leščić Ašler Ivana, Ruđer Bošković Institute, Division of Physical Chemistry, Zagreb, Croatia

LJ

Ljubojević Marija, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

M

Mamić Tomislav, University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia
Marcelić Lucija, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia
Marinković Mija, University of Split, Faculty of Science Department of Physics, Department of Biology, Split, Croatia
Marš Tomaž, University of Ljubljana, Faculty of Medicine Alliance4Life Virtual Research Center, Department of Metabolism and Endocrinology of Skeletal Muscle, Ljubljana, Slovenia
Martin Stanislav, LKB Vertriebs Ges.m.b.H.
Martinić Cezar Tea, University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Chemistry and Biochemistry, Laboratory of Biochemistry, Zagreb, Croatia
Matković Ana, Gorea Plus d.o.o.
Matovina Mihaela, Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia
Mavar Marija, ¹Medical University of Graz Gottfried Schatz Research Centre, Division of Molecular Biology and Biochemistry, Graz, Austria ²Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia
Medić Krševan, ¹University of Zagreb, School of Medicine, Department of Medical Biology, Zagreb, Croatia ²University of Zagreb, School of Medicine Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia
Meštrović Nevenka, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia
Meyer-Tönnies Marleen, Institute of Pharmacology, C_DAT, University Medicine Greifswald Pharmacology, Greifswald, Germany
Miletić Marina, ¹University of Zagreb, School of Medicine, Department of Medical Biology, Zagreb, Croatia ²Centre of Excellence for Reproductive and Regenerative Medicine Centre of Excellence for Reproductive and Regenerative Medicine, Zagreb, Croatia
Mirnics Karoly, University of Nebraska Medical Center Munroe-Meyer Institute, Omaha, United States of America

Mitrović Maja, Medic d.o.o.

Mrak Lara, MEDIPRO, trženje in raziskave d.o.o.

Močibob Marko, University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Molas Kilianek Agnieszka, Greiner Bio-One GmbH

Munitić Ivana, University of Rijeka, Faculty of Biotechnology and Drug Development, Laboratory of Molecular Immunology, Rijeka, Croatia

N

Nakić Petar, Institute for Medical Research and Occupational Health, Department of Toxicology, Zagreb, Croatia

Novak Nakir Ivana, University of Split, School of Medicine, Laboratory for Cancer Research, Split, Croatia

O

Oklopčić Rebeka Monika, Josip Juraj Strossmayer University of Osijek, Faculty of Medicine, Department of Laboratory Medicine and Pharmacy, Osijek, Croatia

Oskomić Marina, Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Laboratory for Protein Biochemistry and Molecular Modelling, Zagreb, Croatia

P

Paić Antonia, University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Chemistry and Biochemistry, Laboratory for Biochemistry, Zagreb, Croatia

Pascua Ana Malen, ¹Danube Private University Laboratory for Life Sciences and Technology (LiST), Department of Medicine, Faculty of Medicine and Dentistry, Krems 3500, Austria
²GISENS BIOTECH Department of Bioelectronics, Berkeley, CA 94704, California, United States of America

Paškvan Kordić Paola, Gorea Plus d.o.o.

Piccinini Esteban, National University of La Plata (UNLP) – CONICET Department of Chemistry – Faculty of Exact Sciences. Institute of Theoretical and Applied Physical Chemistry Research (INIFTA), La Plata, Argentina

Piškor Antonija, Poliklinika Breyer, Department of Molecular Diagnostics and Genetics, Zagreb, Croatia

Pizent Alica, Institute for Medical Research and Occupational Health, Division of Occupational and Environmental Health, Zagreb, Croatia

Plavša Branimir, University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Biochemistry and Molecular Biology, Zagreb, Croatia

Popović Marta, Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia

Pranjić Marija, Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia

R

Raos Dora, Ruđer Bošković Institute, Division of Molecular Medicine, Laboratory for Hereditary Cancer, Zagreb, Croatia

Rokov-Plavec Jasmina, University of Zagreb, Faculty of Science, Division of Biochemistry, Department of Chemistry, Zagreb, Croatia

S

Sagin Ferhan, Ege University Medical Faculty, Izmir, Türkiye

Salopek Sondi Branka, Ruđer Bošković Institute, Department for Molecular Biology, Zagreb,

Croatia

Schinagl Sabine, Greiner Bio-One GmbH

Schnaar Ronald, Johns Hopkins University School of Medicine Department of Physiology, Pharmacology & Therapeutics, Baltimore, Maryland, United States of America

Sinčić Nino, University of Zagreb, School of Medicine, Department of Medical Biology, Zagreb, Croatia

Slade Neda, Ruđer Bošković Institute, Division of Molecular Medicine, Laboratory for Protein Interactions, Zagreb, Croatia

Smith Rosie, Novogene GmbH

Stojan Iva, University of Split, Faculty of Science, Department of Physics, Split, Croatia

Stojanović Nikolina, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Stuparević Igor, University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Biochemistry, Zagreb, Croatia

Suman Iva, University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia

Šutić Maja, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Szekereš Tibor, Novogene GmbH

Š

Šatović Vukšić Eva, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Šeruga Musić Martina, University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

Šestan Marko, University of Rijeka, Faculty of Medicine, Department of Histology and Embryology, Rijeka, Croatia

Šimić Lidija, ¹Clinical Hospital Center Rijeka Emergency Department, Rijeka, Croatia

²University of Rijeka, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Rijeka, Croatia

Šimić Marija, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Šimunić Ena, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Šinko Goran, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

Šprung Matilda, University of Split, Faculty of Science, Department of Chemistry, Split, Croatia

Švob Štrac Dubravka, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

T

Tadijan Ana, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Taylor Cormac, University College Dublin School of Medicine, Dublin, Ireland

Teigen Knut, University of Bergen, Department of Biomedicine, Bergen, Norway

Teparić Renata, University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory of Biochemistry, Zagreb, Croatia

Terzić Janoš, University of Split School of Medicine, Split, Croatia

Tolić Iva, Ruđer Bošković Institute, Zagreb, Croatia

Tomaić Vjekoslav, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Trajković Katarina, Ruđer Bosković Institute, Division of Molecular Medicine, Zagreb, Croatia

Traven Ana, Biomedicine Discovery Institute, Monash University Department of Biochemistry and Molecular Biology and the Infection Program, Melbourne, Australia

Tuksar Petra, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

Tzvetkov Mladen, Institute of Pharmacology, C_DAT Felix-Hausdorf-Str. 3, Greifswald, Germany

V

Verasto Leona, Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia

Villo Ly, Tallinn University of Technology Department of Chemistry and Biotechnology, Tallinn, Estonia

Viljetić Barbara, J. J. Strossmayer University of Osijek, Faculty of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Osijek, Croatia

Vlašić Ignacija, Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia

Volarević Siniša, University of Rijeka, Faculty of Medicine, Molecular Medicine and Biotechnology, Rijeka, Croatia

Vrbančić Lea, ¹Ruđer Bošković Institute, Laboratory for Protein Dynamics, Division of Molecular Medicine, Zagreb, Croatia ²University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

Vučemilo Paripović Nikolina, Ruđer Bošković Institute, Division of Molecular Medicine, Laboratory for Hereditary Cancer, Zagreb, Croatia

Vuković Katja, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

Vuraić Kristijan, University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Biochemistry, Zagreb, Croatia

W

Wagner Kostadinović Jasenka, J. J. Strossmayer University of Osijek, Faculty of Medicine, Department of Medical Biology and Genetics, Osijek, Croatia

Weber Igor, Ruđer Bošković Institute, Division of Molecular Biology, Zagreb, Croatia

Z

Zandona Antonio, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

Zrnić Vladimir, Jasika d.o.o.

Ž

Žagar Mirta, University of Zagreb, Faculty of Science, Department of Biology, Zagreb, Croatia

Žarak Marko, ¹Dubrava University Hospital Clinical Department of Laboratory Diagnostics, Department of Molecular Diagnostics and Genetics, Zagreb, Croatia ²University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

Živković Igor, University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia

Žunar Bojan, University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Chemistry and Biochemistry, Zagreb, Croatia

Žunec Suzana, Institute for Medical Research and Occupational Health, Division of Toxicology, Zagreb, Croatia

