FEBS3+ MEETING

24th-27th September
Hotel M / Belgrade

Serbia, Croatia, Hungary, Slovenia, Bosnia and Herzegovina

" **ADVANCES IN MOLECULAR BIOSCIENCES:** FROM GENES TO PERSONALIZED THERAPIES "

SUPPORTED BY >

FEDERATION OF EUROPEAN BIOCHEMICAL SOCIETIES

BOOK OF ABSTRACTS

ORGANIZED BY >

Хемијски факултет
Универзитет у Београду

SBS

HDBMB
Hrvatsko društvo za biokemiju i molekularnu biologiju

SLOVENIAN BIOCHEMICAL SOCIETY

UBMB U BIH
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Proceedings

Editor: Dragana Robajac
Technical support: Jelica Milošević
Cover design: ARIA.ONE Conference & Consulting
Publisher: Faculty of Chemistry, Serbian Biochemical Society, Belgrade
Year: 2025
Printed by: Faculty of Chemistry, Serbian Biochemical Society, Belgrade
Number of printed copies: 150
ISBN 978-86-7220-136-9

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EXHIBITOR & INDUSTRY LECTURE

ANALYSIS
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DEAR COLLEAGUES AND FRIENDS,

We are delighted to greet you on occasion of the

FEBS3+ MEETING

" ADVANCES IN MOLECULAR BIOSCIENCES:
FROM GENES TO PERSONALIZED THERAPIES "

that is being organized in Hotel M in **Belgrade, Serbia**, from **24th to 27th September 2025.**

On behalf of the Organizing Committee of **FEBS3+ Meeting**, **the Serbian Biochemical Society (SBS)**, and also on behalf of **the Croatian Society of Biochemistry and Molecular Biology (HDBMB), the Hungarian Biochemical Society (HBS), the Slovenian Biochemical Society (SBD)** and **the Association of Biochemists and Molecular Biologists in Bosnia and Herzegovina (ABMBBIH)**, we are pleased to see that you were able to join the upcoming scientific congress.

The congress is **regional gathering of scientists from five participating countries** that work in the fields of biochemistry and molecular biology. FEBS3+ Meeting is planned as a broad conference with a diversity of session topics to cover different aspects of life sciences studied in participating countries.

Scientific Committee defined an array of attractive session topics that help to gather almost 200 lecturers and participants on the event from all five of our cohost countries, but also from more than ten other countries throughout Europe.
We are certain that all colleagues present at the event will use the next three days to be introduced to innovative concepts, exchange experiences, refresh existing and establish new contacts with each other, in the pleasant atmosphere of Belgrade in September 2025.
We look forward to the event and a fruitful exchange among all of us.

Sincerely,

Prof. Natalija Polović
President of the Organizing Committee the FEBS3+ Meeting

FEBS3+ MEETING

24th-27th September 2025
Hotel M / Belgrade

Congress Program

SESSION TOPICS

1. MOLECULAR EVOLUTION
2. SYSTEMS BIOLOGY AND BIOINFORMATICS
3. REGULATION OF GENE EXPRESSION
4. BIOMARKERS AND THERAPEUTICS DEVELOPMENT
5. MOLECULAR BASIS OF DISEASE
6. DESIGN AND STRUCTURE OF PROTEINS
7. GENETICS
8. BIOTECHNOLOGY
9. BIOACTIVE COMPOUNDS AND METABOLISM
10. CELLULAR PROCESSES AND SIGNALING

DAY 1 WEDNESDAY 24th September 2025

13:00 - 14:30 **REGISTRATION**

14:30 - 15:00 **OPENING** CEREMONY

15:00 - 15:15 **FEBS** PRESENTATION

15:15 - 16:00 **FEBS EDUCATION** LECTURE

Chairpersons:
Natalija Polović /Serbia,
Nino Sinčić /Croatia

Ferhan Sagin /Turkey
Publish or perish? Zoom or doom? Rethinking the future of research and education

16:00 - 17:00 **PLENARY** LECTURE

Chairperson:
Beata Lontay /Hungary

Balázs Papp /Hungary
Leveraging genomic surveillance to understand and combat antibiotic resistance

17:00 - 18:00

TOPIC 1

MOLECULAR EVOLUTION
Chairpersons:
Marta Popović /Croatia,
Bálint Csörgő /Hungary

17:00 - 17:30
> **Damir Baranašić /Croatia**
Sequence determinants of transcription initiation and promoter architecture in vertebrates

17:30 - 18:00
> **Bálint Csörgő /Hungary**
Molecular tools for probing bacteriophage-host interactions in clinical pathogens

TOPIC 2

SYSTEMS BIOLOGY AND BIOINFORMATICS
Chairpersons:
Attila Csikász-Nagy /Hungary,
Radivoje Prodanović /Serbia

> **Attila Csikász-Nagy /Hungary**
From yeast cells to digital twins: Charting cellular networks for disease and drug discovery

> **Abdurahim Kalajdžić /BIH**
Innovations in bioinformatics tools for the analysis of archeogenomics data

18:00 - 18:30 | **INDUSTRY** LECTURE *ANALYSIS* | Luka Mihajlović **Recent advances in HRAM-based -omics**

19:00 - 20:30 **WELCOME RECEPTION**

DAY 2 THURSDAY 25th September 2025

09:00 - 10:00 **PLENARY** LECTURE
Chairperson: **Boris Rogelj /Slovenia**
Jernej Ule /Slovenia
Collective homeostasis of condensation-prone proteins via their mRNAs

10:00 - 11:00

TOPIC 3
REGULATION OF GENE EXPRESSION
Chairpersons:
Igor Stuparević /Croatia,
Ágnes Tantos /Hungary

10:00 - 10:30
> **Erna Karalija /BIH**
Priming the future: Harnessing plant memory for climate-resilient crops

10:30 - 11:00
> **Rosa Karlić /Croatia**
Decoding the interplay between the epigenome and mutational processes in cancer

TOPIC 4
BIOMARKERS AND THERAPEUTICS DEVELOPMENT
Chairpersons:
Nino Sinčić /Croatia,
Tamara Saksida /Serbia

10:00 - 10:30
> **Olga Gornik /Croatia**
N-glycosylation of complement component 3 (C3) as a potential target in type 1 diabetes

10:30 - 11:00
> **Jelena Vekić /Serbia**
Aspects of cardiometabolic risk in women: focus on pregnancy

11:00 - 11:30 COFFEE **BREAK**

11:30 - 13:30	SHORT TALK SESSION 1	SHORT TALK SESSION 2
11:30 - 11:45	> **Mehmet Gümüş /Turkey** SACM1L modulates autophagic flux, cellular dynamics and PI3K-AKT-mTOR signalling in MCF7 breast cancer cells	> **Isidora Protić-Rosić /Austria** Redirected killing of allergen-specific CD4+ T cells by pMHC class II CAR NK-92 cells
11:45 - 12:00	> **Agnes Tantos /Hungary** Structural and functional effects of disease-related mutations of KMT2D	> **Zorana Lopandić /Serbia** Glycoengineered H1sD2 as a novel immunomodulatory agent for enhancing allergen-specific immunotherapy
12:00 - 12:15	> **Buse Ceyda Öncel, Turkey** Transcriptome analysis of propranolol and labetalol applied sensitive and doxorubicin-resistant breast cancer cells	> **Loretta Laszlo /Hungary** Stage-Dependent Remodeling of the RAS Network in Colon Adenocarcinoma
12:15 - 12:30	> **Belmina Šarić Medić /BIH** In silico functional assessment and molecular interaction modeling of antimicrobial agents	> **Matilda Šprung /Croatia** Quinuclidine-based quaternary ammonium compounds: a natural scaffold for antimicrobial innovaion

12:30 - 13:30	**PLENARY** LECTURE *Chairperson:* **Marija Gavrović Jankulović /Serbia**	**Ana Cvejić /Denmark** Single-cell multi-omics map of human foetal blood in Down syndrom

13:30 - 16:00	**LUNCH** AND **POSTER PRESENTATIONS**	
13.30	> **LUNCH**	
14:00 - 16:00	> POSTER SESSION **1**	**Biomarkers and therapeutics development /Molecular basis of disease**
	> POSTER SESSION **2**	**Biotechnology/Genetics**

16:00 - 16:30

INDUSTRY LECTURE
LKB

Aleksandra Kukhareva
Simplified tagged protein purification and removal

16:30 - 17:30

TOPIC 5

MOLECULAR BASIS OF DISEASE
Chairpersons:
Boris Rogelj /Slovenia,
Nino Sinčić /Croatia

16:30 - 17:00
> **Naida Lojo-Kadrić /BIH**
Genetic basis of coronavirus associated dysosmia and dysgeusia

17:00 - 17:30
> **Damjana Rozman /Slovenia**
Integrative omics for biomarker discovery in metabolic associated steatotic liver disease and hepatocellular carcinoma

TOPIC 6

DESIGN AND STRUCTURE OF PROTEINS
Chairpersons:
Marta Popović /Croatia,
Roman Jerala /Slovenia

> **Gregor Anderluh /Slovenia**
Engineering of protein nanopore for sensing applications

> **Natalija Polović /Serbia**
Amyloids - from pathological protein aggregates to promising nanomaterials

17:30 - 18:30

SHORT TALK SESSION 3

17:30 - 17:45
> **Alma Herić /BIH**
Monitoring a patient with suspected myelodysplastic syndrome – case report

17:45 - 18:00
> **Antonija Jurak Begonja /Croatia**
Interleukin-1 alpha alters mega-karyocyte maturation, promotes emperipolesis, and induces a distinct proteomic profile

18:00 - 18:15
> **Tamara Saksida /Serbia**
Connecting brain and gut inflammation in the murine model of Alzheimer's disease

SHORT TALK SESSION 4

> **Eszter Nagy-Kanta /Hungary**
Insights on the interaction between the postsynaptic GKAP and Shank scaffold proteins

> **Albert Pál /Hungary**
Engineering non-CG specific DNA methyltransferaseses for functional studies of epigenetic regulation

> **Natalija Andrejević /Serbia**
Unraveling the human serum albumin aggregation pathway: Structural insights into the amyloid/amorphous aggregation shift and oligomerization potential modulated by physiological ligands and thiol content

18:15 - 18:30

> **Özlem Darcansoy İşeri / Turkey**
Cellular and gene regulatory effects of genistein on docetaxel sensitive and resistant breast cancer cells

> **Mujo Hasanović /BIH**
Seed priming of Solanum lycopersicum with Paraburkholderia phytofirmans PsJN enhances nickel stress response and induces intergenerational transcriptomic modulation

20:00 - 23:00 CONGRESS **DINNER**

DAY 3 FRIDAY 26th September 2025

09:00 - 10:00

PLENARY LECTURE
Chairperson:
Antonija Jurak Begonja /Croatia

Ivan Mijaković /Sweden
Molecular biology meets nanotechnology: promise of personalized medicine

10:00 - 11:00

TOPIC 7
GENETICS
Chairpersons:
Emina Kiseljaković /BIH
Đorđe Miljković /Serbia

TOPIC 8
BIOTECHNOLOGY
Chairpersons:
Tanja Ćirković Veličković /Serbia
Zoltan Lipinszki /Hungary

10:00 - 10:30

> **Lejla Kapur Pojskić /BIH**
Novel research insights into biological effects of halogenated boroxine (HB) reconfirm its antitumor potential

> **Balázs Enyedi /Hungary**
Illuminating leukocyte migration: Visualizing chemoattractant gradients with novel fluorescent probes

10:30 - 11:00

> **Uroš Potočnik /Slovenia**
Multi-omic biomarkers for personalized medicine and molecular mechanisms of non-response to biological drugs in chronic immune diseases

> **Marko Živanović /Serbia**
Electrospun scaffolds: Merging technology and biomedicine for scientific progress

11:00 - 11:30 COFFEE **BREAK**

11:30 - 12:30

SHORT TALK SESSION 5

11:30 - 11:45
> **Anita Skakić /Serbia**
CRISPR/Cas9 and iPSC-based platforms to explore pathophysiology and therapeutic approaches in glycogen storage disease type Ib

11:45 - 12:00
> **Cecile Otten /Croatia**
ACRC/GCNA is an essential protease which repairs DNA-protein crosslinks during embryonic development

12:00 - 12:15
> **Anja Pišlar /Slovenia**
Multifunctional roles of γ-enolase in the central nervous system: More than a neuronal marker

12:15 - 12:30
> **Arshi Arshi /Hungary**
Effects of 2-deoxyglucose and diclofenac sodium on MCF7 cell lines

SHORT TALK SESSION 6

> **Luka Blagojević /Serbia**
Characterisation and recombinant expression of a thermostable endoglucanase from Bacillus velezensis for lignocellulosic biomass hydrolysis

> **Tina Vida Plavec /Slovenia**
Engineering Lactococcus cremoris for recombinant protein packaging into extracellular vesicles

> **Nina Tiršo /BIH**
Melatonin modulates lipid metabolism in a rat model of testosterone-induced polycystic ovary syndrome: Comparison with metformin and combination therapy

> **Mario Stojanović /Croatia**
Toll-like receptors as a missing link in Notch signaling cascade during neurodevelopment

12:30 - 13:30

PLENARY LECTURE
Chairperson:
Lejla Kapur Pojskić /BIH

Lada Lukić Bilela /BIH
Lightless, not lifeless: Extreme habitats as sources of novel bioactive molecules

13:30 - 16:00 LUNCH AND POSTER PRESENTATIONS

13.30 > **LUNCH**

14:00 - 16:00

> POSTER SESSION **3** : **Design and structure of proteins/Regulation of gene expression/Systems biology and bioinformatics**

> POSTER SESSION **4** : **Bioactive compounds and metabolism/Cellular processes and signalling**

16:00 - 17:00

TOPIC 9

BIOACTIVE COMPOUNDS AND METABOLISM

Chairpersons:

Nataša Poklar Ulrih /Slovenia

Suzana Jovanović Šanta /Serbia

16:00 - 16:30

> **Zita Bognár /Hungary**

Comparison of mitochondrial and antineoplastic effects of amiodarone and desethyla-miodarone in cancer lines

16:30 - 17:00

> **Ivana Beara /Serbia**

Wine polyphenols: occurrence and biological activity as a biochemical aspect of wine quality

TOPIC 10

CELLULAR PROCESSES AND SIGNALING

Chairpersons:

Igor Stuparević /Croatia

Antonija Jurak Begonja /Croatia

> **Mirta Boban /Croatia**

Degradation-mediated protein quality control in quiescent cells

> **Tea Lanišnik Rižner /Slovenia**

From biomarker discovery to molecular mechanisms: omics approaches in endometriosis

17:00 - 17:30

SHORT TALK SESSION 7

17:00 - 17:15

> **Cene Skubic /Slovenia**

Sterol specific regulation of hepatic signaling distinct roles of lanosterol, desmosterol and lathosterol in liver cell fate and signaling

SHORT TALK SESSION 8

> **Snježana Jurić /Slovenia**

Nanobodies Uncouple Florigen Binding to Membranes and THF1

17:00 - 17:15

> **Dragica Mićanović /Serbia**
The mechanism of action of HYCO-3, an Nrf2 activator/CO releaser hybrid, in inhibiting autoimmune disease development in mouse models

> **Milena Zlatanova /Serbia**
Proteolytically active allergen Act d 1 induces inflammation via TLR4 receptor cleavage

17:15 - 17:30

> **Berfin Doğa Koçkaya /Turkey**
Investigation of the effects of selected miRNAs using bioinformatics approaches on AHNAK protein regulation in drug-resistant breast cancer cells

> **Lana Vujica /Croatia**
Understanding the ecotoxicological role of the Oatp1d1 transmembrane transporter using a zebrafish (Danio rerio) Oatp1d1 mutant line

18:00 - 20:00

SLOVENIAN BIOCHEMICAL SOCIETY SESSION

Chairpersons:
Gregor Anderluh /Slovenia,
Marija Gavrović – Jankulović /Serbia

18:00-18:30 > **Igor Križaj /Slovenia**
From snake venom to therapy: A novel drug candidatefor short-term thrombosis prevention

18:30-19:00 > **Iva Hafner Bratkovič /Slovenia**
NLRP3 inflammasome and pyroptosis: from mechanisms to applications

19:00-19:30 > **Tina Lebar /Slovenia**
Homology-guided engineering of tyrosine recombinase DNA specificities

19:30-19:45 > **Boštjan Petrič /Slovenia**
Alzheimer's dementia and paraoxonase 1: Why it pays off to measure enzyme kinetics and not just activities

20:00 - 21:00 **CLOSING** CEREMONY

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Congress **Abstracts**

FEBS3+ MEETING

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FEBS education lecture

Education

Publish or perish? Zoom or doom? Rethinking the future of research and education

Ferhan G. Sagin

Ege University Medical Faculty, Dept. of Medical Biochemistry, ***Izmir, Türkiye***
FEBS Education and Training Committee Chair

contact: *ferhan.sagin@gmail.com*

KEYWORDS: molecular life sciences, PhD training, supervision, AI, career development

Publish or perish? Zoom or doom? Lab rat or innovator? The pressures facing today's researchers and educators are real — but so are the possibilities for change. This talk will examine key challenges, including hyper-competition, hybrid supervision, and postdoctoral insecurity, while highlighting emerging practices in education and mentoring. It will invite the audience to rethink what it really means to train and support the next generation of life scientists in a world of shifting research culture, digital classrooms, and ain career paths. Together, we will ask: what kind of research culture do we want for the future, and how can we create it? Participants will leave with new perspectives on balancing teaching and research, embracing lifelong learning, and building more sustainable scientific careers.

ORCID: Ferhan G. Sagin *https://orcid.org/0000-0003-1309-6788.*

Plenary lecture

Molecular evolution

Leveraging genomic surveillance to understand and combat antibiotic resistance

Balázs Papp[1,2,3]

1 *HUN-REN Biological Research Centre,* ***Szeged, Hungary***
2 *HCEMM-BRC Metabolic Systems Biology Group,* ***Szeged, Hungary***
3 *HUN-REN Office for Supported Research Groups,* ***Budapest, Hungary***

contact: *papp.balazs@brc.hu*

KEYWORDS: genomic surveillance, antibiotic resistance, resistance evolvability, phage therapy

Antibiotic resistance poses an urgent threat to global health, demanding innovative solutions. In this talk, I present two complementary studies that harness the power of large-scale bacterial population genomics. First, we demonstrate how large-scale genomics combined with high-throughput phage typing enables the design of region-specific phage cocktails to combat carbapenem-resistant *Acinetobacter baumannii*, a top priority pathogen. Crucially, by identifying patients benefiting from the same phages across geographical scales, genomic surveillance provides a scalable framework for precision phage therapy. Second, we map the evolutionary dynamics of resistance gene acquisition throughout the history of *Escherichia coli*, revealing ecological factors and specific virulence genes that predict the emergence of highly resistant clones. We demonstrate that a clone's pathogenic lifestyle is a major determinant of its propensity to acquire resistance. Generalist clones, isolated from diverse clinical and environmental sources, rapidly acquire resistance, whereas specialized pathogens like enterohemorrhagic E. coli accumulate resistance more slowly. Overall, these studies pave the way for genome-based predictions of emerging high-risk clones and the tailoring of precision therapies.

ORCID: Balázs Papp *https://orcid.org/0000-0003-3093-8852*

Systems biology and bioinformatics

Collective homeostasis of condensation-prone proteins via their mRNAs

Rupert Faraway[1,2,3,4], Neve Costello Heaven[1,2], Holly Digby[1,2], Klara Kuret Hodnik[5], Jure Rebselj[5],Oscar G. Wilkins[1,6], Anob M. Chakrabarti[1,7], Ira A. Iosub[1,2], Neža Vadnjal[5], Rhys Dore[2],Lea Knez[1], Stefan L. Ameres[3], Clemens Plaschka[4], Jernej Ule[1,2]

1 *The Francis Crick Institute,* ***London, UK***
2 *UK Dementia Research Institute at King's College London,* ***London, UK***
3 *Max Perutz Labs, University of Vienna, Vienna BioCenter,* ***Vienna, Austria***
4 *Research Institute of Molecular Pathology, Vienna BioCenter,* ***Vienna, Austria***
5 *National Institute of Chemistry,* ***Ljubljana, Slovenia***
6 *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, UCL,* ***London, UK***
7 *UCL Respiratory, Division of Medicine, University College London,* ***London, UK***

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KEYWORDS: interstasis, interstatic mRNA, arginine-enriched mixed charge domains

The concentration of proteins containing intrinsically disordered regions must be tightly controlled to maintain cellular homeostasis. However, mechanisms for collective control of these proteins, which tend to localise to membraneless condensates, are less understood compared to pathways mediated by membrane-bound organelles. I will report on 'interstasis', a homeostatic mechanism in which increased concentration of proteins within RNA-protein condensates induces the sequestration of their own mRNAs. The selectivity of interstatic mRNA capture relies on the structure of the genetic code and conserved codon biases, which ensure that similar multivalent RNA regions encode similar low-complexity domains. For example, arginine-enriched mixed charge domains (R-MCDs) tend to be encoded by repetitive purine-rich sequences in mRNAs. Accumulation of proteins containing R-MCDs increases the cohesion of nuclear speckles, which induces selective capture of their own purine-rich mRNAs, thus preventing further production of the same proteins.

Each type of multivalent RNA regions is bound by specific RNA-binding proteins, which form cooperative RNA-protein complexes through repetitive RNA interactions. TRA2 proteins are the strongest binders of the multivalent purine-rich sequences, and they relocalise to speckles upon interstasis to promote selective capture of purine-rich mRNAs.

Systems biology and bioinformatics

Collective homeostasis of condensation-prone proteins via their mRNAs

Rupert Faraway[1,2,3,4], Neve Costello Heaven[1,2], Holly Digby[1,2], Klara Kuret Hodnik[5], Jure Rebselj[5],Oscar G. Wilkins[1,6], Anob M. Chakrabarti[1,7], Ira A. Iosub[1,2], Neža Vadnjal[5], Rhys Dore[2],Lea Knez[1], Stefan L. Ameres[3], Clemens Plaschka[4], Jernej Ule[1,2]

1 *The Francis Crick Institute,* ***London, UK***
2 *UK Dementia Research Institute at King's College London,* ***London, UK***
3 *Max Perutz Labs, University of Vienna, Vienna BioCenter,* ***Vienna, Austria***
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6 *Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, UCL,* ***London, UK***
7 *UCL Respiratory, Division of Medicine, University College London,* ***London, UK***

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CLK-mediated phosphorylation of TRA2 proteins counters their localisation to speckles, thereby modulating interstasis. Thus, the condensation properties of nuclear speckles act as a sensor for interstasis, a collective negative-feedback loop that co-regulates mRNAs of highly dosage-sensitive genes, which primarily encode nuclear condensation-prone proteins. I will discuss the broad potential of interstasis to support cellular homeostasis in human physiology and disease.

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Molecular basis of disease

Single-cell multi-omics atlas of human fetal blood in Down syndrome

Ana Cvejić

University of Copenhagen, Biotech Research & Innovation Centre – BRIC, ***Copenhagen, Denmark***

contact: *ana.cvejic@bric.ku.dk*

KEYWORDS: haematopoietic stem cells, aneuploidy, single-cell multiome

Down syndrome is associated with a predisposition to hematological abnormalities, including elevated erythrocyte counts and an increased risk of leukemia— conditions that originate prenatally through mechanisms that remain only partially understood. To investigate the disrupted hematopoiesis characteristic of

Down syndrome, we employed an integrative approach combining single-cell transcriptomics from over 1.1 million cells with chromatin accessibility and spatial transcriptomics data.
Our analysis revealed that gene expression changes in Down syndrome varied according to both cell type and microenvironment. Notably, we uncovered multiple lines of evidence indicating that hematopoietic stem cells (HSCs) in Down syndrome exhibit a 'primed' state toward differentiation. Using 10X multiome data we constructed a trisomy-specific regulatory map connecting non-coding elements to target genes in both disomic and trisomic HSCs. Integration of this map with genetic variants linked to blood cell traits revealed that trisomy alters regulatory architecture, disrupting enhancer function and gene expression pathways essential for erythroid differentiation.

Additionally, consistent with prior findings that mutations in Down syndrome are marked by oxidative stress, we confirmed elevated mitochondrial mass and oxidative stress in trisomic samples. These stress-associated mutations were enriched in regulatory regions of actively expressed genes in HSCs. Collectively, our comprehensive single-cell, multi-omic analysis offers a detailed molecular atlas of fetal hematopoiesis in Down syndrome, highlighting extensive regulatory remodeling that contributes to the development of its characteristic blood-related disorders.

ORCID: Ana Cvejić *https://orcid.org/0000-0003-3204-9311*.

Biotechnology

Molecular biology meets nanotechnology: Promise of personalized medicine

Ivan Mijaković[1,2]

1 *Division of Systems & Synthetic Biology, Department of Life Sciences, Chalmers University of Technology, **Gothenburg, Sweden;***
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KEYWORDS: graphene, "green" Au/Ag nanoparticles, antibacterial coatings, drug delivery, biomarkers, diagnostics

The talk will focus on the mechanisms of interactions of nanomaterials and living cells, underpinning various biomedical applications in antibacterial coatings, anti-infection and anti-cancer drug delivery, mitigation of neurodegenerative disease, and precision diagnostics. Unique features of graphene, such as hydrophobicity that is modulable via oxidation state, electrical conductivity, and possibility of functionalization with bioreceptors will be discussed. Metallic nanoparticles of biological origin will be presented, focusing on their anticancer and antibacterial potential. Examples of biomedical applications of nanomaterials developed in the Mijakovic lab will be presented, focusing on the disruption of infectious bacterial biofilms, the potential of graphene oxide and metallic nanoparticles in treatment of cancer and Alzheimers's disease, and the development of miniaturized graphene-based diagnostic sensors for detection of circular tumor DNAs and monitoring of infection progression in chronic wounds. The perspective of molecular diagnostics combining graphene-based sensors and AI will be discussed in the context of the upcoming EU MSCA doctoral network BUG-ID (***biosensors using graphene for infection diagnostics***).

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Bioactive compounds and metabolism

Lightless, not lifeless: Extreme habitats as sources of novel bioactive molecules

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KEYWORDS: microbiodiversity, microbiotechnology, bioprospecting, karst ecosystems, Dinarides

Subterranean ecosystems host diverse microbial communities within a wide range of microniches, often shaped by extreme environmental conditions and complex intra- and interspecies interactions across all three domains of life. This ecological complexity forms the basis for interdisciplinary knowledge exchange and methodological alignment, particularly in the bioprospecting of karst caves for novel bioactive compounds. The Dinaric karst, characterized by exceptional gene, species and ecosystem diversity, is a globally recognized hotspot of subterranean biodiversity. To overcome growth-limiting factors, microbial communities in cave biofilms form highly complex, mutualistic networks. Understanding their ecological roles and survival strategies in such environments is essential for discovering new enzymes and bioactive molecules, developing bioremediation techniques, and conserving fragile ecological balances.

Using an integrative approach, combining cultivation-based methods, metagenomics, and whole-genome sequencing, microbial profiling of cave sediments, particularly moonmilk (a biogenic, nanostructured calcite deposit rich in actinobacteria), has revealed numerous taxa with promising potential for biotechnological applications. Often overlooked in conservation efforts, these microbiomes show remarkable adaptability via horizontal gene transfer and synergistic interactions within biofilm. However, their stability and ecological function are increasingly threatened by climate change, drought, and pollution.

This lecture presents findings from long-term speleological expeditions, molecular, microbiological and mineralogical analyses, and addresses ethical aspects of genetic resource access and benefit-sharing. It advocates for an interdisciplinary framework uniting molecular ecology, biotechnology, and conservation. In the face of rising antimicrobial resistance and biodiversity loss, cave microbiomes emerge as a critical scientific frontier, and a vital global resource, requiring focused research conservation efforts, and responsible stewardship.

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Oral presentations

Molecular evolution

Sequence determinants of transcription initiation and promoter architecture in vertebrates

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KEYWORDS: *Cyprinus carpio, Danio rerio*, DANIO-CODE

Promoters encode the regulatory grammar that dictates when, where, and how strongly vertebrate genes are expressed, so pinpointing their boundaries is critical for both biology and genome engineering. I will first outline our single-nucleotide atlas of transcription start sites (TSSs) for *Danio rerio*, generated within the DANIO-CODE consortium using Cap Analysis of Gene Expression (CAGE). These annotations not only refine gene models but also enable the design of CRISPR-interference guides that silence genes by targeting their exact promoters. Building on this resource, we asked whether DNA sequence alone determines promoter "shape"—a sharp initiation at one base versus a broad cluster of TSSs. Fine-tuning the Nucleotide Transformer on the DANIO-CODE promoters recapitulated classic motifs, most notably the TATA box of sharp promoters. It also highlighted a 10-bp WW dinucleotide periodicity, ~50 bp downstream of the TSS, as the strongest predictor of broad initiation, outcompeting CpG islands.
Finally, I will present new CAGE profiles from eight developmental stages of the common carp (*Cyprinus carpio*), a close relative of zebrafish that underwent recent allotetraploidization. Comparison of its two sub-genomes reveals that only a subset of duplicated promoters retains the ancestral TSS nucleotides; changes in TSS bases are accompanied by complete activation, silencing, or graded changes in promoter output between homologous genes. Together, these studies illustrate how functional genomics, coupled with explainable AI, clarifies the rules of promoter architecture and its rapid evolutionary rewiring following genome duplication.

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Molecular evolution

Molecular tools for probing bacteriophage-host interactions in clinical pathogens

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KEYWORDS: gene-editing, bacterial immunity, phage-host interactions

Recent years have seen the emergence of an entirely new field in microbiology, the comprehensive characterization of the immune systems of bacteria and their relations to the viruses of bacteria, bacteriophages. As with other areas of microbiology, these studies have overwhelmingly focused on a limited number of model organisms for which the proper culturing techniques and genetic tools have been developed. However, by studying only a small number of species, our knowledge regarding these systems suffers great limitation. Furthermore, often what is true for these species may not be the case for other, clinically, or environmentally important bacteria.

In this regard, a great deal of knowledge is lost regarding the identification and functional characterization of unknown systems. We focus on mapping the phage-sensitivity properties of a large collection of clinical isolates of the emerging pathogen *Acinetobacter baumannii* against a diverse panel of *Acinetobacter* bacteriophages we have isolated. Utilizing genomic sequencing, a variety of bioinformatic tools, and genetic manipulation methodologies we have specifically optimized for *Acinetobacter* strains, we aim to identify and characterize anti-phage defense mechanisms in their original backgrounds. We hypothesize that studying these systems within their native contexts will greatly expand our knowledge and lead to discoveries not possible in heterologous settings.

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Systems biology and bioinformatics

From yeast cells to digital twins: Charting cellular networks for disease and drug discovery

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Systems biology and bioinformatics

Innovations in bioinformatics tools for the analysis of archeogenomics data

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KEYWORDS: aDNA comparisons, ancient genomic data simulations, chronopopulations

This study presents a genomic comparison of three chronopopulations—contemporary, medieval, and prehistoric—from Bosnia and Herzegovina, aiming to investigate the presence of a genetic continuum through time. We have implemented next-generation sequencing (NGS). A crucial and technically challenging step involved the isolation of aDNA, especially from archaeological samples where the DNA was highly fragmented and degraded. Specialized extraction protocols were applied to maximize DNA recovery while minimizing contamination, ensuring the integrity of the ancient genetic material.

Following DNA isolation and sequencing, comprehensive bioinformatics pipelines were employed for data processing and variant analysis. Modified and adjusted population-genomic analyses included assessments of intragroup heterogeneity and interpopulation genetic differentiation were implemented. The results showed relatively low levels of differentiation (0.30%) between medieval and contemporary populations, 0.02% between prehistoric and medieval, and 0.22% between prehistoric and contemporary groups. We have simulated gene flow across 150 generations (3000 years) using Monte Carlo model, artificial neural network and machine learning algorithms. Innovative backward simulation was implemented which requires "learning in time" approach. Findings pointed to the presence of a clear genetic continuum, indicating that prehistoric populations significantly contributed to the gene pool of medieval and modern-day inhabitants. The persistence of prehistoric genetic signatures through time suggests deep-rooted demographic continuity in the region, despite historical events and cultural transformations. Overall, this study provides important insights into the long-term genetic history of human populations in Bosnia and Herzegovina and contributes to a broader understanding of genetic continuity and population dynamics in southeastern Europe.

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Regulation of gene expression

Priming the future: Harnessing plant memory for climate-resilient crops

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KEYWORDS: seed priming, plant memory, epigenetics, climate resilience, sustainable agriculture

In the face of climate change and increasing environmental stress, sustainable food production depends on the development of crops with enhanced resilience. Our research focuses on leveraging plant memory—specifically, stress priming and transgenerational epigenetic mechanisms—to improve crop performance in key species such as chickpea (*Cicer arietinum*) and tomato (*Solanum lycopersicum*). By applying controlled priming strategies using abiotic stressors and beneficial microorganisms, we investigated physiological, biochemical, and molecular responses that lead to "stress imprinting" and enhanced tolerance in both immediate and subsequent generations.

The results reveal that primed chickpea and tomato plants exhibit improved photosynthetic efficiency, antioxidant defense, and water-use regulation under drought and heat stress. Notably, transcriptomic and qPCR analyses suggest the persistence of epigenetic marks and gene expression changes associated with stress-responsive pathways, even in unexposed progeny—indicative of transgenerational memory.

This work not only highlights the potential of seed priming and plant memory to enhance crop adaptability but also supports the integration of such strategies into sustainable agriculture frameworks. As a molecular farming innovation, these findings contribute directly to the “Farm to Fork” vision by enabling more efficient, climate-resilient crop systems and reducing input dependency.

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Regulation of gene expression

Decoding the interplay between the epigenome and mutational processes in cancer

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KEYWORDS: cancer genomics, somatic mutations, machine learning, cell-of-origin, epigenomics

The cell-of-origin (COO) plays a crucial role in determining cancer treatment and prognosis yet identifying it remains difficult for a subset of patients diagnosed with cancer of unknown primary. Earlier research demonstrated a correlation between patterns of somatic mutations and the chromatin landscape of the COO. Building on this, we developed a machine learning approach that integrates epigenetic features with somatic mutation data to accurately determine the cancer's primary site. This method is effective across a wide range of common cancer types and subtypes, enabling precise identification of the originating cell type.

Recently, we enhanced our model by combining whole-genome sequencing data from patient samples with single-cell chromatin accessibility profiles. Our model, which managed to predict the COO across multiple cancer subtypes with high accuracy and robustness, validated known anatomical and cellular origins of various cancers and uncovered distinct cellular trajectories during cancer development among different histological subtypes. These findings offer important insights for improving cancer prevention, early detection, and treatment stratification.

Beyond COO identification, models linking epigenetic features of the COO to somatic mutations provide a framework to study mutation accumulation mechanisms across the genome and to identify potential driver genes involved in cancer pathways. Incorporating mutational signatures—distinct patterns arising from different mutational processes—further enhances model accuracy, supporting the notion that the interplay between chromatin and mutation landscapes depends on the underlying mutational mechanisms. Together, these insights highlight the pivotal influence of the cell type of origin and mutational processes in shaping cancer genomes.

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Molecular basis of disease and therapy

N-glycosylation of complement component 3 (C3) as a potential target in type 1 diabetes

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KEYWORDS: N-glycosylation, C3, type 1 diabetes, autoimmunity

N-glycosylation, the attachment of oligosaccharides to proteins, is a crucial post-translational modification that significantly influences immune system functions. Complement component 3 (C3), a central glycoprotein in immunity, exhibits an unusual N-glycosylation pattern dominated by oligomannose glycans, unlike most plasma proteins. In our previous study involving nearly 2,000 children newly diagnosed with type 1 diabetes and their healthy siblings, we identified the C3 gene as a novel genetic regulator of total plasma N-glycosylation.

We further characterized N-glycans on two C3 N-glycosylation sites using LC-MS approach and demonstrated the diagnostic potential of C3 glycopatterns associated with type 1 diabetes. Notably, altered patterns were also observed in children at increased risk of the disease, even before clinical diagnosis. To further investigate the biomarker potential and functional role of C3 N-glycosylation in immune regulation, we analyzed C3 N-glycans in 816 Croatian adults, followed by a genome-wide association study (GWAS). The GWAS identified six loci associated with C3 N-glycosylation, involving genes linked to glycan processing, the secretory pathway, complement regulation, and gene expression. Moreover, we found shared causal genetic associations between C3 N-glycosylation and both rheumatoid arthritis and inflammatory bowel disease. Molecular modeling suggested direct glycan-mediated interactions between iC3b and the CR3 receptor, highlighting C3 N-glycosylation as a regulatory mechanism within the complement pathway and a promising therapeutic target in autoimmune diseases.

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Molecular basis of disease and therapy

Aspects of cardiometabolic risk in women: Focus on pregnancy

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KEYWORDS: pregnancy complications, lipid profile, HDL

Although cardiovascular disease (CVD) remains the leading cause of death for both women and men, significant gender disparities persist in its prevention, diagnosis and management. Throughout life, women are exposed to unique cardiometabolic risk factors, including those linked to pregnancy complications and menopause, which are often under-recognized or under-addressed in CVD risk assessment. Development of pregnancy complications, such as gestational diabetes, gestational hypertension, preeclampsia, premature birth and fetal growth restriction, have various causes, all of which are closely linked to disturbances in maternal metabolism. Physiological changes in lipid metabolism during pregnancy are a normal adaptation to meet fetal demands.

However, emerging evidence suggests that alterations in lipid status occurring during pregnancies with complications, particularly those involving high-density lipoproteins (HDL), may increase the risk of developing CVD later in life. While HDL is known for its role in reverse cholesterol transport, it also exhibits important anti-inflammatory and antioxidant functions through its complex proteomic and lipidomic composition. Recent studies suggest that a suboptimal rise in HDL-cholesterol during the second trimester may be linked to pregnancy complications. Yet, structural and functional changes in HDL during pregnancy, especially in relation to adverse outcomes and future cardiometabolic health, remain underexplored. Updated clinical practice guidelines increasingly recognize the impact of lipid abnormalities during pregnancy for the long-term cardiometabolic health of both mother and child. Investigation of the lipidomic and proteomic aspects of HDL particles in both normal and high-risk pregnancies may help identify novel biomarkers for predicting pregnancy complications, as a step toward personalized CVD prevention.

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Genetics

Genetic basis of coronavirus associated dysosmia and dysgeusia

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KEYWORDS: dysosmia, dysgeuzia, next generation sequencing, coronavirus

Odor and taste dysfunction (dysosmia and dysgeusia) are common symptoms of SARS-CoV-2 infection, often presenting as partial or complete loss, with potential for reversibility influenced by genetic factors. As evolutionarily ancient senses, olfaction and gustation are critical for survival, guiding the search for food and shelter. The human olfactory apparatus, featuring sensory cells in the nasal epithelium, initiates complex neural pathways via the olfactory bulb to brain regions like the limbic system. With over 400 olfactory receptors, humans perceive intricate odors, though a precise predictive model remains elusive. The olfactory system's ~400 OR genes are susceptible to single nucleotide polymorphisms (SNPs) that alter receptor responses, with polymorphisms in 36 OR genes significantly impacting smell perception. Similarly, variations in taste receptor genes (TAS1R and TAS2R families) affect taste function. Sensorineural loss of these senses can arise from neuroepithelial destruction by toxic inflammatory factors or due to genetic factors.

This research investigates the genetic basis of COVID-19-associated dysosmia and dysgeusia through genomic screening using Next-Generation Sequencing (NGS). A custom panel of 66 selected olfactory and taste receptor genes, alongside relevant inflammatory factors and SARS-CoV-2 receptor genes, was designed. In a study involving 96 samples, sequencing of these 66 genes identified 971 polymorphisms that differed from established databases, with 8 of these being statistically significant. These findings suggest a complex genetic landscape influencing sensory dysfunctions, necessitating larger sample sizes for deeper correlation with COVID-19 symptoms.

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Molecular basis of disease

Integrative omics for biomarker discovery in metabolic associatedsteatotic liver disease and hepatocellular carcinoma

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KEYWORDS: MASLD, NAFLD, HCC, fibrosis, machine learning

Metabolic associated steatotic liver disease (MASLD, formerly NAFLD) represents a growing global health challenge and a major risk factor for hepatocellular carcinoma (HCC), even in the absence of viral hepatitis. Identifying molecular signatures that capture the transition from early fibrosis to malignant transformation is critical for timely intervention. We applied transcriptomic profiling of liver tissue across progressive stages of MASLD-related fibrosis and identified sets of differentially expressed genes reflecting extracellular matrix remodeling and metabolic reprogramming. In a larger patient cohort, selected candidates were validated and combined with routine clinical parameters to develop a machine learning–based classification model.

This model enabled reliable stratification of patients with early fibrosis, a stage where clinical decision-making is most impactful. To extend these findings toward HCC, we performed a meta-analysis of transcriptomic datasets and integrated them into a genome-scale metabolic model. Based on differentially expressed genes and network analysis fatty acid metabolism, chemical carcinogenesis and retinol metabolism were identified as key pathways in HCC. Integration of transcriptomics data into a reference human genome-scale metabolic model identified fatty acid activation, purine and vitamin D & E metabolism as key reactions of HCC. Our integrative approach highlights potentially novel transcriptome biomarkers of MASLD and HCC. Further attention is paid to the genes whose protein products can be found in the blood.

Molecular basis of disease

Integrative omics for biomarker discovery in metabolic associatedsteatotic liver disease and hepatocellular carcinoma

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ACKNOWLEDGEMENTS: Supported by the Slovenian Research and Innovation Agency grants P1-0390, P2-0359, IP-0022 (CFGBC), J1-50024, and the Ph.D. grant to E.K. A.W. was supported by H2020 TranSYS grant #860895.

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Design and structure of proteins

Engineering of protein nanopore for sensing applications

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KEYWORDS: protein pores, sensing, planar lipid bilayer

Nanopore sensing is emerging as a direct, rapid, and cost-effective technology with broad potential for high-throughput detection of diverse analytes. Protein nanopores, in particular, have gained significant attention following their successful application in DNA sequencing. However, the number of unique nanopores currently available remains limited.

We are developing sensing strategies that exploit natural protein nanopores in combination with high-throughput biophysical approaches. Specifically, we investigated an actinoporin from the mountainous star coral, which forms stable octameric pores upon extraction from model lipid membranes using detergents. Cryo-electron microscopy revealed that membrane lipids are retained within the pore structure, forming an intricate network of protein–lipid and lipid–lipid interactions. These lipids were essential for pore assembly on the membrane surface and were found to modulate the pore's functional properties. Lipid membrane properties were also found to be the most important factor affecting stoichiometry of the pore.

Guided by the structural model, we performed protein engineering to enhance pore incorporation into artificial membranes, increase stability, and reduce electrical noise during planar bilayer recordings. Using this optimized system, we successfully discriminated between different histone proteins in a high-throughput planar lipid bilayer setup.

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Design and structure of proteins

Amyloids - from pathological protein aggregates to promising nanomaterials

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KEYWORDS: amyloid fibrils, metal-induced aggregation, FTIR spectroscopy, nanomaterials

Amyloid fibrils are highly ordered protein aggregates characterized by a cross-β sheet structure, where β-strands align perpendicular to the fibril axis. Their formation is associated with numerous degenerative diseases, including Alzheimer's, Parkinson's, and type 2 diabetes.
In this work, we present strategies for the detection, production, and characterization of amyloid oligomers and fibrils. The development of sensitive and non-invasive detection methods is crucial for early diagnosis. Our results highlight the use of biophysical techniques, particularly Fourier-transform infrared (FTIR) spectroscopy, for the quantification and structural analysis of amyloid species.

We also investigate the effect of lead (Pb^{2+}) and cadmium (Cd^{2+}) ions on amyloid fibrillation. These heavy metals promote aggregation by destabilizing native protein conformations and inducing clustering, resulting in amyloid fibrils with distinct morphologies.
Furthermore, employing various protein models as starting materials allowed us to generate a heterogeneous population of amyloid fibrils. These fibrils exhibit altered selectivity and enhanced chemical and mechanical resistance, demonstrating their potential as functional nanomaterials.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Biomarkers and therapeutics development

Novel research insights into biological effects of halogenated boroxine (HB) reconfirm its antitumor potential

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KEYWORDS: halogenated boroxine (HB), antitumor potential, apoptosis modulation, selective cytotoxicity, gene expression pathways

Recent research into the biological effects of halogenated boroxine (HB) has further substantiated its promising antitumor potential. Apoptosis modulators are emerging as potential cancer therapies by targeting the cell's natural death mechanism, often disrupted in cancer cells. These approaches aim to selectively induce death in tumor cells while minimizing damage to healthy cells, potentially enhancing the effectiveness of cancer treatments. Extensive studies on HB have provided compelling evidence of its ability to modulate these processes and act as a potent antitumor agent.

Halogenated boroxine exhibits significant antitumor activity across various cancer cell lines. In GR-M melanoma cells, HB substantially inhibits cell growth and downregulates BCL-2 expression, indicating its role as an effective tumor growth inhibitor. Similarly, in UT-7 leukemia cells, HB triggers apoptosis by modulating apoptosis-related genes and inhibiting the NF-κB signaling pathway. Its selective cytotoxicity is attributed to mechanisms such as Bax/Bak-independent mitochondrial depolarization and TRAIL-like activation, which are specific to tumor cells.

A recent genome-wide expression study further confirmed HB's antitumor effects, revealing distinct pathway enrichments in MG-63 osteosarcoma cells over time. At 24 hours post-treatment, the compound induced significant activation of pathways related to protein chaperoning, cholesterol biosynthesis, and the unfolded protein response (UPR), which are vital in managing cellular stress and ensuring cell survival under unfavorable conditions.
These findings reinforce the potential of halogenated boroxine as an effective antitumor treatment, emphasizing its selective cytotoxic effects on cancer cells and its ability to modulate key cellular pathways involved in tumor progression.

Biomarkers and therapeutics development

Novel research insights into biological effects of halogenated boroxine (HB) reconfirm its antitumor potential

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Biomarkers and therapeutics development

Multi-omic biomarkers for personalized medicine and molecular mechanisms of non-response to biological drugs in chronic immune diseasesl

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Keywords: multi-omics biomarkers, personalized medicine, functional cell models, asthma, inflammatory bowel diseases

We apply multi-omics analyses to clinical samples and cell models to uncover mechanisms of non-response to biologics and to identify biomarkers for personalized treatment of chronic immune diseases, including asthma and inflammatory bowel disease (IBD).

Biomarkers and therapeutics development

Multi-omic biomarkers for personalized medicine and molecular mechanisms of non-response to biological drugs in chronic immune diseasesl

In asthma, we have performed genomic (GWAS), epigenomic, transcriptomic, proteomic, metabolomic, breathomic, and microbiome studies, and identified biomarkers associated with asthma exacerbations in patients despite regular use of inhaled corticosteroids. These biomarkers could help prioritize subgroups of asthma patients for early treatment with biologics. We developed a novel pharmacogenomic cell model to predict treatment response to omalizumab (OMA), the first approved asthma biologic. In this model, we integrated the Basophil Activation Test (BAT) with *in vitro* OMA treatment and high-throughput transcriptomic analysis, using primary blood cells from patients before therapy. Basophils were activated with patient-specific allergens, and their cells treated *in vitro* with OMA.

In IBD, we identified genomic and transcriptomic biomarkers for treatment of Crohn's disease (CD), a major IBD subtype, with adalimumab (ADA). Using a cell model, we demonstrated that monocyte-to-macrophage differentiation plays an important role in non-response to ADA, and proposed IL1B as a biomarker for ADA treatment in CD. More recently, we have developed a pharmacogenomic cell model for vedolizumab (VDZ) treatment in CD. As VDZ blocks leukocyte–endothelial interactions and thereby prevents leukocyte trafficking to the gut, we established an immune–endothelial co-culture model that mimics the environment of intestinal blood vessels surrounding the inflamed gut of CD patients.

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Biotechnology

Illuminating leukocyte migration: Visualizing chemoattractant gradients with novel fluorescent probes

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KEYWORDS: chemotaxis, neutrophil swarming, leukotriene

Chemoattractant gradients are essential in directing cellular migration by activating specific receptors, primarily from the G protein-coupled receptor (GPCR) family. While the pathways and signaling events that regulate cell movement are well characterized, visualizing the spatial distribution of chemoattractants in live tissues has remained a significant technical challenge. Previously, we developed GEM-LTB4, a genetically encoded fluorescent biosensor for leukotriene B4 (LTB4), a key lipid mediator of chemotaxis and neutrophil swarming. This biosensor enabled detection of LTB4 release from neutrophils ex vivo, but in vivo measurements have proven to be more challenging.

Here, we present a new platform for the high-throughput development of GPCR-based fluorescent biosensors. By inserting conformationally sensitive fluorescent protein variants (cpEGFP or cpmScarlet) into the third intracellular loop of various GPCRs with linkers of random length and amino acid composition, we can engineer hundreds of thousands of sensor variants simultaneously. This approach has allowed us to successfully convert multiple GPCRs into biosensors, resulting in a suite of sensors capable of detecting extracellular gradients of key chemoattractants and chemokines, including fMLF, C5a, LPA, S1P, CCL19, and CCL21. Ina addition, we have developed an enhanced LTB4 biosensor with a 5-fold increase in signal response upon ligand binding, compared to the previous version.

These tools, termed GEM-sensors, facilitate GPCR-based Extracellular Measurement of chemokine and chemoattractant gradients in our project — “Inflamapping”. This work enables real-time visualization of gradient dynamics in live tissues, providing valuable insights into inflammatory processes.

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Biotechnology

Electrospun scaffolds: Merging technology and biomedicine for scientific progress

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KEYWORDS: electrospun scaffolds, artificial intelligence, wound healing, polycaprolactone, polyethylene glycol

This study presents an innovative approach to the design of biomaterials for biomedical applications by merging electrospinning technology with artificial intelligence (AI). Over a two-year collaboration involving more than 20 researchers from seven institutions across Kragujevac, Belgrade, and Vienna, 125 different polymeric combinations of polycaprolactone (PCL) and poly(ethylene glycol) (PEG) were synthesized. The resulting nanofiber scaffolds were structurally and mechanically characterized using advanced microscopic techniques, and the obtained data were employed to train an artificial neural network. The AI model successfully predicted optimal scaffold formulation, which was further enriched with antibiotics to enhance its biomedical potential.

In vitro assays confirmed biocompatibility, promoted cell proliferation, and demonstrated antibacterial properties. *In vivo* analyses using chick embryo CAM assays and Wistar albino rat burn models revealed that the antibiotic-loaded scaffolds significantly promoted angiogenesis, reduced inflammatory responses, and accelerated wound healing, leading to complete re-epithelization and improved tissue regeneration. This research highlights three key findings: (i) AI-driven methods are highly effective in the optimization of biomaterials, (ii) electrospun PCL/PEG scaffolds loaded with antibiotics represent a promising platform for wound healing, and (iii) the methodological framework provides a blueprint for future integration of AI into material science and tissue engineering. The outcomes suggest that AI-guided electrospinning can model complex material–biological interactions, streamline scaffold development, and open new avenues for regenerative medicine and advanced wound care.

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Cellular processes and signaling

Comparison of mitochondrial and antineoplastic effects of amiodarone and desethylamiodarone in cancer lines

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KEYWORDS: mitochondrial dysfunction, mitochondrial drug, cancer cell energy metabolism, stem cell, drug repositioning

Amiodarone (AM) is a widely used antiarrhythmic drug. Its main metabolite is desethylamiodarone (DEA) produced by a process primarily catalyzed by the CYP3A4 enzyme. Both substances are found in the blood and tissues of patients treated with amiodarone. Additionally, more than fifty years of clinical experience with amiodarone should answer most of the safety concerns regarding both. Previously we demonstrated antineoplastic, anti-metastasizing, and direct mitochondrial effects of desethylamiodarone in B16F10 melanoma, T24 bladder cancer and HeLa cervix carcinoma cells. Breast cancer is the most frequent cancer type in women and the second primary cause of cancer related death worldwide.

Novel compounds significantly interfering with mitochondrial energy production may have therapeutic value. In the present study, we compared AM's and DEA's mitochondrial, antineoplastic effects in breast cancer (MCF7, MDA231) cell lines. Both compounds reduced viability in monolayer and sphere cultures and invasive growth of the breast cancer lines by inducing apoptosis. They lowered mitochondrial trans-membrane potential, increased Ca2+ influx, induced mitochondrial permeability transition and promoted mitochondrial fragmentation. In accordance with their mitochondrial effects, both substances massively decreased overall mitochondrial ATP production as it was determined using a Seahorse live cell respirometer. Both AM and DEA decreased the appearance of the CD44+CD24- phenotype, which is suitable for cancer stem cell identification and has been associated with poor prognosis in breast cancer patients. Our result indicated that DEA was more effective than AM in all experiments indicating that DEA may have higher potential in the therapy of breast than its mother compound.

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Bioactive compounds and metabolism

Wine polyphenols: Occurrence and biological activity as a biochemical aspect of wine quality

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KEYWORDS: wine, polyphenols, biological activity

A substantial body of evidence supports the cardioprotective effects of moderate wine consumption, as exemplified by the "French paradox", a phenomenon characterized by a low incidence of coronary heart disease in the French population despite a diet rich in saturated fats, attributed to wine-derived polyphenolic compounds.
This lecture is a subset of results from a comprehensive study on the polyphenolic profiles and in vitro biological activities of Serbian and other European wines. We provide a summary of the chemical characteristics (22 common polyphenols in red wines by HPLC-UV/VIS) and in vitro biological activities, evaluated through biochemical and cell-based assays assessing enzyme inhibition, antioxidant, and anti-inflammatory effects, of over 50 samples, including Merlot, Cabernet Sauvignon, Dionis, and Cabernet Franc varietals, with a focus on comparing Serbian wines to their European counterparts. Also, case reports on 24 Serbian orange wines and Merlot/Cabernet Sauvignon blend will be discussed.

In general, gallic acid and catechin were the predominant polyphenols in all wines, with malvidin 3-*O*-glucoside as the main anthocyanin and stilbene resveratrol detected in lower amounts. While PCA showed partial clustering, no significant differences in major compound levels were observed. All samples inhibited α-amylase, α-glucosidase, and lipase (local effects). Antioxidant and anti-inflammatory activities varied, including effects on lipid oxidation, ROS generation and eicosanoid production in U937 cells. No clear correlation was found between compound levels and bioactivity. Our current research aims to clarify the mechanisms of polyphenol metabolites responsible for systemic anti-inflammatory effects observed *in vivo*.

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AKNOWLEDGEMENT:
The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125).

Cellular processes and signaling

Degradation-mediated protein quality control in quiescent cells

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KEYWORDS: misfolded proteins, *Saccharomyces cerevisiae*, ubiquitin-proteasome system

In nature many cells spend a major part of their life in quiescence, a reversible state of cell cycle exit characterized by a distinct cellular organization and metabolism. Examples include resting adult stem cells and microorganisms facing unfavorable conditions. Cells can remain quiescent for extended periods of time, while maintaining their viability and reproductive capacity, indicating a need to maintain protein homeostasis. Protein quality control pathways that maintain protein homeostasis have been mostly studied in proliferating cells, however, given the changes in intracellular organization and cell physiology, the operation of this system in quiescent cells has been unclear. We investigated protein quality control pathways in glucose-depleted quiescent cells of yeast *Saccharomyces cerevisiae*, which exhibit a metabolic shift from glycolysis to respiration, induce autophagy, and reorganize a large fraction of proteasomes into cytoplasmic granules containing disassembled particles. We found that quiescent yeast cells retain the ability to target misfolded proteins to selective degradation by the ubiquitin-proteasome system. Furthermore, the efficient clearance of certain misfolded proteins additionally required intact nucleus vacuole junctions and selective autophagy. Together, the data demonstrate that quiescent cells maintain active protein quality control. The necessity of multiple degradation pathways for the removal of misfolded proteins during quiescence underscores the importance of misfolded protein clearance in this cellular state.

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Cellular processes and signaling

From biomarker discovery to molecular mechanisms: Omics approaches in endometriosis

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KEYWORDS: proteomics, metabolomics, transcriptomicsnon-invasive diagnosis, machine learning

Omics approaches, either targeted or non-targeted, have great potential for biomarker discovery. These are hypothesis-generating approaches with the primary aim of providing information on biomolecules associated with a particular pathology and with the secondary aim of contributing to a better understanding of pathophysiology. The discovery of biomarkers for clinical application as non-invasive diagnostic tests is performed in physiological fluids, preferably blood, urine or saliva.

In this talk, I will present omics approaches for discovery of diagnostic biomarkers for endometriosis, a common debilitating gynaecological condition associated with severe pain and infertility. Patients are currently diagnosed with an average delay of 7-12 years as only surgical diagnosis is available, so biomarkers for non-invasive diagnosis are urgently needed. We performed targeted proteomics and metabolomics and non-targeted transcriptomics studies on plasma samples from a group of patients from Slovenia and Austria to find panels of proteins, metabolites and transcripts that represent biomarker candidates for the construction of diagnostic algorithms using machine learning. The best validated logistic regression model includes ratios of metabolites, the combined variable of pain and/or infertility, age and the level of the protein TGFBI with an AUC of 0.89, a sensitivity of 84% and a specificity of 79%. The best discovered but not yet validated SVM model includes six transcripts and shows an AUC of 0.92, a sensitivity of 75% and a specificity of 100%. The identified proteins, metabolites and transcripts point to molecular mechanisms associated with endometriosis, which will also be discussed.

Cellular processes and signaling

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Short talks

Cellular processes and signaling

SACM1L modulates autophagic flux, cellular dynamics and PI3K-AKT-mTOR signalling in MCF7 breast cancer cells

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KEYWORDS: SACM1L, PI3K-AKT-mTOR, breast cancer

SACM1L is a phosphoinositide phosphatase involved in membrane trafficking, but its role in cancer and autophagy is not well understood. This study examined the role of SACM1L in MCF7 breast cancer cells, focusing on autophagy, migration, invasion, and cell cycle regulation. SACM1L levels were modulated via overexpression using lentiviral delivery of SACM1L cDNA or downregulation by CRISPR-Cas9 (KO). Autophagy-related proteins and PI3K-AKT-mTOR pathway components were analysed by Western blotting; migration and invasion by wound healing and on-chip chemotaxis assays; and cell cycle phases by flow cytometry.

Overexpression of SACM1L reduced LC3B and ATG5 expression, suggesting suppressed autophagy, while KO increased both, indicating enhanced autophagic activity. ATG16L1 levels declined under both conditions, suggesting post-transcriptional regulation. PI3K levels (total and phosphorylated) remained unchanged, but mTOR was elevated in both conditions. Total AKT decreased upon overexpression and increased in KO, whereas phosphorylated AKT significantly declined in KO cells.

Phenotypically, SACM1L downregulation enhanced migration and invasion, while overexpression markedly reduced migration with little effect on invasion. KO cells also displayed a cell cycle shift with reduced S-phase and increased G1-phase populations.

Overall, SACM1L appears to suppress autophagy and migration when overexpressed, while its loss promotes invasive behaviour and alters cell cycle dynamics. The data suggest SACM1L modulates breast cancer cell behaviour partly through differential regulation of AKT phosphorylation and mTOR expression. These findings position SACM1L as a potential tumour suppressor and novel regulator of autophagy in breast cancer, meriting further investigation as a therapeutic target.

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Regulation of gene expression

Structural and functional effects of disease-related mutations of KMT2D

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KEYWORDS: histone methyltransferase, RNA binding, NMR, CD, MST

KMT2D (MLL4) is a histone methyltransferase responsible for the monomethylation of the H3K4 residue in active enhancer regions. Mutations of the KMT2D gene have been implicated in the development of Kabuki-syndrome, a serious genetic disorder leading to several developmental defects. While Kabuki-related KMT2D mutations generally lead to the loss of a significant portion of the protein1, resulting in an enzymatically inactive variant, other, seemingly less-disruptive missense mutations have also been implicated in the emergence of serious developmental defects2.

These missense variations exclusively localize to a short segment of KMT2D, a region with RNA binding capacity3 and a predicted coiled-coil formation tendency, and do not affect the enzymatically active SET domain on the C-terminus. To understand how these mutations interfere with the normal activity of KMT2D, we expressed the affected region (RBR-Q) and compared the behaviour of the disease-related variants with the wild type. Microscale thermophoresis (MST), temperature dependent translational diffusion NMR and circular dichroism (CD) spectroscopy results indicate that the wild type RBR-Q region has a predominantly alpha helical structure and possesses a significant tendency to self-associate. The mutations interfere with the native structure of RBR-Q and reduce the self-association capacity, but not in a uniform manner. They also appear to influence the RNA binding capacity of the protein, as the mutants had altered affinity and specificity towards the tested RNAs.

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Molecular basis of disease

Transcriptome analysis of propranolol and labetalol applied sensitive and doxorubicin-resistant breast cancer cells

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KEYWORDS: beta-adrenergic receptor antagonists, drug repositioning, breast cancer, multidrug resistance

Propranolol (non-selective beta-adrenergic receptor antagonist) and labetalol (nonselective $\beta 1/\beta 2$ and selective $\alpha 1$ receptor antagonist) have potential anti-cancer properties. Cancer cells with a multidrug resistance (MDR) phenotype can resist to cytotoxic effects of multiple drugs due to various cellular protection mechanisms. The aim of this study is to investigate the effects of propranolol and labetalol comparatively on parental sensitive (MCF-7/S) and doxorubicin-resistant (MCF-7/Dox) MCF-7 breast cancer cell lines for *in vitro* evaluation of repositioning potential of beta-blockers.
The cytotoxicity of propranolol and labetalol on MCF-7/S and MCF-7/Dox cells was determined by MTT. Flow cytometry was used to analyze cell cycle and induction of apoptosis, and the colony formation and migration capacity were tested after drug application. RNA sequence analysis of propranolol and labetalol applied cells were performed to evaluate changes at transcriptome level. MCF-7/1000Dox cells showed cross-resistance to both drugs. Propranolol and labetalol reduced the colony-forming capacity of cells and caused G2 arrest in MCF-7/Dox cells and increased G1 phase in MCF-7/S, and induced apoptosis of both cell lines. A total of 286 and 62 genes were found to be upregulated in MCF-7/S and MCF-7/Dox cells, respectively, following treatment with 15 μM labetalol. 31 genes were upregulated and 54 genes were downregulated in MCF-7/S, following treatment with 50 µM propranolol. 50 µM propranolol treatment caused upregulation of 326 genes and downregulation of 164 genes in MCF-7/Dox cells. Genes associated with cancer were listed. Amongst were the genes related to apoptosis, cell migration and angiogenesis.

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Bioactive compounds and metabolism

In silico functional assessment and molecular interaction modeling of antimicrobial agents

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KEYWORDS: molecular docking, antibacterial, antiviral, natural compounds, inhibition

The emergence of multidrug-resistant microorganisms has become a significant global public health concern, emphasizing the urgent need for the discovery of novel antimicrobial agents. In recent years, *in silico* methods such as molecular docking have gained considerable attention as efficient, cost-effective tools in early-stage drug discovery. The application of molecular docking techniques offers a precise and predictive modern strategy for evaluating the antimicrobial potential of selected natural and synthetic compounds, facilitating the identification of promising candidates against resistant pathogens.

Molecular docking enables the prediction of interactions between small molecules and biological targets, providing valuable insights into potential mechanisms of antimicrobial action. Insights from our prior in silico studies highlight the effective application of molecular docking methodologies in the identification of natural compounds with potential inhibitory activity against key biological targets of multidrug-resistant bacteria, such as Methicillin-resistant Staphylococcus aureus (MRSA), as well as viral pathogens including Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Rift Valley Fever Virus (RVFV). Our studies revealed that among the tested compounds, grapefruit seed extract (GSE) components, particularly naringin and narirutin, exhibited the highest binding affinity toward the PBP2a receptor of MRSA. Scopoletin demonstrated the strongest binding affinity to key biological targets of SARS-CoV-2. In addition, among the existing antiviral drugs, arbidol—a broad-spectrum agent—showed the greatest binding affinity for the Gn glycoprotein of RVFV. These findings underscore the value of molecular docking as a predictive tool in the early-stage screening of bioactive molecules with promising antimicrobial and antiviral properties.

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Biomarkers and therapeutics development

Redirected killing of allergen-specific CD4 T cells by pMHC class II CAR NK-92 cells

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KEYWORDS: CAR, allergy, peptide-MHC based CAR, CD4+ T cells

The prevalence of IgE-mediated allergies is globally increasing. Allergies are driven by an exaggerated immune response which is governed by $CD4^+$ Th2 cells directed against otherwise harmless environmental antigens. Mugwort pollen represents an increasingly relevant source of weed pollen worldwide. Art v 1, the major mugwort allergen, contains an immuno-dominant $CD4^+$ T cell epitope (Art v 123–36) which is presented in the context of HLA-DR1, which drives allergen-specific T cell responses that contribute to initial disease manifestation and persistence.

A peptide–MHC class II–based chimeric antigen receptor (pMHC-CAR) approach was developed to selectively eliminate Art v 123–36 specific T cells. The recognition domain consists of HLA-DR1 loaded with peptide, which is either fused to intracellular signaling domains (HLA-DR1 CAR) or remains unfused (HLA-DR1wt). NK-92 cells were stably transduced with these constructs, and their proper surface expression was confirmed by flow cytometry. Cytotoxicity of NK-92 CAR cells was assessed using Na-251CrO4-release assays performed with Jurkat T cells and transgenic murine $CD4^+$ T cells expressing an Art v 123-36–specific TCR (TRAV17/TRBV18$^+$) as targets. HLA-DR1 CAR–NK-92 cells specifically lysed allergen-specific but not -nonspecific T cells in a peptide-dependent manner, confirming antigen-specific redirection of cytotoxicity.

Notably, HLA-DR1wt–NK-92 cells also induced moderate target cell lysis upon peptide loading, suggesting that pMHC display alone may facilitate prolonged NK-92 cell– T cell interaction or partial activation of NK-92 cells sufficient to elicit cell-death.

Biomarkers and therapeutics development

Redirected killing of allergen-specific CD4 T cells by pMHC class II CAR NK-92 cells

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These findings highlight the enormous potential of pMHC-based CAR platforms for antigen-specific depletion of pathogenic T cells in hypersensitivity disorders such as allergic diseases but also others, i.e. bona fide autoimmune diseases, and pave the way for personalized immunotherapies with reduced off-target effects.

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Biomarkers and therapeutics development

Glycoengineered H1sD2 as a novel immunomodulatory agent for enhancing allergen-specific immunotherapy

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KEYWORDS: allergy, allergen-specific immunotherapy, adjuvants, N-glycans, hemagglutinin

Allergen-specific immunotherapy (AIT) is the only treatment capable of inducing long-term immune tolerance in allergic individuals. To enhance its efficacy, glycans are being investigated as novel adjuvants due to their ability to modulate immune responses through pattern recognition receptor interactions. Hemagglutinin (HA), a surface glycoprotein of the influenza virus, exhibits immunomodulatory properties, making it a promising adjuvant candidate. This study focuses on H1sD2 glycoforms, fusion proteins combining the receptor-binding domain of HA (H1s) with the major house dust mite allergen Der p 2 (D2). H1sD2 glycoforms were designed in silico, expressed in *Pichia pastoris* and purified.

Their immunomodulatory potential was analyzed in peripheral blood mononuclear cells (PBMCs) from HDM-allergic individuals after stimulation with H1sD2, and cytokine profile was analyzed by ELISA. Additionally, BALB/c and C57BL/6 mice were immunized with Der p 2 via subcutaneous or intranasal routes to induce allergic responses. After allergen challenge, splenocytes were isolated and treated with H1sD2 glycoforms, followed by analysis of IL-4, IL-10, and IFN-γ cytokine expression.

Confocal microscopy and flow cytometry were used to investigate binding of H1sD2 glycoforms to cell membrane receptors on THP-1 derived macrophages. Our results demonstrate that specific N-glycan composition of H1sD2 significantly influences cytokine responses and receptor engagement, both in human and murine models. These findings highlight the potential of glycoengineered HA-based fusion proteins as immunomodulatory tools for improving the safety and efficacy of AIT.

Biomarkers and therapeutics development

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ACKNOWLEDGEMENTS: Supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia; Contract number: 451-03-136/2025-03/200168 and FEBS Collaborative Developmental Scholarship 2022.

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Biomarkers and therapeutics development

Stage-dependent remodeling of the RAS network in colon adenocarcinoma

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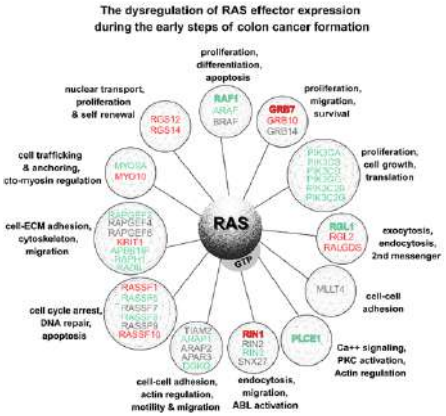
KEYWORDS: lung adenocarcinoma, lung squamous cell carcinoma, PLCE1, RAF1

Cancer arises from disruptions in intricate cellular networks rather than single gene defects. Applying a Network Medicine approach, this study integrated transcriptomic data and patient tissue analysis to unravel RAS-driven molecular changes in colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), and lung squamous cell carcinoma (LUSC). Analysis of RNA-seq data for 43 verified RAS effectors from The Cancer Genome Atlas revealed a marked loss of co-regulation within the RAS network in tumors, particularly in COAD, where this disruption was evident as early as Stage I. This suggests that perturbations in RAS-mediated signaling are early events in colon tumorigenesis.

For COAD, a five-gene RAS effector signature—RAF1, PLCE1, RGL1, RIN1, and GRB7—was identified using logistic regression with cross-validation, demonstrating high accuracy (balanced accuracy > 0.90) for early tumor detection. Validation on patient tissues using qPCR confirmed significant downregulation of PLCE1 and RAF1, along with upregulation of GRB7, mirroring patterns from the TCGA data.



A graphical network model illustrates the dysregulation of 43 RAS-interacting effector molecules in early-stage colorectal adenocarcinoma (COAD) compared to normal tissue, with nodes grouped by functional category and downstream effects. Significant expression changes are color-coded (red for upregulation, green for downregulation), and core RAS effectors with high diagnostic accuracy are highlighted in bold.

Biomarkers and therapeutics development

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Bioactive compounds and metabolism

Quinuclidine-based quaternary ammonium compounds: A natural scaffold for antimicrobial innovation

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KEYWORDS: biodegradibility, zebrafish, membrane disruption

Quinuclidine, a naturally occurring, rigid bicyclic amine, has shown great promise as a scaffold for designing new quaternary ammonium compounds (QACs) with enhanced bioactivity and improved safety profiles. In recent efforts to address the well-known drawbacks of conventional QACs—such as cytotoxicity, environmental persistence, and bacterial resistance—a series of 3-substituted and *N*-quaternized quinuclidine derivatives was synthesized and evaluated.
These compounds displayed strong antimicrobial activity against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains. In addition to their antimicrobial effects, several derivatives also showed notable antioxidative properties, suggesting potential for dual biological functionality. Toxicity assessments revealed lower cytotoxicity in mammalian cell lines and, importantly, reduced embryotoxicity in zebrafish (*Danio rerio*), highlighting their potential for safer use in biological and environmental settings.

Early biodegradability tests indicated a more favorable environmental profile compared to traditional alkyl-based QACs. Mechanistic studies suggest a membrane-disruptive mode of action and point to possible evasion of common resistance pathways. Taken together, these findings support quinuclidine-based QACs as a promising direction for the development of next-generation antimicrobial agents with broad potential applications in healthcare, disinfection, and materials science.

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Molecular basis of disease

Monitoring a patient with suspected myelodysplastic syndrome – case report

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KEYWORDS: MDS, peripheral blood smear, flow cytometry

Myelodysplastic syndromes (MDS) are malignant neoplasms of hematopoietic stem cells, primarily affecting pluripotent stem cells. They result in impaired proliferation, differentiation, and maturation of myeloid lineage cells. MDS is believed to arise due to somatic mutations in genes involved in epigenetic regulation of DNA expression (e.g., *TET2*), RNA splicing (e.g., *SF3B1*), and signal transduction (e.g., *JAK2*). These mutations lead to specific morphological changes observed in both bone marrow and peripheral blood.

The objective of the study was to monitor a patient with suspected MDS. Complete blood count with differential was obtained using the Sysmex 3100 analyzer. Bone marrow was analyzed by flow cytometry with an MDS panel. Peripheral blood smear was stained using the May-Grünwald-Giemsa method. Initial results showed pancytopenia: WBC 2.19 × 10^9/L; RBC 2.90 × 10^{12}/L; PLT 118 × 10^9/L. Peripheral smear revealed 1% metamyelocytes and reactive lymphocytes. Bone marrow flow cytometry showed 1% blasts expressing markers consistent with myeloblasts (CD34+, CD117+, HLA-DR+, CD38+, CD13+), immature myeloid forms, 1% NK cells, 0.1% B cells, and a CD4:CD8 ratio of 1.72. Granulocyte markers were negative (CD10-, CD16-). After six months: WBC 15.08 × 10^9/L; RBC 2.99 × 10^{12}/L; PLT 21 × 10^9/L. The smear showed 5% myelocytes, 8% metamyelocytes, no blasts. All granulocytes were agranular. Bone marrow had 2% blasts, 0.5% NK and B cells, 2.5% plasma cells, CD4:CD8 ratio 1.75.

Peripheral smear and bone marrow flow cytometry provided key insights into disease progression, revealing an increase in immature granulocytes, persistent anemia, and worsening thrombocytopenia.

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Molecular basis of disease

Interleukin-1 alpha alters megakaryocyte maturation, promotes emperipolesis, and induces a distinct proteomic profile

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KEYWORDS: megakaryocytes, platelet biogenesis, interleukin-1α, neutrophils, emperipolesis

Megakaryocytes (MKs) are large polyploid cells and precursors of blood platelets. Interleukin-1 alpha (IL-1α) is a pro-inflammatory cytokine and a known mediator of emergency thrombopoiesis when acute thrombocytopenia occurs. However, effect of IL-1α on MK maturation *in vitro* has not been studied in detail. We aimed to investigate the phenotypical and molecular consequences of IL-1α-based MK maturation. Murine bone marrows were cultured in the presence of IL-1α in addition to thrombopoietin (TPO) in vitro and analyzed kinetics of MK maturation. Furthermore, proteome analysis of MK cultured with IL-1α and/or TPO was performed. IL-1α induced differentiation of a greater number of larger MKs with higher ploidy, increased the release of platelet-like particles (PLP) but had decreased expression of maturation markers. Interestingly, we found a significantly higher rate of emperipolesis, transiently present Ly6G+ neutrophils within the MK cytoplasm in early cultures, dependent on IL-1α. At later stages, IL-1α-cultured MKs were morphologically undistinguishable from TPO-cultured MKs, although they kept the ability to produce more PLP. Proteome analysis further revealed a significantly higher abundance of neutrophil-related proteins and antimicrobial peptides in early-stage-IL-1α-cultured MKs, a pattern that persisted throughout late maturation. We found C-X-C motif chemokine 5 (CXCL5), a neutrophil chemoattractant, as one of the most significantly upregulated proteins in early and late-stage MKs. Taken together, these results shed light on the modulatory role of IL-1α on MK maturation, influencing not only platelet biogenesis but also promoting emperipolesis and an immune-driven proteomic MK phenotype.

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Molecular basis of disease

Connecting brain and gut inflammation in the murine model of Alzheimer's disease

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KEYWORDS: inflammation gut immunity, Alzheimer's disease, Alz mice

In the mouse model of Alzheimer's disease, App NL-G-F NL-G-F (Alz) mice that carry the Arctic, Swedish and Beyreuther/Iberian mutations, aggressive Aβ amyloidosis with cortical and subcortical deposition were documented. We investigated the composition and function of immune cells in the gut associated lymphoid tissue in Alz mice and their littermate controls (LC) at the age of 6 months, when they display microgliosis, astrocytosis and memory impairment. The small intestine (SI) lamina propria (LP) of Alz mice harbour fewer ILC3 cells and IL-22-producing ILC3, important for the maintenance of intestinal homeostasis. SI homogenates from Alz mice show lower IL-10 and IL-22, but higher IL-17 levels. In line with these, the frequency of Treg cells in the SI LP is lower. Simultaneously, the cortex and hippocampus of these animals have higher concentrations of TNF and IFN-γ. Alz mice differ from the LC in microbiota composition and the abundance of short chain fatty acids in the SI. Data from SI's TEM shows that there is no difference in the morphological measurements of intestinal barrier integrity. Also, the motility and peristaltic of SI is the same. However, the expression of mRNA for tight junction proteins claudin 4 and cadherin 1 is significantly downregulated in Alz mice, compared to LC.

In conclusion, the observed brain inflammation parallels the loss of intestinal homeostasis. These data show new insights about the relevant compartments in the gut that can be explored for manipulation of gut immunity and related to the pathogenesis of Alzheimer's disease.

Molecular basis of disease

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FUNDING: This research was funded by the Ministry of Science, Technological Development of Republic of Serbia, 451-03-136/2025-03/200007.

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Molecular basis of disease

Cellular and gene regulatory effects of genistein on docetaxel sensitive and resistant breast cancer cells

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KEYWORDS: genistein, docetaxel, epithelial-mesenchymal transition, multidrug resistance

Genistein is an isoflavone that has been known to have various molecular effects, such as inhibition of inflammation, promotion of apoptosis, modulation of steroidal hormone receptors and metabolic pathways. Cancer cells with multidrug resistance (MDR) phenotype may be resistant to more than one drug due to various cellular protection mechanisms. EMT (epithelial-mesenchymal transition) can increase the invasive/metastatic characteristics of cancer cells and elevate resistance to apoptosis. Recent studies have shown that genistein has the potential to inhibit cancer metastasis by regulating the EMT process through various signaling pathways. Our previous research has demonstrated that P-gp mediated drug efflux and EMT is one of the processes associated with docetaxel resistance in MCF-7 cells. The aim of this study was to demonstrate the cellular and multidrug resistance (MDR) related gene regulation effects of genistein on sensitive parental (MCF-7/S) and docetaxel-resistant (MCF-7/Doc) breast cancer cell lines in vitro.

The cytotoxicity of genistein on MCF-7/S and MCF-7/Doc cells was determined using MTT assays at 48 and 72 hours. Expression of P-gp (*MDR1*), E- cadherin (*CDH1*) and vimentin (VIM) were analyzed by qRT-PCR following 48 hours of genistein treatment. The colony formation and migration capacity were tested following genistein treatment. Genistein caused concentration-dependent reductions in MCF-7/S and MCF7/Doc proliferation, showing higher cytotoxicity in MCF-7/S. Genistein notably reduced the colony-forming capacity and migration of parental and resistant cells, but inducing a lesser reduction in MCF-7/S migration. Genistein treatment of MCF-7/Doc cells caused concentration-dependent down-regulation of MDR1 and VIM expressions.

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Design and structure of proteins

Insights on the interaction between the postsynaptic GKAP and Shank scaffold proteins

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KEYWORDS: postsynaptic density, protein NMR, intrinsically disordered proteins

Intricate and subtle molecular mechanisms are behind the processes of memory, learning and synaptic plasticity. The immense molecular network responsible for efficient and adaptive signal transduction in the acceptor side of excitatory synapses is called the postsynaptic density (PSD). Essential PSD proteins include NMDA receptors, PSD-95, GKAP, Shank and Homer. Disruption in any of these proteins may lead to a cascade of consequences resulting in diverse neural diseases.
The protein GKAP (guanylate kinase associated protein) is a mostly disordered multivalent scaffold molecule. Several SLiMs (Short Linear Motifs) have been described in GKAP to interact with other PSD proteins. One of these motifs is the C terminal QTRL motif, recognized by the Shank PDZ domain. We have shown previously (in the case of the LC8 dynein light chain binding motif of GKAP) that neighboring residues of SLiMs can contribute to complex formation. We performed NMR measurements to acquire data about the chemical environment of a longer GKAP C terminal region to describe the structural properties in free form and when bound to the Shank PDZ domain. We will complete the analysis of the emerging complex with experimental methods and molecular dynamics calculations. Uncovering residue-level, subtle details of these interactions will lead to better understanding of the postsynapse assembly.

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Design and structure of proteins

Unraveling the human serum albumin aggregation pathway: Structural insights into the amyloid/amorphous aggregation shift and oligomerization potential modulated by physiological ligands and thiol content

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KEYWORDS: misfolding, aggregation, amyloid, HSA, hydrophobic collapse

Proteins are prone to misfolding, commonly forming unstructured amorphous aggregates or highly structured amyloid fibrils. Human serum albumin (HSA), the most abundant protein in human blood, has a serum half-life of 21 days and interacts with various biomolecules. Containing 35 cysteine residues in its native sequence, HSA plays a key role in maintaining plasma's redox status and is prone to oligomerization under oxidative stress. Given its abundance and physiological importance, investigating its aggregation pathway under variable thiol content and natural serum ligands presence is of great relevance.

The aggregation pathway of serum-derived, ligand-saturated and ligand-free HSA was examined under prolonged thermal treatment to accelerate the aggregation kinetics. HSA's thiol count was varied using DTT as a reducing agent to assess the role of free thiols in oligomerization potential and amyloid/amorphous aggregation shift. Both untreated HSA and the resulting aggregates were characterized using spectroscopic techniques, including ATR-FTIR, DLS, ANS and ThT fluorimetry, atomic force microscopy, as well as electrophoretic techniques. The experimental findings were further supported by theoretical analysis of HSA's amyloidogenic peptides and their mapping within its 3D structure. These findings demonstrate that HSA, despite its high α-helical content, has a strong capacity to form amyloid-like structures, with aggregation kinetics profoundly influenced by thiol redox state, while the presence of natural serum ligands emerges as the dominant factor that redirects aggregation pathway from amyloid-like structures toward the formation of amorphous aggregates and suppresses HSA's oligomerization potential.

AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Design and structure of proteins

Unraveling the human serum albumin aggregation pathway: Structural insights into the amyloid/amorphous aggregation shift and oligomerization potential modulated by physiological ligands and thiol content

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Design and structure of proteins

Engineering non-CG specific DNA methyltransferaseses for functional studies of epigenetic regulation

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KEYWORDS: DNA methylation, non-CG methylation, protein engineering, epigenetics, directed enzyme evolution

Cytosine methylation in mammals predominantly occurs at CG dinucleotides and is catalyzed by the de novo DNA methyltransferases Dnmt3a and Dnmt3b. However, non-CG methylation at CA, CC and CT dinucleotides have also been observed in specific cell types such as neurons and embryonic stem cells. Although emerging evidence suggests that non-CG methylation may play important biological roles, its functional analysis is limited by the lack of tools capable of selectively targeting non-CG sites without perturbing canonical CG methylation.

To address this, we aimed to convert the bacterial CG-specific DNA methyltransferase M.MpeI into enzymes that methylate non-CG sites. We generated by structure-guided mutagenesis a triple mutant M.MpeI that, in addition to its original CG target, can also methylate CA and CC dinucleotides. This broadened-specificity variant served as starting point for directed evolution experiments using random mutagenesis and selection with methylation-sensitive restriction enzymes. These experiments yielded novel M.MpeI variants whose specificity shifted toward non-CG sites, with minimal residual activity on CG dinucleotides. One quadruple mutant is a CC specific DNA-MTase with strong preference for CCA and CCC sequences. Using further mutagenesis and selection, we also isolated CA-specific variants, which are currently characterized biochemically.
These engineered enzymes have the potential to become valuable research tools for dissecting the biological roles of non-CG methylation.

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Molecular basis of disease

Proteolytically active allergen ACT D 1 induces inflammation via TLR4 receptor cleavage

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KEYWORDS: actinidin, Act d 1, kiwifruit allergen, bone marrow-derived macrophages

Many allergens exhibit protease activity, which can disrupt epithelial barriers and activate immune responses via non-allergic mechanisms. Actinidin (Act d 1), a major kiwifruit allergen from the cysteine protease family, retains its enzymatic activity even under harsh digestive conditions. While the effects of allergen proteinases on epithelial cells are well known, their interaction with immune cells and specific receptors needs further investigation. This research investigates the interaction of Act d 1 with macrophages and the role of TLR4 in the initiation of the pro-inflammatory response. Native Act d 1 induced concentration-dependent NFκB activation in Raw blue macrophages and secretion of IL-6 and TNFα in bone marrow-derived macrophages (BMDMs).

Using primary BMDMs (wild-type and TLR4 knock-outs), we observed a noticeable decrease in IL-6 and TNFα secretion in the absence of TLR4. Similarly, in HEK293 cells (wild-type and TLR4-transfected), activation of NFκB and IFNβ promoters, as well as IL-8 secretion after Act d 1 treatment, occurred only in TLR4-transfected cells, highlighting the role of TLR4 in initiating the immune response to Act d 1. Heat-treated and E64-inhibited Act d 1 induced significantly lower NFκB activation. Also, using N-terminally Myc-tagged TLR4 in HEK293 cells, we showed the cleavage of TLR4 on the ectodomain in the presence of native Act d 1, but not with E64-inhibited Act d 1. These findings highlight the importance of the proteolytic activity for TLR4-mediated immune response and can significantly advance the understanding of allergen-mediated inflammation and the versatile role of allergen proteases in both allergic and non-allergic diseases.

ACKNOWLEDGMENT: This work was supported by the FEBS Short-term fellowship 2025 and the Ministry of science, technological development and innovation of the Republic of Serbia.

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Molecular basis of disease

CRISPR/Cas9 and iPSC-based platforms to explore pathophysiology and therapeutic approaches in glycogen storage disease type Ib

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KEYWORDS: GSD Ib, CRISPR/Cas9 gene editing, iPSCs reprogramming, ER stress-induced apoptosis, disease modeling

Glycogen Storage Disease type Ib (GSD Ib) is a rare autosomal recessive disorder caused by pathogenic variants in the *SLC37A4* gene, which encodes the glucose-6-phosphate translocase (G6PT). In addition to metabolic and immunological disturbances, GSD Ib is associated with elevated endoplasmic reticulum (ER) stress and increased apoptosis, contributing to cellular dysfunction in affected tissues. Despite recent progress in clinical management, disease-specific treatments remain lacking, in part due to the absence of adequate human model systems.

To investigate these mechanisms and establish a platform for evaluating novel therapeutic approaches, we developed two complementary human cell-based models. First, we generated *SLC37A4* knockout Flp-In HEK293 cells using the CRISPR/Cas9 gene editing method. This model provides a simplified system to study the functional consequences of G6PT deficiency. Molecular characterization using the RT-qPCR method confirmed alterations in the relative expression of ER stress (*ATF4, DDIT3, HSPA5, XBP1s*) and apoptosis (*BCL2/BAX, CASP3, CASP7*) markers, supporting its relevance for disease modeling. In parallel, we reprogrammed peripheral blood mononuclear cells (PBMCs) from healthy controls and GSD Ib patients into induced pluripotent stem cells (iPSCs) using a non-integrating Sendai virus system expressing OCT4, SOX2, KLF4, and c-MYC. Pluripotency was validated via trilineage differentiation, RT-qPCR for key transcription factors (*OCT4, SOX2, KLF4, NANOG*), and immunocytochemistry for OCT4 and NANOG.

Molecular basis of disease

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Together, these models offer a reliable platform to study the pathophysiology of GSD Ib, including ER stress and apoptosis, and provide a foundation for future studies involving lineage-specific differentiation and targeted therapeutic interventions.

ACKNOWLEDGEMENT: This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 6999, GlucoAdjust.

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Cellular processes and signaling

ACRC/GCNA is an essential protease which repairs DNA-protein crosslinks during embryonic development

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KEYWORDS: metalloprotease, SprT-like N-terminal domain, ACidic repeat containing/germ cell nuclear antigen

DNA-protein crosslinks (DPCs) are frequent lesions that hamper all DNA transactions, thereby impairing cell function. Defective repair of DPCs has been associated with cancer, neurodegeneration and premature ageing. Specialized DNA-Protein-Crosslink Repair (DPCR) mechanisms have evolved to cope with the variety of proteins that can be crosslinked to DNA. The main DPC repair pathways are (1) proteolysis followed by removal of the peptide remnant from the DNA backbone by nucleotide excision repair (NER) or by the tyrosyl-DNA phosphodiesterases TDP1 and TDP2 and (2) the nucleolytic pathway, where the part of DNA which holds the crosslinked protein is excised. So far, the proteolytic pathway has been explored through the role of the metalloprotease SPRTN (SprT-Like N-Terminal Domain) which can directly cleave the protein part of DPCs. Here we present our findings on the role of the putative protease ACRC/GCNA (ACidic Repeat Containing/Germ Cell Nuclear Antigen) in DPCR in zebrafish. We created zebrafish mutant lines and showed that the protease activity of Acrc is absolutely vital during first days of embryonic development.

We isolated cellular DPCs from 6 hour-old embryos and identified histone H3, topoisomerases 1 and 2, Dnmt1, Parp1, Polr3a and Mcm2 as DPC substrates of Acrc. Furthermore, using transient gene silencing of DPCR genes during the vertebrate development, we recently identified a novel DPC repair pathway (nucleophagy) at the crossroad of autophagy and DNA repair in the nucleus. Our findings have potential therapeutic implications, since chemotherapy resistance is frequently due to improving repair of topoisomerase- and Parp1-mediated DNA damage.

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Cellular processes and signaling

Multifunctional roles of -enolase in the central nervous system: More than a neuronal marker

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KEYWORDS: neurotrophic support, γ-enolase, neuroprotection, neurodegeneration

The function of the nervous system depends on highly specific connections formed between neurons during their development, and their proper arrangement in the central nervous system is ensured by neuroglia. Neurotrophic proteins—also known as neurotrophic factors—are crucial in this process, as they enable the survival, differentiation, regeneration, and maintenance of neurons in the central nervous system. Due to their activity, they are important in neurodegenerative diseases because they protect damaged neurons from degeneration and promote their regeneration. Similar to neurotrophic factors, the glycolytic enzyme γ-enolase also exhibits such activity. Enolase, a multifunctional protein with diverse isoforms, has generally been recognized for its primary roles in glycolysis and gluconeogenesis. The shift in isoform expression from α-enolase to neuron-specific γ-enolase extends beyond its enzymatic role. Enolase is essential for neuronal survival, differentiation, and the maturation of neurons and glial cells in the central nervous system. Neuron-specific γ-enolase is a critical biomarker for neurodegenerative pathologies and neurological conditions, not only indicating disease but also participating in nerve cell formation and neuroprotection and exhibiting neurotrophic-like properties. The neurotrophic activity of γ-enolase depends on the proteolytic action of the cysteine carboxypeptidase cathepsin X, which, by cleaving two amino acids from the C-terminal end of γ-enolase, prevents its transport to the plasma membrane and its interaction with the tyrosine kinase receptor, thereby eliminating its neurotrophic function. Recent findings indicate a protective role of γ-enolase against toxic processes in the central nervous system that led to neurodegeneration. This positions γ-enolase not only as a glycolytic enzyme and neuronal marker but also as a protein whose intact function significantly contributes to the prevention of neurodegenerative processes and the promotion of regeneration of damaged neurons through its neurotrophic activity.

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Biomarkers and therapeutics development

Effects of 2-deoxyglucose and diclofenac sodium on MCF7 cell lines

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KEYWORDS: 2-deoxyglucose, diclofenac sodium, MCF7

Resistance of breast cancers to chemotherapy remains a global challenge to date. Drug combination studies between anti-cancer agents are increasingly becoming therapeutic strategies, geared towards alleviating breast cancers. Previously, 2-deoxyglucose has been shown to target and interrupt glycolysis. Available evidence also suggests that diclofenac, which was originally designed as a pain reliever, could inhibit the proliferation of breast cancer cells. However, the reverse Warburg effect and other metabolic reprogramming mechanisms in breast cancers limit the pharmacological application of both 2-deoxyglucose and diclofenac as mono-therapeutic agents. The present study explores the additive anti-cancer effects of 2-deoxyglucose and diclofenac sodium on breast cancer cells. In this study, MCF7 cells were treated with 2-deoxyglucose and diclofenac sodium in single and combination doses before being evaluated for viability, and additionally, immunoblotting of pro-apoptotic proteins, Caspase-3 and Caspase-9, and a hypoxia-inducible factor-1 alpha, was also performed. The results showed that combination treatments of the cells with the drugs exhibited additive anti-cancer effects by limiting proliferation and suppress the expression of HIF-1 alpha in MCF7 these results reveal the combined effects of 2-deoxyglucose and diclofenac sodium on breast cancer cells, hence potentially elevating their pharmacological profile in the overall breast cancer therapy.

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Biotechnology

Characterisation and recombinant expression of a thermostable endoglucanase from *Bacillus velezensis* for lignocellulosic biomass hydrolysis

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KEYWORDS: endoglucanase, Bacillus velezensis, thermostable enzyme, lignocellulosic biomass, recombinant expression

Lignocellulosic biomass is a promising alternative to starch for sustainable fuel ethanol production. Its economic viability depends on efficient enzymatic processes that hydrolyse cellulose into fermentable sugars. While extensively studied fungal cellulases have been utilised in industrial applications, thermophilic microorganisms present an underexplored avenue for robust and stable enzymes, offering potential advantages for industrial contexts due to their heat tolerance and catalytic efficiency. In this study, strains of thermophilic bacilli were screened for cellulolytic activity using a gel diffusion assay on selective medium at 55 °C.

Strain 16B, identified as *Bacillus velezensis*, demonstrated significant activity against carboxymethyl-cellulose. The most prominent enzyme, with a molecular mass of approximately 55 kDa, was purified 60-fold, yielding a 27% yield and exhibiting a specific activity of 180 IU/mg. Biochemical characterisation indicated an optimal temperature of 65°C, while the enzyme remained highly stable at 60°C, maintaining over 90% of activity after one hour. The enzyme exhibited functionality over a broad pH range, with a maximum activity at pH 6.0, sustaining 70% activity at pH 5 and 45% at pH 9.
Whole genome sequencing of strain 16B identified the gene for this enzyme, annotated as eglS. To facilitate production and application, the eglS gene was successfully cloned and expressed in *Escherichia coli* utilising the pQE_Ek expression vector. The recombinant enzyme was obtained in its active form, demonstrating biochemical properties comparable to the native enzyme, thereby validating the potential of this thermophilic *Bacillus strain* as a source of industrially relevant cellulolytic enzymes.

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ACKNOWLEDGMENTS: This research was supported by the Science Fund of the Republic of Serbia, 14884, Maximising sustainability: tailoring cellulolytic enzyme cocktails for high-value nanocellulose and fermentable sugar extraction from agro-waste - NANOCELLMAX; and by the Ministry of Science, Technological Development and Innovation of Republic of Serbia (Contract No. 451-03-136/2025-03/200026).

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Biotechnology

Engineering *Lactococcus cremoris* for recombinant protein packaging into extracellular vesicles

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KEYWORDS: *Lactococcus cremoris*, extracellular vesicles, recombinant proteins, protein delivery

Lactococcus cremoris is a Gram-positive bacterium widely recognized as a model system for recombinant protein expression. Recently, extracellular vesicles (EVs) produced by Gram-positive bacteria have attracted increasing interest due to their potential physiological roles. Leveraging recombinant protein-producing bacteria as cell factories offers a promising strategy for packaging functional and bioactive proteins into EVs. In this study, we investigated how the expression of various recombinant proteins in *L. cremoris* influences EV formation and alters their protein composition. EVs were isolated following a standard protocol using an ultracentrifuge at 130.000 × g for 2 hours, followed by removal of the supernatant and collection of the EVs. EVs were characterised by transmission electron microscopy (TEM), flow cytometry, polydispersity index measurement and proteomic analysis. Characterization by TEM and flow cytometry revealed differences in both the quantity and heterogeneity of the secreted EVs, depending on the recombinant protein that was expressed in *L. cremoris*. The size of the isolated EVs was within the expected nano-scale range, mostly 50 - 200 nm. The presence and quantity of individual recombinant proteins inside the vesicle was confirmed by proteomic analysis, e.g. western blotting and mass spectrometry. In summary, we have shown that recombinant *L. cremoris* can be used to successfully produce EVs. The recombinant proteins expressed by *L. cremoris* were detected in the EVs-containing ultracentrifuged pellets. We have therefore shown the potential of using *L. cremoris* for the production of EVs and for loading them with recombinant proteins.

FUNDING: ARIS J3-60060 Extracellular vesicles from recombinant *Lactococcus lactis* as novel protein delivery vectors for treatment of intestinal inflammation and prevention of colorectal cancer; ARIS N3-0184 Small protein blockers of IL-23/IL-17 axis as intestinal inflammation inhibitors secreted by probiotic bacteria; ARIS P4-0127 Pharmaceutical biotechnology: Science for health.

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Cellular processes and signaling

Toll-like receptors as a missing link in Notch signaling cascadeduring neurodevelopment

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KEYWORDS: neuronal progenitor cells, neural cells, brain development

The fascinating intricacy of cellular and molecular infrastructure during neurodevelopment emerges from a sequence of cellular proliferation patterns entangled with migration waves directed by neuronal progenitor cells. Each of the neurodevelopmental phases is empowered by linked signaling cascades that are activated in a specific spatiotemporal manner and driven by complex protein machineries. Two signaling pathways—Notch and Toll-like receptor (TLR) signaling cascade—described as pivotal in neurodevelopment, have so far been unrelated to the literature.

However, by reviewing the current knowledge on Notch and TLR signaling, we found that their effects on the differentiation and maturation of neural cells are remarkably overlapping, which led us to propose a theory based on structural models. We advocate that TLRs are an integral part of the Notch signaling cascade by presenting an overview of similar and overlapping functions during brain development, building models of probable protein interactions, and suggesting the cascade flow of the Notch signal from TLRs localized within membranes at the cellular surface to the endosomal TLRs. Additionally, we discuss the interplay between immune and developmental aspects of TLR signaling and how environmental challenges, such as pathogens, impact cellular communication. Lastly, we discuss an intriguing thought about whether specific human behavior patterns may be evidence of the dysregulated TLR signaling during early phases of brain development.

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Biomarkers and therapeutics development

Melatonin modulates lipid metabolism in a rat model of testosterone-induced polycystic ovary syndrome: Comparison with metformin and combination therapy

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KEYWORDS: melatonin, PCOS, lipid metabolism, chronic inflammation

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine produced by the pineal gland and peripheral tissues, including the ovary, known for its potent antioxidant and metabolic regulatory properties. Acting through MT1 and MT2 receptors, melatonin plays a critical role in modulating steroidogenesis and follicular development, while simultaneously scavenging reactive oxygen species and enhancing endogenous antioxidant enzyme activity.

This study aimed to investigate the effects of melatonin on lipid metabolism and its role in reducing low-grade chronic inflammation in a testosterone-induced polycystic ovary syndrome (PCOS) rat model. Additionally, we compared its efficacy with that of metformin and the combination of both treatments.

Prepubertal female Wistar rats were randomized into five groups (n=6 each): control, PCOS, melatonin-only, metformin-only, and melatonin+metformin. PCOS was induced with testosterone over 35 days, with treatment regimens based on established literature. PCOS was confirmed by irregular estrous cycles and bilateral oophorectomy on day 56. Evaluations included serum lipid profile (TGC, TC, HDL, LDL, VLDL), CRP, IL-1, TNF-α, and visceral fat accumulation.

Anthropometric parameters differed significantly among groups, with abdominal circumference notably higher in the PCOS group compared to melatonin-treated groups ($p<0.05$). The melatonin+metformin group showed the lowest TGC and VLDL levels, significantly reduced compared to either treatment alone ($p<0.05$). Total cholesterol levels did not differ significantly. IL-1 levels were significantly lower in the combination group than in melatonin-only ($p=0.041$) and metformin-only ($p=0.026$) groups. No significant changes were observed in TNF-α or CRP.

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These findings support melatonin, particularly in combination with metformin, as a promising adjunct therapy in managing PCOS-associated dyslipidemia and inflammation.

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Bioactive compounds and metabolism

Sterol specific regulation of hepatic signaling distinct roles of lanosterol, desmosterol and lathosterol in liver cell fate and signaling

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KEYWORDS: lanosterol, desmosterol, *CYP51A1*, sterol, sterol intermediates

Cholesterol biosynthesis intermediates are increasingly recognized as bioactive lipids with regulatory functions beyond cholesterol production. This study dissects the sterol-specific effects of lanosterol, desmosterol, and lathosterol on hepatic gene networks and cellular behavior. Using CRISPR–Cas9, we engineered HepG2 cells to disrupt *CYP51A1, DHCR24* or *SC5D*, resulting in selective accumulation of each sterol. Complementary inducible shRNA systems enabled temporal modulation of sterol flux in HepG2, Huh7 and Snu449 lines.

Despite a shared blockade in cholesterol synthesis, the transcriptional responses were largely unique to each sterol (<10% overlap in differentially expressed genes). Lanosterol accumulation promoted G_2/M transition, LEF1 induction, and WNT/NF-κB activation, enhancing proliferation. In contrast, desmosterol and lathosterol induced G_0/G_1 arrest, suppressed mitotic genes, and favored hepatocyte differentiation. Temporal profiling revealed early versus late shifts in signaling networks, underscoring the value of dynamic sterol control. These findings highlight discrete sterol-mediated regulation of cell cycle, differentiation, and metabolic gene networks. Our inducible models offer a platform to explore sterol signaling in liver disease, regeneration, and drug metabolism, and suggest that targeting specific sterol intermediates may have important physiological consequences.

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Biomarkers and therapeutics development

The mechanism of action of HYCO-3, an Nrf2 activator/CO releaser hybrid in inhibiting autoimmune disease development in mouse models

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KEYWORDS: autoimmunity, CORM, Nrf2, immunomodulation

The novel hybrid compound HYCO-3, consisting of a CO-releasing molecule conjugated to a fumaric ester derivative, is known to release CO and simultaneously induce Nrf2-dependent expression of the CO-producing enzyme HO-1. We have previously shown that HYCO-3 ameliorates EAE, the animal model of multiple sclerosis1. The immunomodulatory potential of HYCO-3 was tested on myeloid-derived cells, microglial cells and T lymphocytes obtained from EAE-immunized mice. HYCO-3 exerted immunomodulatory effects on all the examined cell populations by inhibiting the generation of pro-inflammatory cytokines and NO, and downregulated antigen-presenting capacity of these cells2.

In the animal model of type 1 diabetes, HYCO-3 inhibited the development of the disease by halting infiltration of immune cells in the pancreas and maintaining normoglycaemia. HYCO-3 decreased the frequency of IL-17+CD8+ cells in the pancreatic draining lymph nodes and IL-17+CD4+ cells in the pancreas, which are key pathogenic populations in disease progression. Also, HYCO-3 increased the frequency of Treg cells, displaying a shift towards the protective Treg axis. Furthermore, we determined the kinetics of activation of Nrf-2 and CO levels in blood, pancreas, spleen and liver during oral application, thus correlating the effect of HYCO-3 with its mechanism of action.

FUNDING: This research was funded by the Ministry of science, technological development, and innovation, Republic of Serbia (Contract No. 451-03-66/2024-03/200007) and by grants from the Société d'Accélération de Transfert Technologique (SATT)-Ile de France Innovation (Project HYCOs) and ANR (ANR-18-CE18- 0032 and ANR-23-CE14-0080-01).

Biomarkers and therapeutics development

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Cellular processes and signaling

Nanobodies uncouple florigen binding to membranes and THF1

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KEYWORDS: flowering, florigen, Arabidopsis, plastid-nucleus communication

Flowering is a critical stage of plant development that is strongly regulated by environmental factors such as temperature. FLOWERING LOCUS T (FT), also known as florigen, is a mobile protein that promotes flowering and binds to both negatively charged phospholipid membranes (e.g. phosphatidylglycerol, PG) and the chloroplast-localised protein thylakoid formation 1 (THF1). These dual interactions are temperature-dependent and influence FT mobility and the onset of flowering. Our recent results suggest that the FT-PG and FT-THF1 interactions act in parallel to delay flowering at low temperatures.

To decipher these intertwined binding events, we propose the use of nanobodies targeting FT (nanFT) and THF1 (nanTHF1) to selectively inhibit or monitor specific interactions without interfering with FT transport. We will use native PAGE, liposome sedimentation, surface plasmon resonance (SPR) and BiFC assays to assess the effects of nanobodies *in vitro* and *in vivo*, including in transgenic Arabidopsis plants. By generating and expressing nanobody-protein fusions in chloroplasts and whole plants, we aim to uncouple the interactions of FT with membranes and THF1. This approach will elucidate early FT-mediated signalling pathways and improve our understanding of plastid-nucleus communication. The results could provide potential strategies to mitigate the flowering shifts caused by climate change.

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Biotechnology

Seed priming of *Solanum lycopersicum* with *Paraburkholderia phytofirmans* PsJN enhances nickel stress response and induces intergenerational transcriptomic modulation

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KEYWORDS: PGPB, abiotic stress tolerance, molecular memory, progeny analysis, gene expression, RNA-seq, stress imprinting

Seed priming with plant growth-promoting bacteria (PGPB) can enhance plant stress resilience and induce lasting physiological and molecular changes. In this study, *Solanum lycopersicum* seeds were primed with *Paraburkholderia phytofirmans* PsJN and grown under three conditions: chronic nickel (Ni) stress (10 μM throughout the experiment), acute Ni stress (50 μM for three weeks), and a control with no Ni exposure. Non-primed plants served as controls and were grown in same conditions. After phenologial cyclus was completed by all plants, seeds were harvested from all treatment groups and subjected to RNA sequencing.

The progeny of each treatment was grown in a stress-free substrate, and RNA-seq was performed to assess the persistence of priming effects. Results show that bacterial priming influenced gene expression patterns from the parent plants are imprinted in seeds and their progeny, particularly in genes associated with metal homeostasis, oxidative stress response, and regulatory pathways. These findings indicate that *P. Phytofirmans PsJN* priming induces molecular memory and intergenerational modulation of stress-responsive pathways, supporting its potential in enhancing crop performance under environmental stress.

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Bioactive compounds and metabolism

Understanding the ecotoxicological role of the Oatp1d1 transmembrane transporter using a zebrafish (*Danio rerio*) Oatp1d1 mutant line

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KEYWORDS: ADME, *Danio rerio*, Oatp1d1, diclofenac, PFOS

Organic anion-transporting polypeptides (OATPs) mediate the uptake of various compounds into eukaryotic cells. *In vitro* studies on the zebrafish (*Danio rerio*) Oatp1d1 transporter strongly suggest its ecotoxicological importance, while the physiological consequences of Oatp1d1 deficiency are still largely unknown. Therefore, this study aims at a detailed in vivo characterisation of Oatp1d1 using the zebrafish as a model organism.

We exposed the embryos to diclofenac and PFOS, model contaminants that have been shown to be Oatp1d1 substrates, to observe differences in the phenotypic and toxic responses of the WT and Oatp1d1 mutant embryos. Exposure experiments with diclofenac and PFOS have revealed a possible dual role of Oatp1d1 in embryonic development, which can be both protective and potentially harmful when exposed to different Oatp1d1 substrates. Diclofenac-induced malformations were delayed and less frequent in Oatp1d1 mutants compared to WT embryos. In addition to pericardial oedema, cardiac hemorrhage and body curvature, WT embryos exhibited pronounced muscle degeneration. PFOS-induced phenotypes, such as reduced swim bladder size, abnormal positioning, scoliosis and necrosis, were more severe and occurred earlier in Oatp1d1 mutants. The mutants also showed significantly higher levels of oxidative stress and apoptosis as well as reduced locomotor activity, indicating increased neurotoxicity of PFOS in the absence of Oatp1d1. In addition, we have designed two morpholino oligonucleotides to knockdown Oatp1d1 protein in WTs and monitor phenotypic changes due to exposure. Our results suggest that in the absence of Oatp1d1, hepatotoxic metabolites such as those of diclofenac are not transported to the liver, increasing the resistance of the mutants. Conversely, compounds with limited metabolism, such as PFOS, show reduced excretion, leading to toxic accumulation in mutant embryos.

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Slovenian biochemical society session: Biomarkers and therapeutics development

From snake venom to therapy: A novel drug candidate for short-term thrombosis prevention

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KEYWORDS: antiplatelet drugs, snake venom, snaclecs, thrombocytopenia, short-term thrombosis prevention

Contemporary antiplatelet drugs exert their antithrombotic effects by reducing platelet count and inhibiting platelet function, often resulting in excessive bleeding—a major side effect that complicates procedures in interventional cardiology and angiology. Profound and transient thrombocytopenia of functional platelets without bleeding was observed in patients envenomed by *Vipera a. ammodytes (Vaa*). This condition was rapidly reversed by Fab fragments of antibodies raised against the whole venom, leaving platelets fully functional. It was proposed that snake venom C-type lectin-like proteins (snaclecs) were responsible for this effect. To test this hypothesis, we purified snaclecs from crude venom, biochemically characterized them and studied their interaction with platelets. Six Vaa-snaclecs were isolated from the venom using a combination of five consecutive liquid chromatography steps and structurally analyzed. Platelet count, agglutination and aggregation assays, along with standard blood coagulation tests, identified *Vaa*-snaclec-3/2 as the most potent antiplatelet molecule.

This snaclec is a covalent heterodimer composed of Vaa-snaclec-3 (α-subunit) and Vaa-snaclec-2 (β-subunit). Flow cytometry revealed that Vaa-snaclec-3/2 induces thrombocytopenia by binding to the platelet receptor GPIb thus triggering platelet agglutination.

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Importantly, this effect was reversible leaving platelets functionally competent. We further evaluated the antithrombotic efficacy of Vaa-snaclec-3/2 in a murine model of ferric chloride-induced carotid artery thrombosis. This substance induced profound thrombocytopenia in a dose-dependent manner, with a median effective dose of 4.7 μg/kg.

Although it prolonged tail bleeding time, bleeding remained within the physiological range, and no spontaneous hemorrhage was observed. Histological analysis also showed no signs of acute bleeding. *Vaa*-snaclec-3/2 efficiently protected mice from carotid artery occlusion. The lowest dose that induced severe thrombocytopenia and completely inhibited ferric chloride-induced thrombus formation was 20 μg/kg. Our findings highlight the potential of Vaa-snaclec-3/2 as a promising agent for short-term thrombosis prevention in interventional cardiology and angiology.

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Slovenian biochemical society session: Molecular basis of disease

NLRP3 inflammasome and pyroptosis: From mechanisms to applications

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KEYWORDS: inflammasome, NLRP3, gasdermin, ROS, cancer

Inflammasomes are central components of early immune responses. Upon sensing infection or disrupted tissue homeostasis, inflammasome sensors lead to the assembly of inflammasomes and activation of inflammatory caspases. Caspase-1 subsequently proteolytically activates proinflammatory cytokines IL-1beta and IL-18 and gasdermin D into its N-terminal pore-forming domain that causes pyroptosis. Proinflammatory cytokine release combined with necrotic cell death induces potent inflammatory responses underlying different pathologies such as neurodegenerative and metabolic diseases. There are still major gaps in our understanding of how inflammasomes are activated and the regulatory processes involved. Particularly enigmatic is NLRP3, which, upon sensing chemically and morphologically diverse triggers, forms an inflammasome. We are investigating the regulation of the initial steps of NLRP3 inflammasome assembly1,2 and downstream responses that are common to all inflammasomes3,4.

While the current emphasis of NLRP3 inflammasome research is focused more on its detrimental action as the major driver of inflammation, the canonical role of inflammasomes is to protect the organism against threats. Cancer is an example of a disease where the immune system fails, and several types of tumors downregulate different cell death pathways. Using the synthetic biology approach combined with the mechanistic insight into inflammasome assembly, we are currently developing inflammasome-inspired approaches to provide efficient priming of immune responses in cancer immunotherapy5.

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Slovenian biochemical society session: Biotechnology

Homology-guided engineering of tyrosine recombinase DNA specificities

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KEYWORDS: genome editing, computational prediction, Xer-family tyrosine recombinases

Tyrosine recombinases represent attractive genome editing tools due to their ability to catalyze precise rearrangements of large DNA fragments without inducing double-stranded breaks. However, their complex sequence recognition and limited programmability have constrained their use. Here, we use comparative analysis of natural diversity to modulate the specificity of Xer-family tyrosine recombinases. By transplanting a seven-residue motif across structurally similar orthologs, we achieved predictable shifts in specificity for half of the monomer target. This strategy, combined with a high-throughput specificity profiling assay, enabled computational prediction of candidate native targets for previously uncharacterized archaeal Xer-like recombinases, validated by functional recombination in HEK293T cells. Finally, we predicted Xer-accessible sites in the human genome and demonstrated scarless integration of a 4.8-kb plasmid payload into endogenous genomic loci without pre-inserting recognition sites. These results represent a groundwork for rational engineering of Xer recombinase specificity highlighting their potential as compact genome editing tools.

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Slovenian biochemical society session: Molecular basis of disease

Alzheimer's dementia and paraoxonase 1: Why it pays off to measure enzyme kinetics and not just activities

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KEYWORDS: paraoxonase 1, Alzheimer's disease, mild cognitive impairment, cerebrospinal fluid, enzyme kinetics

Paraoxonase 1 (PON1) is an enzyme present in human blood plasma within HDL particles. The biological substrates of PON1 are still debated, but it is clear that PON1 plays an antioxidative role, and that its activity is decreased in many oxidative-stress-related conditions, including Alzheimer's dementia (AD). However, it is unclear whether PON1 activity is reduced in AD patients compared to the precursor stage of AD, mild cognitive impairment (MCI). Part of the problem is that PON1 activity is measured with different substrates; most groups only report specific activity for one or two substrates. To get a clearer understanding of PON1's role in MCI and AD, we measured PON1 plasma concentration as well as lactonase, arylesterase and aryldialkylphosphatase activities, and the corresponding kinetic parameters, for both blood plasma and cerebrospinal fluid (CSF) for a group of 73 patients with AD and 32 patients with MCI.

We found no difference in any of the measured parameters between AD and MCI, which strongly implies that after the onset of MCI, PON1 status does not change during disease progression. At the same time, we noticed that in CSF, patient age correlated with PON1 activity and with enzyme kinetics, but not with PON1 concentration. From this we can conclude that during the process of aging, there is a decrease in PON1 activity caused mostly by enzyme damage rather than by a decrease in its concentration.

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Poster presentations

Systems biology and bioinformatics

Exploring the function of conoporins, actinoporin-like proteins from cone snails

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Cone snails are marine gastropods that rely on venom to capture prey and defend against predators1. Their venom is rich in small, bioactive peptides known as conotoxins, which primarily target membrane receptors. In addition, a smaller part of the venom contains larger proteins believed to aid in conotoxin maturation and the envenomation process2. Among these are conoporins, which are similar to the α-pore-forming toxins actinoporins from sea anemones3. Due to the high expression of conoporins in the venom glands of many cone snail species, they are hypothesized to play a significant role in envenomation, although their exact function is still unknown. We propose four hypotheses for the function of conoporins: (i) permeabilization of epithelial barriers, (ii) facilitation of conotoxin membrane translocation through pores, (iii) antimicrobial activity, and (iv) involvement in digestion.

Our bioinformatic search yielded 95 unique conoporin sequences from 27 species. Compared to actinoporins, conoporins feature extensions at the N- and C-termini, while the predicted structure of the β-sandwich core appears conserved, similar to other molluscan actinoporin-like proteins4,5. Phylogenetic analysis revealed at least three distinct clades of conoporin sequences. Experimentally, we have expressed and purified three conoporins: ConM3 and ConM6 from the piscivorous *Conus magus*, and ConEb1 from the vermivorous *Conus ebraeus*. Interestingly, preliminary results indicate that ConM3 has hemolytic activity on bovine erythrocytes, while ConM6 and ConEb1 do not.

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Regulation of gene expression

Design of a modular miRNA-sensing based genetic circuit

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KEYWORDS: microRNA sensor, synthetic circuit, protease-based sensor

MicroRNAs (miRs) are a class of small non-coding, endogenous RNAs that regulate gene expression by interacting with target mRNAs. Their expression levels dynamically respond to cellular conditions thus serving as real-time biomarkers, providing a genetic snapshot of the cell's state. This unique feature makes miRs valuable for diagnostic applications, where specific miRs can be detected as a biomarker. Leveraging this mechanism, miR-based sensors can be developed for precise diagnostics. Traditional miR sensors often rely on loss-of-signal output, limiting their utility for dynamic or live-cell applications. We present a synthetic miR sensor platform that delivers a quantitative positive readout without facing the limitations of the previous existing systems.

Our system utilises a bidirectional promoter to co-express a reporter containing a specific protease cleavage site and a mammalian compatible protease. The miR-regulated expression of the protease controls cleavage of the reporter protein, tunable via small-molecule inhibition. During the absence of miRs, the protease cleaves and destabilizes the reporter, resulting in low signal. However, by placing the protease under negative regulation of miRs, miR expression leads to reduced protease levels, thereby preserving the integrity of the reporter and enhancing signal output, corresponding to the miR levels. Constructs were generated using molecular cloning and transfected into HEK cells, with miR overexpression confirmed by qPCR and reporter activity quantified using dual luciferase assays. This system is thought to offer improved specificity, tunability, and translational potential for live-cell monitoring and therapeutic applications.

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Regulation of gene expression

Investigation of the effect of selected miRNAs using bioinformatics approaches on AHNAK protein regulation in drug-resistant breast cancer cells

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KEYWORDS: AHNAK, breast cancer, drug resistance

AHNAK protein plays a role in various cellular processes and can exhibit both tumor-promoting and tumor-suppressing properties through different pathways in cancer. Examining these dual effects of AHNAK, particularly in cancer cells with a multidrug resistance phenotype is crucial for revealing its therapeutic potential. The aim of this study is to identify miRNAs whose expression is altered in drug-resistant cells and target AHNAK protein by bioinformatic analysis and to reveal the regulation of AHNAK in drug-resistant cells in relation to candidate miRNAs.

For this purpose, AHNAK gene expression was examined in drug-sensitive parental MCF-7 (MCF-7/S), docetaxel-resistant MCF-7 (MCF-7/RDoc), and doxorubicin-resistant MCF-7 (MCF-7/RDox) cell lines. An 8-fold increase in MCF-7/RDoc cells, and a 3-fold increase in MCF-7/RDox cells was detected. To identify the miRNAs associated with this increase, dataset "GSE237873" from Gene Expression Omnibus was used to identify miRNAs expressed differently in drug-resistant breast cancer cells. Among the identified miRNAs, those targeting the AHNAK were identified by comparing data from MirDB, miRTargetLink, and TargetScan databases with Venny2.1. In line with our experimental results showing that AHNAK expression increases in resistant cells, 3 miRNAs (miR-222-5p, miR-5194, miR-30a-3p) targeting AHNAK and whose expression decreases in resistant cells were selected for further analysis. When miRNA gene expression was examined, an alteration was observed that was inversely proportional to the increase in AHNAK, and these results showed that AHNAK regulation in drug-resistant cells may be associated with altered miRNA expression.

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Biomarkers and therapeutics development

EryA-mCherry as a specific molecular probe for detecting surface-exposed cardiolipin in apoptotic mammalian cells

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KEYWORDS: aegerolysin, annexin, apoptosis marker, cardiolipin, *Pleurotus*

Erylysin A (EryA), an aegerolysin protein produced by the edible king oyster mushroom (*Pleurotus eryngii*) interacts strongly with an invertebrate-specific membrane sphingolipid ceramide phosphoethanolamine. Recently, a fluorescently fused variant of EryA was shown to bind to artificial and bacterial lipid membranes containing cardiolipin (CL). This tetra-acylated glycerophospholipid, present in bacteria and in inner mitochondrial membranes of eukaryotic cells, was shown to be externalized to the plasma membrane surface during the process of apoptosis. In this work, we evaluated the interaction of EryA-mCherry with CL-containing artificial lipid vesicles and with mammalian cells undergoing apoptosis and compared its binding affinity and specificity to that of the well-established apoptosis marker, annexin V-FITC. Our results show that, in contrast to annexin V-FITC which binds different negatively charged glycerophospholipids, EryA-mCherry specifically recognizes and binds CL in artificial membrane systems. Experiments using mammalian cells showed the ability of EryA-mCherry to selectively label the surface of apoptotic cells, exhibiting the same labeling pattern as anti-CL antibodies. Our data suggest that EryA-mCherry might be used as marker of early apoptosis, as well as a marker of CL in biological and artificial lipid membranes.

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Biomarkers and therapeutics development

Differentiation therapy as a new multidisciplinary approach to the treatment of human brain glioma

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KEYWORDS: brain glioblastoma, differentiation therapy, aptamer, small molecules

Glioblastoma is one of the most severe human tumors. Today there is no effective treatment for glioblastoma. With radiation therapy or chemotherapy most tumor cells die, but a small part of the cells is resistant and initiate a relapse of the tumor. Therapeutic failures require a search for new approaches to the treatment of glioblastoma. We have developed a fundamentally new approach to the treatment of glioma coined as "differentiation therapy". It is based on a cytostatic effect of the DNA aptamer bi(AID-1-T), capable of blocking a proliferation of tumor cells, in combination with the subsequent treatment with inducer molecules that control cascades of neurogenesis - SB431542, LDN-193189, Purmorphamine, BDNF. Aptamer bi(AID-1-T) stops division of tumor cells for a short time, making the tumor cell to be sensitive to agents promoting their differentiation. Studies on cells of continuous cultures of gliomas developed from patients with a high degree of malignancy have shown the success of this approach *in vitro*. *In vivo* studies using an animal model (rat) with implanted tissue glioblastoma 101/8 have shown the effectiveness of this approach as well.

ACKNOWLEDGMENT: The work has financial support from grant No. 075–15-2024-561.

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Biomarkers and therapeutics development

Expressions of *IL10* and *IL6* are upregulated and correlated with miR-21 expression in peripheral blood mononuclear cells of women with gestational diabetes

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KEYWORDS: IL6, IL10, miR-21-5p, gestational diabetes, inflammation

Continuous low-level inflammation (IFM) accompanies gestational diabetes (GDM) and contributes to the development of severe pregnancy complications associated with hyperglycemia. Inflammatory pathway-related genes are targeted by microRNAs heavily dysregulated in GDM, including miR-21. The aim of the conducted research was to evaluate the potential changes in the expression of miR-21-related cytokine genes *IL10* and *IL6* in peripheral blood mononuclear cells (PBMCs) in GDM patients versus healthy normoglycemic controls. PBMCs were extracted from peripheral blood samples obtained from 50 women diagnosed with GDM and 50 controls (pregnancy weeks 24-30). Relative quantification of mir-21-5p, as well as of *IL10* and *IL6* mRNA, was conducted by quantitative real-time PCR. A significant increase in the level of expression of IL10 and IL6 was observed in GDM patients, compared to normoglycaemic controls ($p=0.001$ and $p=0.041$, respectively). Furthermore, the expression of these mRNAs positively correlated with miR-21 level in PBMC, while IL10 also showed correlation with the newborn weight. The presented results highlight the specific changes in the expression of immune system modulators in GDM and illustrate their relation with the IFM-associated miR-21, as well as their biomarker potential in hyperglycemic pregnancies.

AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200019).

Biomarkers and therapeutics development

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Biomarkers and therapeutic development

Olaparib alleviates Diclofenac induced-hepatocellular toxicity via targeting mitochondrial function

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KEYWORDS: ROS, mitochondrial membrane potential, apoptosis inducing factor, poly (ADP-ribose) polymerase (PARP1)

Diclofenac is a widely used non-steroidal anti-inflammatory drug (NSAID) for the treatment of pain and inflammation. However, its use is often associated with serious health risks including liver toxicity, kidney damage, and heart problems. Diclofenac-induced hepatotoxicity is characterized by the increased production of reactive oxygen species (ROS) and mitochondrial dysfunction, both of which are critical factors in liver injury. Diclofenac elevates oxidative stress in hepatic tissues by enhancing the generation of ROS and malondialdehyde (MDA), a marker of lipid peroxidation, while simultaneously reducing the levels of glutathione (GSH), a key intracellular antioxidant. This imbalance leads to oxidative damage in hepatic cells. Olaparib, a targeted cancer therapy agent, acts primarily as a poly (ADP-ribose) polymerase (PARP1) inhibitor, impairing the repair of single-strand DNA breaks.

This inhibition leads to accumulation of DNA damage, which trigger cancer cell death. In addition to inhibitory role in DNA repair, olaparib exhibits cytoprotective effects by reducing cellular damage and promoting cell survival under stress conditions. In this study, we demonstrated that olaparib confers protection to the HepG2 cells against diclofenac-induced cytotoxicity in vitro. The inhibition of PARP1 by olaparib mitigates parthanatos by blocking the excessive activation of PARP1, which is typically triggered by elevated ROS levels.

Biomarkers and therapeutic development

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Olaparib reduces mitochondrial respiration, which, in turn, lowers diclofenac-induced intracellular ROS levels. We demonstrated, for the first time, that inhibition of PARP1 by olaparib protects HepG2 cells from diclofenac-induced toxicity by enhancing mitochondrial polarization. Given its hepato-protective properties and its ability to attenuate damage in HepG2 cells, olaparib has significant potential for clinical applications beyond oncology.

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Biomarkers and therapeutics development

Engineering of the intrinsically fluorescent tetravalent cytotoxic conjugate TetraFHER2-vcMMAE for efficient elimination of HER2+ breast cancer cells

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KEYWORDS: HER2, breast cancer, targeted therapy, cytotoxic conjugate, endocytosis

Breast cancer is the most prevalent malignancy among women worldwide. The HER2 receptor is overexpressed in approximately 20-30% of all breast tumors and is considered one of the major oncogenic drivers in breast cancer. HER2 is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (RTKs). HER2 is involved in the regulation of pivotal cellular processes, such as cell division, motility, differentiation and plays a fundamental role in the physiological growth and differentiation of breast tissue. Several HER2-targeted cytotoxic conjugates have been developed, but their efficacy is limited. One of the main obstacles restraining the effectiveness of HER2-specific cytotoxic conjugates is their very low internalization, as HER2 is immobile mainly on the cell surface. Therefore, there is a need to develop novel HER2-selective cytotoxic conjugates that will overcome HER2 immovability and, by this, ensure efficient drug delivery into HER2-overexpressing cancer cells. Here, we present a novel system for generating high affinity, self-assembling, inherently fluorescent, multivalent HER2 ligands.

The developed HER2-specific ligands largely overcome the innate stability of HER2 in the plasma membrane by triggering clathrin-independent, aggregation-dependent endocytosis of the receptor. We selected TetraFHER2 as a drug carrier for the engineering of a fluorescent cytotoxic conjugate. Our data indicate that TetraFHER2-vcMMAE serves as a highly selective and efficient drug carrier for targeted treatment of HER2-positive breast cancer cells.

ACKNOWLEDGMENT: This research was funded by a PRELUDIUM grant (2022/45/N/NZ1/00088) from the National Science Centre.

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Biomarkers and therapeutics development

Epigenetic biomarkers associated with offspring mental health following parental exposure to war trauma

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KEYWORDS: NR3C1 gene, DNA methylation, epimutations

DNA methylation, the most biochemically stable epigenetic modification regulating gene expression, plays a crucial role in shaping an individual's phenotype and is directly influenced by environmental factors, including social interactions. Moreover, DNA methylation biomarkers can be inherited across generations, encoding phenotypic traits in offspring born long after initial exposure. This has led to a growing hypothesis that warrelated events and consequent social disruptions may leave a lasting imprint on the epigenome, contributing to mental health challenges and behavioural impairments in future generations.

One of the most prominent genes associated with traumarelated epigenetic inheritance is NR3C1, which epimutations have been associated with psychiatric conditions such as depression, PTSD, and anxiety, as well as neurodevelopmental disorders. To investigate the intergenerational transmission of wartraumarelated epigenetic biomarkers, an interdisciplinary study integrating biomedical and psychosocial research was conducted, funded by the Croatian Science Foundation (IP2020025967). A targeted participant recruitment protocol resulted in a sample of 76 mothers and 45 fathers who survived war trauma during the Croatian War of Independence in the 1990s, along with 80 of their children born in the postwar era. Buccal swabs were collected, DNA isolated, and DNA methylation analysis of the NR3C1 gene was performed using pyrosequencing. The results revealed comparable average DNA methylation levels of the NR3C1 gene in both parental and offspring generations, suggesting a strong intergenerational transmission of wartraumarelated epigenetic biomarkers. Additionally, a significant positive correlation was observed between maternal and offspring DNA methylation, indicating a more pronounced maternal inheritance of warrelated epigenetic biomarkers.

Biomarkers and therapeutics development

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Biomarkers and therapeutics developement

Proteomic identification of biomarker proteins for *Campylobacter jejuni* detection in poultry

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KEYWORDS: Campylobacter jejuni, hippuricase, LC-MS/MS, biomarker proteins, poultry safety

Campylobacter jejuni is a leading cause of bacterial gastroenteritis, frequently linked to poultry consumption. In this study, we applied a combined biochemical and proteomic workflow for the identification of C. jejuni in retail chicken meat (breast, liver, legs, wings, and backs), with the goal of identifying protein biomarkers for sensitive detection. C. jejuni were enriched on selective charochal-agar based medium and tested by the biochemical assay based on hypuricase activity of C. jejuni. Eleven samples (16.9%) were positive, with the highest prevalence in liver (30.7%) and backs (20%).

Mass spectrometry identified 7–9 characteristic C. jejuni proteins per positive sample, including major outer membrane protein (P80672), flagellin B (P56964), catalase (Q59296), cysteine synthase B (P71128), threonine-tRNA ligase (A1VXT5), fumarate hydratase class II (O69294), succinate-CoA ligase subunit beta (A8FKV6), large ribosomal subunit protein uL3 (Q9PLX1), and probable histidine-binding protein (Q46125). These biomarkers, associated with motility, energy metabolism, and nutrient transport, provided strong molecular confirmation of C. jejuni presence in enzymatically positive samples. Eight assay-negative samples were further analyzed by proteomics; one revealed multiple C. jejuni-specific proteins and was confirmed positive upon re-plating and enzymatic testing, demonstrating the assay's specificity and the importance of colony selection. The remaining assay-negative samples yielded proteins from other bacterial species (e.g., Escherichia coli, and Pseudomonas fragi), supporting the methods specificity for hippuricase-positive C. jejuni. Our study demonstrates that combining the colorimetric assay with LC-MS/MS biomarker identification provides a sensitive and confirmatory approach for C. jejuni detection in poultry. This workflow enhances specificity, allows detection of both metabolically active and certain inactive bacterial cells, and supports its application in food safety monitoring.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Biomarkers and therapeutics development

Redox and immunomodulatory effects of silver nanoparticles synthesized using *Salvia verticillata L.* extracts in human myeloid leukemia cells

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KEYWORDS: silver nanoparticles, Salvia verticillata L., redox status, human myeloid leukemia cell line K562, immunomodulatory effect

The objective of the study was to examine in vitro the effects of silver nanoparticles synthesized using Salvia verticillata L. aerial part (SVAAgNPs) and root (SVTAgNPs) extract on the proliferation rate, redox status, migratory potential, concentration of metalloproteinases (MMP-2 and MMP-9) and MMP-9 gene expression of the human myeloid leukemia cell line K562, as well as the immunomodulatory effect through cytokine production (IL-1β and IL-6). The treatments with SVAAgNPs and SVTAgNPs at concentrations range from 5 to 100 μg/mL induced an increase in the concentration of superoxide anion radicals and nitrite production after 24 and 72 h of treatments, indicating a pro-oxidative effect of the nanoparticles.

The concentrations of total and reduced glutathione in K562 cells were also elevated, confirming oxidative stress induction and de novo glutathione synthesis. This response may contribute to a degree of tumor cell resistance to silver nanoparticle action but could also help preserve healthy cell viability and mitigate the adverse effects of cytostatics. The migration capacity, MMP-2 level, and MMP-9 expression were significantly reduced compared to the control in both time treatments. Additionally, IL-6 concentrations increased, while IL-1β levels decreased, indicating notable immunomodulatory activity of the silver nanoparticles. The findings from this study may support the development of combined therapeutic strategies incorporating conventional cytostatics and silver nanoparticles to enhance antitumor efficacy while preserving the viability of healthy cells and reducing the harmful side effects associated with chemotherapy.

Biomarkers and therapeutics development

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Molecular basis of disease

Molecular basis of identification *Mycobacterium tuberculosis*

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KEYWORDS: M. tuberculosis, PCR, tuberculosis

In our work, we determine the presence of viable bacillus Mycobacterium tuberculosis compl. (M. tuberculosis compl.) in patient samples.

After admission, patient samples were processed using the NALC method. We used RT-PCR molecular methods: BD MAX MDR-TB test (BD MAX, Becton Dickinson, USA) and Xpert MTB/RIF Ultra test (GeneXPERT, Cepheid, USA). We analyzed the samples in which the presence of the gene M. tuberculosis compl. were detected by molecular methods in a small number of copies. The presence of viable bacilli in these clinical samples was confirmed by the growth of colonies on solid Jansen-Löwenstin agar (LJ; made in the laboratory) and liquid mycobacterial growth indicator tube (MGIT; Becton Dickinson, USA). Phenotypic results were interpreted after 6 and 8 weeks of incubation, respectively.

During 2024, out of 30 positive samples by molecular method (Xpert MTB/RIF Ultra test) with the result ''in trace'', after incubation, the presence of M. tuberculosis compl. was phenotypically confirmed in 2 samples on MGIT, in 1 on LJ medium, while 19 samples were negative (no growth). Out of 11 positive samples with the result ''low pos'' on BD MAX MDR-TB test, after incubation on MGIT and LJ medium, the presence of M. tuberculosis compl. was phenotypically confirmed in 3 samples.
The molecular tests have shown the ability to detect the M. tuberculosis compl. gene in a very low number of copies, which enables rapid diagnosis of tuberculosis. However, the results of the molecular test must be phenotypically confirmed by cultivation, since the presence of the gene does not indicate the viability of the bacilli.

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Molecular basis of disease

Crosstalk between androgen receptor and hedgehog-GLI signaling pathways regulates PSA expression in prostate cancer

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KEYWORDS: hedgehog-GLI signaling pathway, GLI2, prostate cancer, PSA regulation

Prostate cancer (PC) is the commonly diagnosed cancer in men and is associated with rising mortality. Prostate-specific antigen (PSA) is a key biomarker for PC, and its expression is regulated by the androgen receptor (AR), which is often overexpressed in PC. Recent studies have highlighted a potential interaction between AR and GLI2, a transcription factor in the Hedgehog-GLI (HH-GLI) signaling pathway, which is also active in various cancers, including PC.

This study investigates whether the expression of PSA is influenced by AR, GLI2, or their interaction. Using LNCAP prostate cancer cells overexpressing GLI2, researchers observed increased PSA (KLK3) levels compared to controls. Analysis of the KLK3 gene revealed multiple GLI2 binding sites, suggesting direct regulation. The study further examined the effects of various treatments—enzalutamide (AR inhibitor), sonidegib (HH-GLI inhibitor), and dihydrotestosterone (DHT, an AR activator)—on PSA expression. qPCR analysis showed that PSA levels decreased with both enzalutamide and sonidegib treatment, indicating that both AR and GLI2 play roles in PSA regulation. DHT treatment increased PSA and AR expression, but this effect was reversed with sonidegib, pointing to crosstalk between the AR and HH-GLI pathways. These findings provide new insight into the molecular mechanisms controlling PSA expression in prostate cancer. Understanding how AR and GLI2 jointly regulate PSA may lead to novel therapeutic targets for managing PC.

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Molecular basis of disease

Evaluation of barrier dysfunction in Caco-2 and T84 cells exposed to peanut allergens

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KEYWORDS: allergen, peanut, Caco-2, T84, barrier

The human intestinal barrier serves as a first line of defense and plays an essential role in the regulation of immune response to food antigens1. Alterations to this barrier caused by food allergens, such as peanut allergens may lead to increased permeability, immune activation and allergic sensitization2. Therefore, understanding the interactions between peanut allergens and the intestinal epithelium is important for determining the underlying allergic mechanism. Caco-2 and T84 are frequently used models for such investigation of different intestinal barrier aspects. In the present study, the effects of peanut allergen exposure on the barrier integrity as well as mucosal innate immunity were investigated in Caco-2 and T84 cell lines. qRT-PCR was performed to determine the baseline expression of tight junction proteins (ZO-1, Claudins, and E-cadherin), mucins (MUC2, MUC5B) and Toll-like receptors (TLR2, TLR5). Both cell lines were treated with whole peanut extracts derived from raw and roasted peanuts, with lipopolysaccharide (LPS) serving as a positive control.

Barrier integrity was assessed post treatment using transepithelial electrical resistance (TEER), Lucifer Yellow permeability assays and FITC-Dextran transport studies. ZO-1 and MUC2 expressions at protein level were assessed using immunofluorescence microscopy. An inhibition ELISA was performed to assess the translocation of Ara h 1 across the epithelial layer. Findings have shown that the peanut allergen exposure transiently reduced TEER in cells treated with whole peanut extracts. Regardless of the drop in TEER, the results from the Lucifer yellow assay did not show much difference after peanut allergen exposure. Ara h 1 transport to the basolateral compartment was detectable in basolateral compartment of cells treated with raw extract but to less degree with roasted extract. These results suggest that peanut allergens impair epithelial barrier function, highlighting barrier dysfunction as a key early event in allergic responses.

Molecular basis of disease

Evaluation of barrier dysfunction in Caco-2 and T84 cells exposed to peanut allergens

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Acknowledgment: The ALLPreT project has received funding from the European Union's research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101072377.

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Molecular basis of disease

Cathepsin L inhibition enhances microglial CX3CR1 expression in EAE

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KEYWORDS: cathepsin, multiple sclerosis, experimental autoimmune encephalopathy, microglia

Cathepsin L (CatL), a lysosomal cysteine protease, is highly expressed in antigen-presenting cells and plays a role in the processing of autoantigens. It is widely expressed throughout the CNS and is associated with microglia-driven neuroinflammatory responses. Previous studies suggest that inhibition of catL activity may offer therapeutic potential for the treatment of multiple sclerosis. The aim of this study was to investigate the effects of a selective catL inhibitor [CC(=O)c1cccc(NC(=O)c2cnc3sccn3c2=O)c1] on microglia in mice immunized for experimental autoimmune encephalomyelitis (EAE).

The catL inhibitor was administered intraperitoneally for five consecutive days from the onset of clinical signs of disease. Mononuclear cells were isolated from the spinal cord (SC) of EAE rats and analysed by flow cytometry. Given that this was the first in vivo application of this inhibitor, hepatotoxicity and nephrotoxicity were also investigated. The administered catL inhibitor reduced the infiltration of the SC by CD4+ T lymphocytes. On the other hand, the proportion of CD11b+ cells among the mononuclear cells isolated from the SC was higher in the treated EAE mice. CatL inhibitor decreased the proportion of CD45high cells among CD11b+ cells, most likely monocyte-derived macrophages that infiltrated the SC and increased the proportion of CD45low/int cells among CD11b+ cells, which correspond to microglia. The proportion of neuroprotective CX3CR1+ cells among microglial cells was significantly higher in treated mice than in untreated EAE mice. These findings reinforce the hypothesis that catL could serve as a potential therapeutic target for treating multiple sclerosis.

Molecular basis of disease

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Molecular basis of disease

Salivary immune response to Candida albicans in children with early childhood caries

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KEYWORDS: early childhood caries, immune responses, IgG and IgA immunoglobulins, C. albicans

Early childhood caries (ECC) is a common chronic condition in young children characterized by rapid tooth destruction. While oral streptococci are the main pathogens, fungi like C. albicans may also contribute to disease progression. C. albicans is more frequently found in children with ECC, but the role of local immune responses, especially salivary immunity to C. albicans, remains unclear. This study aimed to analyse total salivary proteins and the titre and avidity of IgG and IgA immunoglobulins, including their subclasses, specific to C. albicans in children with ECC compared to caries-free children. The study included 30 children with ECC and 15 caries-free children (control group). Salivary total protein concentration was measured by BCA assay, and titres and avidities of C. albicans-reactive IgG, IgA, and their subclasses (IgG1–IgG4; IgA1–IgA2) were assessed using an in-house ELISA. Data analysis was performed using SPSS.

Results showed a significant increase in titre of total IgG, IgA, and IgG1 and IgA2 subclasses in the ECC group compared to controls (p=0.020; p=0.035; p=0.013; p=0.036). Strong correlations were observed between titres of IgA1 and IgA2, and titres between IgG1 and IgG4 in ECC (r=0.59, p=0.01; r=0.67, p=0.001). In controls, a significant correlation was found between anti-Candida IgG2 and IgG3 titres (r=0.618, p=0.001). No differences were found between ECC and control group in total salivary protein concentration, antibody avidity, or other subclass titres.
These findings indicate enhanced humoral immune responses against C. albicans in children with ECC, potentially reflecting immune adaptation or increased antigenic stimulation. Further research is needed to explore their role in ECC pathogenesis.

Molecular basis of disease

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Molecular basis of disease

The role of p97 ATPase and SPRTN protease in the repair of DNA-protein crosslinks in human cells and animal model

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KEYWORDS: DNA damage, DNA-protein crosslinks, SPRTN protease, p97 ATPase, zebrafish

Proteins that interact with DNA can become irreversibly covalently bound to DNA and form lesions called DNA-protein crosslinks (DPCs). At the cellular level, impaired DPC repair can lead to DNA double-strand breaks, genomic instability and cell death, resulting in cancer, premature aging and neurodegenerative diseases at the organismal level. p97 (VCP) is an AAA+ ATPase that unfolds and segregates target proteins from various cellular structures, including chromatin. SPRTN (DVC1) is an essential protease involved in DPC repair in metazoans. In human cells, SPRTN and p97 form a stable complex and may cooperate in DPC repair. The aim of our study was to determine whether the interaction of SPRTN with p97 is important for DPC repair using human cell cultures and a zebrafish animal model. We used gene silencing to reduce endogenous SPRTN levels, chemical inhibitors to inhibit p97, overexpression of SPRTN with mutated p97 interaction motif and the modified RADAR (rapid approach to DNA adduct recovery) method to isolate cellular DPCs. Our results showed that knockdown of endogenous SPRTN and inhibition of p97 in human cells and zebrafish embryos lead to increased levels of cellular DPCs, including crosslinked histone H3, KU80, PARP1, DNMT1 and TOP1. Furthermore, we showed that overexpression of the SPRTN mutant which cannot bind to p97 leads to an increase in specific cellular DPCs in human cells and zebrafish embryos lacking SPRTN-WT. Our study demonstrates that SPRTN and p97 act separately in the repair of most cellular DPCs under physiological conditions and after exposure to anticancer drugs, whereas the SPRTN:p97 complex is important for the repair of specific DPCs.

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Molecular basis of disease

Identifying the molecular causes of neurodegenerative diseases using new animal models created with CRISPR-Cas gene editing

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KEYWORDS: DNA repair, DNA-protein crosslinks, topoisomerases, brain, animal model

Human neurological disorders SCAN1 (spinocerebellar ataxia with axonal neuropathy type 1) and SCAR23 (spinocerebellar ataxia, autosomal recessive 23) are caused by mutations in tyrosyl-DNA phosphodiesterases 1 and 2 (TDP1 and TDP2). These enzymes remove crosslinked topoisomerases and thus prevent genomic instability. Topoisomerases 1 and 2 (TOP1 and 2) are essential for cell survival as they release the torsional stress of DNA during transcription and replication. If crosslinked topoisomerases are not repaired, replication and transcription is stalled which eventually leads to DNA breaks. How the impairment of enzymatic TDP activity leads to neurological dysfunction and why the effects only occur in the brain is still unknown.

To address these questions, we have used CRISPR-Cas editing to create new zebrafish lines deficient in TDP1 and/or TDP2. I will present our recent discoveries where we biochemically characterized DNA protein crosslinks in mutant strains. We have found that (1) TDP1 is a key factor for the repair of TOP1- and histone H3-DPCs and TDP2 for the removal of TOP2 and KU70/80-DPCs; (2) TDP1 and SPRTN protease function in separate pathways in the repair of endogenous TOP1-DPCs and in the same pathway after TOP1-DPC induction by chemotherapeutics and (3) TDP2 deficiency leads to an increase in DSBs, likely due to its role in the error-free NHEJ. In summary, we have identified some of the molecular causes of the neurodeficient phenotypes caused by TDP deficiencies, while our ongoing work will provide further in-depth analyses of neurological disorders in the aging brain.

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Molecular basis of disease

Blood serum biochemical parameters related to cardiovascular health in peripubertal rats as markers for evaluating the effectsof *in-utero* exposure to ELF-MF

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KEYWORDS: ELF-MF, in-utero exposure, cardiovascular function, peripubertal rats

Although the effects of acute or chronic exposure to extremely low-frequency magnetic field (ELF-MF) generated by domestic installations, household appliances, and power grids on the cardiovascular system are inconclusive, there are serious indications in this direction. Blood tests are one of the approaches that can be used as markers for evaluating the health of the cardiovascular system. Thus, this study addresses the effects of continuous in-utero exposure to ELF-MF (50 Hz, 0.5 mT) on serum concentrations of creatine kinase, total cholesterol, triglyceride, and low-density lipoprotein-cholesterol in peripubertal rats.

Adult male and female rats (Wistar IGS) were placed near an on/off electromagnet generating ELF-MF (50 Hz, 0.5 mT) until conception, and pregnant females until the offspring were born. Immediately after birth, the mothers and their offsprings were removed from the ELF-MF exposure system. Between 09:00 and 10:00 on postnatal days 40-42, blood samples were taken from the trunk of each animal, and the sera were isolated and stored at -80°C until analysis. Serum parameters of cardiovascular function were analyzed using an automated biochemical analyzer (BS-240 Vet). The Kruskal-Wallis and Mann-Whitney tests were used to evaluate the changes in each parameter.

Molecular basis of disease

Blood serum biochemical parameters related to cardiovascular health in peripubertal rats as markers for evaluating the effectsof in-utero exposure to ELF-MF

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Continuous in-utero exposure to ELF-MF causes the following significant effects in peripubertal offspring: (1) decreased creatine kinase in males and increased creatine kinase in females, (2) decreased total cholesterol in males, (3) increased triglyceride in both sexes, and (4) decreased low-density lipoprotein-cholesterol in both sexes. These results suggest that continuous in-utero exposure to ELF-MF may influence cardiovascular function in later life.

ACKNOWLEDGMENT: This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200007).

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Molecular basis of disease

Investigating long non-coding RNA lnc-RNMT-2:5 indicates stromal origin and putative regulatory function in rectal cancer

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KEYWORDS: colorectal cancer, gene expression regulation, long non-coding RNA, transcriptome

Recent pan-cancer transcriptome analysis revealed differential activity of two alternative LDLRAD4 gene promoters in malignant vs. non-malignant rectal tissue. The promoter upregulated in rectal cancer (RC) drives expression of the long non-coding RNA lnc-RNMT-2:5, previously annotated as LDLRAD4-217 transcript (ENST00000592657). This study aimed to investigate the relevance of lnc-RNMT-2:5 for malignant transformation using in silico and in vitro approaches.

Promoter binding site prediction using TFBIND, Motiflab, and CIIIDER identified several transcription factors, including GATA1/2/3, YY1, ARNT, and STAT3, which are known to regulate key cancer-related processes such as proliferation, invasion, metastasis, and therapeutic response in cancer. Additionally, miRNA target analysis predicted binding sites for miR-214-5p and members of the miR-30 family, both recognized as tumor suppressors in colorectal malignancies.

These findings suggest that lnc-RNMT-2:5 may act as a competing endogenous RNA (ceRNA), promoting tumor progression by sequestering suppressive miRNAs. lncLocator predicts its predominant localization in the cytoplasm, further supporting its potential sponge-like function. Although previous studies reported differential expression of lnc-RNMT-2:5 in rectal tissue, its absence in gut epithelial cell lines (HCEC-1CT, HCT116, DLD1, and SW620) in our transcriptomic analysis suggests a stromal-specific expression pattern. This stromal origin is further supported by our qPCR validation, which confirmed its presence in the fibroblast cell line CCD-18Co and in healthy and inflamed colon mucosa.

The lnc-RNMT-2:5 likely represents a stromal-derived sponge lncRNA with a potential role in promoting colorectal cancer progression. Future studies should confirm its stromal-specific expression and further explore its molecular function and therapeutic potential.

Molecular basis of disease

Investigating long non-coding RNA lnc-RNMT-2:5 indicates stromal origin and putative regulatory function in rectal cancer

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FUNDING: This research was supported by the Science Fund of the Republic of Serbia, PROMIS, #6052315, SENSOGENE and IMGGE Annual Research Program for 2025, Ministry of Science, Technological Development and Innovation of the Republic of Serbia, 451-03-136/2025-03/200042.

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Molecular basis of disease

Establishment of an insulin resistance model in HepG2 cells using combined high glucose and insulin treatment

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KEYWORDS: insulin resistance, type 2 diabetes, insulin signaling cascade, HepG2 cell line

Insulin resistance, a key feature of type 2 diabetes, is characterized by impaired cellular response to insulin and dysregulation of the insulin signaling cascade. The HepG2 cell line is widely used as a model for investigating the pathological mechanisms of insulin resistance. However, establishing a consistent and reliable model remains challenging due to differences in treatment protocols and inherent limitations of the HepG2 cell line itself.

In this study, we treated HepG2 cells with high concentrations of glucose (3, 4.5 and 10 g/L) and high doses of insulin (10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M), both individually and simultaneously, to establish an insulin resistance model. Cell viability was assessed using the MTT test, and qPCR was used for determination of expression of IRS1, GLUT2, AKT1 and G6PC1 genes in treated cells.

Our results demonstrated a significant decrease in cell viability at the highest insulin concentration (10^{-5} M). Notably, neither insulin alone nor high glucose concentrations individually induced insulin resistance in HepG2 cells. However, the combined treatment with 4.5 g/L glucose and 10^{-6} M insulin effectively induced insulin resistance while maintaining cell viability. As expected, the expression of IRS1, AKT1 and GLUT2 was decreased, while the expression of G6PC1 was increased in the model.
The established model of insulin resistance will be further used for drug screening purposes

ACKNOWLEDGEMENT: This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 6999, GlucoAdjust.

Molecular basis of disease

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Design and structure of proteins

Extracellular protease of Aspergillus ochraceus VKM F-4104D: Properties and medical application promises

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KEYWORDS: proteases of snake venom, Aspergillus ochraceus protease, protein C activators

In recent years, extracellular proteinase from Aspergillus ochraceus was shown to possess activating activities for protein C, one of the most important plasma proenzymes. The need to determine the content of this protein in human plasma has led to the development of corresponding diagnostic approaches and preparations. Diagnostic kits for the colorimetric detection of the protein C concentration include protein C activating proteinases obtained from snake venom from the Agkistrodon contortrix contortrix.

The present study aimed to isolate and investigate the properties of plasma protein C activating proteinase from the culture fluid of micromycete A. ochraceus VKM-4104D in comparison with the protein C activator obtained from venom of Agk. contortrix. Based on inhibitory analysis, the protein C–activating proteinase from A. ochraceus appeared to be a serine proteinase - protein C activator, together with that isolated from the venom of Agk. contortrix contortrix. The isolated enzyme was a nonglycosylated protein with a molecular weight of about 33 kDa, pI 6.0 with an observed optimal activity under a pH of 8.0–9.0 and 37°C. A comparison of the properties of the protein C–activating proteinase formed by A. ochraceus and the enzyme derived from the venom of Agk. contortrix contortrix demonstrated a similarity in their properties; however, proteinase from the micromycete appeared to be in the nonglycosylated state and possessed the ability to hydrolyze the chromogenic plasmin substrate H-D-Val-Leu-Lys-pNA.
The in vitro anticoagulant action of proteases secreted by the micromycete A. ochraceus and contained in snakes' venoms (Protac® and RVV-X® preparations) was studied. The severity of the action was shown of micromycete protease in relation to plasmas of humans and warm-blooded animals, as well as human plasmas deficient in certain factors of the hemostasis system, in comparison with snake proteases in reactions with chromogenic peptide substrates of activated protein C and factor X as well as using the activated partial thromboplastin time (APTT) test.

Design and structure of proteins

Extracellular protease of Aspergillus ochraceus VKM F-4104D: Properties and medical application promises

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Therefore, the protein C–activating proteinases produced by A. ochraceus might appear to be more available and cheaper compared to the venom-derived ones, which makes this producer a promising alternative source of protein C activators for their use in diagnostics.

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Design and structure of proteins

Amyloid fibrillation and toxic/essential trace elements in Parkinson's disease

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KEYWORDS: Parkinson disease, amyloid aggregation, essential metals, potentially toxic metals

Parkinson disease (PD) is progressive neurodegenerative disease that mostly affects motor system. Severity of the disease is monitored via the Unified Parkinson's Disease Rating Scale (UPDRS) total score, where higher score indicates more severe symptoms. Amyloid aggregation of misfolded alpha-sinuclein into insoluble beta-sheet rich fibrils that accumulate as Lewy bodies plays a crucial role in progression of the disease. PD is associated with altered amyloid protein structure and altered levels of circulating trace elements, which could influence the outcome and progression of PD. In this regard, we aimed to examine the presence of amyloid in 33 sera from PD patients with different UPDRS using thioflavin T (ThT) fluorescence, and the propensity of proteins to misfold and aggregate using infrared (IR) analysis of the Amide I, II, and III regions. In the same serum samples, the concentration of essential (Cr, Mn, Co, Cu, Zn, Se, Mo) and selected toxic trace elements (Ni, Al, As, Cd, Pb) was determined using inductively coupled plasma mass spectrometry (ICP-MS).
We established the following findings: a) serum Cu, Zn and Se levels were significant for PD progression, b) Cu, Zn and Se promoted fibril accumulation and overall protein conformational changes, and c) all analyzed trace elements (except Cu, Zn, and Se) promoted a tendency towards β-sheet-specific aggregation. Our findings indicate that serum levels of essential and toxic trace elements had synergistic or antagonistic effects on amyloid aggregation/conformation, highlighting the role of metallomics in the pathophysiological cascade of PD.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Design and structure of proteins

Papain and ficin: Environmental drivers of amyloid formation

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KEYWORDS: amyloid fibrillation, aggregation, papain, ficin

Due to their complex yet metastable three-dimensional structure, proteins are susceptible to aggregation as a result of environmental changes. Aggregates range from amorphous forms to highly ordered amyloid fibrils. Although fibrillation is thought to be possible for all proteins, the specific conditions for many are still unclear.

Our work investigates the conditions promoting amyloid fibrillation of two cysteine proteases, papain and ficin, that have been widely studied for their enzymatic properties and applications in various industries, including food processing, pharmaceutical production, and biotechnology. The effects of pH, temperature, ethanol, and protein purity on fibril formation were examined. Structural changes during aggregation were monitored by infrared spectroscopy, distinguishing intramolecular β-sheets from intermolecular aggregation-specific β-sheets. The kinetics and efficiency of amyloid fibril formation were also tracked using changes in the fluorescence of the dye thioflavin T, which specifically binds to amyloid fibrils. As a final confirmation of amyloid fibril presence, the samples were analyzed using atomic force microscopy.

Results show that both enzymes exhibit the highest amyloid-forming potential in 90% ethanol, 10 mM DTT, at 90 °C, following initial complete denaturation with 8 M urea in a reducing environment. It was found that papain forms amyloid fibrils with more difficulty compared to commonly studied model systems, most likely due to its kinetic stability, while in the case of ficin, the likely cause is the higher stability of the ficin isoform mixture. This is a consequence of the reduced ability of polypeptides with different sequences to form nucleation cores.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Design and structure of proteins

Engineering of highly stable bryoporin nanopores using genetic code expansion

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KEYWORDS: non-canonical amino acids, nanopore sensing, protein design

Genetic code expansion (GCE) enables the engineering of proteins containing one or more non-canonical amino acids (ncAAs) which give them diverse properties. In particular, halogenated ncAAs are known to increase the stability of proteins so that they can be used in methods and processes that require harsh conditions. One of the potential applications for this approach is the stabilization of protein nanopores used in nanopore sensing, where the analysis of protein analytes requires the presence of denaturants during data acquisition.

Using GCE, we have produced several variants of the pore-forming protein bryoporin (Bry) containing either m-Cl-tyrosine (ClY) or p-pentafluorosulfanyl phenylalanine (SF5). We tested their ability to oligomerize by native PAGE and their stability in guanidinium chloride (GdmCl) by nano-differential scanning fluorimetry (nanoDSF). Pores of the most stable variants were then purified and further characterized by cryo-EM and single channel recordings on planar lipid membranes.

SF5-containing pores exhibited high stability in nanoDSF experiments, but due to their low solubility and high aggregation tendency, we could not use them for single channel recordings or cryo-EM. In comparison, only certain ClY-containing pores were equally stable, but were still preferred for further experiments as they were easier to produce. For single channel recordings, the most stable ClY-containing pores could be used at GdmCl concentrations of up to 3 M, which is twofold higher than the pores of the Bry variants without ncAAs. Based on these promising results, we expect our next goal of translocation and identification of full-length protein analytes to be within reach.

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Design and structure of proteins

Driving forces for the aggregation of misfolded proteins

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KEYWORDS: misfolding, aggregation, thermodynamics, hydrophobic effect

During the 1990s, structural transitions of human and hen egg-white lysozyme were explored while developing methodologies and paving the way for understanding complex protein dynamics. Along with lysozyme, ovalbumin has also been extensively studied in the context of unfolding and aggregation. These proteins, despite originating from the same source, share very few features in terms of pI, molecular weight, and folding. We identified gaps in the understanding of their unfolding/misfolding, so we further investigated the existence of intermediate states in hen egg-white protein denaturation under various conditions, as well as the role of these intermediates in the aggregation process. We applied different approaches to destabilize proteins to maintain prolonged solubility, giving preference to ordered rather than amorphous aggregation.

The conditions tested included pH changes and alterations in solvent polarity through the addition of ethanol, with the aim of exploring the possibility of stimulating different aggregation-driving processes, both dependent on and independent of the hydrophobic effect. By measuring intrinsic fluorescence and ANS binding of misfolded monomers as well as aggregated states, we examined the driving forces of conformational changes and aggregate formation. Infrared spectroscopy was used to study secondary structure changes in parallel with changes in hydrophobic exposure. The results showed that proteins maintained solubility for longer periods upon heating at extreme pH values compared to their pI (pH 2 for lysozyme and pH 2 and 10 for ovalbumin), which prevented disordered aggregate formation. ANS binding demonstrated that hydrophobic stabilization varied among aggregates, supporting different aggregation pathways for a single protein. ThT binding and infrared spectroscopy, with a pronounced aggregation-specific β-sheet band, clearly indicated amyloid-like aggregation under low-pH conditions in both proteins. For ovalbumin, which forms monomeric intermediates designated as S- and I-ovalbumin in early literature, our data support the hypothesis that their formation correlates with the pathway toward further stabilization into polymorphic aggregated states.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Design and structure of proteins

Structural characterization of factors involved in DNA-protein crosslink repair

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KEYWORDS: DNA-protein crosslinks, DNA-protein cross-link repair, DNA-dependent metalloprotease, hexameric AAA+ ATPase, characterization of the molecular interplay

DNA-protein crosslinks (DPCs) are DNA lesions that occur endogenously at high frequency and are triggered by by-products of cellular processes as well as by exogenous agents including anticancer drugs. Any protein near DNA can be crosslinked after exposure to DPC inducers, or DNA transaction enzymes can be trapped during their catalytic cycles. DPCs interfere with all DNA transactions, including replication and transcription. At the cellular level, impaired DNA-protein cross-link repair (DPCR) leads to double-strand breaks, genomic instability and cell death, and at the organismal level, impaired DPCR is associated with cancer, ageing and neurodegeneration.

Research into DPCR has made significant progress in the last decade with the discovery of a specialized replication-coupled DPCR pathway based on proteolysis in yeast and vertebrates, including the proteases Wss1 and SPRTN, respectively. These enzymes cleave the protein component of the DPC, and the remaining DNA-peptide fragment is further processed by different DNA repair pathways. Biallelic mutations in the SPRTN gene cause Ruijs-Aalfs syndrome (RJALS), a genetic disorder characterized by genome instability, premature aging, and early-onset hepatocellular carcinoma.
SPRTN is a DNA-dependent metalloprotease. While it can cleave certain DPCs independently, its efficiency and substrate range are enhanced by the hexameric AAA+ ATPase/unfoldase p97 and other p97 protein adaptors, which help p97 to unfold bulky or ubiquitinated DPCs, making them more accessible to SPRTN.
In our group, we are investigating the molecular mechanisms of DPCR. In my talk, I will present our recent characterization of the molecular interplay between SPRTN and p97 at the high-resolution level.

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Design and structure of proteins

Assessing size and aggregation of paraproteins of different isotypesusing photon correlation spectroscopy

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KEYWORDS: monoclonal immunoglobulin, paraprotein, correlation spectroscopy, protein aggregation, protein size

In monoclonal gammopathies, an overexpanded clone of plasma cells secretes a notable amount of monoclonal immunoglobulin, detectable in peripheral blood as an electrophoretically homogeneous fraction—paraprotein. Paraproteins represent a valuable source of natural variability for studying the structure of immunoglobulins, with important implications for understanding disease pathology. Paraproteins were assessed from sera with IgG paraprotein (n=96), IgA paraprotein (n=52), and one serum with extremely high concentration of IgM paraprotein. IgG and IgA were purified using protein G and peptide M affinity chromatography, while IgM was purified by size-exclusion chromatography. IgG and IgA subclasses were identified by dot blot, and monoclonality was confirmed by isoelectric focusing. After confirmation of monoclonality, both native and heat-aggregated paraproteins were analyzed by photon correlation spectroscopy (PCS) on a Zetasizer Nano ZS.

PCS enabled differentiation of hydrodynamic size between IgG subclasses, with IgG3 having a larger diameter compared to other IgG subclasses. Purified IgA1 and IgA2 had larger hydrodynamic diameters than IgG1, IgG2, and IgG4, and the diameter of IgA2 was comparable to that of IgG3. The hydrodynamic diameters of different IgM fractions isolated via size-exclusion chromatography did not align with the molecular weight/purity profiles obtained by SDS-PAGE. This discrepancy may reflect the occurrence of molecular interactions highlighting limitations in analyzing protein mixtures.

Design and structure of proteins

Assessing size and aggregation of paraproteins of different isotypesusing photon correlation spectroscopy

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Finally, the tendency of paraproteins to form aggregates varied according to antibody class and subclass, even in the absence of heat treatment. These findings emphasize the need for further studies to investigate the pathogenetic relevance of differences in paraprotein aggregability.

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Design and structure of proteins

Structural adaptation of the potyviral coat protein to accommodate species-specific residues in virions and virus-like particles

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KEYWORDS: potyviruses, helical filaments, virus-like particles

Potyviruses are important plant pathogens worldwide, yet their infection mechanisms remain poorly understood. Their virions are flexible, helical filaments composed of genomic single-stranded RNA (ssRNA) encapsidated by thousands of copies of the coat protein (CP). When CP is expressed in bacteria, it self-assembles into filamentous virus-like particles (VLPs). In our study, we analyzed the three-dimensional structure of potato virus A (PVA) and VLPs from several potyvirus species, including PVA, potato virus Y (PVY), tobacco etch virus (TEV), pepper mottle virus (PepMoV), and Johnsongrass mosaic virus (JGMV) using cryo-EM. We observed several architectural types of VLPs, including virion-like RNA-containing filaments and polymorphic RNA-free filaments. We show that RNA-containing VLPs have the same overall structure as potyviral virions regardless of CP origin. However, we observed species-specific structural features in three regions of each CP unit, one of which is involved in CP-CP and two others in CP-ssRNA interactions.

These deviations in the otherwise highly conserved 3D fold of the CP units are apparently tolerated as long as the CP-CP and CP-RNA interactions maintain the overall integrity of the virion particle. In contrast, RNA-free VLPs exhibited species-specific architectures. We identified specific CP residues contributing to this structural variability. Their identity appears to be important because mutations alter the architectural parameters of RNA-free VLPs, which are generally larger in diameter than those produced by wild-type CPs. Our results show how a balance between conserved and variable CP residues enables diversification without compromising the conserved capsid of native virions.

Design and structure of proteins

Structural adaptation of the potyviral coat protein to accommodate species-specific residues in virions and virus-like particles

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This work provides a structural basis for future studies of multiple CP functions in the potyvirus infection cycle.

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Design and structure of proteins

Characterization of the molecular interactions between the DNA-protein cross-link repair proteins p97 and SPRTN

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KEYWORDS: DNA-protein crosslinks, ATPase p97, SPRTN, SHP motif, p97:SPRTN complex

DNA-protein crosslinks (DPCs) are DNA lesions that occur when a protein is irreversibly bound to DNA. DPCs have harmful effects on the organism, including cancer, premature aging and neurodegenerative diseases. Due to their bulky nature, DPCs interfere with all DNA transactions such as replication, transcription and repair, making DPC repair an essential cellular pathway. Cells utilize multiple mechanisms to remove DPCs, including the relatively recently discovered pathway which involves the joint action of a hexameric AAA+ ATPase p97, whose function is to unfold DPC substrates, and metalloprotease SPRTN, whose function is to cleave DPCs into smaller peptides whose covalent connection with DNA is then resolved by other downstream mechanisms. As is typical for many other known p97 adaptors, a large part of SPRTN is intrinsically disordered, with only the N-terminal protease domain adopting a globular fold. The C-terminal disordered region of SPRTN contains several different interaction motifs, including several DNA-binding motifs and a SHP motif that is thought to mediate the p97:SPRTN interaction. The main goal of our study is to qualitatively and quantitatively characterize molecular interactions between p97 and SPRTN. I will present the results of biochemical and biophysical approaches such as pull-down assays, size exclusion chromatography, microscale thermophoresis (MST) and isothermal titration calorimetry (ITC). We have also performed a structural analysis of the p97:SPRTN complex using cryo-electron microscopy, which provides high-resolution insight into the interactions between these two important DPC repair proteins.

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Design and structure of proteins

Heterologous expression of human glucose-6-phosphate translocase (SLC37A4) in prokaryotic and yeast systems

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KEYWORDS: glucose-6-phosphate translocase, membrane protein, expression

Deficiency of the human glucose-6-phosphate translocase (G6PT), encoded by the SLC37A4 gene, leads to glycogen storage disease 1b. We report a study of the heterologous expression in both bacterial and yeast hosts. The wild-type gene was cloned into the pET22b(+) vector with and without a pelB signal sequence to facilitate expression in the periplasmic space or intracellularly in various Escherichia coli strains. In parallel, a codon-optimized mutant variant (N27K) was constructed for yeast expression and cloned into the pPIZalphaA vector, employing the Saccharomyces cerevisiae α-mating factor prepro-peptide and the native G6PT propeptide to target expression to the endoplasmic reticulum membrane of Pichia pastoris. Additionally, the same codon-optimized gene was re-cloned into the pCTCON2-GFP vector to produce a surface-displayed G6PT-GFP fusion chimera in S. cerevisiae, utilizing the AGA2 anchoring system. Protein expression was tested by SDS-PAGE, immunoblotting, and flow cytometry. Optimization of inducer concentration, fermentation temperature, and duration was performed. The findings from this work give additional knowledge necessary for the expression of G6PT endoplasmic reticulum membrane transporter for structural, functional, and drug screening studies.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Design and structure of proteins

Molecular characterisation of *Ostrinia nubilalis* antimicrobial peptides

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KEYWORDS: antimicrobial peptides, bioinformatics, peptide structure, qPCR

Antimicrobial peptides (AMPs) are biologically active peptides classified by conserved structural features crucial for insect immune defence. They are broadly grouped into α-helical (cecropins), cysteine-rich (defensins), proline-rich, and glycine-rich peptides. To detect AMP sequences, characterise peptides in *Ostrinia nubilalis*, and gather data for LC-MS/MS analyses, a combined wet lab and in silico approach was implemented. Selected AMP genes were analysed by qPCR to monitor expression throughout *O. nubilalis* larval development. In-house databases of six insect AMPs—cecropin, moricin, lebocin, defensin, attacin, and gloverin—were created using reference sequences from public nucleotide databases. These were queried against the *O. nubilalis* genome (RefSeq: GCF_963855985.1) with BLASTn to identify homologous regions, which were then mapped to chromosomes to locate putative AMP genes. Genomic regions were analysed with NCBI ORFfinder to predict open reading frames. Translated protein sequences were validated by BLASTp against NCBI RefSeq and nr databases to verify AMP identity. Confirmed peptides were modelled with AlphaFold to predict 3D structures, verifying group-specific features and inferring function. Structural predictions confirmed conserved motifs, such as two α-helices separated by a disordered hinge in cecropin and moricin, the CS$\alpha\beta$ motif in defensin, the Pro-X-Pro motif in lebocin, and glycine-rich regions with R-XX-R motifs in attacin and gloverin. Expression analysis revealed significant upregulation of AMP genes at the end of larval dormancy in *O. nubilalis*. This identification and structural validation of AMPs provide insights into their conserved immune roles and establish a foundation for further functional studies.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125) and the Visegrad Fund Scholarship no. 52310337

Design and structure of proteins

Selection of redesigned pullulanase variants for enhanced industrial biocatalyst

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KEYWORDS: Bacillus paralicheniformis, α-1,6-glycosidic bond cleavage, starch-processing

Pullulanase (EC 3.2.1.41) is a debranching enzyme that cleaves α-1,6-glycosidic bonds with widespread industrial use in starch saccharification and resistant starch formation. However, most naturally occurring variants lack the thermostability and solubility required for efficient function at elevated process temperatures, limiting broader application.

To improve the performance of Bacillus paralicheniformis 9945a pullulanase (BliPull), a mesophilic enzyme with a temperature optimum of 40 °C, a comprehensive in silico pipeline was applied. Structural models were generated using AlphaFold21 and SWISS-MODEL2. Stabilizing and solubility-enhancing mutations were predicted through evolutionary and structural analysis using tools developed by the Loschmidt Laboratories including FireProt3, FireProt-ASR4, HotSpot Wizard5 and AggreProt6 and similar tools such as PROSS7, MAESTROweb8 and Disulfide by Design9. Literature-guided N- and C-terminal truncations were also introduced to support secretion and expression. Eighteen single-point mutants were selected based on predicted improvements in thermostability and solubility. Experimental validation will guide the selection of the most robust candidates for further optimization.

The selected variants were generated and expressed in E. coli, purified by IMAC and underwent biochemical characterization to assess improvements in thermostability, solubility, and catalytic performance under industrially relevant conditions. Future efforts will focus on potential enzyme immobilization of the most promising variants to enhance operational stability, enable reusability in continuous biocatalytic systems, and support integration into modern starch-processing workflows.

Design and structure of proteins

Selection of redesigned pullulanase variants for enhanced industrial biocatalyst

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ACKNOWLEDGMENTS: This research was supported by the Science Fund of the Republic of Serbia, #7347, Unique enzyme solutions for obtaining added value products from corn – UniCorn and COST Action COZYME. This project was also supported by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 101136607.

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Genetics

The significance of the spermatoproteasome during *Drosophila melanogaster* spermatogenesis

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KEYWORDS: Drosophila, spermatoproteasome, spermatogenesis, testis

Drosophila melanogaster is a valuable model organism for the exploration of spermatogenesis, primarily because the critical stages of this process are easily observable and show significant similarity to those in mammals. The basic structures and key organelles of sperm in both Drosophila and humans are notably alike. Our research is directed towards understanding the identity and organization of cellular organelles during spermatogenesis in Drosophila. The principal protein complexes that are crucial for spermatogenesis, including the spermatoproteasome, are present in both mammals and fruit flies, however, their specific roles, compositions, and substrates require further investigation. More than one-third of the 26S proteasome subunits have distinct forms in the testis, which are encoded by paralogous genes in Drosophila.

We characterized the function and subcellular localization of the testis-specific proteasome (spermatoproteasome) by analyzing the testis-specific catalytic subunits Prosβ5R1 and Prosβ5R2, and the regulatory subunits Rpn12R and Rpt3R. We found that homozygote mutants of Prosβ5R2, Rpn12R and Rpt3R are male sterile, suggesting an essential function for both catalytic and regulatory subunits of spermatoproteasome. We followed the localization of tagged reporter constructs for testis-specific proteasome subunits during spermatogenesis, tracking the distribution of spermatoproteasome in Drosophila.

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We demonstrate the essentiality of testis-specific proteasome in spermatogenesis and its critical role in the nuclear rearrangement and spermatid elongation after the meiotic stages of spermatogenesis. The diverse localization patterns of different proteasomal subunits highlight a complex and essential role in directed protein degradation during late stages of spermatogenesis.

FUNDING: This work was supported by grants from Cluster of the Centre of Excellence for Interdisciplinary Research, Development and Innovation of the University of Szeged (IKIKK) to AV, JZ, VV, DL, and NKFIH (2022-2.1.1-NL-2022-00008) to ZL.

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Genetics

Association between FADS rs174602 polymorphism and fatty acid profile in plasma of breast cancer patients on aromatase inhibitors

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KEYWORDS: FADS polymorphism, fatty acids, breast cancer

Polyunsaturated fatty acids (PUFAs), including both pro-inflammatory and anti-inflammatory mediators, exhibit altered metabolism in breast cancer (BC) patients receiving aromatase inhibitor (AI) therapy. Fatty acid desaturases (FADS) are key enzymes in the metabolic pathways of omega-3 and omega-6 PUFAs, and polymorphisms in FADS genes have been associated with variations in circulating PUFA levels. This study investigated the association between the FADS gene polymorphism rs174602 and the plasma PUFA profile in 48 BC patients undergoing AI therapy. Genotyping was performed using real-time PCR, and plasma fatty acid composition was analyzed via gas chromatography. The results demonstrated that heterozygous carriers of rs174602 exhibited significantly lower plasma levels of γ-linolenic acid (GLA), arachidonic acid (AA), docosapentaenoic acid (DPA), and Δ6-desaturase activity (all $p < 0.05$) compared to homozygotes of the major allele.
While lower AA levels are generally associated with reduced inflammatory risk, GLA and DPA are known for the anti-inflammatory properties. Therefore, individuals with genotypes associated with reduced GLA and DPA levels may benefit from increased dietary intake of those fatty acids. These findings underscore the impact of genetic variation on PUFA metabolism and support the potential role of personalized nutritional strategies in enhancing therapeutic efficacy and clinical outcomes in breast cancer patients receiving aromatase inhibitor therapy.

ACKNOWLEDGMENTS: This research was supported by the Science Fund of the Republic of Serbia, #5050 AID.

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Genetics

Caveolin-1 as potential biomarkers of prostate cancer: Evidence from Serbian population

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KEYWORDS: exosomes, liquid biopsy, prostatic hyperplasia

Caveolin-1, a membrane scaffolding protein, plays a key role in regulating cell proliferation, migration, and angiogenesis. Its overexpression in prostate cancer (PCa) cells is significantly associated with increased tumor aggressiveness. The aim of this study was to investigate whether caveolin-1, found in plasma-derived exosomes, could serve as a liquid biopsy biomarker for PCa.

Exosomes were isolated from plasma samples of 39 patients with PCa and 33 patients with benign prostatic hyperplasia (BPH), used as the control group, using the Total Exosome Isolation Kit (from plasma) according to the manufacturer's instructions. Morphological characterization of the isolated exosomes was performed using transmission electron microscopy (TEM), scanning electron microscopy (SEM), nanoparticle tracking analysis (NTA), and flow cytometry. Western blot analysis confirmed the presence of the typical exosomal marker CD63, as well as caveolin-1 in plasma exosomes.The results showed no statistically significant difference in exosomal caveolin-1 levels between PCa and BPH patients (p=0.349). However, when PCa patients were stratified according to standard prognostic parameters and tumor aggressiveness scores, the relative protein levels differed between subgroups.

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Notably, exosomal caveolin-1 levels were significantly higher in PCa patients with elevated PSA levels (p=0.033), clinical stage T3 or T4 (p=0.0083), and in those with aggressive PCa (p=0.0061), compared to patients with favorable clinicopathological features. These findings suggest that while exosomal caveolin-1 may not distinguish PCa from BPH overall, it holds promise as a non-invasive biomarker for identifying aggressive forms of prostate cancer, warranting further investigation.

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Biotechnology

Manganese accumulation in the microalga *Haematococcus pluvialis*

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KEYWORDS: *Haematococcus pluvialis*, manganese, microalgae, water remediation

Haematococcus pluvialis is a unicellular Eukaryotic alga (microalga) of tremendous commercial and environmental importance. It is extensively used in the food and feed industry, and represents a pioneering photosynthetic organism, well suited to survive extreme conditions in polluted waters. Herein, we analyzed the capacity of this microalgal species to accumulate manganese, a common pollutant of water bodies, from mining and industrial drainage, and from natural deposits of Mn-oxides. *H. pluvialis* cultures were treated in the green phase with 1 mM Mn2+.

After one and three days of treatment, Mn accumulated up to 7% and 10% of the dry biomass, with no significant impact on cell viability. X-ray fluorescence microscopy co-localized Mn distribution with O and Na, whereas TEM microscopy showed that in response to Mn cells develop large vacuoles. Altogether, this implies that Mn is most likely accumulated in the vacuoles. X-ray absorption fine structure spectroscopy showed that Mn predominately remained in the Mn2+ redox form and was coordinated to sulfates.

Pertinent to this, free sulfates, and phytochelatins, which contain thiol groups that may be oxidized, are accumulated in vacuoles.

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Low T EPR spectroscopy of the biomass revealed the presence of multiple transitions that arise from the high-spin Mn2+ with large zero-field splitting, indicating low symmetry around the metal, and possibly suggesting that Mn is coordinated to ligands with mixed donor atoms or bulky chelates, such as phytochelatins. H. pluvialis showed a significant capacity to accumulate Mn through an intricate sequestration mechanism, demonstrating its potential application in remediation of Mn-infested waters.

ACKNOWLEDGMENT: This research was financially supported by the Science Fund of the Republic of Serbia, #7078, Microalgae for biosynthesis of metal cluster compounds – BioSynthClust.

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Biotechnology

A highly optimized protocol for the isolation and purification of transferrin from human serum

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KEYWORDS: transferrin, anion-exchange chromatography, protein purification

Different methods for the isolation and purification of human transferrin (Tf) from human plasma and serum have been described in the literature. However, none provide a highly pure final product in a time-efficient manner, whereas the method described in this work accomplishes both. It consists of two separate steps—a sample preparation and an anion-exchange chromatography step.

Our initial methodology used rivanol for protein precipitation in the first step and activated charcoal for the removal of excess rivanol from the remaining supernate. This provided Tf of >99% purity while retaining Tf function, analyzed by immunofluorescent staining for transferrin-transferrin receptor 1 interaction and ferrozine method for iron binding capacity, and structure, confirmed by recording and comparing fluorescent spectra of purified Tf and commercially available pure Tf. However, the method produced a relatively low Tf yield. The initial method was then modified to use potassium bromide for excess rivanol removal, increasing yield by approximately 82% compared to the initial protocol. We then tested if saturating Tf with iron(III) ions before precipitation with rivanol would increase yield. This increased yield by a further 20% compared to potassium bromide rivanol removal, and a final 119% compared to the initially proposed method. The improved method was additionally evaluated for the purification of Tf from the serum of patients with different iron-associated pathologies, showing its applicability on different types of samples.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200019).

Biotechnology

Antioxidative activity of extracts from rocket and spinach biofortified using selenium nanoparticles

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KEYWORDS: selenium nanoparticles, biofortification, antioxidants

Selenium nanoparticles (SeNPs) present a novel selenium formulation that can be used for plant biofortification with the aim of increasing the content of Se and antioxidants. In this work, rocket and spinach were biofortified with different forms of selenium selenate, SeNPs stabilized with polyvinylpyrrolidone (PVP SeNPs), polysorbate 20 (PS SeNPs), and humic acid (HA SeNPs). The plants were sampled, dried by lyophilization and the extraction of antioxidants was performed. The extracts were characterized using Trolox equivalent antioxidant capacity and Folin Ciocalteu assays. Antioxidative activity of the extracts was investigated in vitro on HepG2 and Caco2 cells. The cells were incubated with rocket or spinach extracts in a concentration of 0.5 mg/mL for 24h.

Oxidative stress was then induced by adding prooxidant tertbutyl hydroperoxide (tBOOH, 100 μM) and cell viability was measured by performing MTT assay. Extracts obtained from rocket and spinach biofortified using HA SeNPs had the strongest antiradical (Trolox equivalents) and total reductive capacity (gallic acid equivalents). In vitro antioxidative activity assessed by measuring cell viability was shown in HepG2 cells in the case of rocket biofortified using PS SeNPs and spinach biofortified using PS SeNPs and HA SeNPs. Caco2 cells were more responsive to treatment with rocket extracts compared to spinach, in terms of their protective activity against tBOOH. All tested rocket extracts had protective effects, while in the case of spinach, only extract biofortified with PS SeNPs and control extract exhibited significant effect, under lower concentration level. This research demonstrated that SeNPs could be utilized for the development of novel functional foods with improved antioxidative characteristics. It also showed that different plant species can exhibit distinct responses to biofortification with SeNPs.

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Biotechnology

Adaptation of Pseudomonas sp. enzymatic activity in response to PFOA exposure

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KEYWORDS: PFOA, enzymes, biodegradation

Perfluorooctanoic acid (PFOA) is a persistent environmental pollutant with high chemical stability and toxicity, raising concern due to its bioaccumulation and resistance to conventional degradation processes1. Microbial adaptation, particularly via enzymatic responses, represents a promising strategy for mitigating its impact2. This study investigates the influence of PFOA on selected enzymatic activities of Pseudomonas chlororaphis isolated from PFOA polluted environment and compares the response with commercial enzyme preparations. Cells were cultivated in minimal medium supplemented with 500ppm PFOA and harvested after 72h.

Protein extracts were prepared, and enzymatic assays were performed for catalase (CAT), superoxide dismutase (SOD), peroxidase, laccase and lipase. Commercial catalase, peroxidase, and lipase were additionally tested in vitro after preincubation with 10–500μM PFOA. PFOA showed a concentration-dependent effect on enzymatic activities. Commercial peroxidase was completely inhibited at 500μM, whereas catalase remained unaffected. Lipase activity increased markedly, reaching 197% of control at 500μM. In P. chlororaphis exposed to 500ppm PFOA (1207μM), SOD and peroxidase activities were strongly reduced to 25% and 41% of control, respectively, while catalase activity decreased moderately to 75%. Laccase activity was not detected under these conditions. These results highlight that PFOA can selectively inhibit or activate enzymes, potentially reshaping the oxidative stress response and metabolic adaptation of Pseudomonas sp. The activation of lipase and partial resilience of catalase suggest possible adaptive mechanisms, whereas strong inhibition of SOD and peroxidase reflects enzymatic vulnerability to PFOA stress. Understanding these shifts provides valuable insights into microbial strategies for coping with perfluorinated pollutants and informs future approaches for their bioremediation.

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ACKNOWLEDGMENT: This work was supported by the European Union's Horizon Europe Framework Programme, Twinning Western Balkans, under grant agreement No. 101059534 (PFAStwin).

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Biotechnology

Nanobody-based capture of extracellular vesicles as a diagnostic strategy for prostate cancer

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KEYWORDS: extracellular vesicles, prostate cancer cell lines (PC3), prostate (RWPE-1) cell lines

Extracellular vesicles (EVs) are small membrane-enclosed entities released from different cell types. EVs are promising biomarkers for the diagnosis and monitoring of various malignancies, including prostate cancer1. From the heavy-chain-only VHH library, we selected three different nanobodies targeting EVs from the PC3 cell line. Nanobody genes were cloned into a vector and fused with the GFP protein. Production was optimized in E. coli cells, where nanobodies mainly accumulated in inclusion bodies. They were purified from these fractions. SDS-PAGE confirmed the purity of the protein samples obtained. The selected nanobodies were immobilized on a polymethylacrylate polymer, which was used for the isolation of EVs from prostate cancer cell lines (PC3) and epithelial cells of prostate (RWPE-1) cell lines. Their ability to discriminate between EVs from PC3 and RWPE-1 cells was evaluated using fluorescence-activated cell sorting (FACS). EV samples were tested for protein and lipid content.

This work highlights the potential of nanobodies as selective tools for the recognition of EVs. The obtained candidates may serve as a basis for novel diagnostic strategies targeting tumor-derived vesicles in prostate cancer. Furthermore, the integration of nanobody-based capture with standardized EVs characterization could provide robust platforms for clinical biomarker discovery. Future studies will focus on validating binding specificity in complex biological samples and exploring their application in liquid biopsy approaches.

ACKNOWLEDGEMENTS: This research was supported by the Science Fund of the Republic of Serbia, Grant PRISMA No. 4747, Project title: Advancing REversible immunocapture toward SCALablE EV purification—RESCALE-EV. This research was supported by the European Union under Grant Agreement No. 101182851.

Biotechnology

Nanobody-based capture of extracellular vesicles as a diagnostic strategy for prostate cancer

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Int J Mol Sci. 2024 Mar 21;25(6):3533. doi: 10.3390/ijms25063533.

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Biotechnology

Recombinant production and application of endo-polygalacturonase II in fruit juice processing

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KEYWORDS: endo-polygalacturonase, Pichia pink, recombinant technology, fruit juice processing, antioxidant capacity

Producing native enzymes from filamentous fungi often requires complex culture conditions, low yields, and a difficult purification process. These limitations have been overcome by using recombinant DNA technology to produce endo-polygalacturonase II (PG II), a pectin-degrading enzyme. Pectin is a polysaccharide found in the middle lamella of the plant cell wall, where it plays a key role in cell adhesion. In fruit juice processing, pectin is responsible for juice turbidity and limits efficient extraction.

In this study, the pgaII gene from Aspergillus tubingensis FAT 43 was cloned into the Pichia pink expression system using the pPinkα-HC vector. The use of the Pichia pink expression system enabled efficient secretion of the recombinant enzyme with minimal background proteolytic activity, simplifying downstream purification and ensuring high yield and activity of PG II.

PG II was expressed extracellularly, subsequently purified utilising Ni-NTA affinity chromatography, and validated to possess a molecular mass of 39 kDa via SDS-PAGE. The recombinant enzyme exhibited significant thermal and pH stability, comparable to that of the native enzyme. Because of its catalytic efficiency and substrate specificity, this enzyme has substantial industrial relevance, notably in fruit juice processing, where it contributes to higher extraction yield. Enzymatic treatment of various fruit juices was optimized. The recombinant PG II enzyme shows strong potential in pulp degradation and clarification, particularly in strawberry, apple, and orange juices. Enzymatic treatment with PG II led to an increase in antioxidant capacity, especially in quince (47%) and apple (20%) juices, which reduced pulp sedimentation, enhancing juice stability during storage.

Biotechnology

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Biotechnology

Immobilization of glucose oxidase by biomineralization using different imidazole derivatives

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KEYWORDS: Aspergillus niger, oxidoreductases, ZIF-8, ZIF-zni

Glucose oxidase (GOx) from Aspergillus niger belongs to oxidoreductases and has many applications in different commercial processes. Due to wide applications in industrial processes, which require harsh conditions such as non-optimal pH and temperature, immobilization of enzymes represents a good strategy to overcome this problem. In this work, we immobilized glucose oxidase in various ZIFs like ZIF-8 and ZIF-zni, that belongs to the group of metal organic frameworks (MOFs). These biocomposites were synthesized using optimized ratios of zinc and imidazole derivatives. The obtained results show that composites with the highest specific activity are obtained in reaction conditions where the ratio of zinc and imidazoles were 1:10, for 10 mM and 60 mM final concentrations of zinc. However, for a final concentration of 20 mM zinc, the highest specific activity was obtained in the case of ZIF-zni and for ratio of 1:50. The biocomposites synthesized in this way also showed stability towards detergents (SDS), which indicates that part of the enzyme is immobilized inside ZIFs. A specific activity for GOx@ZIF-zni biocomposite was two times higher at a ratio of 1:50 for a final concentration of 20 mM zinc, compared to our earlier research in which we immobilized glucose oxidase in ZIF-8. After optimizing the ratio of zinc and imidazole in the biomineralization reaction, the results obtained show that the highest specific activity was obtained for a ratio of 1:10 at a final zinc concentration of 10 mM. This specific activity was 2.5 times higher compared to the one reported in our previous work with GOx@ZIF-8.

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Biotechnology

A functional screening approach for the identification of gluten-degrading microorganisms

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KEYWORDS: gluten removal, proteases, screening assay, Bacillus

Gluten is a major protein component of wheat, rye and barley bran and consists of glutenin and gliadin fractions, some of which are resistant to gastrointestinal digestion and pose a health risk to individuals with gluten-related disorders. The only remaining treatment option is strict elimination of gluten, but this is difficult to implement due to the economic burden of such a diet and the inefficient routine strategies for removing gluten from processed foods. Enzymatic hydrolysis has proven to be a promising alternative to traditional methods of gluten removal in food processing. Therefore, there is an urgent need for the discovery of new efficient proteases suitable for the targeted degradation of immunogenic gluten motifs. This study aimed to identify local producers of microbial gluten-degrading proteases. For this purpose, a rapid and cost-effective screening test for the detection of gluten hydrolysis was developed. A total of 70 soil-derived Bacillus isolates from all over Serbia were simultaneously analysed for their proteolytic activity by diffusion tests with gluten-containing gels at different temperatures and pH conditions. The gluten hydrolysis pattern of selected promising protease producers was further analysed by SDS-PAGE, and the enzyme specificity for other proteinaceous substrates, including zein, gelatin and casein, was elucidated. With this comprehensive screening approach, promising Bacillus strains (12B, 16B, 22A, 24B, WTB13, WTB18) were identified and selected for future characterisation and potential application in enzymatic strategies for gluten removal at alkaline pH, indicating the presence of robust, potentially industrially relevant proteases.

ACKNOWLEDGEMENTS: This research has been financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-136/2025-03/200026 and No. 451-03-136/2025-03/200168).

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Biotechnology

Production of xylooligosaccharides from corn cob using a novel endoglucanase A from *Aspergillus tubingensis*

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KEYWORDS: endoglucanase A, XOS, GH11, waste valorisation

The sustainable recovery of bioactive compounds from agro-industrial waste is crucial for developing value-added bioproducts. This study investigated a novel enzymatic approach for producing xylooligosaccharides (XOS) from corn cob, an abundant agricultural residue. A xylanase was isolated from the fermentation liquid of *Aspergillus tubingensis* grown on SSF corn cob and identified via mass spectrometry (LC-MS/MS) as Endoglucanase A, an enzyme previously unreported for xylan hydrolysis. The enzyme exhibited remarkable activity against xylan isolated from corn cob, efficiently generating XOS without detectable xylose formation, characteristic of GH11 endoxylanases.

The resulting XOS displayed diverse degrees of polymerisation and showed strong antioxidant activity (according to DPPH and FRAP test), suggesting their potential as functional food ingredients. This enzymatic process provides a green and efficient alternative for XOS production, avoiding the need for harsh chemical treatments and reducing processing costs. Moreover, the unique capability of Endoglucanase A to act on hemicellulosic substrates expands its biotechnological relevance beyond its classical classification. Our findings highlight a new application of *A. tubingensis* endoglucanase A in agro-waste valorisation and contribute to the broader goals of circular bioeconomy and sustainable food innovation.

ACKNOWLEDGMENTS: This research was supported by the Science Fund of the Republic of Serbia, #7347, Unique enzyme solutions for obtaining added value products from corn – UniCorn and the Ministry of Science, Technological Development and Innovation of Republic of Serbia (Contract numbers: 451-03-136/2025-03/200026 and 451-03-136/2025-03/200168).

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Biotechnology

A fluorescence-based method for monitoring the proteolysis of native proteins in real time

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KEYWORDS: native protein, proteases, enzymatic activity

Proteases play a crucial role in numerous biological processes and industrial applications, necessitating efficient methods to monitor their enzymatic activity. The existing assays for continuous real-time protease activity measurements are often based on peptide substrates and lack the ability to monitor proteolysis of native proteins. Here, we introduce a novel fluorometric assay based on the fluorescent dye SYPRO Orange, which enables continuous monitoring of native protein hydrolysis in a single reaction. This assay was tested with two subtilisin enzymes exhibiting different thermostabilities and four diverse protein substrates to assess its versatility.

Utilizing the capabilities of a real-time PCR instrument, the method facilitates fluorescence measurements in small-volume samples, even at elevated temperatures. The fluorescence increase corresponds directly to the proteolytic breakdown of the protein substrate, allowing real-time kinetic analysis. Compared to conventional methods, this assay offers simplicity, sensitivity, and adaptability, making it particularly well-suited for high-throughput screening of thermostable proteases. Its compatibility with standard laboratory equipment and minimal sample requirements further enhances its practical utility. Overall, this assay provides a valuable tool for both fundamental protease research and industrial enzyme optimization, enabling faster and more accurate characterization of protease activity.

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Biotechnology

Optimization of one pot synthesis of laccase@ZIF for highest enzymatic activity achievement

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Among all oxidoreductases, laccases present large group of multi copper enzymes. These widespread enzymes are occurring in diverse organisms like bacteria, fungi, plants and animals and their function is attached to their origin. This situation led to possibility of catalyzing different reaction types which emphasize commercial potential of these enzymes in various industries like food, cosmetic and bioremediation industry. Reaction that could be laccase catalyzed are very diverse and one of great benefits of laccase catalyzed reactions is green approach because only side product in these reactions are molecules of water. To accomplish better results in potential commercial usage it is necessary to provide reusability and acceptance of more sever reaction conditions, and that can be gained by immobilization. In our research, we used zeolitic imidazolate frameworks as a carrier which has great potential because of undemanding synthesis and manipulation.

To achieve best enzymatic activity we varied few parameters, like zinc (Zn^{2+}) concentration, imidazolate linker type (imidazole and 2-methylimidazole), zinc to imidazolate linker ratio, and time of synthesis. Besides this we examined the impact of two laccases from different sources. We discovered that the lowest zinc concentrations are beneficial for higher activity, and when two laccases are taken into account, there is no unambiguous conclusion when comparing which ratio of zinc to imidazolate linker is the most favorable for better enzymatic activity, so the T. versicolor laccase favors 1:3, and S. cyaneus laccase favors 1:10 zinc to imidazolate linker ratio in the case of laccase@ZIF-8.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Biotechnology

Changes in plant enzymatic activity in response to PFOA

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KEYWORDS: PFAS, oxidative stress, antioxidant enzymes, phytoremediation

Per- and polyfluoroalkyl substances (PFAS), including perfluorooctanoic acid (PFOA), are highly persistent environmental pollutants that induce stress responses in plants1. Understanding plant adaptation mechanisms under PFAS exposure, and the interaction between PFAS and plant systems is crucial for assessing the risks of PFAS contamination to plants, and the understanding of underlaying mechanisms can be translated to the plant application in environmental monitoring and phytoremediation2. Hydroponic experiments were conducted with cucumber (Cucumis sativus) and zucchini (Cucurbita pepo L.) cultivated in nutrient solutions containing 0.1 mg/L PFOA.

Plant responses were evaluated through chlorophyll content, Normalized Difference Vegetation Index (NDVI), and the activity of oxidative stress-related enzymes. Leaf reflectance spectra (330–1100 nm) were recorded using a SpectraVue Leaf Spectrometer. Antioxidant response was analyzed by various enzymatic assays. Zucchini exposed to PFOA showed enhanced superoxide dismutase (SOD) activity in leaves, stems, and roots, with minor increase in leaf absorption at 670 nm. NDVI values declined slightly compared to controls. In cucumbers, responses were stronger: SOD activity increased by 43 % in aerial parts and 52 % in roots, while catalase (CAT) activity in roots rose nearly tenfold. Notably, laccase activity, absent in controls, was strongly induced in PFOA-treated cucumber roots.

Exposure to PFOA disrupted redox homeostasis, increasing reactive oxygen species (ROS) production and activating antioxidant defenses. The induction of laccase in cucumbers suggests a dual role in oxidative stress mitigation and potential PFAS degradation. These findings highlight cucumbers and zucchini as bioindicators of PFAS exposure, with cucumber showing particular promise for phytoremediation applications.

Biotechnology

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Biotechnology

Green light photopolymerization of L-DOPA-modified alginate for hydrogel formation and microbead-basedenzyme immobilization

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KEYWORDS: alginate, photopolymerization, hydrogel, immobilization, glucose oxidase

Alginate is a natural polysaccharide derived from brown algae, known for its biocompatibility, biodegradability, and non-toxicity. These properties make it highly attractive for various biomedical applications. Through chemical modification, the functionality of alginate can be significantly expanded. By introducing photopolymerizable groups into its backbone, alginate acquires the ability to undergo photopolymerization.

This enables spatial and temporal control over hydrogel formation, which is crucial for applications such as tissue engineering, 3D bioprinting, and controlled drug delivery. In our study, alginate was modified by periodate oxidation, followed by reductive amination with the L-DOPA molecule. The resulting alginate derivative demonstrated the ability to form hydrogels in the presence of Eosin Y as a photoinitiator and green light irradiation. This system was used to optimize photopolymerization in an emulsion, resulting in the formation of microbeads. Glucose oxidase was added to the polymerization mixture and successfully immobilized within the hydrogel matrix during photopolymerization. The immobilized enzyme exhibits potential for applications in biosensing, biocatalysis, and biomedical diagnostics.

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AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

Bioactive compounds and metabolism

A gut microbiota metabolite: Trimethylamine induces M1-like activation in macrophages

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KEYWORDS: TMA, macrophage activation, mitochondria

Trimethylamine (TMA) is a metabolite produced by gut bacteria, including Clostridium species, Escherichia coli, Desulfovibrio species, Proteus species, and Acinetobacter, under anaerobic conditions. It is derived from dietary quaternary amines such as L-carnitine, phosphatidylcholine, choline, and betaine. After its formation in the intestine, TMA is absorbed into the bloodstream and transported to the liver, where it is oxidized into trimethylamine N-oxide (TMAO). Although TMAO has been identified as a cardiovascular risk factor, surprisingly little is known about the biological effects of TMA itself, which directly contacts intestinal epithelial and immune cells during absorption in the gut. Therefore, this study aimed to investigate the effects of TMA on colonic epithelial cells and macrophages.

RAW264.7, RAW BLUE macrophages, and co-cultures of RAW264.7 and Caco2 human intestinal epithelial cells were treated with 5 and 7.5 mM TMA. The effect of TMA on barrier integrity was assessed in co-cultures using the xCELLigence Real-Time Cell Analyzer. Macrophage activation was evaluated by the measurement of TNF-α expression (ELISA), NF-κB activity (QuantiBlue), and metabolic parameters (mitochondrial oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)) using the Seahorse XFp Analyzer. Flow cytometry was used for analyzing cell viability.

TMA significantly decreased Caco2 barrier integrity in co-culture. It increased NF-κB activity and TNF-α production in macrophages. At 7.5 mM, TMA increased ECAR while reducing basal/maximal respiration, ATP production, and spare respiratory capacity. TMA also triggered apoptosis in macrophages.
TMA induces a metabolic change in macrophages that mimics inflammatory (M1-like) activation, thereby undermining epithelial barrier integrity in co-culture. These results suggest that TMA may contribute to the development of inflammatory diseases in the colon.

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Bioactive compounds and metabolism

CRISPR-Cas9 knockout of Oatp1d1 reveals its role in zebrafish pigmentation and development

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KEYWORDS: Oatp1d1, zebrafish, ADME, pigments, pteridines

Organic anion-transporting polypeptide 1d1 (Oatp1d1) is a membrane transporter with important function in the absorption, distribution, metabolism, and excretion (ADME) of endogenous and exogenous compounds in zebrafish. Its tissue-specific expression in the liver and brain, along with known substrate specificity, indicates a potential role in regulating hormonal homeostasis. Newly developed CRISPR-Cas9 Oatp1d1 functional mutant (Oatp1d1-/-) showed viable phenotype of developing embryos, however deviations from normal development were noticed in pigment production as well as in the changes of gene expressions involved in pigmentation of developing zebrafish embryos.

A marked reduction or absence of embryo coloration during early development was clearly visible in comparison to WT embryos. Quantitative analysis of methanol extracts from embryos (24–120 hpf) and larvae (12–18 dpf) revealed significantly decreased fluorescence signals at wavelengths corresponding to pteridines. Staining with methylene blue further confirmed the absence of pteridines in the nasal epithelium, inner ear, and along the body axis of mutant embryos. The pigment-related phenotype became evident around 25 hpf, correlating with the onset of pigment cell differentiation from cranial neural crest cells. Interestingly, adult Oatp1d1-/- zebrafish displayed darker pigmentation and an increased number of xanthophores compared to WT. To confirm that the observed phenotype is due to the Oatp1d1 mutation, we crossed a mutant female with a WT male and obtained a rescue of the WT phenotype. We hypothesize that the Oatp1d1 could have a role in the transport of pigment cell precursors or hormones that coordinate their formation.

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Bioactive compounds and metabolism

Iron ion-binding algal chromopeptides as alternatives to transferrin in cell growth media

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KEYWORDS: algae, peptides, iron, transferrin

Transferrin, an iron-binding serum protein, is present in fetal bovine serum (FBS), commonly added to cell growth media. A non-animal-derived alternative to transferrin is necessary to lower the cost of growing cell cultures. Peptides obtained via protein hydrolysis from various sources are a viable but unexplored alternative to transferrin. Phycobiliproteins (PBPs) are abundant algal proteins with unique covalently attached tetrapyrrole chromophores with the ability to bind biologically relevant metal ions. The aim of this study is to probe the potential of algal-derived chromopeptides to bind Fe3+ ions affinity for specific transferrin replacement. Chromopeptides obtained through the enzymatic digestion of phycobiliproteins of the red macroalgae Porphyra haitanensis using pepsin exhibit moderate to high Fe3+ binding affinities, with a Ka in the range of 104-106 M-1, determined using the fluorescence quenching approach. Cell culture experiments using QM7 and CHO-K1 cells were conducted to determine if these chromopeptides could serve as a replacement for transferrin. It was found, using cell viability and live-dead staining assays, that cell growth in chromopeptide-supplemented media was comparable to cell growth in media supplemented with transferrin and a control culture containing FBS. Further studies involving iron uptake assays are necessary to study the mechanism of iron transfer mediated by algal chromopeptides.

ACKNOWLEDGMENTS: This research was supported by the Science Fund of the Republic of Serbia, Grant No. 10302, Exploring the potential of algal-derived (chromo)peptides as serum replacement components for meat cultivation — PEP4MEAT.

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Bioactive compounds and metabolism

Primed to protect: Intercropping with bioprimed Silene sendtneri enhances tomato resilience to cadmium

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KEYWORDS: seed priming, intercropping, Silene sendtneri, cadmium stress, tomato, stress memory

Seed priming is a promising technique for inducing stress memory and enhancing plant adaptation to adverse environmental conditions. However, its potential benefits in intercropping systems remain underexplored. This study aimed to evaluate whether biopriming of the cadmium (Cd)-accumulating species Silene sendtneri induces molecular and physiological memory effects, and how this influences mineral dynamics and stress responses in co-cultivated tomato (Solanum lycopersicum 'Micro Tom') under Cd stress.

Bioprimed S. sendtneri seeds were grown in control or Cd-contaminated soil. Transcriptomic analysis identified differentially expressed genes and enriched GO categories associated with priming. Tomato was cultivated either in monoculture or intercropped with primed S. sendtneri. Mineral content (Cu, Mn, Fe, Cd, Zn, Ni) and stress-related metabolites (proline and soluble sugars) were measured in roots and leaves.

Primed S. sendtneri exhibited significant upregulation of stress-responsive genes and improved growth under Cd stress. Intercropping with primed S. sendtneri reduced Cd accumulation in tomato and enhanced Fe and Zn homeostasis. Additionally, proline and sugar levels in tomato were modulated by intercropping, indicating improved physiological responses to stress.

These results demonstrate that biopriming induces a stress memory in S. sendtneri that not only improves its own tolerance to Cd but also benefits neighboring tomato plants through intercropping. The study highlights the potential of integrating primed plants into cropping systems to enhance phytoremediation and crop resilience in contaminated soils.

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Bioactive compounds and metabolism

Optimization of cell-based assays for identifying compounds with anti-inflammatory and anti-allergic activity

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KEYWORDS: RAW 264.7 macrophages, RBL-2H3 basophil/mast cell degranulation, Arthrospira platensis

With the global rise in allergy cases, there is a pressing need for efficient and cost-effective methods to screen therapeutic compounds. We optimized two cell-based assays in 48-well format for this purpose: (1) activation of RAW 264.7 macrophages for testing potential anti-inflammatory compounds, and (2) RBL-2H3 basophil/mast cell degranulation assay for evaluating compounds with potential allergy-relief properties. RAW 264.7 cells, upon lipopolysaccharide (LPS) stimulation (1 μg/mL), produce pro-inflammatory mediators such as nitric oxide, TNF-α, and IL-6, allowing assessment of anti-inflammatory activity. RBL-2H3 cells, which express high-affinity IgE receptors, when sensitized with antigen-specific IgE and triggered to degranulate, release β-hexosaminidase and histamine—mimicking allergic responses. Arthrospira platensis extract (10 and 100 μg/mL) and budesonide (Pulmicort) (2, 10, 20 μM), a corticosteroid with known anti-allergic properties, were tested for their anti-inflammatory activity.

Algae are a rich source of bioactive compounds, including proteins, vitamins, and minerals, making them promising candidates for therapeutic applications across various fields of medicine. As controls, ketotifen fumarate (10 and 50 μM) and calcium ionophore (1 and 10 μM) were included. Ketotifen fumarate is an antihistamine and mast cell stabilizer, whereas calcium ionophore functions by artificiallyincreasing intracellular calcium levels, thereby inducing mast cell or basophil degranulation. To optimize the RBL-2H3 degranulation assay, DNP-BSA (albumin from bovine serum, 2,4-dinitrophenylated) and anti-DNP IgE were used. DNP-BSA serves as the antigen, stimulating a cellular response in the study of IgE-mediated allergic reactions. These optimized assays provide reliable, scalable tools for identifying new compounds with therapeutic potential in inflammation and allergy.

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AKNOWLEDGEMENT:
Project was supported by the Alliance of International Science Organizations - ANSO-TP-2022-06 "Comparison of seafood and house dust mite sensitization profiles in allergenic patients from Asia and Europe" and Ministry of Education, Science and Technological Development and Innovation of Republic of Serbia, contract number 451-03-136/2025-03/200168; 451-03-136/2025-03/200288.

Bioactive compounds and metabolism

A database of interactions of modified steroids with human cytochromes P450

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KEYWORDS: modified steroids, cytochromes P450, database, deep learning

Understanding the metabolism of small molecules is of great importance in drug discovery to avoid possible side effects. In the context of metabolism prediction, silico tools are commonly used for identification of ligands of the enzymes. To build efficient and predictable in silico model one must use diverse dataset of experimental data to train the model, but current datasets have several problems that restrict their usage in AI-based models.

It is well-known that modified steroids are widely used at present time as lead compounds for creation of novel high-efficient antitumor or antihormone therapeutics. On the other hand, human CYP enzymes are taking part in different important processes in the body (hormone and bile acid synthesis, xenobiotic transformation etc.).This study introduces a dataset of interactions of a palette of modified steroids (estrane, androstane and bile acids analogs) with human steroid metabolizing enzymes CYP7A1, CYP7B1, CYP17A1, CYP19, CYP21A2. The freely accessible relational dataset is based on our previous results obtained in the frame of several bilateral projects. For each entry, the following information is provided: common information (molecule name, formula, etc.), interaction data (Kd and activity values, details of the experimental setup (buffer, concentration etc.)), a set of molecular descriptors (Chemopy (1135), CDK (275), RDKit (196), Pybel (24), BlueDesc (174), PaDEL (1875), molecular fingerprints (59)) and reference to the original publication.

The database can be used in QSAR studies and for the training of the deep learning-based models for the identification of novel ligands of CYPs in diverse datasets of chemical compounds.

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AKNOWLEDGEMENT:

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (SRO 200125) and Belarus-Serbia bilateral projects (Projects No X18S-RBG- 002 / 51-03-003036/2017-09/02, No X20SRBG-004 / 337-00-00612/2019-09/04 and 337-00-00230/2022-09/09) which were being realized between Institute of Bioorganic Chemistry of NAS of Belarus and Faculty of Sciences University of Novi Sad.

Bioactive compounds and metabolism

In vitro evaluation of encapsulated elderberry extracts as tyrosinase inhibitors

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KEYWORDS: *Sambucus nigra* L., encapsulation, polyphenolic profiles, tyrosinase inhibitory activity

Elderberry (*Sambucus nigra* L.) is rich in polyphenolic compounds known for their antioxidant, anti-inflammatory, and therapeutic properties. Due to these benefits, elderberry has gained increasing attention in the pharmaceutical, food, and cosmetic industries. However, its polyphenols are chemically unstable and prone to degradation during processing and storage, which limits their functional application. Encapsulation represents a promising strategy to protect these bioactive compounds and enhance their usability. In this study, lyophilized elderberry fruits were used to obtain extracts via microwave-assisted extraction. The resulting extracts were encapsulated using various carriers, including maltodextrin (MD), gum Arabic (GA), sodium alginate (ALG), whey protein concentrate (WPC), and inulin (IN) through freeze-drying over a 48-hour period. Two different carrier-to-core ratios (1:1 and 6:1) were employed. To assess variations among the encapsulated formulations, polyphenolic profiles were evaluated by HPLC-UV/VIS, while total phenols, tannins, flavonoids, and anthocyanins were analyzed spectrophotometrically. The tyrosinase inhibitory activity of the encapsulated extracts was evaluated in vitro, using kojic acid as positive control. Inhibitory effects were expressed as kojic acid equivalents (mg KA eq/g extract). Among the 40 analyzed compounds, 13 polyphenols were quantified, with cyanidin-3-O-glucoside and rutin identified as the most abundant. Distinct quantitative differences were observed depending on the type of carrier and ratio used. Notable differences in tyrosinase inhibitory activity were also recorded between formulations (from 3.6. to 10.4 mg KA eq/g extract). These results suggest that targeted encapsulation can contribute to polyphenols stability and preserve their bioactivity, supporting the potential use of elderberry-derived formulations in cosmetic applications.

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AKNOWLEDGEMENT:
The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-137/2025-03/ 200125 & 451-03-136/2025-03/ 200125).

Bioactive compounds and metabolism

Antidiabetic potential of *Gentiana dinarica* root extracts and their active component norswertianin and its glycoside norswertianin-1-O-primeveroside

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KEYWORDS: *Gentiana dinarica*, xanthones, antidiabetic properties, norswertianin, norswertianin-1-O-primeveroside

Xanthones, the main secondary metabolites found in *Gentiana dinarica*, display various biological activities. In in vitro cultured and particularly in genetically transformed *G. dinarica* roots, there is a higher content of xanthones. The aim of this study was to investigate and compare antidiabetic properties of secondary metabolites prepared from *G. dinarica* roots collected from nature, cultured in vitro or genetically transformed. We compare HPLC secondary metabolite profiles and the content of the main extract compounds of *G. dinarica* methanol root extracts with their ability to scavenge DPPH free radicals and inhibit intestinal α-glucosidase and α-amilase in vitro. Anti-hyperglycemic activity of extracts was tested further in vitro by glucose uptake test and in vivo on glucose-loaded Wistar rats. Our findings reveal that the most prominent radical scavenging potential and potential to control the rise in glucose level, detected in xanthone-rich extracts, were in direct correlation with an accumulation of xanthones norswertianin and norswertianin-1-O-primeveroside in roots cultured in vitro and genetically transformed.

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AKNOWLEDGEMENT:
This research was funded by the Ministry of Science Technological Development and Innovation of the Republic of Serbia (registration No. 451-03-66/2024-03/200007).

Bioactive compounds and metabolism

Association of the molybdenum serum concentration with the disease severity in COVID-19 patients

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KEYWORDS: molybdenum, COVID-19, disease severity

Molybdenum plays a significant role in all cells of the human body because it ensures energy generation and detoxification of the body. It achieves these functions because it is a cofactor of several important enzymes: xanthine oxidase, aldehyde oxidase and sulfite oxidase. Our main goal was to examine association of molybdenum concentrations in serum in Covid-19 patients with disease severity.

The study included 210 SARS-CoV-2 positive participants who were recruited at the General Hospital in Tešanj and based on clinical manifestations, laboratory and radiological (imaging) findings, oxygen saturation values and general condition of the patient, were divided into four different groups: mild clinical picture, moderate clinical picture, severe clinical picture, and ex. letalis (dead) patients. Serum samples were analyzed at the Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Portugal, using duly validated inductively coupled plasma mass-spectrometry (ICP-MS) analytical procedures.
Significant trend of decreasing molybdenum serum concentration was determined according to the severity of the clinical picture (from 1.270 ± 0.075 in the "mild" group to 0.946 ± 0.092 μg/L in the "exitus lethalis" group).

Bioactive compounds and metabolism

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A statistically significant difference was observed between several groups: "mild" and "severe" groups (p=0.006); "mild" and "exitus lethalis" (p<0.001) and "moderate" and "exitus lethalis" groups (p=0.020). A significant decrease of molybdenum serum levels was observed according to the disease severity Covid-19 patients. These findings reinforce the importance of monitoring molybdenum levels in COVID-19 patients and conducting relevant interventions.

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Cellular processes and signaling

Magnesium-dependent-protein phosphatase 1b regulates the protein arginine methyltransferase 5 through the modulation of myosin phosphatase in cervical carcinoma

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KEYWORDS: PRMT5, gene expression, symmetric dimethylation, cervical carcinoma, retinoblastoma protein

Dysregulation of the expression levels and the activity of kinases/phosphatases is an intrinsic hallmark of tumor transformation and progression, as either as a primary cause or consequence. The myosin phosphatase (MP)/protein arginine methyltransferase 5 (PRMT5)/histone (H4) pathway is an oncogenic signaling pathway downregulating the gene expression of tumor suppressors. However, the upstream regulators of the pathway are unknown. We show that the Mg2+-dependent protein phosphatase 1 B (PP2Cb or PPM1B) interacts and regulates MP through the MYPT1 regulatory subunit, and this interplay results in the inactivation of the tumorigenic pathway driven by PRMT5. The phospho-Thr696 inhibitory residues of the MYPT1 regulatory subunit of MP was dephosphorylated by PPM1B.

The inhibition of PPM1B by sanguinarine (SNG) resulted in the deactivation of MP and the increased activity of PRMT5 leading to increased symmetric dimethylation of histone H4 in HeLa cells. The overexpression of PPM1B had the opposite action. The overexpression of PPM1B decreased the colonization activity of HeLa cells through modulation of MP. Finally, human cervical carcinoma biopsies showed almost complete elimination of PPM1B compared to their healthy control counterparts. The phosphorylation of the inhibitory MYPT1pT696 and the regulatory PRMT5pT80 residues and the symmetric dimethylation of H4 were elevated in the cancer biopsies and it resulted in a decrease in retinoblastoma protein expression. The results indicate a tumor suppressor role of the PPM1B/MP axis via inhibition of PRMT5, thereby regulating gene expression through H4 arginine dimethylation. Collectively, PPM1B is a tumor suppressor and a possible tumor marker for cervical carcinoma.

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Cellular processes and signaling

The role of ninjurins in plasma membrane rupture

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KEYWORDS: plasma membrane rupture, NINJ1, NINJ2

The regulation of cell death plays a vital role in ensuring tissue homeostasis, influencing the progression of tumors and modulating inflammatory responses. Cells can undergo different cell death pathways, yet the process of plasma membrane rupture has long been considered a passive event. Recently, ninjurin-1 (NINJ1) was identified as a key molecule mediating the plasma membrane rupture after lytic types of cell death1. The ninjurin family consists of two members, NINJ1 and NINJ2, which were initially characterized as adhesion molecules2. NINJ1 and NINJ2 share high sequence similarity and were both shown to make fibrils in the plasma membrane, yet only NINJ1 potently breaks the membrane apart, leading to the release of large damage-associated molecular patterns and contributing to excessive inflammation3. The main aim of this study is to elucidate the role of NINJ2 in the plasma membrane rupture. To achieve this, we made a panel of NINJ1/NINJ2 chimeras identifying segments crucial for mediating PMR and tested the variants in HEK293 overexpression systems as well as in biologically relevant cell death induction systems. In agreement with recently published studies, we identified the crucial role of NINJ1 N-terminus. Furthermore, we revealed an unexpected regulatory role of NINJ2 that can suppress NINJ1 mediated release of large cellular cargo. These results highlight NINJ1 as a key mediator of immunogenic cell death and suggest that modulation of the NINJ1–NINJ2 axis may represent a novel therapeutic strategy in inflammatory diseases.

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Cellular processes and signaling

An analysis of the relationship between the deformability of erythrocytes and clinical biochemical indicators in patients diagnosed with diabetes mellitus

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KEYWORDS: erythrocyte hemorheology, tissue oxygenation, elongation index, shear stress

It is well known that long-term hyperglycemia in patients with diabetes mellitus (DM) leads to a series of successive changes in erythrocyte biochemical and biophysical traits, which further impact erythrocyte hemorheology and overall microcirculation, resulting in DM complications1. This research investigates the relationship between clinical biochemical parameters and red blood cell deformability, crucial for tissue oxygenation, in DM patients.

Red blood cells (RBCs) were extracted from DM patients' blood (n=13) who regularly underwent check-ups at the Clinic for Endocrinology, Diabetes, and Metabolic Diseases at the University Clinical Center of Serbia, along with matched controls (n=11) of similar age and sex. RBC deformability was evaluated using the RheoScan-D300 ektacytometer (RheoMeditech Inc., Korea), and results were presented as the elongation index (EI) in response to increasing shear stress (SS). For the quantitative parameters correlated with the clinical biomarkers, we used EI at 3Pa (3 Pa represents mean vascular pressure), ½ EI (EI value at half the maximum of the deformability curve), and dEI/dSS, the first derivative of the EI at the one-half value of the deformability curve, as a more sensitive metric for assessing RBC response to SS in DM than the conventional EI parameters2.

Cellular processes and signaling

An analysis of the relationship between the deformability of erythrocytes and clinical biochemical indicators in patients diagnosed with diabetes mellitus

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The results showed a statistically significant correlation ($p<0.05$) between dEI/dSS and serum cholesterol levels. Although no significant correlation was found between EI 3Pa and cholesterol, a direct relationship was observed between EI 3Pa and serum urea levels. In addition, ½EI exhibits a positive correlation with the level of the enzyme creatine kinase. Overall, these insights can be beneficial for advancing clinical practices and improving outcomes for DM patients with complications.

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Cellular processes and signaling

Interaction between human stem cell-derived neural progenitor cells and microglia

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KEYWORDS: microglia-NPC interaction, iPSC-derived neural progenitor cells, neuroinflammation, cytokine signaling, neural regeneration

Neural progenitor cells (NPCs), playing a crucial in neural development and regeneration, hold great potential for stem cell-based therapies for conditions affecting neural cells or tissues. These include various neurodegenerative diseases, ischemic stroke, as well as traumatic brain and spinal cord injuries. A major obstacle of the stem cell-based therapies is the limited integration of transplanted cells into neural tissue. Application of human induced pluripotent stem cell (iPSC)-derived NPCs or partially differentiated neural cells may alleviate these difficulties. The initial step of NPCs' differentiation is the outgrowth of neurites, which is determined by a combination of internal and external factors. Microglia, the brain-resident macrophages, patrolling the central nervous system, may also affect NPC differentiation. However, cell migration is key to the establishment of the NPC-microglial interaction. Our aim is to elucidate the molecular mechanisms that determine microglial migration and the neurite outgrowth and mobilization of NPCs.

We have previously demonstrated that BV2 cells (murine microglia) stimulated either toward pro- or anti-inflammatory direction enhanced elongation of neurites of human iPSC-derived NPCs, and that pro-inflammatory microglia even promoted NPCs' proliferation. Now we analyzed the effect of pro- (IFN γ, LPS) and anti-inflammatory (IL 4, IL 13) stimulation on BV2 microglia morphology and motility using high content screening and analysis (HCS) combined with wound healing assay. We also investigated the major cytokines secreted by BV2 cells using a flow cytometry-based method. The most prominent observation from the cytokine analysis is the strong activation of BV2 cells by LPS, leading to massive cytokine release, in particular secretion of IL 6, TNF α, G CSF, CCL5, and CCL2.

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We also found that anti-inflammatory stimulation had only a minor effect on the secretion of the cytokines tested, except for CCL2, whose release was stimulated by IL 13. In addition, we analyzed how microglia affected the numbers and branching of neurites of NPCs, as well as their migratory properties using HCS approach and GFP-expressing human iPSC-derived NPCs. Our further studies aim to identify the particular cytokines responsible for influencing motility and neurite formation of NPCs.
The results from our study may help to elucidate the molecular details of the complex interactions between microglia and NPCs, which may contribute to optimizing the therapeutic use of stem cell-derived NPCs in the treatment of neurodegenerative diseases and neurological injuries.

ACKNOWLEDGMENT: This work has been supported by the Stipendium Hungaricum to KGA and the National Research, Development and Innovation Office to LH (grant number: K 128123).

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Cellular processes and signaling

Studies on the coordinated binding between a hexameric ATPase p97 and its three adaptor proteins involved in DNA–protein crosslink repair

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KEYWORDS: AAA+ ATPase p97, DNA–protein crosslinks, protein characterization, molecular interactions

DNA–protein crosslinks (DPCs) are frequent and harmful DNA lesions that interfere with essential cellular processes such as DNA replication and transcription. These crosslinks occur when proteins are covalently attached to DNA. Inefficient DPC repair has been linked to various diseases such as cancer and neurodegeneration. In humans, the metalloprotease SPRTN plays a key role in DPC repair by degrading DPC protein substrates. It has been proposed that loosely folded DPCs can be processed directly by SPRTN, whereas more tightly folded DPCs require ubiquitylation and assistance of a hexameric ATPase and unfoldase p97, as well as two other proteins, Ufd1 and Npl4. It is proposed that the Ufd1:Npl4 complex recognizes these lesions and recruits p97 to unfold the protein component so that SPRTN can reach and degrade it. SPRTN, Ufd1 and Npl4 have been classified as p97 adaptor proteins.

Each of these adaptors has a characteristic motif in its amino acid sequence to bind p97. However, there is no information on whether all three bind to p97 simultaneously and if so, how this is achieved. Our project aims to uncover the structural mechanisms underlying DPC repair by investigating the molecular interactions between p97 and the Ufd1:Npl4 complex and SPRTN. Using a bottom-up approach, we have successfully produced single recombinant proteins of human origin in a bacterial expression system. I will present our results of the biochemical and biophysical analysis of these proteins and their interactions, which provide an important basis for the future structural characterization of these complexes.

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Cellular processes and signaling

MIF tautomerase inhibitor TE-91 inhibits inflammatory cytokine production and activation of ETS2 transcription factor in the chronic inflammatory macrophage model

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KEYWORDS: chronic inflammation, macrophage, MIF, ETS2

Inflammatory bowel diseases (IBD) are chronic autoimmune diseases with an unknown origin. The two main types of IBD are Crohn's disease and Ulcerative Colitis. Both diseases are characterised by the disruption of the intestinal epithelial barrier ("leaky gut"), which leads to immune activation and chronic inflammation. The transcription factor ETS2 has been shown to play a key role in macrophage activation during chronic inflammation, and elevated ETS2 activation was found in patients with IBD. Macrophage migration inhibitory factor (MIF) plays a key role in numerous acute and chronic inflammatory diseases. The protective effect of MIF inhibition has also been described in various inflammatory models. In this work, we investigated the impact of a small molecule MIF tautomerase inhibitor TE-91 on the activation of ETS2 transcription factor, along with its effect on various inflammatory cytokines in a chronic inflammatory macrophage activation model.

Raw264.7 and RawBlue cells were pre-treated with 20 μM TE-91, then induced with GTPP treatment including 50 ng/ml GM-CSF and mouse TNFα, along with 2 μg/ml PGE2 and Pam3CSK4. After 72 hours of induction, various inflammatory parameters (ROS, nitrite, inflammatory cytokine, and AKT production) of the macrophages were determined, along with the activation levels of the transcription factors NF-κB, HIF-1α, and ETS2.

GTTP treatment significantly increased all inflammatory parameters of the macrophages compared to control cells. In contrast, TE-91 significantly reduced the production of ROS, nitrite, IL-6, CCL-2, and AKT, as well as the activation of the transcription factors NF-κB, HIF-1α, and ETS2, compared to chronically treated cells.

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The pharmacological MIF tautomerase inhibitor TE-91 reversed inflammatory macrophage activation with the inhibition of NF-κB and ETS2 transcription factors in a chronic inflammatory model. These findings raise the possibility of testing TE-91 as a first step in animal models of diverse inflammatory disorders.

FOUNDING: The research was supported by EKÖP-24-4-I-PTE-8.

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Cellular processes and signaling

Uptake of Ara h 1 from raw and thermally processed peanuts, cytokine response, and the modulatory role of peanut lipids in macrophages

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KEYWORDS: peanut allergy, Ara h 1, lipids, macrophages, cytokine

Peanut allergy is a growing global health concern, with Ara h 1 recognized as a major allergen alongside Ara h 2 and Ara h 31. Macrophages, as key antigen-presenting cells, contribute to allergic inflammation through phagocytosis, antigen presentation, and cytokine release2. This study aimed to isolate and characterize Ara h 1 from raw and thermally processed peanuts, assess its interaction with cell culture macrophages, measure subsequent cytokine production, and investigate the influence of peanut lipids on these cytokine responses, both independently and in combination with allergens. The methodology included the purification of Ara h 1 using a combination of precipitation and chromatographic techniques, with purity confirmed by SDS-PAGE. The allergen was fluorescently labeled to monitor its binding/uptake by macrophages using flow cytometry after cell culture monocyte differentiation. After the treatment of macrophages with allergen alone or in combination with lipids, levels of proinflammatory and regulatory cytokines were quantified using ELISA. Results demonstrated the successful purification of Ara h 1 from both raw and thermally processed peanuts, revealing isoforms. Cell culture macrophages showed concentration and time-dependent binding/uptake of Ara h 1 from both sources. Furthermore, the study identified specific cytokine production profiles induced by Ara h 1 and demonstrated the modulatory effects of lipids on these inflammatory responses, both alone and in combination with allergens. These findings underscore the active role of macrophages in allergen processing and immune modulation, even after thermal treatment, and emphasize the critical impact of dietary components like lipids on allergic inflammation.

AKNOWLEDGEMENT:
This study was supported by the Ministry of Science, Technological Development and Innovation of Serbia (contract number 451-03-136/2025-03/200168).

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Cellular processes and signaling

Mineral content and fatty acid composition in different kind of dietin Wistar rats liver-correlations

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KEYWORDS: fatty acids, minerals, correlations

We investigated the effects of 4-weeks treatment milk- and fish-based diet, in female 4 months old Wistar rats fed with different types of diets: standard S, milk-based M, fish-based F. Fatty acid were analyzed by GC. Analysis of elements was performed by ICP-MS. Data were processed by SPSS, determination normality by Kolmogorov-Smirnov test and Univariate ANOVA analysis for differences between groups, Post Hoc test between groups. Pearson correlation determined correlation between groups and variables ($p<0.05$). Results of separate minerals showed differences between groups for Ca, Fe, Cu, Zn, As, Hg, Pb with huge effects ($\eta p2= 0.32-0.63$), microelements with huge effect $\eta p2= 0.604$, differences between almost all fatty acids.

Post Hoc showed Ca concentration differences between F-S 44.9 (95% CI 17.0-72.9), Fe differences Fish-Milk group 138.6 (95% CI 45.4-231.7), M-S 210.15 (95% CI 121.9-298.4), since Cu concentration differences between M-S -1.26 (95% CI -1.9, -0.6), and between F-S -1.4 (95% CI -2.1, -0.7). Zn concentration showed differences between M-F as 7.9 (95% CI -12.7 - -3.1), and M-S -5.5 (95% CI -10.1 - -1.0). Differences in As between M-F -.07 (95% CI -0.1 - 0.0), and between Fish-Standard .060 (95% CI 0.0 - 0.1), Hg between M-F -.00258 (95% CI 0.0 - 0.0), F-S .00259 (95% CI 0.0 - 0.0), Pb concentrations differences between M-S were -.03 (95% CI -0.1 - 0.0). Differences in toxic between M-F group -.09 (95% CI -0.1 - 0.0), and microelements between M-F 130.9 (36.5 - 225.4), between M-S 203.3 (95% CI 113.9 - 292.8).

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AKNOWLEDGEMENT:
This research was funded by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (No. 451-03-66/2024-03/200015).

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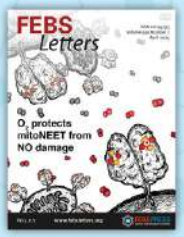

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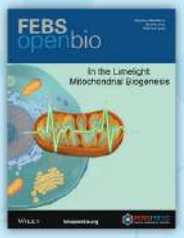

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60(048)(0.034.2)
577(048)(0.034.2)

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Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 150.

ISBN 978-86-7220-136-9

a) Биотехнологија -- Апстракти b) Биохемија -- Апстракти

COBISS.SR-ID 179724041

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