2026

FEBS YSF

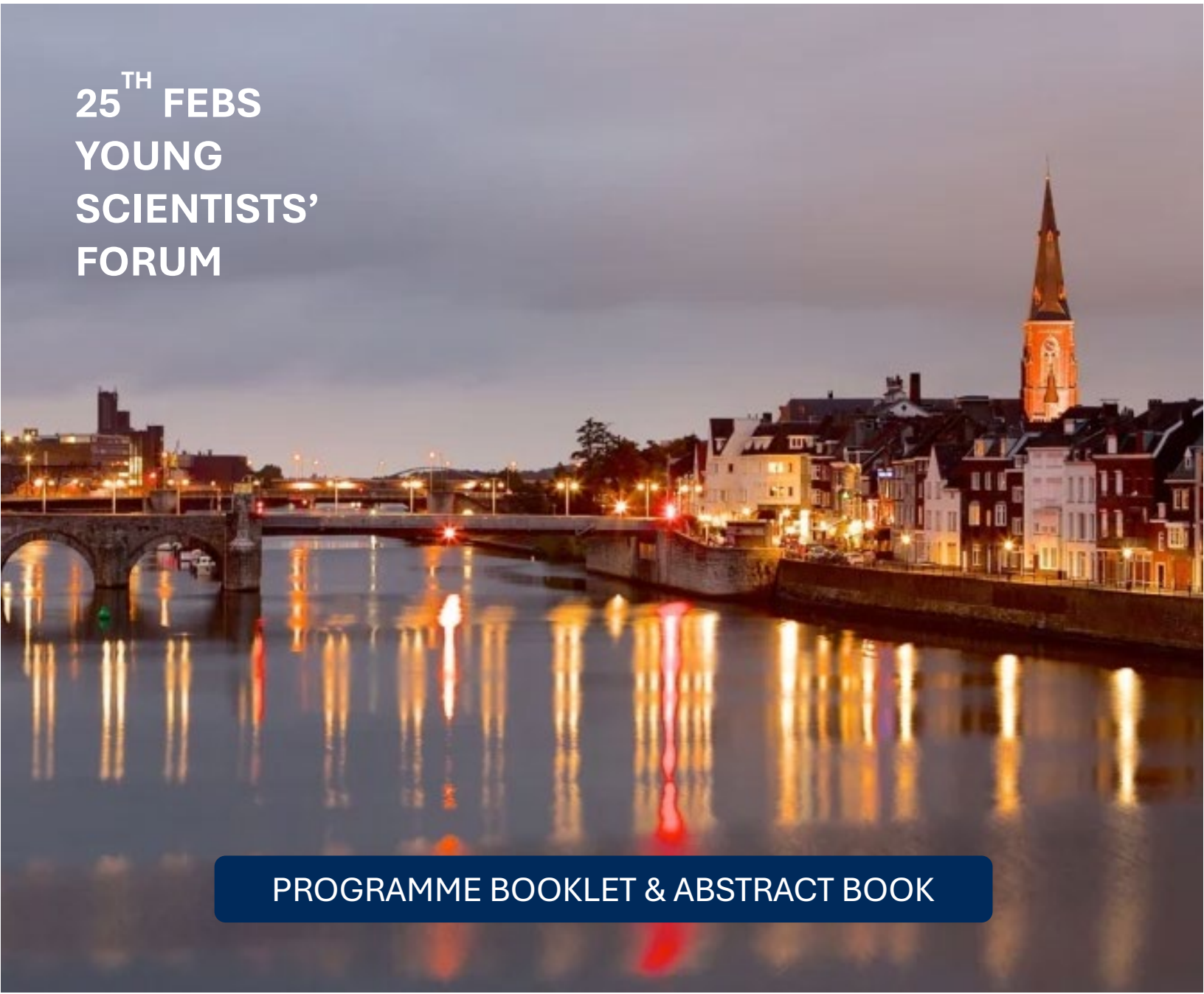
2–4 July • Wageningen • The Netherlands

FEBS

NVBMB

Nederlandse Vereniging voor Biochemie en Moleculaire Biologie

25TH FEBS YOUNG SCIENTISTS' FORUM



PROGRAMME BOOKLET & ABSTRACT BOOK

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Welcome Note

Dear participants,

Welcome to the 25th edition of the FEBS Young Scientists' Forum (YSF), taking place from July 2nd to 4th, 2026, in the beautiful and picturesque city of Wageningen, the Netherlands. This YSF edition will highlight the importance of interdisciplinary collaborations in science and deliver a diverse and interesting programme.

The YSF 2026 will bring together approximately 100 selected PhD students and early-career postdoctoral researchers from across Europe and beyond. Over the days of the YSF, participants will have the opportunity to:

- Present and discuss their research with peers
- Explore topics across diverse scientific fields
- Attend lectures by leading scientists
- Join workshops, including scientific and soft-skill focused themes
- Network in a social, friendly and supportive setting

Set in Wageningen, a city known for its contributions to life sciences and sustainability, the YSF 2026 offers a unique backdrop for promoting interdisciplinary connections. Located in the province of Gelderland, this historic yet dynamic city is surrounded by lush greenery and breathtaking landscapes, including the Veluwe National Park and the Nederrijn River floodplains.

YSF2026 will be held at the Fletcher Hotel-Restaurant De Wageningsche Berg, a 4-star superior hotel nestled in the serene, wooded area between the Utrechtse Heuvelrug and the Lower Rhine. The venue's excellent convention facilities are perfectly designed to foster scientific exchange in sync with nature. In addition to making professional connections, participants will have ample opportunity to socialize, relax, and enjoy the tranquil surroundings.

Following the YSF, participants will continue their scientific journey at the 50th FEBS Congress in Maastricht, one of the biggest bioscience conferences in Europe.

We look forward to welcoming you to YSF 2026, where science meets collaboration, and new connections take root.

Kind regards,
The YSF 2026 Organizing Committee

YSF2026 Chair

Ricfrid W.G.N. van der Marel

Chair of the FEBS Careers of Young Scientists Committee

Riccardo Miggiano

Practical Information

VENUE

Fletcher Hotel-Restaurant De Wageningsche Berg
Generaal Foulkesweg 96
6703 Wageningen, the Netherlands

REGISTRATION

Registration for the YSF will take place at the registration desk in the Fletcher Hotel lobby from 15:30–18:00 on 2nd July. For the 50th FEBS Congress, you can get your badge from the congress registration desk at the Maastricht Exhibition & Conference Centre on 4th July.

CERTIFICATE OF ATTENDANCE

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

POSTER PRESENTATION

Posters should be mounted according to the provided "List of Posters". The same poster will be displayed also at the 50th FEBS Congress according to the instructions for the Congress.

AWARDS

There will be three awards sponsored by FEBS and IUBMB journals: two sponsored by *FEBS Open Bio* (for the best short oral communication and best poster) and one by *BioFactors* (best poster).

TRANSFER FOR THE FEBS CONGRESS

After the closing on 4th July, all participants will be transferred by organized transport to the Green Elephant Hostel and the Townhouse hotel in Maastricht. Accommodation includes breakfast.

CONTACT

YSF organising committee: ysf2026@febs.org
FEBS Congress secretariat | MCI Suisse SA: febs@mci-agency.com

Organizing Committee and Committee Advisors

Organizing Committee

Ricfrid W.G.N. van der Marel - *Chair*
Netherlands Cancer Institute (Amsterdam), The Netherlands

Bram Boon
European Institute for the Biology of Ageing (ERIBA), UMC Groningen, The Netherlands

Eva D. Tunderman
UMC Utrecht Brain Center, The Netherlands

Ivar van Galen
Maastricht University, The Netherlands

Letizia Cornaro
Wageningen University & Research, The Netherlands

Max W. van Baalen
UMC Utrecht, The Netherlands

Committee Advisors - FEBS Careers of Young Scientists Committee (CYSC)

Riccardo Miggiano - *Acting Chair*
University of Piemonte Orientale, Italy

Irene Díaz-Moreno -
CYSC Chair until 31.12.25
Institute for Chemical Research, University of Seville, Spain

Vlastimil Kulda
Charles University, Czech Republic

Anna Jagusiak
Jagiellonian University, Poland

YSF Programme

Thursday 2 July 2026	
15:30 – 18:00	**ARRIVAL AND REGISTRATION**
18:00 – 18:30	**OPENING REMARKS** Ricfrid van der Marel, Chair of the 25th FEBS YSF Organizing Committee Jan Willem Borst, Co-Chair of the 50th FEBS Congress Riccardo Miggiano, Acting Chair of the FEBS Careers of Young Scientists Committee Miguel A. De la Rosa, FEBS Secretary General
18:30 – 19:15	**OPENING LECTURE** **Luca Scorrano**, *Italy* 'Keeping mitochondria in shape: a matter of life and death'
19:30	**WELCOME RECEPTION**

Friday 3 July 2026	
08:30 – 09:15	**KEYNOTE LECTURE 1** **Joanna Sułkowska**, *Poland* 'Topology in biological matter: new ways to use entanglement in structural biology'
09:15 – 10:30	**SELECTED SHORT TALKS 1** **Ilse Lagerwaard**, *The Netherlands* 'Delineating the protein network regulating early termination/elongation of RNA Polymerase II' **Ludovic Poiré**, *France* 'Discovery of novel immunity systems against plasmids in the opportunistic pathogen Acinetobacter baumannii' **Regina Mengual Fenollar**, *Spain* 'Multidirectional neuron-glia mitochondrial transfer and metabolic rewiring' **Jakub Kaňka**, *Czech Republic* 'Enlightening the quietness – The search for early silenced proviruses' **Gabriela Guedes**, *United Kingdom* 'Exploiting the versatility of engineered consensus tetratricopeptide repeat proteins as multivalent theranostic agents'
10:30 – 10:35	**SPONSORED TALK – PreLights** preLights Preprint highlights, selected by the biological community
10:35 – 11:00	**COFFEE BREAK**
11:00 – 11:35	**CAREER SKILLS 1** **Alain van Gool**, *The Netherlands* 'Sliding doors in my career as translational biomarker scientist'
11:35 – 12:10	**CAREER SKILLS 2** **Jana Christopher**, *Germany* 'Navigating image integrity, paper mills, and the impact of generative AI'
12:10 – 12:30	**CAREER SKILLS 3** **Marta Reyes-Corral**, *Spain* 'Funding opportunities from the FEBS Fellowships Committee'
12:30 – 14:00	**LUNCH**

14:00 – 14:45	**KEYNOTE LECTURE 2** **Tom de Greef**, *The Netherlands* 'Physical and molecular strategies for secure DNA data storage'
14:45 – 16:00	**SELECTED SHORT TALKS 2** **Pablo Ortega Martínez**, *Finland* 'Extracellular electron transfer in cyanobacteria' **Susana Marques Costa**, *Portugal* 'A pair of 'false twins' ncRNAs in Pseudomonas putida: study of their role and regulation' **Tõnis Laasfeld**, *Estonia* 'Unlocking systems biology and pharmacology of biochemical reactions and interactions through structured approach to data' **Marianna Genta**, *Italy* 'Bacterial DNA nucleotide excision repair: a multi-approach investigation of the damage recognition process' **Agienszka Będzińska**, *Poland* 'Crosstalk between SLAMF7 and NF-κB signalling pathway in inflammation'
16:00 – 18:30	**POSTER PARTY**
19:00	**SOCIAL PROGRAMME AND DINNER**

Saturday 4 July 2026	
09:00 – 09:35	**CAREER SKILLS 4** **Jason Perret**, *Belgium* 'The most underrated soft skill: the lab book'
09:35 – 10:10	**CAREER SKILLS 5** **Keith Elliott**, *United Kingdom* 'Preparing your CV: how to make the most of yourself'
10:10 – 10:45	**COFFEE BREAK**
10:45 – 11:30	**CLOSING LECTURE** **Philippe Glaser**, *France* 'Emergence and dissemination of antibiotic resistance'
11:30 – 12:00	**CLOSING REMARKS AND PRIZES** **Sara Fuentes**, *Spain* 'Early Career Reviewer Hub: a new initiative to build your skills as a peer reviewer' **Anna Jagusiak**, *Poland* 'FEBS-IUBMB-ENABLE 2026 Conference' **Tõnis Laasfeld**, *Estonia* 'FEBS YSF and Congress 2027' **Péter Deák**, *Hungary* 'FEBS Junior Section' Prizes sponsored by *FEBS Open Bio* and *BioFactors*
12:30	**TRANSFER TO MAASTRICHT** (lunch in transit)

Keynote Speakers

Keeping mitochondria in shape: a matter of life and death

Luca Scorrano

University of Padua, Italy

Luca Scorrano earned an MD and a PhD from the University of Padua, Italy. From 2000 to 2003, he was a HFSP postdoctoral fellow in Stan Korsmeyer's lab at the Dana-Farber Cancer Institute, Harvard Medical School, Boston. In 2003, he was recruited by the Dulbecco-Telethon Institute, Italy, as Assistant Scientist. In 2006, he became Full Professor at the University of Geneva Medical School, Switzerland, until 2013 when he was named "Outstanding Recognition (Chiara fama)" Professor of Biochemistry at the University of Padua. From 2014 to 2020, he was Scientific Director at Veneto Institute of Molecular Medicine. Luca discovered the cristae remodeling pathway, foundational to mitochondrial dynamics. His laboratory elucidated mechanisms of cristae architecture, remodeling, and mitochondrial fusion-fission regulation. His lab revealed the impact of mitochondrial shape on bioenergetics, angiogenesis, cardiac function, adipocyte differentiation, infection, and cancer. His group identified the first molecular tether between endoplasmic reticulum and mitochondria and showed that it depends on Mitofusin 2 alternative splicing, advancing membrane contact sites research. He is an elected member of EMBO and Academia Europaea. He received several awards, including the 2006 Eppendorf/Nature European Young Investigator Award, the 2013 European Society for Clinical Investigation Award, and the 2024 International Society for Heart Research (ISHR) Research Achievement Award.

Topology in biological matter: new ways to use entanglement in structural biology

Joanna Sułkowska

University of Warsaw, Poland

Joanna Sułkowska received her PhD in biophysics from the University of Warsaw in 2007, with distinction. After postdoctoral research at the University of California, San Diego, she joined the Centre of New Technologies at the University of Warsaw, where she now leads the Interdisciplinary Laboratory for Modeling Biological Systems. She also held visiting positions at MIT and the California Institute of Technology. She specializes in structural biology, focusing on the theoretical and experimental study of protein free energy landscape and topology. Sułkowska identified and characterized various forms of non-trivial protein topology, such as knots, slipknots, and lassos, and discovered new protein folds with complex topological architectures. Her research also includes work on GPCR-type proteins. She has authored over 90 publications in journals. Sułkowska has received numerous honors, including the EMBO Installation Grant, EMBO YIP, the UNESCO–L'Oréal "Rising Talent" award, and the National Science Centre Award in Life Sciences Poland and Fulbright STEM. She was named "Person of the Year" by RMF Classic (MocArty).

Keynote Speakers

Physical and molecular strategies for secure DNA data storage

Tom F.A. de Greef

Eindhoven University of Technology, Netherlands

Tom F.A. de Greef is Full Professor of Synthetic Biology in the Department of Biomedical Engineering at Eindhoven University of Technology (TU/e), Netherlands, where he leads research at the intersection of synthetic biology, molecular computing, and engineered living systems. He received his MSc degree cum laude in 2004 and his PhD in 2009, both in Biomedical Engineering from TU/e. His research group develops next-generation living technologies focusing on DNA-based data storage, biological computing devices, synthetic cell engineering, and mammalian synthetic biology. Notable contributions from his laboratory include the development of thermoresponsive microcapsules enabling reliable random access to DNA-encoded information for sustainable data storage, and the engineering of scalable synthetic communication platforms in mammalian cells using designed coiled-coil peptides for programmable cell-to-cell signaling with applications in cell therapeutics. De Greef has published over 100 peer-reviewed articles in leading journals and has secured multiple Dutch Research Council grants (VENI, VIDI, VICI) and European Research Council (StG and CoG) grants. He is the recipient of the 2017 Cram-Lehn-Pedersen Prize in Supramolecular Chemistry and the 2022 Groundbreaking TU/e Researcher Award, and was appointed Fellow of the Netherlands Academy of Engineering in 2024.

Emergence and dissemination of antibiotic resistance

Philippe Glaser

Institut Pasteur, France

Philippe Glaser is a Professor at the Institut Pasteur in Paris, France, and heads the Ecology and Evolution of Antibiotic Resistance Unit. He is an expert in bacterial genomics and the evolution of antibiotic resistance. He is renowned for his genomic epidemiology studies of Group B Streptococcus (GBS), in both humans and animals. He has demonstrated that the widespread use of tetracycline from the 1950s onwards led to the emergence of neonatal GBS infection in both Europe and the US. He is deciphering the evolution and dissemination of carbapenemase and ESBL-producing Escherichia coli and Klebsiella pneumoniae. His multidisciplinary One Health research ranges from field investigations to insights into mechanisms.

Sliding doors in my career as translational biomarker scientist

Alain van Gool

Radboud University Medical Center, Netherlands

Alain van Gool is Professor of Personalized Healthcare at the Radboud University Medical Center, with a strong passion for the application of biomarkers in translational medicine and personalized healthcare. After his study (biochemistry, 1991) and PhD (molecular biology, 1996) Alain worked at a mix of academia, pharmaceutical industries (Organon, Schering-Plough, MSD), applied research institutes (CancerUK, TNO) and university medical centers (Radboudumc) in Europe, Asia and USA. He has been leading technology-based biomarker laboratories, cross-functional expert teams, therapeutic project teams and public-private consortia, many of which were focused on the discovery, development and implementation of translational biomarkers in a variety of therapeutic areas. His technical expertise resides most strongly in molecular profiling (various Omics approaches), analytical biomarker development, and applications in translational scientific research. Alain currently coordinates several biomarker/omics/data/AI programs as part of the department of Human Genetics including Lead PI of the Netherlands X-omics Initiative, Domein Leader MedTech & Data Sciences of Radboudumc's Research Institute for Medical Innovation, Chair of the Data & AI board of Radboudumc, Co-coordinator of the Radboudumc Technology Centers, Scientific director of the Radboud Healthy Data program, Lead of the sectorplan team on AI, e-health and medical technology, Co-coordinator of the NWO Large Scale Research Infrastructures group Life Technologies & Enabling Technologies, Chair of the Biomarker Platform of EATRIS (the European Infrastructure for Translational Medicine). Previously, Alain co-initiated Health-RI (the Netherlands Health Research Infrastructure for Personalized Medicine and Health) and DTL (the Dutch Techcenter for Life Sciences), thus contributing to the organisation and coordination of local, national and European technology infrastructures. Complementing his daily work, he enjoys contributing to scientific advisory boards of start-up enterpreneurs, multinational companies, translational organisations, funding agencies and conference organisers.

Navigating image integrity, paper mills, and the impact of generative AI

Jana Chrisopher

FEBS Press, Germany

Jana Christopher is the Image Data Integrity Analyst at FEBS Press. She has also been a freelance consultant for The Royal Society since 2019. Jana chairs the STM Working Group on Image Integrity. She is an experienced trainer who has worked with Integrity teams at eLife, Elsevier, PNAS and others, and frequently speaks to early-career scientists. Jana obtained an MA in Linguistics from Westminster University in London, UK, in 2002 and has worked in Scientific Publishing for over 20 years.

Career Speakers

The most underrated soft skill: the lab book

Jason Perret

Free University of Brussels, Belgium (Emeritus)

Jason Perret is Professor Emeritus of the Free University of Brussels (ULB) Medical Faculty, Belgium. Besides 35 years of basic and translational medical research, he also spent 4 years in industry as head of the Molecular Biology lab (Baxter Healthcare research facility in Belgium). He spent 10 years supervising and managing Biochemistry wet labs and 15 years teaching Molecular Biology to medical, biomedical and veterinary students and to Biomedical Civil Engineers. He has also supervised or co-supervised PhDs, MD clinician-researchers, and Master's thesis. Jason is currently President of the Belgian Biochemical. He has also been with FEBS since 2003 as a member of the FEBS Education Working Group and then Committee, and thereafter actively contributing to FEBS education activities, presenting talks and workshops on various topics. Outside the lab there is a life and Jason plays music, plays tennis, nordic walking and swimming, and most of all still remains passionate about all things in life as much as possible.

Preparing your CV: how to make the most of yourself

Keith Elliott

University of Manchester, UK (Emeritus)

Keith Elliott has spent 40 years teaching and researching, mainly in the areas of metabolism and enzymology at the University of Manchester, UK, developing a particular interest in education and career development. He has chaired the Education Committee and been Careers Advisor for the UK Biochemical Society. He was a founder member of the FEBS Education Committee and has run workshops on educational methods and career development in 30 FEBS countries. He has been running CV support sessions at the YSF since 2007 and was awarded the FEBS Diplôme d'honneur in 2014 for his contributions.

List of Posters

Poster #	Poster
1	D. Abella López, [1]*University of Santiago de Compostela, Santiago de Compostela, Spain* **MiST-IC Tagging: Self-assembling protein-only nanospheres with tunable surfaces for diagnostic and therapeutic applications**
2	F. Alabiso, [1]*Department of Biomedical and Neuromotor Science, University of Bologona, Bologna, Italy* **Dissecting novel sncRNAs signatures in iPSC-derived cardiac organoids for the functional modeling of heart failure**
3	M. Anghelache, [1]*Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania* **Immune cell-specific uptake of functionalized lipid nanoparticles in experimental cardiac fibrosis**
4	K. Athanasopoulou, *National and Kapodistrian University of Athens, Athens, Greece* **Dem6A-Vec: A robust type VI CRISPR-based approach for targeted m6A demethylation in human mRNAs**
5	J. Babulicová, [1]*Biomedical Research Center of Slovak Academy of Sciences, Slovakia* **Altered gene expression, proliferation, and metabolic reprogramming in colorectal cancer cells induced by miR-133b**
6	M. D. C. Banqueri-Pegalajar, [1]*Molecular Biology and Biochemistry, University of Málaga,* [2]*Group B-04, IBIMA Bionand Platform, Málaga, Spain* **Natural compound C3, an antioxidant and autophagy modulator, reduces proliferation and metastasis in triple-negative breast cancer models**
7	A. Będzińska, [1]*Laboratory of Experimental Biology and Department of Biochemistry & General Chemistry, Rzeszów University, Medical Faculty, Rzeszow, Poland* **Crosstalk between SLAMF7 and NF-κB signalling pathway in inflammation**
8	B. Bellová, [1]*Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Slovakia* **Synergistic effects of host selection, promoter control, and optimization of production conditions on the production of soluble mutated MMLV RT**
9	R. Borosta, [1]*Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences,* [2]*Doctoral School of Biology, Structural biochemistry, Eötvös Loránd University, Hungary* **Therapeutic targeting of an immuno-oncologically relevant exonuclease: development and characterisation of novel inhibitors**
10	A. Bubanja, [1]*Gene Regulation in Cancer Group, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia* ***In silico* evaluation of the transcript LDLRAD4-213 suggests putative regulatory role in colon cancer**
11	Y. Bukhonska, [1]*Laboratory of Molecular Mechanisms of Cell Metabolism Regulation, V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine* **Brassinosteroid perception via BRI1 controls defense hormone homeostasis and resistance to *Pseudomonas syringae* in *Arabidopsis thaliana***
12	C. Calaud, [1]*Team 14: Cancer Epigenetics and Immunotherapie, Mediterranean Center for Molecular Medecine, C3M, Inserm U1065, Nice, France* **Investigation of combinatorial epigenetic therapies to treat lymphoma**
13	E. Caldero Escudero, [1]*Department of Biochemistry and Molecular Biology and Physiology, Universidad de Valladolid, Valladolid, Spain* **A newly identified mitochondrial calcium uniporter activator enhances cardiac excitation–bioenergetics–contraction coupling**
14	M. Carro Martin, [1]*Department of Biochemistry and Molecular Biology, University of Salamanca,* [2]*Biomedical Research Institute of Salamanca, Salamanca, Spain* **Implication of NOX2 in glutathione homeostasis in chronic myeloid leukemia**
15	W. Chebil, [1]*Department of Clinical Biology, Laboratory of Medical and Molecular Parasitology-Mycology, Faculty of pharmacy, Monastir, Tunisia* **Negative effect of *Malassezia* on *Candida auris* biofilm formation and antifungal susceptibility profiles**

= Selected short talk

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16	A. Cuche, [1]*Biology, University of Mons (UMONS), Mons, Belgium* **Exploring QRICH2 interactome to understand its role in sperm flagellar assembly and function**
17	J. Curcic, [1]*Microbiology and Plant Biology, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia* **Functionalization of titanium alloy with ST1-YtnP lactonase reveals glycerol-dependent toxicity and antivirulence efficacy**
18	J. Daniluk, [1]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Poland* **Mitochondrial potassium channels as sensors and regulators of redox signaling**
19	P. Deák, [1]*Department of Biochemistry and Medical Chemistry, University of Pécs, Medical School, Pécs, Hungary* **MIF tautomerase inhibitor TE-91 prevents inflammatory cytokine production and activation of transcription factors in GTPP mouse macrophage model**
20	E. Dearlove, [1]*Cancer Research UK Scotland Institute,* [2]*School of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom* **Characterising the ubiquitination of nucleic acids by DELTEX E3 ligases**
21	S. Digiovanni, [1]*Department of Oncology,* [2]*Molecular Biotechnology Centre "Guido Tarone", Italy* **IRAK1 regulates the ABC transporter balance defining chemo-immuno-resistant NSCLC**
22	L. Eixerés, [1]*Metabolism, Inflammation and Aging, Instituto de Biomedicina de Valencia, Valencia, Spain* **Human UPRT structure suggests a non-enzymatic moonlighting role in pyrimidine metabolism**
23	M. E. Erol, [1]*Medical Biochemistry, Gazi University, Institute of Health Sciences, Türkiye* **Low-grade systemic inflammation in metabolic dysfunction-associated steatotic liver disease (MASLD)**
24	N. Fabianová, [1]*Pavol Jozef Safarik University, Košice, Slovakia* **Impact of hyaluronic acid metabolism on endometrial receptivity and embryo implantation**
25	H. Ferreira, [1]*Doctoral Program in Experimental Biology and Biomedicine, Interdisciplinary Research Institute,* [2]*Coimbra Institute for Biomedical Imaging and Translational Research, University of Coimbra, Coimbra, Portugal* **Neural and behavioral diversity in a Nlgn3 mouse model: linking sensory processing to atypical behavior**
26	L. Galvez-Larrosa, [1]*CIC bioGUNE, Derio, Spain* **Engineering a conformational signaling receptor for the detection of rapamycin**
27	S. Gasparyan, [1]*L. A. Orbeli Institute of Physiology NAS RA, Yerevan, Armenia* **A simple and cost-effective system to Induce CSC-like states via metabolic stress**
28	S. S. Gelen, [1]*Department of Biochemistry, Ege University, Faculty of Science, Türkiye* **Glyconanoparticles deposited surfaces to develop an electrochemical immunosensor for the detection of C-reactive protein (CRP) in pleural fluid**
29	M. Genta, [1]*Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy* **Bacterial DNA Nucleotide Excision Repair: a multi-approach investigation of the damage recognition process**
30	D.-M. Ghetu, [1]*"Nicolae Simionescu" Institute of Cellular Biology and Pathology, Bucharest, Romania* **Human cardiac organoids as tridimensional platforms mimicking cardiac impairment for pharmacological testing**
31	G. Guedes, [1]*Department of Pharmacology, University of Cambridge, Cambridge, United Kingdom,* [2]*CIC biomaGUNE, San Sebastian, Spain* **Exploiting the versatility of engineered consensus tetratricopeptide repeat proteins as multivalent theranostic agents**
32	E. Heffnerová, [1]*IMTM, Olomouc, Czech Republic* **Repurposing artemisinins as anti-cancer agents: investigating covalent interactions and cytotoxicity profiles**

= Selected short talk

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Poster #	Poster
33	I. Justo, [1]*Macromolecular Biochemistry, Leiden University, Leiden, Netherlands* **Biophysical characterization of the extracellular electron transfer pathway in *Listeria* monocytogenes, a potential antimicrobial target space**
34	J. Kaňka, [1]*Laboratory of Viral and Cellular Genetics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic* **Enlightening the quietness – the search for early silenced proviruses**
35	P. Kenfack Ymbe, [1]*Paul Pascal Research Center (CRPP), University of Bordeaux, Pessac, France* **Bifunctional myeloperoxidase and glucose oxidase chimeras: addressing the limitations of coupled enzymatic system for antimicrobial coatings**
36	C. Kiss, [1]*Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, [2]Department of Physics of Complex Systems, Eötvös Loránd University, Budapest* **Uncovering functional non-coding variants in colorectal cancer using single-cell multiomics**
37	G. Kováčová, [1]*Department of Medical and Clinical Biochemistry, Pavol Jozef Šafárik University, Košice, Slovakia* **Hypoxia, angiogenesis and mitochondrial activity in the dynamics of endometriosis**
38	M. Kovács, [1]*Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary* **Testing molecular processes identified by ribo-sequencing in a neurodegenerative disease model**
39	K. Kozal, [1]*Department of Cytobiochemistry, Faculty of Biology and Environmental ProtectionFaculty of Biology and Environmental Protection, University of Lodz, [2]The BioMedChem Doctoral School of the UL and Lodz Institutes of the Polish Academy of Sciences, [3]Polish Biochemical Society, Lodz, Poland* **Nutrient-dependent, non-canonical stabilization of the hypoxia inducible factor and its role in breast cancer metabolism**
40	A. Kraus, [1]*Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria* **Development and optimization of a SARS-CoV-2 virus antibody-dependent cell mediated cytotoxicity assay**
41	E. Kučerová, [1]*University of Chemistry and Technology, Prague, [2]Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic* **Optimizing an in vitro human blood–brain barrier model and inducing pharmacoresistance for antiepileptic drug screening**
42	T. Laasfeld, [1]*Institute of Chemistry, University of Tartu, Tartu, Estonia* **Unlocking systems biology and pharmacology of biochemical reactions and interactions through structured approach to data**
43	I. Lagerwaard, [1]*Genome Biology & Epigenetics, Utrecht University, Utrecht, Netherlands* **Delineating the protein network regulating early termination/elongation by RNA Polymerase II**
44	R. Leon Foun Lin, [1]*Université Paris Cité, Paris, France* **Molecular dynamics study of how environmental parameters affect the aggregation rate and dimerization of monoclonal antibodies: n-glycosylation, low-temperature cryoprotectants, and pH variation**
45	N. Limberger, [1]*Department of Cell Biochemistry, Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, [2]Doctoral School of Exact and Natural Sciences, Krakow, Poland* **The impact of regnase-2 on stress granule formation in response to different types of cellular stress in glioblastoma cells.**
46	A. Lluch, [1]*Institute for Tumor Immunology, Center for Tumor Biology and Immunology, Marburg University, Germany* **Elucidating adipocyte-NK cell crosstalk in the ovarian cancer microenvironment**
47	N. Majkowska, [1]*Doctoral School of Exact and Natural Sciences, [2]Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland* **Unlocking intracellular targets: a liposomes library for intracellular delivery of monoclonal antibodies targeting undruggable oncoproteins**

= Selected short talk

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48	M. Maniak, [1]*Centre for Inflammation Research,* [2]*Institute for Regeneration and Repair, The University of Edinburgh, Edinburgh, United Kingdom* **Defining the role of hydrostatic pressure in shaping macrophage phenotype**
49	L. Marcelić, [1]*Division of Toxicology, Institute for Medical Research and Occupational Health, Zagreb, Croatia* **Structural modification of nicotinamides and its impact on the modulation of the cholinergic and serotonergic neurotransmission**
50	S. Marques Costa, [1]*MOST-MICRO, Instituto de Tecnologia Química e Biológica António Xavier – Universidade NOVA de Lisboa (ITQB NOVA), Oeiras, Portugal* **A pair of "false twins" ncRNAs in *Pseudomonas putida*: study of their role and regulation**
51	T. Mazza, *Department of Biologia, Ecologia e Scienze della Terra (DiBEST), University of Calabria, Rende, Italy* **Cholesterol controls ASC1 function and structural stability**
52	R. Mengual Fenollar, [1]*Instituto Cajal, CSIC., Madrid,* [2]*Instituto de Biología Funcional y Genómica, CSIC, Universidad de Salamanca, Salamanca, Spain* **Multidirectional neuron-glia mitochondrial transfer and metabolic rewiring**
53	A. Minasyan, [1]*Department of Biochemistry, Microbiology & Biotechnology, Yerevan State University, Research Institute of Biology, 1 A. Manoogian Str., 0025 Yerevan, Armenia* **Modulation of redox homeostasis and inflammatory responses by *Vaccinium myrtillus* extract in microglial cells**
54	A. Molina-Teba, [1]*University of Seville - CSIC, Institute for Chemical Research - CicCartuja, Seville, Spain* **Exploring molecular dynamics and ligand interactions of nucleophosmin C-terminal domain in acute myeloid leukemia**
55	R. J. Moreira, [1]*LAQV-REQUIMTE, Department of Chemistry,* [2]*Institute of Biomedicine, Department of Medical Sciences, University of Aveiro, Aveiro, Portugal* **Aerobic physical exercise under a persistent obesogenic diet reverses biometric alterations and partially mitigates sperm quality loss in a mouse model**
56	K. Myslínová, [1]*Department of Biochemistry, Palacky University Olomouc, Olomouc, Czech Republic* **Metabolic consequences of artificial diets in honey bees**
57	D. Nedeljković, [1]*Applied Bioenergetics Lab, Center for Health, Exercise and Sport Sciences, Belgrade,* [2]*Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia* **Creatine hydrochloride and creatine ethyl ester supplementation: an 8-week evaluation of clinical biomarkers in midlife women**
58	F. Noventa, [1]*University of Padova, Padova, Italy* **Proteomic profiling of FAM20C interactors: investigating the biological functions of the master kinase of the secretory pathway**
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MiST-IC Tagging: Self-assembling protein-only nanospheres with tunable surfaces for diagnostic and therapeutic applications

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Rationale: The IC-Tagging system supports the *in cellulo* production of nanospheres (NS) encapsulating proteins of interest. In this work, we engineered this tool to achieve targeted and modulable surface modification of purified NS.

Methods: NS are produced in bacteria and purified by mechanical disruption (lysis and centrifugation). Surface modifications are introduced using sortase-mediated ligation. The functionality of the modified NS is characterized through multiple techniques, including SDS-PAGE, Western blotting, DLS, microscopy, kinetic assays, and cell-based assays.

Results: The IC-Tagging system is an advanced single-step *in cellulo* strategy for producing NS-encapsulated proteins with enhanced stability. Encapsulated proteins retain their native folding and activity within the NS, where quaternary interactions and complex enzymatic reactions can take place. This system has recently been applied to vaccine development and the stabilization of therapeutic proteins, with particular interest in oral enzyme delivery due to the strong protection provided by NS against the gastrointestinal environment [1]. In this work, we expanded this tool to enable simple, covalent, and targeted attachment of diverse components (small molecules, fluorescent probes, peptides, entire proteins and enzymes, nanobodies, etc.) to the NS surface through sortase-mediated ligation. This new technology, termed MiST-IC, builds on the strengths of IC-Tagging to streamline the design and production of therapeutic proteins. It also broadens the platform's applicability to biosensing, diagnostics, and targeted therapies.

Conclusions: MiST-IC Tagging is a novel nanobiotechnology platform for the one-step production of NS loaded with any type of protein and featuring a highly functionalizable surface. This technology represents a new approach to designing protein nanostructures with advanced functionalities and broad applicability in the biomedical field.

References: [1] Abella et al. (2025) Mater. Today Bio, 101987.

Dissecting novel sncRNAs signatures in iPSC-derived cardiac organoids for the functional modeling of heart failure

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Rationale: Heart failure (HF) remains a major global health challenge and arises from complex molecular disturbances that are difficult to model *in vitro*. To address this, we used 3D iPSC-derived cardiac organoids that preserve human-like organization and stress responses, providing a suitable system to investigate related molecular mechanisms.

Methods: We used 3D iPSC-derived cardiac organoids exposed to ET-1 to model maladaptive remodellng. NGS enabled parallel profiling of two regulatory sncRNA classes— isomiRs and tRNA-derived fragments (tRFs). IsomiRs were annotated and quantified using an isomiRmap-based workflow, followed by class-resolved differential expression, canonical and non canonical seed sequences analysis and functional enrichment through predictive and machine learning-based tools. tRFs were classified using MINTmap and further analysed with tRF-tar and catRAPID to assess their predicted miR-like activity and protein-binding features.

Results: IsomiR profiling uncovered defined subclasses, including abundant Lv5p and Lv3p forms and a smaller NucVar population. ET-1 altered multiple isomiRs, notably reducing miR-126 and miR-30d-5p-C13U variants and increasing miR-1246 and miR-92b-3p. Their predicted targets converged on transcriptional control, calcium handling, metabolism and proteostasis. tRF analysis revealed a distinct HF-associated signature comprising induction of 5'-tRFs, tiRNAs and internal fragments, with concurrent loss of 3'-tRFs; their predicted pathways overlapped with isomiR-regulated processes. Among these, 5'-tRF$^{Glu(CTC)}$ correlated with NPPB expression and, based on catRAPID, interacted with RNA-binding proteins implicated in stress-granule dynamics.

Conclusions: This study delivers the first integrated isomiR–tRF landscape in a 3D human cardiac model, revealing coordinated sncRNA programmes underpinning maladaptive HF-related remodelling. Functional studies are currently underway to dissect the contribution of the identified sncRNAs to these processes.

Immune cell-specific uptake of functionalized lipid nanoparticles in experimental cardiac fibrosis

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Rationale: Cardiac fibrosis is driven by excessive ECM deposition and chronic inflammation. While targeted nanoparticles hold therapeutic potential, immune-nanoparticle interactions in fibrotic conditions remain poorly defined. This study examined how circulating and splenic immune cells internalize peptide-functionalized nanoparticles in an Ang II-induced cardiac fibrosis model to support the design of targeted anti-fibrotic therapies.

Methods: Cardiac fibrosis was induced in male C57BL/6 mice via Ang II pumps, with sham controls. Lipid nanoemulsions (LN) containing a 700 nm NIR fluorophore were functionalized with peptides targeting fibrotic myocardium. Three formulations were tested: untargeted (UN-LN), ECM-targeted (ECM-LN), and VCAM-1-targeted (V-LN). Flow cytometry profiled circulating and splenic immune cells ($CD11b^+$ myeloid cells, $Ly6G^+$ neutrophils, $Ly6C^+$ monocytes) and quantified nanoparticle uptake globally and by subset. Spleen localization was evaluated using the Pearl Trilogy Imaging System.

Results: Ang II increased circulating $CD11b^+$ myeloid cells and neutrophils, indicating systemic immune activation, whereas splenic populations were not significantly altered. Blood immune cells from Ang II-treated mice showed markedly elevated nanoparticle uptake, especially for targeted LN. In the spleen, only UN-LN exhibited increased uptake. Neutrophils were the primary circulating population internalizing nanoparticles. Splenic neutrophils consistently internalized more ECM-LN than UN-LN, while enhanced UN-LN uptake involved additional immune subsets.

Conclusions: Ang II-induced cardiac fibrosis reshapes immune-nanoparticle interactions, increasing uptake of targeted formulations. These findings suggest immune cells may serve as “therapeutic couriers” to fibrotic tissue. Further studies are required to determine how nanoparticle cargo influences immune cell function and trafficking.

Dem6A-Vec: A robust type VI CRISPR-based approach for targeted m6A demethylation in human mRNAs

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Rationale: N6-methyladenosine (m6A) is the most prevalent and dynamically regulated mRNA modification, governing splicing, stability, and translation. Despite its recognized significance, the effective, targeted manipulation of specific m6A sites remains a key challenge. To overcome this, we designed Dem6A-Vec, an innovative, "all-in-one" non-viral plasmid vector for site-specific m6A demethylation.

Methods: Dem6A-Vec is designed for streamlined targeted epitranscriptome engineering, integrating the expression of a catalytically inactive RfxCas13d fused to the major m6A eraser, ALKBH5, along with a customizable guide RNA driven by a U6 promoter, within a single construct. The fusion protein is engineered with HA and FLAG epitope tags and a nuclear export signal to ensure efficient cytoplasmic targeting toward mRNAs. High-confident m6A targets were detected through nanopore direct RNA sequencing and the performance of Dem6A-Vec was investigated through SELECT-qPCR.

Results: Dem6A-Vec was optimized investigating two random m6A target sites of the *EEF2* (m6A:2886) and *RRAGA* (m6A:418) gene, leading to significant demethylation, without affecting adjacent m6A sites or other mRNAs. Moreover, targeted demethylation of these sites significantly increased the mRNA stability of the corresponding mRNAs. Furthermore, Dem6A-Vec efficiently demethylated multiple m6A sites with diverse stoichiometries, highlighting its adaptability to a wide range of transcriptomic contexts. The system also supports reversible RNA modification, as methylation levels are gradually restored post-transfection, highlighting the plasticity of m6A.

Conclusions: By integrating all necessary components into a single vector, Dem6A-Vec represents a robust, scalable and simplified system for precise m6A engineering. This transformative tool opens new avenues for deciphering the functional roles of m6A in gene regulation and disease, providing a foundation for future epitranscriptomic research.

Altered gene expression, proliferation, and metabolic reprogramming in colorectal cancer cells induced by miR-133b

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Rationale: Colorectal cancer (CRC) is the third most common malignancy worldwide. CRC cells show gene and microRNAs dysregulation, including miR-133b, whose basal expression is low and functional role insufficiently understood. Here, we studied the effect of miR-133b overexpression on HCT116 CRC cell proliferation and regulation of energy metabolism.

Methods: Cancer cells were transfected with a miR-133b mimic (30nM), inhibitor (50nM), or their negative controls. Cell proliferation was evaluated by BrdU and MTT assays at 0h, 24h, 48h, and 72h post-transfection. Glycolysis and mitochondrial respiration rate were analysed using Seahorse XF Analyzer. Western blot was used to assess protein levels of oxidative phosphorylation complexes and histone deacetylase 4. Expression of genes related to proliferation, apoptosis and metabolism (*HDAC4, BCL-2, LIF, SIRT1, SRF, TP53, CS, PRKAA1)* was examined 48h post-transfection (qPCR).

Results: Overexpression of miR-133b significantly reduced cancer cell proliferation at 72h, as shown by both MTT and BrdU assays. Decreased basal glycolysis, as well as basal and ATP-coupled mitochondrial respiration rate, while increasing ATP-synthase protein was observed, too. Protein abundance of other respiratory chain complexes remained unchanged. Additionally, it reduced expression of genes related to cell proliferation *BCL-2*, *SIRT1, CS* and *TP53*, and upregulated *LIF*, *PRKAA1* and *SRF*. We also detected a highly significant reduction in *HDAC4* mRNA, however, its protein levels were unchanged. In contrast, miR-133b inhibitor had no effect on HCT116 cell proliferation, metabolism, glycolysis, or respiration, likely due to inherently low levels of miR-133b in cancer cells.

Conclusions: These findings suggest that elevated miR-133b suppresses proliferation and metabolism in HCT116 cells, likely *via* modulation of transcription factors, deacetylases and genes linked to cancer proliferation, apoptosis and energy metabolism.

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Natural compound C3, an antioxidant and autophagy modulator, reduces proliferation and metastasis in triple-negative breast cancer models

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Rationale: The urgent need for new therapeutic options for triple-negative breast cancer (TNBC) is driven by its aggressiveness, high metastatic potential, and the limited treatment strategies currently available. C3, a natural compound derived from the olive tree among other plant sources, exhibits antioxidant activity and the ability to modulate autophagy.

Methods: Given the critical role of oxidative stress and autophagy in tumor progression, C3 was evaluated as a potential antitumor agent in this context. In vitro cytotoxicity assays were performed in human MDA-MB-231 and murine 4T1 cells to determine viability and IC_{50} values, while a wound-healing assay was conducted in MDA-MB-231 cells to assess the effects of C3 on cell migration. Based on the promising results, an in vivo model of TNBC was established in BALB/c mice inoculated with 4T1 cells and tumor volume, growth rate, proliferation (Ki-67), metastatic burden, and potential systemic toxicity were evaluated over time.

Results: In vitro C3 reduced cell viability in both cancer cell lines and partially inhibited MDA-MB-231 migration in a significant manner, suggesting a combined effect on proliferation and motility. In vivo, C3 treatment led to a slight, non-significant reduction in tumor volume and growth rate; however, Ki-67 staining showed a significant decrease, and metastasis was significantly reduced, demonstrating its impact on tumor proliferation and dissemination. Importantly, no signs of toxicity were detected in major organs, supporting a favorable safety profile.

Conclusions: Overall, these findings indicate that C3 exerts antiproliferative and antimetastatic effects in both in vitro and in vivo TNBC models, highlighting its potential as a safe and effective candidate for further preclinical development and future therapeutic applications in aggressive breast cancer.

Crosstalk between SLAMF7 and NF-κB signalling pathway in inflammation

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Rationale: We previously demonstrated SLAMF7 activation via two mechanisms: p53-dependent and p53-independent induction by paclitaxel, accompanied by NF-κB activation. Here, we investigate the mechanism of SLAMF7 activation by an unknown factor, likely via the NF-κB pathway, in epithelial cells—representing the first observation of SLAMF7 activation in non-hematologic cell lines and suggesting potential therapeutic applications of Elotuzumab beyond the hematopoietic system.

Methods: A549 and HaCaT epithelial cell lines, including CRISPR/Cas9-engineered SLAMF7-knockdown and control models, were used in this study. SLAMF7 surface expression and its secreted form were analyzed by FACS and Western blot. The NF-κB pathway components were assessed by Western blot and a luciferase reporter assay. To evaluate the functional role of SLAMF7, CRISPR-Control and CRISPR-SLAMF7 cells were co-cultured with NK-92 cells.

Results: Paclitaxel (PTX) did not induce the secretion of soluble SLAMF7, suggesting potential compatibility with SLAMF7-targeting therapies such as Elotuzumab. Reduced SLAMF7 expression was associated with increased p65/RelA levels and enhanced NF-κB pathway activation, as confirmed by component profiling. Co-culture with NK-92 cells revealed treatment-dependent changes in epithelial cell viability; however, PTX responses were comparable between CRISPR-Control and CRISPR-SLAMF7 cells, indicating that SLAMF7 status does not significantly affect NK-mediated cytotoxicity under these conditions.

Conclusions: Our findings reveal a link between SLAMF7 expression and NF-κB activation in epithelial cells and provide new insights on SLAMF7 therapeutic strategies beyond hematopoietic contexts.

Synergistic effects of host selection, promoter control, and optimization of production conditions on the production of soluble mutated MMLV RT

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Rationale: Moloney murine leukemia virus reverse transcriptase (MMLV RT) is widely used in molecular biology, but its application is often restricted by inherently low solubility and modest expression levels in standard hosts. As demand grows for high-performance variants, improving overall production efficiency and soluble yield becomes increasingly important to support their broader use.

Methods: We evaluated the effects of host organism, expression vector, promoter strength, and cultivation parameters on the soluble production of a mutated MMLV RT. Expression was tested in *E. coli* and two *Vibrio natriegens* strains using plasmids containing T7 or T5 promoters. GroES/GroEL chaperones were systematically assessed to enhance solubility. Soluble and total protein fractions were analyzed by SDS–PAGE, Western blotting, and densitometry, and selected samples were purified by affinity chromatography. Enzymatic activity was confirmed by RT PCR.

Results: Solubility and yield varied markedly across host and vector combinations. The highest production was achieved using a prophage-free *V. natriegens* strain with the pJexpress404 vector under a T5 promoter, combined with GroES/GroEL co-expression, resulting in 435 mg/L of soluble mutated MMLV RT. Chaperone co-expression substantially increased solubility and reduced aggregation. Optimal cultivation was achieved with 2YT medium and a 3-hour expression window post-induction.

Conclusions: The integration of a prophage-free *V. natriegens* host, T5-driven pJexpress404 expression, and GroES/GroEL co-expression provides a highly effective strategy for producing large amounts of soluble and active mutated MMLV RT. Optimized cultivation parameters further enhance yield. These findings establish *V. natriegens* as a robust platform for expressing aggregation-prone recombinant enzymes. The Slovak Research and Development Agency supported the research through grants APVV-19-0196, APVV-20-0284, APVV-21-0215, APVV-22-0161.

Therapeutic targeting of an immuno-oncologically relevant exonuclease: development and characterisation of novel inhibitors

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Rationale: Our study aims to find novel inhibitors of ExoA, an exonuclease that suppresses cytosolic DNA-sensing pathways, and limits anti-tumor immune responses. By blocking ExoA activity, we aim to boost immune activation and improve the efficacy of cancer therapies, offering a potential new strategy in immuno-oncology.

Methods: We employed a FRET-based activity assay developed in our laboratory to screen a small-molecule library and identify potent ExoA inhibitors. IC_{50} values were determined for human and murine ExoA to assess potency across species. To characterize binding interactions, we used a tryptophan-containing ExoA variant and intrinsic fluorescence measurements to determine dissociation constants for enzyme-substrate-inhibitor complexes. These biophysical data guided our crystallization trials using X-ray crystallography to resolve inhibitor-bound structures. In parallel, preliminary cellular assays were also performed on different human and murine cancer cell lines, to assess cell viability and monitor cGAS-STING pathway activation.

Results: Screening identified 11 highly potent and ExoA-specific inhibitors, most exhibiting nanomolar IC_{50} values against both human and mouse ExoA. Binding studies revealed dissociation constants consistent with strong and selective inhibitor engagement. We successfully crystallized the apo enzyme and the enzyme-substrate complex, providing a structural foundation for inhibitor-bound studies. In viability assays, high cellular tolerance to our inhibitors was observed. Furthermore, we optimized cGAS-STING activation in multiple cancer cell lines, where ExoA depletion resulted in an increased cellular response following dsDNA delivery.

Conclusions: Our findings demonstrate that the identified compounds are potent and specific ExoA inhibitors, supported by biochemical, biophysical, and structural data. These results provide a strong foundation for subsequent mechanistic and therapeutic investigations.

In silico evaluation of the transcript LDLRAD4-213 suggests putative regulatory role in colon cancer

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Rationale: Recent pan-cancer transcriptomic analysis has revealed distinct activity patterns of alternative LDLRAD4 promoters in malignant compared with non-malignant colon tissues, with the downregulated promoter giving rise to transcript LDLRAD4-213 (ENST00000587905), which encodes a truncated transmembrane protein shorter than the canonical full-length protein that serves as a negative regulator of the TGF-β signaling pathway. The aim of this study was to use an in silico approach to characterize this transcript, its promoter and to evaluate their potential relevance for cancer.

Methods: Core promoter motifs were identified using YAPP, Promoter 2.0, and ElemeNT, while transcription factor binding sites were predicted with TFBIND, CIIIDER, and MOTIFLAB. GC content and CpG islands were analyzed using CpGplot EMBOSS and CpGProD. Correlation expression analyses of LDLRAD4-213 were performed with TGF-β signaling pathway scores and promoter methylation scores, using TCGA/XENA datasets. All statistical analyses were conducted in R and GraphPad Prism.

Results: The promoter lacks a typical promoter sequence but contains a GC island, with predicted binding of transcription factors including RORA_1, SP1, ERG1 and MYB, known regulators of cell proliferation. Correlation analysis showed no correlation with promoter methylation status and a positive correlation with the TGF-β signaling pathway, specifically with the pathway components involving SMAD7 and NEDD4.

Conclusions: These results suggest that LDLRAD4-213 expression in colorectal cancer is probably not governed by DNA methylation or other epigenetic mechanisms. Beyond this, our result results suggest that LDLRAD4-213 may shape the tumor environment by enhancing TGF-β–related responses, potentially counteracting the function of the canonical isoform.

Brassinosteroid perception via BRI1 controls defense hormone homeostasis and resistance to *Pseudomonas syringae* in *Arabidopsis thaliana*

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Rationale: Brassinosteroids (BRs) are known as regulators of plant growth and stress adaptation, but it is still unclear how the BRI1 receptor specifically contributes to hormonal homeostasis during pathogen defense. This study investigates how BRI1 signaling affects cross-talk between BRs and defense hormones under biotic stress conditions.

Methods: *Arabidopsis thaliana* wild-type (WT) and *bri1-6* plants were treated with 24-epibrassinolide (EBL) and/or the BRs synthesis inhibitor brassinazole (BRZ). Two infection techniques were used to examine resistance to *Pseudomonas syringae* pv. *tomato* DC3000: flood inoculation (sterile medium) and no-needle syringe infiltration (soil-grown). Endogenous levels of abscisic acid (ABA) and its metabolites (PA, DPA), salicylic acid (SA), benzoic acid (BzA), and jasmonic acid (JA) derivatives were quantified via HPLC-MS/MS.

Results: The *bri1-6* plants demonstrated 4–5 times higher susceptibility to *P. syringae* compared to WT across both inoculation methods. This susceptibility was not altered by EBL or BRZ treatments. In WT plants, BRZ treatment resulted in a significant rise in bacterial populations, which was lessened by EBL co-treatment. In terms of hormonal profile, *bri1-6* plants had significantly lower levels of stress-associated hormones—ABA (56% lower), SA (50% lower), and JA (47% lower)—compared to WT. *bri1-6* plants also accumulated significantly higher levels of the SA precursor BzA and ABA catabolites. In WT plants, EBL treatment induced BzA accumulation and restored ABA and DPA levels that were suppressed by BRZ [1].

Conclusions: BRI1 signaling is important in biotic stress tolerance regulation. The accumulation of phytohormone precursors and catabolites in *bri1-6* suggests that functional BRI1 is required for effective immune responses. Brassinosteroid biosynthesis and perception regulate hormone networks differently, suggesting that receptor mutants and chemical inhibition are not functionally equivalent

References: [1] Bukhonska Y et al. (2025) Int J Mol Sci 26, 9644.

Investigation of combinatorial epigenetic therapies to treat lymphoma

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Rationale: Diffuse Large B-cell Lymphomas (DLBCL), which derive from germinal center (GC) B cells, are common and aggressive lymphomas. Standard immuno-chemotherapy does not work for 40% of DLBCL patients; thus, new therapies are needed. Since epigenetic deregulation (DNA methylation, histone modifications) drives GC B-cell transformation into DLBCL, epigenetic therapies have been developed. However, their use remains limited due to modest efficacy in monotherapy and high toxicity linked to low specificity. Based on our previous studies, we hypothesized that combining two epigenetic inhibitors: an hypomethylating agent (5-Azacytidine, 5-Aza) and a specific histone deacetylase 3 inhibitor (HDAC3i) will improve their efficacy against DLBCL without inducing toxicity.

Methods: We treated DLBCL cell lines *in vitro* and *in vivo* with vehicle, 5-Aza, HDAC3i or 5-Aza+HDAC3i and we analyzed survival and proliferation by flow cytometry and western blot. We also analyzed transcriptomic changes by RNA-seq and confirmed by RT-qPCR; and to analyze epigenomic changes we used ChIP-seq (histone acetylation) and reduced representation bisulfite sequencing, RRBS (DNA methylation). Finally, we measured the effect on normal PBMC.

Results: We observed a synergic effect of 5-Aza+HDAC3i, reflected by higher apoptosis and growth inhibition compared to single agents or vehicle *in vitro*, and a significant delay in tumor growth *in vivo*. Mechanistically, 5-Aza+HDAC3i induced increased histone acetylation and DNA hypomethylation, which resulted in upregulation of GC B cell differentiation genes that are aberrantly repressed in DLBCL, and led to lymphoma cell death. Importantly, 5-Aza+HDAC3i did not impact T cell survival.

Conclusions: My results confirm the superior efficacy of combining 5-Aza and HDAC3i against DLBCL, and they suggest that the enhanced anti-tumor effect of the epigenetic combinatorial treatment is associated with an epigenetic reprogramming that leads to the re-expression of genes involved in GC-B cell differentiation.

A newly identified mitochondrial calcium uniporter activator enhances cardiac excitation–bioenergetics–contraction coupling

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Rationale: CGP7930 is a potent MCU activator that enhances mitochondrial Ca^{2+} uptake, ER–mitochondria contacts, and Ca^{2+}-dependent metabolism in cardiomyocytes, boosting cardiac contractility in an MCU-dependent manner.

Methods: A library of 1,280 bioactive compounds was screened to identify modulators of ER–mitochondria Ca^{2+} signaling. Mitochondrial Ca^{2+} uptake was measured in intact and permeabilized cells under controlled Ca^{2+} conditions. Genetic analyses of MCU and MICU1 dependence, fluorescence-based assessment of ER–mitochondria contacts, and computational docking were performed. Functional effects were evaluated in primary cardiomyocyte cultures and Langendorff-perfused mouse hearts.

Results: The screen identified CGP7930 as the strongest enhancer of mitochondrial Ca^{2+} uptake. CGP7930 increased ER–mitochondria contacts, directly activated MCU, and required MICU1 for its effect. It boosted Ca^{2+}-stimulated mitochondrial metabolism in cardiomyocytes and increased left ventricular developed pressure ex vivo, an effect absent in MCU-deficient hearts.

Conclusions: This study identifies CGP7930 as a potent and specific activator of the mitochondrial calcium uniporter (MCU). High-throughput screening and mechanistic analyses demonstrate that CGP7930 directly enhances mitochondrial Ca^{2+} uptake, requires MICU1 for its action, and strengthens ER–mitochondria communication. Functional assays reveal that CGP7930 boosts Ca^{2+}-dependent mitochondrial metabolism and improves cardiac contractile performance in an MCU-dependent manner. These findings position CGP7930 as a promising tool compound to probe MCU regulation and as a potential candidate for modulating excitation–bioenergetics–contraction coupling in physiological and pathological contexts.

Implication of NOX2 in glutathione homeostasis in chronic myeloid leukemia

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Rationale: NADPH oxidases (NOX) regulate reactive oxygen species (ROS) signaling in hematopoiesis, and aberrant NOX activity contributes to elevated ROS in chronic myeloid leukemia (CML). Our studies revealed a functional NOX2–NOX4–mitochondria axis that controls CML cell metabolism, and demonstrated that NOX2 deletion or inhibition reduces glutathione, a major antioxidant supporting leukemic cell survival.

Methods: To further investigate this connection, we generated NOX2 knockout K562 cells ($K562^{NOX2/KO}$) using CRISPR-Cas9 and analyzed alterations in signaling pathways (ABL, STAT5A, mTOR), oxidative stress regulators (NRF2, p62, xCT, CTH), and mitochondrial proteins (VDAC, OPA1, Tim23, DRP1, MFN2). Glutathione was quantified under basal conditions and following treatment with NOX inhibitors (GSK2795039, GKT136901, APX-115). The effect of GSK2795039 was combined with Erastin (a cystine transporter inhibitor), Sulfasalazine, and RSL3 (both ferroptosis inducers via xCT and GPx4 inhibition, respectively) in K562 and KCL22 cells. Finally, the combination of GSK2795039 with Sulfasalazine was also tested in tyrosine kinase inhibitor (TKI)-resistant cell lines (Baf/3, E255K, and T315I).

Results: We observed that NOX2 deletion decreased glutathione, increased CSE expression, and reduced OPA1 L. GSK2795039, a selective NOX2 inhibitor, lowered glutathione, while APX-115, that can inhibit both NOX2 and NOX4, increased it, highlighting that specific inhibition of NOX2 can reduce glutathione levels in CML cells. Hence, combining GSK2795039 with Sulfasalazine synergistically inhibited proliferation in K562, KCL22, and resistant lines, whereas GSK2795039 plus Erastin had an additive effect in K562.

Conclusions: These results uncover the NOX2-glutathione interplay in CML and highlight redox-targeted strategies as promising therapies for both sensitive and TKI-resistant cells.

Negative effect of *Malassezia* on *Candida auris* biofilm formation and antifungal susceptibility profiles

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Rationale: Recent studies show that *Malassezia* plays a multifunctional role in human health including preventing infections by competing with skin pathogens such as *Candida auris*, an emerging multidrug-resistant fungus. This study aimed to evaluate the effect of *Malassezia* on *C. auris* antifungal susceptibility and to assess its impact on *C. auris* biofilm formation *in vitro*.

Methods: Antifungal susceptibility testing of 14 *Malassezia furfur* strains was performed using a modified CLSI microdilution method. Susceptibilities of *C. auris* (clade I), *M. furfur–C. auris* co-cultures, and a *C. parapsilosis* control were assessed against fluconazole (FLC), amphotericin B (AMB), and caspofungin (CAS). MICs were determined visually and spectrophotometrically after 48–72 h, and relative growth and dose–response curves were generated using GraphPad prism. Biofilm formation was evaluated with 18 *M. furfur* strains using XTT reduction assay and total biomass produced by single and mixed cultures was analyzed with GraphPad.

Results: Regarding antifungal susceptibility testing, MIC_{50} values for FLC and CAS and the MIC_{90} for AMB were one dilution higher in *C. auris* monocultures than in co-cultures. Two-way ANOVA showed significant growth differences between conditions for all antifungals ($p < 0.0001$ for FLC and AMB; $p = 0.02$ for CAS). As for biofilm formation, most *M. furfur* strains reduced *C. auris* biofilm metabolic activity in co-culture, with significant differences across conditions confirmed by two-way ANOVA ($p < 0.0001$).

Conclusions: Our findings suggest that *M. furfur* can modulate the antifungal susceptibility of *C. auris* and alter its biofilm formation capacity potentially limiting its ability to colonize the skin. Further investigations including *in vivo* models, are required to validate this effect and clarify the underlying mechanisms.

Exploring QRICH2 interactome to understand its role in sperm flagellar assembly and function

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Rationale: Mutations in QRICH2 cause complete male infertility through severe flagellar defects, but how this protein orchestrates sperm flagellar assembly remains largely unknown. Only six QRICH2 interactors have been identified so far, which likely represents a small subset given the protein's distinct localizations throughout spermatogenesis. We aim to map QRICH2's interaction network during spermatogenesis and in mature spermatozoa to understand its role in flagellar assembly and function.

Methods: We use complementary strategies to identify QRICH2 interactors. Three QRICH2 fragments covering conserved domains are screened against a universal human cDNA library using yeast two-hybrid (Y2H). In parallel, immunoprecipitation coupled to mass spectrometry (IP-MS) is performed on human sperm extract to capture interactions in mature spermatozoa. Selected interactions are validated using Gaussia princeps complementation assay in HEK293T cells.

Results: Y2H screening with QRICH2 fragment 3, the most highly conserved region across mammalian species, yielded 345 positive colonies. These include proteins involved in flagellar assembly, transcriptional regulation, and post-translational modifications, with 36 showing testis-enriched expression. Screening with the two other QRICH2 fragments and IP-MS are ongoing.

Conclusions: These findings reveal an extensive QRICH2 interaction network, expanding beyond the six previously known partners. Complete characterization of these interactions will advance our understanding of molecular mechanisms governing sperm flagellar assembly and provide insights into male fertility.

Functionalization of titanium alloy with ST1-YtnP lactonase reveals glycerol-dependent toxicity and antivirulence efficacy

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Rationale: Implant-associated infections represent a growing clinical challenge, highlighting the need for biomaterials that prevent bacterial virulence while remaining safe for host tissues. Quorum-quenching enzymes that disrupt quorum sensing and biofilm formation offer a promising antivirulence strategy.

Methods: Titanium alloy (Ti6Al4V) discs were chemically pre-treated, functionalized with recombinant ST1-YtnP lactonase (200–1000 µg/mL), and evaluated for biocompatibility in *Caenorhabditis elegans* AU37. Effects on clinical isolate *Pseudomonas aeruginosa* MMA83 were assessed by quantifying quorum-sensing (QS) and virulence-related gene expression using real-time quantitative PCR after direct contact with functionalized surfaces.

Results: Soluble ST1-YtnP caused acute toxicity above 100 µg/mL, while the identical mortality observed in worms exposed to the glycerol-containing buffer, used as a cryoprotectant and protein stabilizer, demonstrated that glycerol, rather than the enzyme, was responsible for the toxic effect. Functionalized titanium discs exhibited no significant toxicity over 72 hours, even at 1000 µg/mL enzyme loading. Direct exposure of *P. aeruginosa* MMA83 to ST1-YtnP-coated titanium significantly reduced the expression of key QS regulatory genes (*lasI, lasR, rhlI, rhlR, pqsH, mvfR*) and virulence genes (*lasB, algK, phzM, pvdS, rhlC*), demonstrating effective interference with bacterial communication and pathogenicity pathways.

Conclusions: Immobilization of ST1-YtnP on titanium eliminates glycerol-associated toxicity and enables high, biologically active enzyme loading. The functionalized surfaces maintain excellent *in vivo* biocompatibility while markedly suppressing QS and virulence gene expression in a clinically relevant *P. aeruginosa* strain. These findings support ST1-YtnP-functionalized titanium as a promising, safe antivirulence biomaterial for preventing implant-associated infections without promoting antimicrobial resistance.

Mitochondrial potassium channels as sensors and regulators of redox signaling

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Rationale: Mitochondria are essential for ATP synthesis, metabolic regulation, and apoptosis. Dysregulated mitochondrial function and redox homeostasis contribute to a variety of diseases, highlighting the importance of maintaining mitochondrial health. MitoK is also part of a redox signaling loop, as their activity is influenced by redox signals. However, the detailed mechanisms of mitoK channel redox regulation remain unknown.

Methods: Our goal is to identify key redox-regulating domains and amino acids within the proteins forming mitoK channels in different models of mitochondrial bioenergetic states, such as the succinate overabundance or the lack of specific components of the respiratory chain. We perform patch-clamp electrophysiology on U-87 or HEK293 cells to evaluate channel activity upon introduction of modulatory compounds, such as respiratory chain substrates, as well as site-directed mutagenesis to generate mitoBKCa channel variants.

Results: We observe a robust inactivation of BKCa channel with mitchondrial substates in transfected HEK293 cells, as well as of the native BKCa in U-87 cells.

Conclusions: Our reults show point out interaction of the BKCa channel with the cellular respiratory chain even in case of transfection of the cells with the channel, therefore positioning the HEK293-BKCa system as a valid tool to study the effect of mitochondrial substrates on the BKCa channel activity. This allows for future experimental work employing transfection of variant channels with specific structural features, as well as investigation of the channel activity in the absence of the respiratory chain proteins.

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MIF tautomerase inhibitor TE-91 prevents inflammatory cytokine production and activation of transcription factors in GTPP mouse macrophage model

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Rationale: MIF (macrophage migration inhibitory factor) plays a key role in many inflammatory diseases, and the transcription factor ETS2 (ETS proto-oncogene 2) has recently been shown to be involved in macrophage activation in inflammatory bowel diseases. In this work, we investigated the effect of a novel small-molecule MIF tautomerase inhibitor, TE-91 (IC_{50}: 1,94 µmol/L), on ETS2 activation in conjunction with other inflammatory transcription factors and tested the role of TE-91 in cytokine production in a chronic inflammatory macrophage activation model.

Methods: Raw264.7 were pre-treated with 20 µM TE-91, then induced with 50 ng/ml GM-CSF (granulocyte-macrophage colony-stimulating factor) and mouse TNFα (tumor necrosis factor α), as well as with 2 µg/ml PGE2 (prostaglandin E2) and Pam3CSK4 (Pam3CysSerLys4), i.e., GTPP treatment was applied. After 72 hours of induction, ROS (reactive oxygen species) and nitrite concentrations were determined by using DHR123 (dihydrorhodamine 123) fluorescent dye and Griess reagent, respectively. We also measured IL-6 (interleukin-6), CCL-2 (C-C chemokine ligand 2), and HIF-1α (hypoxia-inducible factor-1 α) production with ELISA-kits; AKT phosphorylation and ETS2 expression with flow cytometry. Activation of the transcription factor NF-κB (nuclear factor-kappa B) was determined using Raw-Blue™, NF-κB reporter Raw264.7 cells in the same GTTP-induced model.

Results: Chronic GTPP treatment significantly increased all investigated inflammatory parameters in macrophages compared with vehicle-treated cells. TE-91 significantly reduced the production of ROS, nitrite, IL-6, CCL-2, and the phosphorylation of AKT, as well as the activation of the transcription factor NF-κB and protein expression of HIF-1α and ETS2, compared to GTPP-treated cells.

Conclusions: The new pharmacological MIF tautomerase inhibitor TE-91 reversed M1 macrophage activation in the GTPP model. These results advance the possibility of testing TE-91 in animal models of various inflammatory disorders, including experimental colitis.

Characterising the ubiquitination of nucleic acids by DELTEX E3 ligases

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Rationale: The repertoire of ubiquitination substrates has expanded in recent years with various non-proteinaceous substrates of ubiquitination being discovered. The diversity of possible ubiquitination substrates outside of proteins enhances the potential for ubiquitination to play a part in numerous functions beyond its conventional role as a post-translation modification. This study aims to characterise the novel nucleic acid ubiquitination activity of the RING E3 ligase DTX3L.

Methods: Fluorescence polarisation assays coupled with NMR were utilized to investigate nucleic acid binding to DTX3L. Biochemical assays were employed to interrogate the requirements for ubiquitination of nucleic acids and the site of modification. Mutagenesis of residues in the proposed binding pocket validated a trio of residues important for catalysis.

Results: We discover both single-stranded DNA and RNA can be ubiquitinated, identifying them as novel non-proteinaceous substrates of ubiquitination. We determine that DTX3L, a RING E3 ubiquitin ligase, is responsible for the *in vitro* ubiquitination of single stranded nucleic acids and determine the site of ubiquitin modification to be 3'hydroxyl of the 3' end. In addition, we show DTX3L is capable of ubiquitinating double stranded DNA with at least a 2-nucleotide overhang. Despite the presence of several nucleic acid binding motifs in its N-terminus, the RING and DTC domains of DTX3L are the minimal unit required to conduct this reaction. Through NMR analyses we determine the RING and DTC domains bind single-stranded DNA. We find this novel ubiquitination activity to be specific to DTX3L and DTX3 within the DELTEX family.

Conclusions: Overall, we present the direct ubiquitination of nucleic acids by DTX3L and, by exploring the interactions of nucleic acids with DTX3L, open up new avenues for the functional implications of direct ubiquitination of nucleic acids.

IRAK1 regulates the ABC transporter balance defining chemo-immuno-resistant NSCLC

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Rationale: In NSCLC, the high expression of the ABC transporters ABCB1 and ABCC1, together with reduced ABCA1, defines a chemo-immuno-resistant phenotype [1]. However, the molecular mechanisms that orchestrate this deregulated transporter profile remain largely unclear.

Methods: CRISPR-KO kinome screening, targeted gene silencing, immune-killing assays, single-cell RNA-seq, and phosphoproteomics were integrated with in silico drug design and applied in NSCLC cell lines, patient-derived organoids, and immuno-xenograft models.

Results: The kinome screen performed on the NCI-H2228 cell line identified IRAK1 as a top regulator of chemo-immuno-resistance in NSCLC. Transcriptomic analyses of TCGA and early/advanced-stage patients confirmed the IRAK1^{high}ABCB1/ABCC1^{high}ABCA1^{low} signature as a predictor of poor prognosis and low therapy response. Mechanistically, IRAK1 loss suppressed IL-1R/NF-κB and ERK1/2–AP-1 signalling, lowering ABCB1/ABCC1 expression and enhancing chemosensitivity, while activating the LXRα–ABCA1 axis. The latter increased ABCA1-mediated efflux of IPP^2, boosting Vγ9Vδ2 T-cell activation, immunokilling and T-cell recruitment. These effects were observed both *in vitro* and *in vivo* following pharmacological inhibition or silencing of IRAK1. Phosphoproteomics combined with INKA scoring uncovered additional IRAK1-associated kinase networks, including MET, EGFR, and MAPK1/3, and guided the development of a promising multidrug strategy. In particular, low-dose dual inhibition of EGFR and ERK synergistically restored platinum sensitivity in NCI-H2228 cells

Conclusions: IRAK1 emerges as a central driver of NSCLC chemo-immuno-resistance. Targeting IRAK1 and its kinase network offers a promising precision oncology strategy in resensitizing NSCLC tumours refractory to current therapies.

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Human UPRT structure suggests a non-enzymatic moonlighting role in pyrimidine metabolism

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Rationale: Pyrimidine nucleotides are essential for nucleic acid synthesis and as activating molecules for glycosylation and lipid synthesis. In humans and higher eukaryotes, the salvage of pyrimidine nucleotides occurs via uridine phosphorylation to uridine 5-monophosphate (UMP), whereas microorganisms use uracil phosphoribosyltransferase (UPRT) to convert uracil and phosphoribosyl pyrophosphate (PRPP) into UMP. UPRT is essential in some pathogens, and the assumption that humans lack this activity supports antimicrobial strategies targeting this enzyme. However, a putative human UPRT is expressed in many tissues, and although it shows no detectable enzymatic activity *in vitro*, homologs of this "inactive" UPRT are conserved across animals and plants, and its loss in *Drosophila* impairs growth and lifespan, which can be rescued by pyrimidine supplementation.

Methods: Here, we undertook a structural approach to elucidate the important yet unknown function of this conserved and "inactive" UPRT. Human UPRT was engineered, expressed in bacteria, purified and crystallized free or bound to different ligands for X-ray analysis.

Results: The structures, determined at resolutions higher than 2 Å, showed that human UPRT forms a homotetramer closely resembling microbial homologs despite low sequence identity. The protein binds PRPP in a non-hydrolyzable manner and lacks a uracil-binding site, explaining enzymatic inactivity. Unexpectedly, we identified a UTP-binding site that promotes tetramer assembly.

Conclusions: Contrary to our initial hypothesis that human UPRT required missing co-factors or proteins to complete the active site, structural data suggest a non-enzymatic function mediated by UTP-induced oligomerization and PRPP binding. Ongoing studies in model organisms and CRISPR-edited cells aim to clarify this moonlighting function.

Low-grade systemic inflammation in metabolic dysfunction-associated steatotic liver disease (MASLD)

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Rationale: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a common condition characterized by fatty deposition in the liver within the context of cardio-metabolic risk factors. This study evaluated the relationship between inflammatory biomarkers, the presence of MASLD, and the severity of ultrasonographic steatosis, and examined whether inflammatory load reflects disease burden.

Methods: Eighty-four adults were divided into two groups: control (grade 0, n = 28) and MASLD (grades 1-3, n = 56). An Inflammatory Load Index (ILI) was constructed from standardized IL-6, CRP, and TNF-α values (Cronbach's α=0.41). Group differences were assessed using Mann-Whitney U tests or t-tests, and steatosis grades were analyzed using the Kruskal-Wallis test. Spearman correlations and ROC analyses were performed to evaluate the discriminative ability of metabolic and inflammatory markers for MASLD.

Results: IL-6, ILI and sCD163 were significantly higher in the MASLD group ($p < 0.05$), consistent with low-grade systemic inflammation. However, none of these inflammatory markers differed across steatosis grades ($p > 0.10$), indicating that inflammatory activity does not parallel ultrasonographic severity. In contrast, metabolic indicators showed stronger disease relevance: both the TyG index and the ALT/AST ratio were significantly associated with MASLD and steatosis grade, with TyG demonstrating the highest discriminative performance (AUC = 0.767). These findings indicate that, although inflammatory markers are elevated in MASLD, metabolic load markers more accurately reflect disease burden.

Conclusions: In conclusion, while low-grade systemic inflammation is present in MASLD, it does not correlate with steatosis severity. Metabolic load markers, particularly the TyG index and ALT/AST ratio, more strongly reflect both the presence and constitutive severity of the disease. Inflammatory biomarkers add dimension to the biology of MASLD but are not determinants of steatosis severity.

Impact of hyaluronic acid metabolism on endometrial receptivity and embryo implantation

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Rationale: Endometrial receptivity is essential for successful embryo implantation, and its impairment contributes to recurrent implantation failure (RIF). Hyaluronic acid (HA), a key extracellular matrix component, supports epithelial remodeling, stromal communication, and cell–cell interactions during the implantation window. However, the molecular mechanisms linking HA metabolism to endometrial receptivity remain incompletely understood.

Methods: To investigate HA-related pathways, we reanalyzed publicly available bulk RNA-seq data from human endometrial biopsies (GSE106602) and two single-cell RNA-seq datasets covering the mid-secretory phase and RIF samples (GSE183837, GSE250130). Additionally, we employed an in vitro model using Ishikawa endometrial epithelial cells co-cultured with trophoblast surrogates derived from human induced pluripotent stem cells to evaluate the impact of receptivity modulation on HA-associated gene expression.

Results: Bulk RNA-seq analysis revealed downregulation of key HA metabolism genes, including HAS2, HAS3, HYAL2, CEMIP, and the HA receptor CD44, in RIF samples compared to fertile controls. Single-cell analyses demonstrated cell-type-specific regulation, with epithelial, stromal, and endothelial compartments showing distinct HA pathway expression patterns. In fertile endometrium, HAS2, HAS3, and CD44 were elevated during the implantation window, whereas RIF samples showed disrupted HA synthesis–degradation balance and reduced receptor-mediated signaling. In vitro, cytokine- and hormone-induced modulation of receptivity reproduced these alterations, confirming reduced epithelial receptivity markers under impaired HA metabolism conditions.

Conclusions: These findings suggest that dysregulated HA turnover and signaling may contribute to non-receptive endometrial states and implantation failure, highlighting HA pathways as potential targets for improving endometrial function and assisted reproduction outcomes. Study was supported by VEGA No. 1/0747/24

Neural and behavioral diversity in a Nlgn3 mouse model: linking sensory processing to atypical behavior

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Rationale: Autism spectrum disorder (ASD) encompasses social-communication challenges, restrictive patterns of behavior, and frequent sensory disturbances. Individuals with ASD often experience atypical sensory reactivity and exhibit challenging behaviors, such as heightened stereotypies and episodes of aggression. The substantial clinical variability in symptom severity and putative biological mechanisms makes it essential to dissect how sex, genotype, and developmental stage shape the underlying neural networks in ASD.

Methods: Here, we characterized the sensory and behavioral landscape of *Nlgn3* mutant mice, a well-established model of ASD, to clarify how altered sensory processing may contribute to abnormal behaviors. We performed a longitudinal assessment of sensory skills, repetitive behaviors, and aggression, from infancy through adulthood. Furthermore, we introduced a novel sensory-overload paradigm to evaluate whether intensified sensory stimulation precipitates abnormal behavioral responses.

Results: Our findings reveal distinct alterations in visual and olfactory responses in *Nlgn3* mutants, and genotype-dependent differences in repetitive behaviors in adolescence and adulthood. Both male and female mutants displayed aggression, though with strikingly sex-specific behavioral patterns that warrant deeper study. Under sensory-overload conditions, preliminary data suggest that behavioral reactions depend on both sex and genotype, with mutant females additionally showing reduced communicative output.

Conclusions: Overall, these results highlight how sensory capacities and behavioral phenotypes are influenced by sex, genotype, and developmental timing. Next, we aim to identify the neural network architecture that links sensory input to behavioral output in ASD, focusing on the basal ganglia nuclei. In the near future, we will perform behavior experiments using optogenetics, fiber photometry, fMRI, and in vivo electrophysiology to dissect the circuit-level mechanisms that drive this heterogeneity.

Engineering a conformational signaling receptor for the detection of rapamycin

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Rationale: Biosensing technologies for continuous monitoring of biomolecules can improve precision medicine by enabling better control of diagnosis and therapy. However, current biosensors are limited to a few metabolites and rely on enzymatic electrochemical detection, restricting their use to targets with suitable enzymes. Conformational signaling represents a promising alternative, in which a recognition element undergoes structural changes upon ligand binding, enabling a direct signal transduction without additional reagents. To expand this strategy and address the need for continuous immunosuppressant monitoring, we aim to engineer a biosensor capable of real-time rapamycin detection-

Methods: To develop a biosensor for rapamycin, we will exploit its ability to induce dimerization of FKBP and FRB, two proteins that interact exclusively in the presence of rapamycin. We engineered a conformational receptor, building a protein chimera composed by FKBP and FRB joined by a flexible linker. We implemented this receptor in an electrochemical biosensor, modifying the proteins with a redox reporter for electrochemical monitoring and a surface linker for attachment to a gold electrode. We then evaluated receptor stability, ligand binding, and electrochemical responses after rapamycin addition.

Results: The engineered receptor remained stable and showed rapamycin dependent dimer formation that produced reproducible electrochemical signal changes. The sensor responded to rapamycin in a concentration dependent manner and enabled reagentless, continuous detection under buffer conditions.

Conclusions: These results demonstrate that engineered conformational receptors provide an effective strategy for rapamycin biosensing. They show that engineered conformational receptors can function as modular, reagentless biosensors capable of transducing binding events into electronic signals, enabling continuous biomolecular monitoring.

A simple and cost-effective system to induce CSC-Like states via metabolic stress

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Rationale: Cancer Stem Cells (CSCs) are a small but crucial tumor subpopulation driving therapy resistance and progression. How CSC-like states arise under microenvironmental stress remains unclear. This study examines whether metabolic stress can induce CSC-like phenotypes in vitro.

Methods: MCF-7, HepG2, RKO, HeLa, B2V, and MDA-MB-231 cells were cultured for one week under serum deprivation (DMEM+0.1% FBS). CSC-like traits were assessed by CD133 expression using flow cytometry. Cell viability, metabolic activity, and preliminary chemoresistance were assessed by Annexin V/PI staining and MTT assay after 72 h treatment with 5-fluorouracil (5-FU) and cisplatin.

Results: CD133 analysis, using one of the commonly applied CSC-associated surface markers, revealed ~1–3% positive cells in MCF-7 and HepG2, while HeLa and RKO showed <0.2%, directing further studies toward MCF-7 and HepG2 with planned CD133-based enrichment. Serum-deprived MCF-7 cells maintained high viability (>80%) compared with controls (<60%), whereas stressed HepG2 cells showed low viability (<25%). MTT assays revealed low metabolic activity in MCF-7 (<20%) under both serum conditions after 7 days. HepG2 cells displayed a strong decrease under serum deprivation (~26% of control at day 7) with partial recovery (~65%) by day 14. To extend the analysis, B2V and MDA-MB-231 cells were included and preliminary assessment showed 1–2% CD133 expression. Short-term 5-FU and cisplatin treatment caused dose-dependent metabolic inhibition, with residual viability at high doses.

Conclusions: Detectable CD133-positive subpopulations in MCF-7, HepG2, B2V, and MDA-MB-231 support their use for studying stress-induced CSC-like states. Minimal responses in HeLa and RKO indicate that serum deprivation alone is not a universal method for CSC induction and that responses are cell-line specific. Future work will expand cell line testing and apply functional, enrichment, and chemoresistance assays (building on preliminary data) to validate stress-induced CSC-like features.

Glyconanoparticles deposited surfaces to develop an electrochemical immunosensor for the detection of C-reactive protein (CRP) in pleural fluid

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Rationale: Timely and accurate C-reactive protein (CRP) measurement in pleural fluid is crucial for distinguishing infectious, inflammatory, and malignant effusions, highlighting the need for rapid, sensitive, point-of-care detection tools [1,2].

Methods: The study aimed to develop a sensitive electrochemical immunosensor for CRP detection in pleural fluid. Mannose-4-aminophenyl L-alanine (Man-4APA) was synthesized and characterized. Silver nanoparticles (AgNPs) were synthesized in the presence of Man-4APA. The AgNPs/Man-4APA nanocomposite was electrodeposited onto electrode surfaces, and anti-CRP antibodies were immobilized. Selectivity, stability, and reproducibility were tested. Clinical pleural fluid measurements were compared with enzyme-linked immunosorbent assay (ELISA) to confirm accuracy and % recovery.

Results: The immunosensor showed enhanced electrochemical performance, a linear CRP detection range of 0.5–100 ng/mL, a detection limit of 0.38 ng/mL, high specificity against common interferents, and maintained over 90% signal after 12 days. CRP measurements in clinical pleural fluid closely matched ELISA, with recoveries between 97% and 109%.

Conclusions: The AgNPs/P(Man-4APA)/Anti-CRP immunosensor demonstrated rapid, sensitive, and selective detection of CRP in pleural fluid, achieving high accuracy, stability, and concordance with ELISA results. Some of these findings build upon our previously published work within the last 18 months, as required by FEBS guidelines [3].

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Bacterial DNA Nucleotide Excision Repair: a multi-approach investigation of the damage recognition process

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Rationale: Nucleotide Excision Repair (NER) represents one of the major molecular machineries that control chromosome stability in all living species. In Eubacteria, this pathway is carried out by the UvrABC excinuclease complex, comprising the UvrA, UvrB and UvrC proteins. Despite extensive study, NER pathway still lacks a full understanding, and many molecular details about the initial damage recognition process remain controversial.

Methods: In the past years, we carried out a Cryo-EM-based structural characterization of different complexes between UvrA, UvrB and damaged DNA; to then validate our structural observation, biochemical and biophysical investigations were performed. Recently, we approached optical tweezers in combination with single-molecule fluorescence imaging to determine the molecular forces acting in the damage identification process.

Results: The structural results have been recently published [1], describing a novel mechanism of damage recognition, in which we revealed that the DNA lesion is first recognized by UvrA thanks to melting, unwinding and flipping-out mechanisms; then, through conformational changes and rearrangements, one UvrB molecule at a time is recruited, to then lead to the pre-incision complex formation, where one UvrB molecule only remains bound on the DNA, with the final aim to recruit UvrC. Recently, we collected new and interesting data about the molecular forces that UvrA applies to the DNA, using optical tweezers: from our preliminary data, it is possible to distinguish different behaviors that UvrA has when interacting with different types of DNA damage (e.g., UV-damaged and Cisplatin-damaged DNA).

Conclusions: We are currently working on confirming the preliminary data we collected with optical tweezers. Together with the biochemical and structural characterization, the description of the molecular forces involved in damage recognition will widen the understanding of one of the major bacterial systems.

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Human cardiac organoids as tridimensional platforms mimicking cardiac impairment for pharmacological testing

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Rationale: Mitochondrial dysfunction is a hallmark of cardiovascular diseases and hyperglycemia suppresses mitochondrial energy metabolism. Thus, drugs capable of correcting this impairment are highly needed. Hence, our **aim** was to evaluate the effects of phenoxazine (Phx) and iminostilbene (Imi) on high glucose (HG)-induced mitochondrial dysfunction using self-assembling human cardiac organoids (HCOs).

Methods: Beating HCOs generated from human induced pluripotent stem cells were exposed to 33.3 mM glucose for 6 days in order to generate an experimental model that mimics the *in vivo* hyperglycemic conditions. HCOs were characterized by immunofluorescence and the effect of HG was assessed by quantification of beats/minute and transcriptomic analysis (RNA-seq); the latter was also employed to evaluate the effects of the tested drugs.

Results: Immunofluorescence analysis revealed that HCOs were positive for TnnT2, CD31, vimentin, collagen type I and III, an indication for the presence of the major cardiac cell populations: cardiomyocytes, endothelial cells and fibroblasts. HG decreased the contractility of HCOs. Interestingly, exposure of HCOs to HG downregulated genes involved in oxidative phosphorylation, ATP synthesis, and mitochondrial matrix complexes, indicating substantial mitochondrial impairment. These genes were upregulated by Phx treatment. Moreover, the drug induced the downregulation of extracellular matrix related genes, suggesting an anti-fibrotic activity. In contrast, Imi downregulated the collagen-containing extracellular matrix related genes, with no impact on mitochondrial genes.

Conclusions: Phx, but not Imi, reversed glucose-induced mitochondrial gene dysregulation. These findings underscore Phx as a promising therapeutic candidate for cardiac dysfunction associated with mitochondrial impairment.

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Exploiting the versatility of engineered consensus tetratricopeptide repeat proteins as multivalent theranostic agents

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Rationale: Proteins are fundamental to life, playing structural, transport, and regulatory roles. Recently, they have been explored as nanomaterial stabilizing scaffolds, enabling the development of stable, biocompatible hybrids. While ferritin nanocages and bovine serum albumin have been widely used, their full engineering potential remains underexplored.

Methods: Consensus tetratricopeptide repeat proteins (CTPR) offer a robust and modular alternative. Herein, we rationally designed metal-coordination CTPRs and used them to scaffold iron oxide nanoparticles (IONPs), aiming to develop improved magnetic resonance imaging (MRI) contrast agents. The versatility of the platform was exploited by further engineering the protein scaffold to display one or three KEAP1-binding loops. KEAP1-NRF2 pathway was chosen as target in this proof-of concept since it has emerged as a compelling therapeutic target due to its pivotal role in protection against a broad spectrum of diseases marked by oxidative stress and inflammation.

Results: Protein-stabilized IONPs showed comparable MRI properties when compared to the IONPs approved in the clinical practice with enhanced stability and biocompatibility. Interestingly, the grafting of different number of binding modules did not affect the metal coordination, IONPs formation or MRI properties. The incorporation of three binding loops (3NRF2-IONPs) enhanced by roughly threefold the apparent binding affinity compared to the monovalent hybrids (1NRF2-IONPs), which shows the coordinated and potent binding capability of the multi-loop protein constructs. In agreement with these findings, 3NRF2-IONPs showed a more potent activation of the pathway in vitro. Finally, NRF2-IONPs were demonstrated to be effective MRI contrast agents in a preclinical scanner.

Conclusions: This work demonstrates the potential of protein-stabilized nanomaterials as theranostic agents, in which protein engineering allows the tailoring of the nanohybrid properties and the enhancement of the therapeutic potency.

Repurposing artemisinins as anti-cancer agents: investigating covalent interactions and cytotoxicity profiles

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Rationale: Artemisinins are a group of natural compounds and their synthetic derivatives traditionally recognized as essential anti-malarial drugs. Recently, growing evidence suggests that these compounds possess promising anti-cancer properties, making their drug repurposing a highly attractive strategy.

Methods: To gain insight into the molecular mechanism of their observed cytotoxicity, we utilized BODIPY-tagged artemisinin derivatives as chemical probes. These fluorescent probes allow us to track their cellular localization and identify interacting protein targets using gel-based methods and LS-MS.

Results: Our screening results demonstrated that the synthesized derivatives exhibit robust cytotoxicity, predominantly against the CCRF-CEM T-cell acute lymphoblastic leukemia cell line. Furthermore, select compounds also show significant activity against other solid tumor models, such as the A549, non-small cell lung cancer line. Our preliminary electrophoretic results suggest a specific covalent interaction with proteins displaying an approximate molecular weight of 20 kDa in treated cancer cells.

Conclusions: Our findings demonstrate the selective anti-cancer potential of artemisinin derivatives, primarily against the CCRF-CEM line. The evidence from fluorescence detection following SDS-PAGE strongly suggests covalent modification of a 20 kDa protein. Further studies, including mass spectrometry and microscopy, are underway to definitively identify these protein targets and confirm the underlying mechanism of action, which is crucial for advancing artemisinins into effective cancer therapeutics.

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Biophysical characterization of the extracellular electron transfer pathway in *Listeria* monocytogenes, a potential antimicrobial target space

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Rationale: Several bacteria, including the foodborne pathogen *Listeria monocytogenes*, utilize extracellular electron transport (EET) pathways to maintain cellular redox balance under extreme environmental conditions, such as anaerobiosis. Previous studies have demonstrated that *L. monocytogenes* requires expression of the EET locus for survival and virulence, but the individual components of this pathway remain largely uncharacterized, and the underlying molecular mechanism of EET is still unclear.(1) One of the key proteins involved in the EET pathway is a type II NADH dehydrogenase (NDH2b), which serves as the electron entry point into the electron transport chain.(2) NDH2b is unique among type II NADH dehydrogenases because it possesses a membrane domain but is believed to lack proton or ion-pumping activity.

Methods: To elucidate the role of this membrane domain, Cryo-EM, electrochemical, and biophysical analyses were performed, including NADH activity assays and Michaelis–Menten kinetics, in order to characterize the structure, stability, and catalytic properties of NDH2b. Possible interactions with other EET proteins of unknown function are also being investigated.

Results: NDH2b was recombinantly expressed and characterized for the first time. Cryo-EM structures confirmed the protein's dimeric state in its active form and the location of the substrate binding site in relation to the membrane. Michaelis–Menten kinetics and NADH activity assays showed preferential affinity toward specific biologically relevant quinones, confirming NDH2b's catalytic role and substrate specificity.

Conclusions: Given *L. monocytogenes'* increasing capacity to develop resistance to multiple antibiotics and its severe impact on immunocompromised and pregnant individuals, understanding the regulation and function of NDH2b and other proteins in the EET pathway is not only of fundamental scientific relevance but may also inform future antimicrobial strategies against this pathogen.

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Enlightening the quietness – the search for early silenced proviruses

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Rationale: After integrating into the host genome, retroviruses persist as proviruses that either remain transcriptionally active and drive ongoing replication or become silent, creating a latent reservoir that currently blocks efforts to achieve a functional cure for HIV. A significant proportion of silenced proviruses becomes transcriptionally suppressed shortly after integration; however, whether silenced proviruses were transiently active at any time after integration remains to be known.

Methods: We introduce a novel detection system that should register even a short-term pulse of provirus transcriptional activity: First, we generate a sensor cell line by inserting a detection cassette with a reporter gene. Replication-defective retroviral vector transducing Cre recombinase gene is then used as an activator. In this system, any cell in which Cre-mediated recombination is detected in the absence of the proviral reporter signal should contain an early silenced provirus.

Results: We employed our detection system to study early silencing dynamics of several retroviruses in Jurkat and K562 sensor cell lines. As expected, the proportion of cells harboring early silenced proviruses usually increases during the initial 3 days post-infection (dpi), illustrating the gradual silencing pattern. Strikingly, in some retroviruses the proportion of early silenced proviruses in both sensor cell lines decreases during the first 3dpi. This suggests that most of these proviruses exhibit very low, sub-detection reporter expression that increases markedly after 1–2 dpi.

Conclusions: Our approach allows to detect and characterize a previously not described population of proviruses that undergoes rapid silencing after integration. Our workflow could be further applied to other models of retroviral infection, thereby helping to elucidate the silencing dynamics, the conditions under which temporal proviral expression occurs, and to identify the post-integration processes integral to it.

Bifunctional myeloperoxidase and glucose oxidase chimeras: addressing the limitations of coupled enzymatic system for antimicrobial coatings

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Rationale: To address the major heathcare challenge of device-associated infections, we engineered Glucose Oxidase (GOx) and bacterial Myeloperoxidase (RbMPO) systems to generate antimicrobial hypochlorous acid from glucose and NaCl.

Methods: We engineered bifunctional chimeras' template by fusing the open reading frames of RbMPO and GOx, then designed variants with diverse linker sequences including anionic LEGGEAEA (chim-G) or proline-rich LEKRPEAEA (chim-P). We expressed them in *E. coli* as inclusion bodies and purified by gel filtration chromatography. Their activities were evaluated using Megazyme (GOx activity) and chlorination (RbMPO or combined activity) assays. We examined their structural organization and topology using Small-Angle X-ray Scattering and Atomic Force Microscopy, explored their surface immobilization through Quartz Cristal Microbalance measurements using the complementary PDADMAC polycation, and assessed layer thickness with spectroscopic ellipsometry.

Results: Among the designs [1] tested, the chim-G monomer emerged as the best overall performer showing the strongest GOx specific activity. Moreover, the chlorination catalytic efficiencies of chim-P and chim-G were the highest (36 ± 22 and 34 ± 19 $M^{-1}.s^{-1}$, respectively). The Immobilized chim-P monomer reached a maximum surface coverage (16.2 ± 0.9 $mg.m^{-2}$), threefold that of the trimer, but with lower apparent binding affinity. Furthermore, free GOx:RbMPO mixtures require a 1:10 ratio for antimicrobial activity [2]. Here, unlike the negligible surface activity observed with GOx and RbMPO adsorbed individually, the immobilized chim-P show significant activities of 1.8 ± 0.03 (monomer) and 7.2 ± 1.1 $mm^2.s^{-1}$(trimer).

Conclusions: By ensuring optimal active site proximity, our resilient chimera overcomes the constraints of enzyme mixtures, positioning it as a strong candidate for creating self-sustaining, antimicrobial medical surfaces.

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Uncovering functional non-coding variants in colorectal cancer using single-cell multiomics

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Rationale: Genome-wide association studies (GWAS) have identified numerous colorectal cancer (CRC) risk loci in non-coding regions, yet the biological mechanisms and specific cell types driving these associations remain poorly understood. This study aimed to dissect how germline non-coding variants functionally modulate gene regulation across the heterogeneous cellular landscape of the colon.

Methods: Leveraging publicly available scATAC-seq and scRNA-seq data, we utilized deep learning frameworks, such as scANVI (single-cell ANnotation using Variational Inference) and scGLUE (single-cell Graph Linked Unified Embedding), to integrate multi-omics profiles and refine cell type annotations. We further characterized chromatin co-accessibility and allelic imbalance. This computational approach assessed transcription factor binding and epigenetic regulation to identify functional variants within distinct cell populations, including epithelial, stromal, and immune compartments. We will bridge the gap between association and function by validating the regulatory potential of these elements through orthogonal experimental frameworks, specifically CRISPR/Cas9 editing and massively parallel reporter assays (STARR-seq).

Results: Our analysis pinpointed specific candidate variants exhibiting significant allelic imbalance, indicating cell-type-specific regulatory potential. Furthermore, the data highlighted variants with potential non-cell-autonomous effects, suggesting a role in mediating intercellular interactions and alterations within the tumour microenvironment.

Conclusions: These results define a functional map of non-coding CRC risk, revealing that GWAS variants act not only within the epithelium but also through previously overlooked non–cell-autonomous effects from stromal and immune lineages. As these cross-lineage mechanisms remain largely unexplored, our work highlights the necessity of evaluating non-coding variation across the full tissue microenvironment to understand CRC initiation.

Hypoxia, angiogenesis and mitochondrial activity in the dynamics of endometriosis

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Rationale: Endometriosis is a chronic and multifactorial disease with complex pathophysiology that significantly affects women's reproductive health. Its onset and progression are determined by the interaction of inflammatory, angiogenic, and hypoxic processes.

Methods: To investigate these mechanisms, gene expression was quantified using real-time PCR, and protein expression was assessed by ELISA. The aim of this study was to characterize selected biomarkers related to inflammatory activation, vascularization regulation, and mitochondrial function in tissues affected by endometriosis.

Results: Analysis of the expression of selected genes revealed increased activity of the transcription factor HIF-1α, accompanied by the induction of the angiogenic markers transcription mRNA level of VEGFA, ANG1 and ANG2, indicating an adaptive vascular response mediated by hypoxia. Concurrently, the overexpression mRNA of COX-2 and TGF-β1 was observed, reflecting the interplay between chronic inflammation and fibrotic processes. Protein-level analysis of COX-2, TGF-β1 and VEGFA further supported these transcriptional findings. Given the high metabolic demands of angiogenic remodelling, these results suggest that cellular mechanisms ensuring adequate energy production and redox homeostasis are essential for the survival and expansion of the newly formed vascular network. At the level of mitochondrial regulation, activation of the mRNA of PPARGC1A, MCU, and POLRMT genes was observed, indicating reprogramming of cellular bioenergetics. Decreased NRF2 activity also signals a weakened antioxidant response of cells.

Conclusions: The results confirm that endometriosis is a dynamic, systemically conditioned disease shaped by the complex interaction of hypoxic, inflammatory, angiogenic, and mitochondrial mechanisms, which also represent promising targets for future diagnostic and therapeutic strategies.

Testing molecular processes identified by ribo-sequencing in a neurodegenerative disease model

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Rationale: Alzheimer's disease (AD) is a slowly progressive neurodegenerative disorder characterized by amyloid-β deposition, tau pathology, and neuronal loss. Disruption of protein homeostasis, oxidative stress, and inflammation play central roles in disease progression. AGE–RAGE signaling, through the interaction of advanced glycation end-products (AGEs) with their receptor RAGE, enhances these damaging processes and contributes to neurodegeneration. In AD, AGE accumulation and RAGE activation are increased, further exacerbating amyloid-β- and tau-induced pathology. The enzyme Glyoxalase-1 (Glo-1) reduces AGE formation by detoxifying reactive dicarbonyl compounds, potentially mitigating harmful effects along the AGE–RAGE pathway.

Methods: We first performed ribo-sequencing on an AD model of *Drosophila melanogaster*. Based on these results, we achieved neuronal expression of the human amyloid-β peptide using the QF2-QUAS system, in which the QF transcription factor from *Neurospora crassa* binds to QUAS regulatory elements and activates transcription of the amyloid-β transgene in neurons. Using this system, we then examined the effects of Glo-1 overexpression.

Results: In our preliminary experiments, ribo-sequencing identified a total of 1,591 dysregulated RNAs, of which 65% were upregulated and 35% downregulated in AD *Drosophila melanogaster* compared to healthy controls. Based on KEGG pathway analysis, the AGE–RAGE pathway was identified and was represented in both dysregulation groups. In genetic interaction tests, Glo-1 overexpression had beneficial effects on both neurodegeneration and motor activity, and it also increased lifespan compared to AD flies.

Conclusions: In summary, ribo-sequencing enabled the identification of the AGE–RAGE pathway as dysregulated in amyloid-β-expressing flies. Results from genetic interaction tests indicate that overexpression of Glo-1, a key enzyme of the AGE–RAGE pathway, increased lifespan and exerted positive effects on both neurodegeneration and motor activity.

Nutrient-dependent, non-canonical stabilization of the hypoxia inducible factor and its role in breast cancer metabolism

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Rationale: Hypoxia-inducible factor (HIF) regulates the transcription of key genes associated with malignant features of cancers. Although its activity is usually induced by insufficient oxygenation, a few non-canonical pathways that enable stabilization beyond hypoxia are already recognised. Existing research predominantly examines the HIF-1 isoform in conditions of abundant nutrient availability. Our study aims to differentiate between the HIF-1 and HIF-2 isoforms and to explore how variations in nutrient availability affect their stabilization and downstream gene expression.

Methods: We employed *in vitro* siRNA silencing of *HIF2* in breast cancer cell lines (MCF-7 and MDA-MB-231) cultured for 48 hours under normoxic conditions with various glucose (25 mM vs 0.5 mM) and glutamine (2 mM vs 0.2 mM) concentrations. The levels of HIF-1 and HIF-2, and downstream targets associated with metabolism (GLUT1, HK2, PKM2, PDK1), were assessed at the mRNA level using PCR and at the protein level using Western Blot. Results were referred to cells treated with the scrambled siRNA.

Results: Decreased glucose and glutamine non-canonically stabilized HIF, with a more prominent effect for the HIF-2 isoform. Changes following HIF-2 silencing in downstream targets associated with metabolism suggest that, despite current reports of HIF-1's predominant role in glucose metabolism, HIF-2 might play a role in metabolic plasticity of breast cancer.

Conclusions: We report a new, nutrient-dependent, non-canonical pathway of hypoxia-inducible factor's stability induction while evidencing the potential role of the HIF-2 isoform in breast cancer metabolism.

Development and optimization of a SARS-CoV-2 virus antibody-dependent cell mediated cytotoxicity assay

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Rationale: Antibody dependent cellular cytotoxicity (ADCC) plays a key role in antiviral immunity, including that against SARS-CoV-2. However, robust and reproducible *in vitro* systems for assessing ADCC remain scarce.

Methods: Herein, we developed a NK-92 cell line stably expressing the high-affinity variant F158V of CD16a and either IL-2 or IL-15 to enable high-levels of ADCC and cytokine-independent proliferation of NK effector cells. Target cells were generated using HEK-293T and NIH-3T3 cells expressing GPI-anchored SARS-CoV-2 antigens. NK cell lines were characterized for CD16 expression, binding of heat-aggregated IgG, elaboration of distinct cytokine profiles, and cytotoxic activity.

Results: Both NK cell lines maintained high CD16 expression and good endogenous production of the transgenic cytokine, but only IL-2-transduced cells showed strong binding of heat-aggregated IgG and efficient ADCC function. NIH-3T3 cells expressing GPI-anchored SARS-CoV-2 receptor-binding domains (RBDs) lead to less frequent non-specific killing and therefore proved to be superior compared to HEK-293T target cells. The ADCC assay was established and optimized using a SARS-CoV-2 specific monoclonal antibody and sera obtained from 26 convalescent individuals at 10 weeks and 10 months after SARS-CoV-2 infection. The part of the SD1 domain recognized by the serum antibodies (aa 519 - 571) emerged as the most important ADCC target. The resulting interindividual variations in ADCC levels may be attributed to differences in the underling IgG subclasses.

Conclusions: We have established a reproducible and rapidly adaptable ADCC assay using SARS-CoV-2 antigens as a model. The developed platform enables direct functional antibody analysis and provides a valuable tool for investigating antiviral immune responses and vaccine-induced immunity. The ease with which the test system can be adapted, will help us to quickly and efficiently overcome the challenges posed by future dangerous human pathogens.

Optimizing an in vitro human blood–brain barrier model and inducing pharmacoresistance for antiepileptic drug screening

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Rationale: Effective neuropharmacological treatment depends on a drug's ability to cross the blood–brain barrier (BBB), which becomes even more restrictive with pharmacoresistant properties. Despite substantial advances in *in vitro* BBB engineering, models that accurately reproduce pathological features remain limited; therefore, we aim to develop a pharmacoresistant BBB model that could provide a more predictive platform for screening antiepileptic drugs.

Methods: The BBB model is composed of three human cerebral cell lines - endothelial cells (ECs), pericytes, and astrocytes, cultured on Transwell inserts. Optimization parameters include cellular configuration, insert size and membrane composition, surface coating, cultivation time, and tight-junction promoting supplements. Barrier formation is evaluated *via* transendothelial electrical resistance, fluorescein permeability, and fluorescence microscopy of tight junctions. In parallel, ECs are exposed long-term to sub-toxic concentrations of antiepileptic drugs to induce pharmacoresistant properties through physiological adaptation.

Results: Several key factors for successful BBB formation have been identified, including larger (≥ 12well) inserts with polycarbonate or polytetrafluoroethylene membranes, and collagen–fibronectin coatings. Higher barrier tightness was achieved in a contact co-culture arrangement with ECs cultured apically and a mixture of pericytes and astrocytes co-cultured basolaterally. Pharmacoresistant properties were induced by valproate after four weeks of exposure, as demonstrated by altered toxicity profiles and increased efflux transporter expression.

Conclusions: These findings define key requirements for *in vitro* BBB formation and confirm the feasibility of inducing pharmacoresistance, supporting the model's use within a more predictive disease-tailored screening platform.

Unlocking systems biology and pharmacology of biochemical reactions and interactions through structured approach to data

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Rationale: Systems biology seeks internally consistent, holistic descriptions of biological systems rather than isolated, reductionist facts. However, experimental data are often fragmented, differently formatted, and published mainly as secondary parameters, complicating reanalysis and model building. Minimum-information standards such as MIDAS can mitigate these issues by enabling consistent storage and reuse of raw kinetic data.

Methods: We adopted the MIDAS format to standardize kinetic data from receptor–ligand assays. Fluorescence polarization, luminescence, and live-cell fluorescence microscopy were performed in microplate format using BMG PheraStar, BioTek Neo, and BioTek Cytation 5 instruments. Data import from plate readers, deep-learning-based image processing, MIDAS generation, and export were implemented in the open-source MATLAB software *Aparecium (https://www.gpcr.ut.ee/aparecium.html)*. Kinetic modeling was conducted with IQMTools using ODE-based mechanistic models.

Results: For M1, M2, and M4 muscarinic receptors interacting with TAMRA-labeled ligands UR-MK342 and UR-CG072, simple first-order reaction models failed to fully describe the data. Kinetic profiles indicated multi-step binding for all receptors and suggested homodimer formation for M1 and M2. The M2 receptor was modeled most completely: a tandem dimer model with allosteric modulation and symmetry breaking accounted for observed qualitative and quantitative behaviors.

Conclusions: Complex kinetic fingerprints required multi-year, multi-person iterative analysis. Consistently annotated MIDAS datasets enabled reprocessing of old and new data, ultimately allowing construction of a coherent mechanistic model. The study demonstrates the value of standardized data handling and the Aparecium workflow for systems-biology modeling in long, nonlinear research efforts.

Delineating the protein network regulating early termination/elongation by RNA Polymerase II

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Rationale: Tight regulation of the balance between transcription termination and elongation by RNA polymerase II (RNAPII) is crucial for proper gene expression and, consequently, for cell function. Transcription at non-coding loci is generally termination-prone, whereas elongation is more favored at coding loci. Although most RNAPII transcription events terminate early, within ~1 kb after initiation, the molecular mechanisms underlying this fundamental process remain incompletely understood.

Methods: The aim of this study is to discover proteins that regulate the early termination/elongation balance by conducting a pooled CRISPR screen in mouse embryonic stem cells expressing fluorescent reporters at termination-prone and elongation-prone loci. Guide RNAs (gRNAs) altering reporter expression will be identified using Sort-seq: Fluorescence-Activated Cell Sorting followed by DNA sequencing. A selection of screen hits will be functionally characterized.

Results: In a proof-of-principle experiment, subunits of the Restrictor complex and the endonuclease core of the Integrator complex were included as targets in a small library. These subunits are known to mediate early termination of predominantly non-coding transcription. As expected, all gRNAs targeting these proteins were significantly enriched in cells showing increased expression of the reporter at the termination-prone locus, demonstrating the validity of our approach. In future work, the library will be expanded to ~20,000 gRNAs to allow screening of the nuclear proteome. A promising protein for follow-up was found even in the small library, suggesting that a larger screen will yield many additional relevant hits.

Conclusions: By identifying proteins that drive early termination at non-coding loci, or productive elongation at coding loci, and uncovering their mode of action, this study will deepen our fundamental understanding of gene expression control. These novel regulators may also offer new avenues to modulate transcription in disease contexts.

Molecular dynamics study of how environmental parameters affect the aggregation rate and dimerization of monoclonal antibodies: N-Glycosylation, low-temperature cryoprotectants, and pH variation

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Rationale: Monoclonal antibodies (mAbs) are key biotherapeutics, yet aggregation still causes losses and functional issues. The underlying mechanisms remain unclear and are influenced by factors such as temperature, cryoprotectants [1], N-glycosylation [2], and pH [3] during purification.

Methods: Using molecular modeling and MD, we reveal how environmental factors shape mAb conformation, flexibility, and aggregation pathways at atomistic resolution. Pembrolizumab (PMB) (IgG4, anti–PD-1) is our primary system, with extensions to additional mAbs.

Results: We quantified, at atomic resolution, how temperature reshapes PMB's aggregation propensity and conformational dynamics, altering its "Y"-shape and exposing aggregation-prone residues. Cryoprotectants differentially shield key interfaces—especially in the Complementary Determining Regions—and redirect interaction pathways. We examined glycosylation effects on PMB and on the IgG2 mAb231, showing that glycans modulate conformation and flexibility, with mAb231 adopting λ-like states. Finally, we show that solvent pH shifts interface stability within aggregates.

Conclusions: This comprehensive computational study reveals important mechanistic details of antibody association. Our results provide initial actionable insights to mitigate aggregation through targeted molecular or formulation strategies at the protein level.

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The impact of regnase-2 on stress granule formation in response to different types of cellular stress in glioblastoma cells.

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Rationale: Previous studies on the RNase Regnase-2 (Reg-2) have focused mainly on its roles in regulating inflammatory, apoptotic, and cell cycle control factors in glioblastoma (GBM), yet its involvement in cellular stress responses has not been explored. Since stress granules (SGs) support proliferation, inhibit apoptosis, and promote invasion of cancer cells, elucidating whether Reg-2 contributes to SG formation represents an important and unexplored aspect of GBM progression.

Methods: Murine (KMWT1) and human (U87-MG) glioblastoma cell lines were engineered using the Sleeping Beauty system to generate doxycycline-inducible variants expressing Reg-2WT or a ribonuclease-dead Reg-2mut, with matched controls. Where needed, CRISPR–Cas9/CRISPRi/CRISPRa perturbations were also applied. After dox induction, cells were exposed to oxidative (sodium metaarsenite), osmotic (sorbitol), thermal (heat shock), ER (thapsigargin), or mitochondrial (clotrimazole) stress under optimized conditions. Stress granules were quantified by G3BP1 immunofluorescence and fluorescence microscopy with ImageJ (percentage of SG-positive cells, ≥1 granule). Additional readouts included RT-qPCR, RIP and polysome profiling.

Results: Dox-induced Reg-2WT overexpression reduced the fraction of SG-positive cells under oxidative stress (−45% in KMWT1 and −20% in U87-MG) compared with control and Reg-2mut, with no effect under other stress conditions. Mass spectrometry identified SG-related proteins downregulated upon Reg-2WT overexpression, and RT-qPCR, RIP, and polysome profiling indicated that Reg-2 decreases the stability and translation of the corresponding transcripts.

Conclusions: These results suggest a specific effect of Reg-2 on the oxidative stress response pathway, as evidenced by the reduction in SG formation. This indicates a previously unrecognized role of Reg-2 in the cellular stress response, potentially contributing to mechanisms relevant to glioblastoma progression.

Elucidating adipocyte-NK cell crosstalk in the ovarian cancer microenvironment

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Rationale: Adipocytes are increasingly recognized as modulators of immune responses, including the activity of natural killer (NK) cells, key players in tumour immunosurveillance. However, the functional interplay between adipocytes and NK cells remains poorly understood. In ovarian cancer (OC), the accumulation of malignant ascites and preferential metastasis to omentum leads to the reprogramming of adipocytes into cancer-associated adipocytes (CAAs). This tumour microenvironment (TME) becomes enriched with adipocyte-derived extracellular vesicles (EVs). This study aimed to characterize the expression of NK cell receptor ligands within adipocyte-derived EVs in the OC TME.

Methods: Murine 3T3-L1 and 3T3-F442A adipocytes were reprogrammed into CAAs through exposure to ascites. Total secretome was collected, and EVs were isolated by size-exclusion chromatography or ultracentrifugation. Human adipocytes were reprogrammed using the same approach, and their total secretome was analysed by affinity-based proteomics (Olink Explore HT). Data analyses were performed using R.

Results: Proteomic profiling identified multiple NK cell receptor ligands in both EVs and total secretome of murine and human adipocyte in vitro models. In general, ascites exposure downregulated activating ligands and upregulating inhibitory ones, such as PVR (CD155). Interestingly, CAA-like human adipocytes displayed high levels of MICA/MICB in their secretome, which are known to exert immunosuppressive effects in OC.

Conclusions: Our results highlight the increased presence of NK cell inhibitory ligands in the secretome of CAAs, including EVs. These results show a promising research avenue for understanding how adipocytes modulate the cytotoxic activity of NK cells in OC.

Unlocking intracellular targets: a liposomes library for intracellular delivery of monoclonal antibodies targeting undruggable oncoproteins

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Rationale: The study aims to develop liposomes for the intracellular delivery of monoclonal antibodies. The antibodies used in this research target oncoproteins that are difficult to inhibit with small molecules (such as c-Myc protein) and can be used for targeted protein degradation in cancer therapy.

Methods: The primary methods used in this research include lipid film hydration for antibody encapsulation in liposomes, size exclusion chromatography to separate free antibodies from liposomes, an ELISA test to confirm antibody encapsulation and the biological activity of the antibody after the process, flow cytometry to demonstrate intracellular uptake of liposomes with fluorescently labeled mAb, fluorescence microscopy to monitor endosomal escape of liposomes after internalization, CryoEM to visualize liposome structure and verify mAb encapsulation, and viability tests on cancer cells following incubation with the prepared liposomes.

Results: This study characterizes a “liposomes library” composed of liposomes with various lipid compositions. We assessed cytotoxicity, encapsulation efficiency, cellular uptake, and endosomal escape for each formulation, followed by viability tests in cancer cells. Additionally, CryoEM was used to confirm the presence of antibodies within the selected formulation. Overall, this research provides a comprehensive analysis of a liposome library, highlighting the most and least promising candidates for antibody delivery into cells.

Conclusions: The research offers practical, detailed data crucial for delivering macromolecules to cells and shows potential for increasing the use of monoclonal antibodies in targeted cancer treatment.

Defining the role of hydrostatic pressure in shaping macrophage phenotype

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Rationale: Macrophages are innate immune cells that perform diverse functions in inflammation, development, and regeneration by dynamically adapting to the tissue microenvironment. However, how macrophage functions are regulated by mechanical forces within tissues is poorly understood, particularly the role of hydrostatic pressure, which is frequently elevated during inflammation, cancer, and oedema.

Methods: We set out to investigate how macrophage functions and biophysical properties change under pressure using digital holographic microscopy coupled with a custom-built microfluidic system to image cells exposed to hydrostatic pressure in real time, together with complementary biochemical assays.

Results: Both human blood monocyte-derived macrophages and mouse bone marrow-derived macrophages dynamically change their cellular volume and upregulate pro-inflammatory genes upon cyclic hydrostatic pressure.

Conclusions: Macrophages respond to elevated cyclic pressure and shift towards a pro-inflammatory phenotype, highlighting potential biomechanical regulation in innate immunity.

Structural modification of nicotinamides and its impact on the modulation of the cholinergic and serotonergic neurotransmission

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Rationale: Nicotinamides, derivatives of vitamin B3, are known as essential molecules for the body function as critical precursors for NADH and NADPH, participating in major redox reactions, cellular homeostasis, metabolism, as well as neurotransmission. In this study we investigated the effects of 22 synthetic nicotinamide derivatives (NAs) to determine how their structural change guide their effects on the cell level and the level of receptors associated with acetylcholine and serotonin neurotransmission. These receptors are key regulators in conditions such as addiction, depression, ADHD, schizophrenia and Alzheimer's disease.

Methods: All derivatives share a common core derived from a niacin-based scaffold, while differing in the substituents and their position on the rings. We assessed the compounds' cytotoxicity, mitotoxicity, ROS level modulation, as well as the inhibition of nicotinic acetylcholine receptors (nAChR) and serotonin receptors (5-HTR) in human neuronal cell line (SH-SY5Y) and human primary muscle cells.

Results: The majority of the compounds didn't show any cytotoxicity on selected cell lines up to 500 µM, although a few induced notable toxicity. Some of these nicotinamide derivatives reduced ROS with the strongest effect observed for the compound containing methoxy moiety (NA-4OCH_3). Our data show that structural modifications strongly influence receptor activity. The most promising compound from the series was the *para*-phenyl-substituted nicotinamide (NA-Ph), and its corresponding isonicotinamide analogue (INA-Ph) having activity in micromolar concentration. Both compounds exhibited selectivity for nAChR modulation. On the other hand, the compound with the highest potency for 5-HTR modulation was the simplest nicotinamide in the series with no substituent (NA-H).

Conclusions: Together, these findings highlight several NAs and INAs as promising leads for the development of selective inhibitors within cholinergic or serotonergic receptor systems.

A pair of "false twins" ncRNAs in *Pseudomonas putida*: study of their role and regulation

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Rationale: *Pseudomonas putida* is a robust Gram-negative bacterium, highly attractive for Biotechnology. It can be used to sustainably produce valuable compounds with enhanced yield using genetic engineering.

However, there is limited knowledge on key players of gene expression regulation in *P. putida*, namely post-transcriptionally: only few small non-coding RNAs (ncRNAs) are functionally characterized; the main ribonucleases (RNases), which process and degrade all classes of RNA, have been studied, but their interplay with ncRNAs remains largely unexplored.

We aim to identify and characterize ncRNAs relevant for stress adaptation of *P. putida* KT2440 (a highly attractive production strain) under conditions mimicking industrial production.

Methods: We collected bacterial samples to extract total RNA for RNA-Seq along a lab-scale industrial bioreactor with two compartments (reference vs. glucose starvation). We performed differential gene expression analysis and predicted novel ncRNAs. Promising novel ncRNAs will be experimentally validated by Northern blot. Deletion mutants of selected known ncRNAs and main RNases will be constructed to study their roles in bacterial stress adaptation.

Results: Multiple ncRNAs (newly and previously identified) were differentially expressed between compartments, indicating a potential role in stress response in industrial context. Amongst the known ncRNAs being expressed throughout the bioreactor, we found a pair of “false twins” ncRNAs that showed distinct expression patterns between each other. Work is underway to identify their mRNA targets and the RNases that regulate their cellular levels.

Conclusions: The “false twins” ncRNAs, despite having a similar sequence, seem to have a different behavior and regulation, being both required for normal cell functioning. The overexpression or deletion of ncRNAs relevant in the bioreactor setting could be used as a tool to fine-tune gene expression towards improving bacterial stress tolerance and production capability.

Cholesterol controls ASC1 function and structural stability

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Rationale: The human ASC1 (SLC7A10) forms a functional heterodimer with the ancillary protein CD98, with a key role in maintaining neutral amino acid homeostasis in neurons, astrocytes and adipose tissue. Due to the high cholesterol content of cell membranes, we sought to elucidate the mechanisms by which ASC1 interacts with cholesterol and oxysterols, using *in silico*, *in vitro* and *ex vivo* models.

Methods: We produced recombinant hASC1 in *E. coli*, performed IMAC purification and reconstitution in proteoliposome enriched with defined sterols. We then analysed protein orientation and kinetics, performed docking simulations, and depleted cholesterol in intact cells to study thermal stability of ASC1 and CD98.

Results: Recombinant hASC1 requires cholesterol-enriched proteoliposomes for full functionality. Transport Vmax showed a cholesterol dependence with 10 fold activation at 250 µg Chol/mg PL. Whereas, Km or protein incorporation and orientation in the proteoliposomes were not affected by cholesterol. Non-enzymatic or enzymatic oxysterols cannot substitute for cholesterol: 7-ketocholesterol or 7α-, 25- and 27-OH-cholesterol present in proteoliposome membrane strongly impaired transport activity. In agreement, depletion of cholesterol in the native cell membrane reduced the thermal stability of both ASC1 and CD98. The latter protein is only required for ASC1 trafficking. Blind docking on the ASC1 3D structure identified four cholesterol-binding clusters. Targeted docking confirmed that both cholesterol and 7-ketocholesterol can fit into one of these cavities. The experimental and in silico data support a direct sterol-protein interaction and provides a structural rationale for the functional effects.

Conclusions: Cholesterol revealed a modulator of ASC1 function and stability in both artificial and native membranes, paving the way for understanding the role of ASC1 also in pathological context characterized by cholesterol oxidation.

Multidirectional neuron-glia mitochondrial transfer and metabolic rewiring

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Rationale: Mitochondrial homeostasis is essential for neuronal function, and neuron–astrocyte metabolic communication plays a central role in brain physiology and pathology. However, the mechanisms governing intercellular mitochondrial transfer and its functional consequences remain poorly understood.

Methods: We analyzed mitochondrial transfer between neurons and astrocytes using fluorescence microscopy, spectral flow cytometry, and qPCR. The state of transferred mitochondria was assessed using the MitoQC mitophagy reporter mouse model. In vivo mitochondrial exchange was studied through viral hSyn(neuronal)-driven expression of mitochondria-targeted (mtYFP, mtmCherry) reporters and retro-orbital systemic administration of exogenous mitochondria.

Results: Neurons and astrocytes internalize exogenous and transferred mitochondria, where mitochondrial integrity is conserved. Systemically administered mitochondria preferentially accumulated in specific brain regions. The persistence of transferred mitochondria is determined by the balance between their integration into the host mitochondrial network and selective processing towards mitophagy, as revealed by MitoQC organelles. In vivo analyses revealed hierarchical patterns of mitochondrial transfer between neurons and astrocytes, that reconfigure a metabolic rewiring of astrocytes towards ketogenesis at the expense of an unexpected lowered mitochondrial respiration whereby TCA is bypassed.

Conclusions: Our findings show how neural cells spontaneously and hierarchically exchange mitochondria, or acquire them as when transplanted. Mitochondrial incorporations metabolically rewire acceptor astrocytes to produce and release keto bodies. Altogether, we provide evidence of mitochondrial transfer as a tightly regulated mechanism that reshapes the functional and metabolic phenotype of neural cells.

Modulation of redox homeostasis and inflammatory responses by *Vaccinium myrtillus* extract in microglial cells

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Rationale: The regulation of neuroinflammation and oxidative stress is critical in the onset and progression of neurodegenerative and metabolic disorders. Identifying natural therapeutic agents that can modulate these processes is therefore of major scientific interest. This study investigates the antioxidant and anti-inflammatory effects of *Vaccinium myrtillus* (VM) extract using *in vitro* BV-2 microglial models, including both wild-type and Acox1-deficient lines.

Methods: Antioxidant activity was assessed using the DPPH assay. Cytotoxicity was evaluated by the MTT assay, peroxisomal enzyme activities (ACOX1 and catalase) were measured spectrophotometrically or fluorometrically, nitric oxide production by the Griess assay, protein expression by Western blot, and mRNA levels of inflammatory markers by RT-qPCR.

Results: Enzymatic activity measurements showed that lipopolysaccharide (LPS) stimulation led to an approximate 40% reduction in ACOX1 activity, while co-treatment with VM extract restored enzyme activity to baseline levels. Similarly, the LPS-induced increase in catalase activity and expression was significantly attenuated by the extract, suggesting a role in maintaining redox homeostasis. Additionally, the extract modulated the expression of *Abcd1*, a key peroxisomal fatty acyl-CoA transporter, indicating an impact on peroxisomal function and lipid transport. Furthermore, the VM extract exhibited pronounced anti-inflammatory effects, evidenced by reduced LPS-induced nitric oxide (NO) production and downregulation of pro-inflammatory cytokines, including *Tnf-α* and *Il-1β* mRNA expression.

Conclusions: These findings underscore the potential of *Vaccinium myrtillus* extract as a neuroprotective agent capable of mitigating oxidative stress and neuroinflammation, possibly through the modulation of peroxisomal metabolism. Future research should further examine the molecular mechanisms, particularly those involved in redox regulation and peroxisomal signalling, to better elucidate its therapeutic potential.

Exploring molecular dynamics and ligand interactions of nucleophosmin C-terminal domain in acute myeloid leukemia

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Rationale: Mutations in the Nucleophosmin 1 (NPM1) gene are the most common genetic alteration in adult Acute Myeloid Leukemia (AML). These frameshift mutations cause the loss of two key tryptophan residues (Trp288 and Trp290) in the NPM1 C-terminal domain (CTD), leading to its unfolding. As the proper folding of the CTD is critical to mediate NPM1 nucleolar localization, AML-mutated NPM1 ($NPM1^{AML}$) is aberrantly delocalised to the cytoplasm in leukemic blasts. Recently a synthetic microbiota-inspired compound (UCM-13369) has shown promising therapeutic potential for NPM1-mutated AML, as it can specifically bind to the CTD of $NPM1^{AML}$ and restore $NPM1^{AML}$ nucleolar localization.

Methods: Saturation Transfer Difference Nuclear Magnetic Resonance (STD-NMR) experiments were carried out to identify the epitopes of interaction of a UCM-13369-derived compound with $NPM1^{AML}$ CTD, using the wild-type protein as a control ($NPM1^{WT}$). Furthermore, *in silico* equilibrium molecular dynamics simulations were performed to explore the dynamic behavior of both $NPM1^{WT}$ and $NPM1^{AML}$ CTD.

Results: *In vitro* assays indicated that $NPM1^{AML}$ CTD – UCM-13369-derived compound interaction is restricted to the two aromatic rings of the antileukemic molecule, as shown by a higher STD-NMR signal intensity compared to the $NPM1^{WT}$ CTD interaction. *In silico* molecular dynamics simulations revealed structural differences between $NPM1^{AML}$ CTD and $NPM1^{WT}$ CTD, confirming the reduced dynamic stability of the mutant CTD.

Conclusions: Collectively, the results suggest increased dynamics of the NPM1-CTD when the hydrophobic aromatic core is absent in the AML variant, as well as higher interaction affinity with the two aromatic rings of the antileukemic compound. It remains to be explored if the interaction retrains these dynamics to prevent domain unfolding which may constitute the underlying molecular mechanism driving the reversion of the $NPM1^{AML}$ cytoplasmic localization leukemic phenotype.

Aerobic physical exercise under a persistent obesogenic diet reverses biometric alterations and partially mitigates sperm quality loss in a mouse model

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Rationale: Obesity contributes to male infertility, impairing testosterone production and sperm quality. Physical exercise offers a strategy to mitigate/revert reproductive dysfunction in adulthood, preventing lifelong obesity and its consequences, even without obesogenic diet cessation. We investigated the mitigating effects of aerobic exercise initiated in adulthood following early-life obesity, without dietary correction.

Methods: A *Mus musculus* model (n=12/group) was established: a sedentary control group fed with standard diet (CTRL; standard diet: 60% carbohydrates, 21% proteins, 8% fat), a sedentary group fed with high-fat diet (HFD: 36% carbohydrates, 21% proteins, 36% fat), and exercised group fed with high-fat diet subjected to a treadmill protocol (HFD_EX; 60-75 % of maximal speed, 0% inclination, 1 hour/day, 5 days/week). Diets were maintained for 200 days. The exercise protocol began after a 60-day sedentary period and continued until day 200. Biometric characterization included body weight, fasting glycemia, and plasma testosterone. Testis and epididymis were collected for weighing and sperm analyzed following WHO guidelines.

Results: HFD increased body weight and glycemia and decrease plasma testosterone. It also decreased sperm motility, vitality, concentration, and normal morphology, while increasing middle piece abnormalities. Treadmill exercise after 60 days of HFD and sedentarism reduced body weight and glycemia and improved all sperm parameters, even without HFD discontinuation. However, sperm motility was the only parameter that was fully restored to control levels, while the remaining parameters, although improved compared to the HFD group, remained below control.

Conclusions: Our findings suggest that aerobic physical exercise can reverse body weight and glycemia and partially mitigate sperm quality loss caused by obesogenic diets, even without its discontinuation. Next in our work pipeline, we will include metabolomics analysis to elucidate the underlying biochemical pathways.

Metabolic consequences of artificial diets in honey bees

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Rationale: Pollen scarcity driven by habitat loss or monoculture landscapes force beekeepers to use pollen substitutes to support brood rearing. However, the metabolic consequences of these diets for adult bees remain poorly characterised. This study applies targeted metabolomics and lipidomics to determine how natural pollen, commercial substitutes, and microalgae-based diets shape honey bee nutritional physiology.

Methods: Bees were fed sucrose only (CTRL), multifloral pollen (POL), Chlorella microalgae (ALG), or UltraBee (UB) for 10 days. Hemolymph was analysed using targeted LC–MS metabolomics (126 metabolites) and lipidomics (>200 lipid species). Data were QC-normalised and evaluated using multivariate and univariate statistics, and lipid-class enrichment to identify nutritional signatures.

Results: Artificial diets caused marked deviations from the balanced amino-acid profile of pollen-fed bees. ALG bees additionally showed increased 3-methylhistidine, a marker associated with elevated protein turnover. All protein-containing diets led to reduced trehalose (a major carbohydrate energy storage in insects) and increased glucose compared with a sugar-only diet. UB stimulated the strongest monosaccharide mobilisation and ALG the weakest, indicating diet-specific modulation of carbohydrate allocation and energetic metabolism. Lipidomics identified distinct diet-related lipid signatures, with pollen displaying natural balanced profiles and artificial diets showing substantial shifts across several lipid classes.

Conclusions: In conclusion, targeted metabolomics and lipidomics revealed that artificial diets induce metabolic imbalances that do not occur in pollen-fed bees. These results demonstrate that omics approaches provide an informative framework for evaluating the physiological suitability of pollen substitutes, and highlight the need to design formulations that more closely reproduce the metabolic response of natural pollen.

Creatine hydrochloride and creatine ethyl ester supplementation: an 8-week evaluation of clinical biomarkers in midlife women

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Rationale: Growing interest in creatine's role in perimenopausal and menopausal metabolic health highlights its potential to support key metabolic outcomes, with clinical biomarkers offering insight into the effects of new, highly soluble creatine formulations during midlife and menopause-related metabolic changes.

Methods: Thirty-six healthy perimenopausal and menopausal women (mean age 50.1 ± 5.7 years; BMI 23.8 ± 3.03 kg/m^2) were randomized to low-dose creatine hydrochloride (750 mg/day; Group 1), medium-dose creatine hydrochloride (1,500 mg/day; Group 2), creatine hydrochloride plus creatine ethyl ester (800 mg/day; Group 3), or placebo (Group 4). Menopause was defined as ≥12 months of amenorrhea, and perimenopause as ongoing menstruation with typical symptoms. Fasting serum FSH, LH, IL-6, TNF-α, and amyloid-β were measured by ELISA, while GAA, creatine, and creatinine were quantified by HPLC and standard biochemical parameters by automated methods.

Results: Friedman's two-way ANOVA by ranks showed significant interaction effects for LDL cholesterol ($p = 0.01$) and triglycerides ($p = 0.04$), with Group 3 exhibiting the greatest reductions. Serum creatine kinase decreased in the placebo group ($p = 0.04$), and serum glucose decreased in Group 1 ($p = 0.02$). Hormonal and inflammatory markers showed no significant changes, except for a reduction in amyloid-β in the placebo group at 8 weeks ($p = 0.02$). GAA, creatine, and creatinine remained stable overall, though an interaction effect was observed for serum creatine ($p = 0.03$).

Conclusions: Eight weeks of combined creatine hydrochloride and creatine ethyl ester appears to exert pro-metabolic effects on lipid profiles, while low-dose creatine hydrochloride beneficially modulates fasting glucose. Preserved serum amyloid-β levels across intervention groups suggest a possible neuroprotective effect. Overall, these findings highlight the potential metabolic benefits of highly soluble creatine formulations in (peri)menopausal women.

Proteomic profiling of FAM20C interactors: investigating the biological functions of the master kinase of the secretory pathway

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Rationale: This work aims to investigate the biological pathways in which the recently discovered FAM20C is involved. FAM20C is a largely unexplored Golgi kinase of the secretory pathway linked to Raine syndrome, a bone disorder.

Methods: To reach this goal, we profiled the interactome of the kinase performing the proximity-dependent biotin identification method. Briefly, a biotinylating enzyme fused with FAM20C and expressed in cells, biotinylates all the proteins in proximity to the kinase, that are then purified and identified by mass spectrometry. With the aim of obtaining robust results, we analyzed samples using diverse negative controls (biotinylating enzymes with or without different Golgi targeting sequences), using two different biotinylating enzymes (BioID2 and UltraID) and stringent bioinformatic parameters. Moreover, we performed the standard co-immunoprecipitation technique highlighting the complementarity of these two approaches.

Results: We found that one of the most significant biological processes linked to FAM20C is collagen fibril organization, a crucial determinant of the extracellular matrix integrity. Even more intriguingly, we identified a novel relation between FAM20C and the post-translational modification peptidyl-lysine hydroxylation. The hypothesis we are currently exploring is that the influence of FAM20C in collagen organization may be mediated by the hydroxylation, as a post-translational cross-talk. This finding is crucial for a better understanding of tissue structure and function. Finally, the co-immunoprecipitation technique allowed us to discover another novel biological function of FAM20C, opening promising avenues of research: the transport of the zinc ion, a crucial cofactor and regulator of multiple cellular functions.

Conclusions: In conclusion, our robust results highlight novel biological functions linked to FAM20C that we are currently investigating.

BRG1 as an epigenetic regulator of the invasion of colorectal cancer cells

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Rationale: Cancer cell survival in hypoxia is primarily regulated by the hypoxia-inducible factor (HIF). HIF-driven processes enable cancer cells to acquire new properties such as neovascularization, epithelial-mesenchymal transition (EMT), and tissue invasion, which contribute to tumor progression and metastasis. Chromatin accessibility of HIF target genes is a crucial element in the control of the HIF response. The SWI-SNF chromatin remodeling complex, which uses ATP to modify chromatin structure, plays a key role in this process. The central ATPase of this complex is either BRG1 (SMARCA4) or BRM (SMARCA2). This study aimed to evaluate the transcriptional and epigenetic role of BRG1 in the invasion of colorectal cancer cells.

Methods: We employed three-dimensional (3D) culture systems that accurately reflect core hypoxia and other tumor microenvironmental features. Additionally, we performed multiomics analysis to reveal the transcriptional and epigenetic mechanisms by which BRG1 regulates the invasion of cancer cells in a hypoxia-dependent manner.

Results: We found that knockdown of BRG1 in DLD-1 colon cancer cells changed the type of cell movement, increasing their invasiveness and motility. Moreover, next-generation sequencing data revealed that knockdown of BRG1 led to different expression patterns of multiple genes involved in the hypoxia response, EMT, and tumor invasion processes.

Conclusions: Most prior investigations of BRG1 in cancer have relied on two-dimensional (2D) culture systems, which do not recapitulate the spatial complexity of tumors. In contrast, our findings derived from 3D culture models demonstrate a pivotal epigenetic role for BRG1 in regulating cellular invasion under hypoxic conditions and emphasize the necessity of physiologically relevant model systems in cancer research.

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Extracellular electron transfer in cyanobacteria

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Rationale: Cyanobacteria can export electrons to their surroundings through extracellular electron transfer (exoelectrogenesis), a process that has potential for bioenergy applications. However, the molecular mechanisms and biochemical pathways of exoelectrogenesis have not been elucidated

Methods: To map electron transfer outside the cell, we examined exoelectrogenesis in the model cyanobacterium *Synechocystis* sp. PCC 6803 using biofilm photoelectrochemistry. Current generation was measured under different trophic regimes, including photoautotrophy and with exogenous glucose. We analysed mutants impaired in respiratory terminal oxidases (COX, Cyd), cyclic electron transfer (NDH-1), and carbon metabolism (glycogen turnover and the oxidative pentose phosphate pathway). To identify endogenous electron mediators exported by the cells, we are assessing the proteome and metabolome of both cells on the electrode and the extracellular medium post-harvesting current.

Results: Wild-type cells exhibit some current in the dark and an increased current in the light [1]. We observed that glucose supplementation markedly increased dark current and enhanced the initial light-driven current by up to two orders of magnitude compared with photoautotrophic conditions. However, prolonged growth with glucose led to a shift toward cathodic (negative) currents. Mutant strains displayed distinct kinetics of current evolving under dark to light transitions, highlighting the interplay between photosynthetic, respiratory, and metabolic processes during exoelectrogenesis

Conclusions: Together these results suggest that exoelectrogenesis in cyanobacteria is a bidirectional process shaped by organic carbon availability and fuelled by photosynthesis. Ongoing ‘omics analyses will help elucidate the molecular players driving this biochemistry. A deeper mechanistic understanding will support the rational engineering of cyanobacteria for sustainable bioelectrochemical technologies.

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Investigation of the regenerative potential of proteoglycan 4a in the brain in zebrafish models of traumatic brain injury and demyelination

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Rationale: Traumatic brain injury (TBI) is a leading cause of global disability with limited treatment options beyond stabilization. Previous transcriptomic analysis revealed a dramatic increase in *prg4a* expression compared to uninjured controls during zebrafish brain regeneration. Consequently, this study aims to unravel the functional role of *prg4a* in regeneration following TBI and non-traumatic brain injury (NTBI).

Methods: To determine the role of *prg4a*, we employed CRISPR-Cas9 technology to generate loss-of-function mutants and the Tol2 transposase system for gain-of-function overexpression. Adult zebrafish were subjected to TBI via telencephalic stab injury or NTBI via cerebroventricular microinjection. Regenerative capacity was assessed at 3 and 7 days post-injury (dpi) using immunofluorescence to quantify cell proliferation (PCNA), neurogenesis (HuC/D), and glial reactivity (GFAP). Additionally, qPCR and Western blot analyses were performed to quantify the expression of specific signaling pathways, immune response factors, and ECM remodeling markers.

Results: We present immunofluorescence images and quantification, characterizing the regenerative competency of *prg4a*. Our investigation focuses on characterizing the spatial distribution of *prg4a* within the injury microenvironment. Furthermore, qPCR and Western blot data reveal the differential regulation of key signaling pathway components, ECM remodeling, and immune response markers following injury. These molecular profiles define the impact of *prg4a* expression levels on the progression of brain repair.

Conclusions: We anticipate that this study will elucidate the functional role of *prg4a* as a critical ECM-based regulator of the regenerative niche by integrating histological data with transcriptional and protein-level analysis. These findings provide new insights into the molecular machinery of brain regeneration and highlight *prg4a* as a potential target for therapeutic intervention in brain injury.

Development of an *in vitro* model to evaluate aquaporin-5 contribution to immunosuppressive tumor microenvironment in pancreatic ductal adenocarcinoma

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Rationale: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most aggressive and deadly malignancies, characterized by a profoundly immunosuppressive tumor microenvironment (TME). Although immunotherapeutic strategies have advanced considerably, their success in PDAC has been minimal. Aquaporins (AQPs), a family of membrane channels involved in the passive transport of water and some small solutes, are frequently overexpressed in PDAC and have been associated with increased tumor aggressiveness and poorer overall survival. In this context, this work aims to investigate the role of AQPs in cancer immunotherapy by evaluating their impact on modulating TME components. Ultimately, we seek to determine whether AQPs could serve as therapeutic targets or prognostic biomarkers capable of improving outcomes for PDAC patients.

Methods: To achieve this, we established and characterized two murine PDAC cell lines: Panc02 and KPC. qPCR and Western blot analyses identified AQP5 as the most prominent isoform, with expression levels in Panc02 comparable to those typically found in tumorigenic cells. In contrast, immunofluorescence and stopped-flow assays indicated higher AQP5 expression in KPC cells. Given AQP5 relevance in this type of cancer, we decided to generate AQP5-overexpressing variants and validate their efficacy.

Results: Cells overexpressing AQP5 showed a 60-fold higher gene expression compared to the control, detected by qPCR and corroborated by immunofluorescence, and a higher AQP5 expression localized at the membrane. Finally, AQP5-overexpressing cells showed a higher rate of cell proliferation (control cells with 20h of doubling time *vs* 17h for overexpressing cells) and rate of cell migration *(100 ± 15.00* (%) vs *119 ± 16.97* (%)*)*.

Conclusions: These modified lines will be further implanted in murine models to assess tumor progression, immune infiltration, and therapeutic responses, aiming to unveil more effective and personalized immunotherapy strategies for PDAC.

Targeting RNase HI: structure-guided drug design to combat *Neisseria gonorrhoeae*

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Rationale: Neisseria gonorrhoeae is a significant global pathogen, estimated to cause over 80 million new infections annually, with rapidly rising rates of antibiotic resistance that threaten our ability to effectively treat infection. RNase HI is responsible for the resolution of R-loops (RNA:DNA hybrids that are lethal if not effectively resolved), and is essential in N. gonorrhoeae, making it an exciting target for the development of novel antibiotics.

Methods: A compound library was designed for screening, with many incorporating a canonical metal-chelating motif of three oxygens tailored to the enzyme's active site. A FRET-based enzyme assay enabled rapid screening of compound potency against purified N. gonorrhoeae RNase HI, while a complementary live/dead viability assay assessed antibacterial efficacy in cultured cells. X-ray crystallography was used to determine the structure of the enzyme and soaking and co-crystallisation experiments were utilised to determine the interactions of inhibitor compounds with the enzyme.

Results: Several compounds have emerged as dual-activity hits, inhibiting RNase HI catalysis and reducing N. gonorrhoeae viability at low micromolar concentrations. To confirm the specificity of these hits, compounds were then counter-screened against Human RNase H1, and compounds were only progressed if they showed low potency against the human enzyme. We have determined for the first time the apo N. gonorrhoeae RNase HI structure, as well as the interactions of an identified inhibitor compoundwithin the enzyme active site.

Conclusions: Our integrated biochemical, microbiological and structural approach confirms RNase HI as a viable target in N. gonorrhoeae. This work delivers a framework for the iterative optimisation of inhibitor potency and selectivity and gives promise for developing RNase HI inhibitors as a new class of N. gonorrhoeae therapeutics capable of overcoming existing antibiotic resistance mechanisms.

Evaluating photodynamic antibacterial efficacy and protein expression dynamics in *rickettsia* infection models

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Rationale: *Rickettsiae* are small Gram-negative obligate intracellular bacteria that replicate within the cytoplasm of vascular endothelial cells. In Slovakia, they are mainly transmitted by ticks and several species cause spotted fever group rickettsioses. Carbon quantum dots (CQDs) are emerging fluorescent nanomaterials investigated for photodynamic therapy (PDT) as an alternative antibacterial strategy. Under light activation, CQDs generate superoxide radicals that inactivate bacteria while sparing mammalian cells. CQDs are bio- and hemocompatible, supporting their therapeutic potential.

Methods: Infected cells without photodynamic inactivation (PDI) served as controls. DNA/RNA were isolated from PDT samples using the AllPrep DNA/RNA Kit, RNA was reverse-transcribed, and qPCR targeting *ompB* quantified viable *R. conorii* using standards from 3×10^{0}–3×10^{6} copies. PDI efficiency was calculated as R (%) = [(a – b) / a] × 100 [3], and statistical significance assessed by the Mann–Whitney U test ($p < 0.05$). Proteins were precipitated with chloroform/methanol, quantified by Bradford assay, separated by SDS-PAGE, purified by ZipTip, and analyzed by MALDI-MS.

Results: RT-PCR analysis demonstrated approximately 98% antimicrobial efficacy after 72 h of treatment. Proteomic profiling of *R. conorii*-infected Vero cells using Waters Synapt mass spectrometry and ProGenesis QI revealed extensive dysregulation of bacterial proteins linked to growth, replication, and ATP generation. Notably, proteins associated with Rickettsia LPS biosynthesis increased, while those involved in ATP synthesis and DNA repair decreased. In host Vero cells, detoxification pathways and central metabolic processes were the most prominently altered.

Conclusions: These findings provide CQD-based PDT as a potential strategy against intracellular pathogens and antimicrobial resistance. This research was supported by VEGA 02/0011/25 and APVV 23-0325.

Discovery of novel immunity systems against plasmids in the opportunistic pathogen *Acinetobacter baumannii*

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Rationale: Horizontal gene transfer (HGT) plays a crucial role in bacterial evolution, spreading elements that can harbour antibiotic resistance genes. Although multiple mechanisms protecting against phages (HGT vectors) have been identified in recent years, defense against plasmids remains poorly understood, with only a few isolated cases studied so far [2, 3].

Methods: High-throughput assays were used to quantify the transfer of two plasmids by conjugation to a collection of 364 *A. baumannii* strains. Random mutagenesis allowed the identification of genes impairing this transfer. Functional genetics confirmed their role *in vivo*; live-cell imaging (microscopy, flow cytometry) and protein characterization were used to study their molecular mechanism.

Results: Conjugation efficiencies were found highly variable and unrelated to the phylogeny. Multiple transfer-inhibiting genes were identified, including four encoding proteins of unknown function. Phylogenetic analyses indicate that these candidates are very rare, standalone, highly specific anti-plasmid defenses. The defenses do not seem to target conjugation itself as their presence in a cell is incompatible with that of their target plasmid, whatever the mode of entry. Our findings suggest that they act through a DNA-degradation or "Abortive conjugation" (induced cell death) mechanism.

Conclusions: The ability of strains to receive plasmids strongly varies, indicating a rapidly-evolving, non-conserved trait. Rare, multiple, single-gene defenses like the four candidates identified, might be a key component of this variability. Interestingly, the anti-plasmid defenses are plasmid-specific, suggesting the existence of plasmid-borne anti-defenses. This work reveals the existence of a new class of small, monogenic, and widely distributed bacterial defense against plasmid infection.

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Investigating the effects of a small heat shock protein, HSPB1 on glial activation in a mouse model of Alzheimer's disease

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Rationale: Our group previously found that heat shock protein B1 (HSPB1) can improve certain symptoms of Alzheimer's disease (AD). HSPB1 has chaperoning function, which would explain this effect. However, recent findings have been shown that it can influence inflammatory responses. Chronic neuroinflammation and glial activation contribute to the pathology of AD, and we hypothesise that HSPB1 may influence these processes.

Methods: In the present study, we aimed to investigate the role of HSPB1 in chronic neuroinflammation and glial activation in a mouse model of AD. To this end, the AD model (APP/PS1) mouse strain was crossed with an HSPB1-overexpressing line. Mortality was monitored, and the expression pattern of HSPB1 was assessed using immunofluorescence staining. Neuroinflammation and glial activation in the hippocampus and cortex were evaluated by qPCR and immunohistochemistry.

Results: Due to a high incidence of epileptic seizures, female APP/PS1 mice exhibit higher mortality, which was markedly reduced by HSPB1 overexpression. Immunofluorescence staining confirmed HSPB1 accumulation and the presence of activated glial cells in proximity to Aβ plaques. Furthermore, immunohistochemistry revealed that the activation of microglia cells in female APP/PS1/HSPB1 mice increased. Moreover, a significant elevation in the expression of inflammatory, astrocytic, and microglial marker genes was observed in both the cortex and the hippocampus of APP/PS1 mice compared to wild-type controls. However, HSPB1 overexpression reduced cortical TNFα levels in male APP/PS1 mice and raised the expression of anti-inflammatory microglial marker genes in APP/PS1 mice brain.

Conclusions: Taken together, our results indicate that HSPB1 exerts protective effects in the APP/PS1 model, as reflected by reduced mortality in APP/PS1/HSPB1 mice. Moreover, overexpression of HSPB1 may influence the activation of the microglia cells promoting tissue repair.

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IPET: an excellent tool for diagnostic applications for human pathologies

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Rationale: This research focuses on developing a novel redox-biology tool to detect, identify, quantify, and monitor changes in redox-sensitive cysteine modifications in proteins, addressing the current lack of effective, sensitive, and affordable commercially available tools for quantitative and comprehensive redox proteomics analysis. Furthermore, this approach was evaluated for its potential as a biomarker for lung cancer progression.

Methods: This approach involves the design, synthesis, and application of two differentially sized alkylating reagents, Iodo-Peptide Enrichment Tag (IPET), which specifically target reduced cysteine thiols. IPET refers to modified peptides that are chemically synthesized using solid-phase peptide synthesis (SPPS). This strategy enables the analysis of two differentially labeled samples within a single high-resolution LC-MS/MS run. Furthermore, data analysis using computational MSFragger (FragPipe & IonQuant integration) and Perseus software maximizes peptide coverage and enables consistent quantification of redox-sensitive cysteine modifications.

Results: To optimize IPET specificity and cleavage, we tested labeling conditions on a small cysteine-containing peptide, successfully labeling it with light or heavy reagents at a 1:1 ratio. Applying the workflow to yeast achieved >98% labeling efficiency and detected over 90% of cysteines. A panel of IPET variants with different alkylation, cleavage, and enrichment groups was synthesized and validated. Redox-proteomics of purified BSA showed improved identification and selectivity, and preliminary results indicate strong compatibility of IPET for redoxome profiling of serum and plasma from healthy individuals and cancer patients.

Conclusions: Based on our results, we have successfully established a novel protocol that detects over 98% of cysteine residues across diverse samples—including model peptides, purified BSA, yeast, and serum or plasma from both healthy individuals and cancer patients. Furthermore, our computational platform reliably quantifies the redox status of all labeled cysteines.

Integrative text-mining and computational analysis identifies key genes and Src-targeting drug candidates for prostate cancer

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Rationale: Prostate cancer is increasing at an alarming pace globally. Available information in PubMed displays gene alterations in prostate cancer. However, the data is dispersed across multiple publications, making it challenging for mankind.

Methods: In this study, we employed an *in-silico* text-mining strategy using the **pubmed.mineR** package of RStudio to systematically collect, organize and analyze relevant published findings from PubMed. The genes were further examined through the construction of a **protein-protein interaction (PPI) network,** and their biological functions were annotated using **Gene Ontology (GO) analysis** via the **DAVID** tool. Modules were fetched using MCODE by keeping a specific threshold value. Furthermore, hub genes were retrieved by utilizing all the algorithms of cytoHubba. The most frequent hub gene was docked with FDA approved drugs sourced from ZINC database. Two promising drug candidates were then subjected to **molecular dynamics (MD) simulations** to evaluate the stability of their complexes with Src.

Results: By combining automated text mining with manual curation, we identified **2,357 genes** implicated in prostate cancer. Subsequently, **four gene clusters** were extracted from the PPI network using **MCODE** and the **10 hub genes** were identified through comprehensive analysis using all algorithms available in **cytoHubba**. Among these, **SRC, TP53 and CTNNB1** emerged as central players potentially involved in prostate cancer pathogenesis. In the second phase of the study, the **Src protein** (PDB ID: **2H8H**) was selected as the target for **molecular docking** with FDA-approved drugs. Our study highlights **Budesonide** and **ICG** as potential therapeutic agents of clinical interest.

Conclusions: ICG has the potential in improving the diagnosis of prostate cancer by enhancing tumor visualization and clearly demarcating tumor margins [1]. Additionally, the emerging anticancer effects of budenoside in lung cancer [2] and pancreatic cancer [3] further highlight the need to explore its potential as a diagnostic/prognostic/therapeutic agent in prostate cancer.

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Endoplasmic Reticulum oxidoreductin 1 (ERO1) as a key driver of Loss-of-function RYR1 myopathy

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Rationale: Loss-of-function mutations in the RYR1 gene, including the I4898T variant (I4895T in mice), are among the most common causes of congenital myopathies, yet no FDA-approved therapies are available. Previous studies identified a link between this mutation and endoplasmic reticulum (ER) stress, but the underlying mechanism remains unclear.

Methods: Here, we generated a murine model carrying the I4895T RYR1 mutation and lacking CHOP, which lies upstream of ERO1, and we examined ER-stress mediators, mitochondrial function, and sarcoplasmic reticulum (SR)/mitochondrial ultrastructure through biochemical assays and quantitative transmission electron microscopy.

Results: I4895T RYR1 primary myotubes displayed elevated ER-stress markers—including ERO1 upregulation—which were reduced in CHOP-deficient cells. Gene set enrichment analysis revealed downregulation of myogenesis and Oxidative Phosphorylation (OXPHOS) pathways. Treatment with the chemical chaperone TUDCA or a novel ERO1 inhibitor rescued myotube differentiation, linking ER stress to defective myogenesis. ERO1 inhibition increased mitochondrial membrane potential after thapsigargin exposure, suggesting a key role for ERO1 in ER-stress-mediated mitochondrial dysfunction. Current analysis of calcium handling in the ER and mitochondria is examining the contribution of ERO1 upregulation to mitochondrial Ca^{2+} overload, linking ER stress to mitochondrial structural and functional alterations. Ultrastructural analysis of I4895T RYR1 diaphragms showed impaired mitochondrial morphology.

Conclusions: These findings demonstrate that ERO1 upregulation, induced by ER stress, plays a critical role in disease progression in I4895T RYR1 mutants. Thus, we identify ERO1 as a target for the development of pharmacological treatments for RYR1-related myopathies.

Decoding the interaction blueprint of the μ-opioid receptor and β-arrestins

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Rationale: The μ-opioid receptor (MOR) is a G protein-coupled receptor (GPCR) whose signaling is regulated by β-arrestins, which can mediate side effects of opioid-based pain therapies. We aimed to map the MOR–β-arrestin interface at single-residue resolution to provide structural insights for understanding receptor regulation and helping to develop pain medications with reduced side effects.

Methods: Due to the lack of 3D structures for MOR signaling complexes, we employ genetic code expansion to map the β-arrestin–MOR interaction. By incorporating an electrophilic, genetically encoded unnatural amino acid into β-arrestins and selectively replacing cysteines in the receptor, we induce a proximity-driven reaction, allowing us to probe the MOR–β-arrestin interface in live mammalian cells with single-residue resolution.

Results: A systematic scan of the intracellular surface of MOR reveals a complex network of interactions that link the receptor's intracellular loops and C-terminal tail to the N-terminal domains of β-arrestin-1 and β-arrestin-2. These data provide a comprehensive set of structural constraints that will be instrumental in developing an accurate model of the MOR–β-arrestin-1/2 complex, for which no experimental structure is currently available.

Conclusions: We characterized MOR–β-arrestin interactions in live cells, defining structural constraints that support accurate modeling of the complex and improve insight into β-arrestin regulation of GPCRs.

Taylor dispersion analysis reveals how terminal modifications and isoform differences reshape early amyloid-β aggregation pathways

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Rationale: Early amyloid-β (Aβ) aggregation determines the formation of toxic intermediates in Alzheimer's disease, yet the impact of N-terminal modifications and isoform differences on these early pathways remains unclear. Defining these effects is essential for understanding why closely related Aβ variants display distinct aggregation behaviors.

Methods: In this work we used Taylor Dispersion Analysis (TDA) to resolve monomers, oligomers, protofibrils, and large non-diffusing species during the aggregation of Aβ42, pyroglutamate-modified Aβ3–42 (p-EAβ3–42), and C-terminally truncated Aβ40. Defined mixtures enabled assessment of how isoform composition alters intermediate formation.

Results: Aβ42 showed rapid monomer loss followed by oligomers, protofibrils, and large-aggregate spikes. Aβ40 alone aggregated slowly, consistent with earlier findings [1, 2]. p-EAβ3–42 displayed a sigmoidal oligomer increase but no protofibrils and far fewer spikes, indicating a distinct pathway. Coaggregation modified these behaviors: in equimolar Aβ42:p-EAβ3–42 samples, oligomers, protofibrils, and spikes all appeared, but spike abundance was reduced relative to Aβ42 alone, showing that N-terminal modification reshapes both pathway topology and aggregate size distribution. These results align with the known distinct aggregation tendencies of Aβ isoforms, including the enhanced pathogenicity of N-terminally modified species.

Conclusions: TDA thus reveals how terminal modifications and isoform mixing redirect early Aβ assembly routes, altering intermediate populations relevant to pathogenic progression. The distinct detected intermediates may help explain why some Aβ variants accumulate preferentially in plaques. Moreover, the ability of TDA to separate coexisting species underscores its value for dissecting heterogeneous aggregation mechanisms.

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A dual-source biological shield: comparative scavenging mechanisms of plant and mammalian extracellular vesicles against cisplatin neurotoxicity and ototoxicity

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Rationale: Cisplatin triggers oxidative stress–driven neuro- and ototoxicity, yet current protective options are insufficient. We investigated whether bioactive cargo carried by mammalian and plant extracellular vesicles (EVs) can provide a protective effect against cisplatin-induced damage.

Methods: EVs were isolated from NRF2-overexpressing neural progenitor cells and plant cell suspension cultures using ultracentrifugation&ultrafiltration-based protocol; size and purity were confirmed by nanoparticle tracking analysis and TEM. *In vitro* neurotoxicity was induced in mature neural cells exposed to cisplatin, and EVs were pre-applied to assess viability, oxidative stress, and molecular markers. For *in vivo* evaluation, rats received cisplatin followed by transtympanic EV delivery. Auditory function was measured by auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE). Neurocochlear tissues were examined histologically to assess structural injury

Results: *In vitro* analyses demonstrated that cisplatin exposure markedly reduced neuronal cell viability and increased oxidative stress, whereas pre-treatment with both mammalian- and plant-derived EVs significantly attenuated these toxic effects, as previously demonstrated [1]. *In vivo*, cisplatin administration induced clear neuro- and ototoxic impairments, reflected by deteriorated ABR thresholds, reduced DPOAE amplitudes, and structural degeneration in cochlear and neuronal tissues. Transtimpanic delivery of EVs substantially mitigated these functional and histological deficits.

Conclusions: Mammalian and plant EVs demonstrate protective effects against cisplatin-induced neuro- and ototoxicity *in vitro* and *in vivo*. These findings support EVs as promising biological candidates for reducing chemotherapy-related sensory toxicity.

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Targeting spleen tyrosine kinase SH2 domains disrupts survival pathways in acute myeloid leukemia

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Rationale: Spleen Tyrosine Kinase(Syk) is an important oncogenic regulator in Acute Myeloid Leukemia(AML) pathogenesis, supporting survival and drug resistance. Since conventional ATP-competitive Syk inhibitors are limited by low specificity and drug resistance, this highlights the need for new therapeutic strategies, such as targeting the allosteric Src Homology 2(SH2) domains, which mediate critical protein-protein interactions (PPIs).

Methods: Z2155444005(-66.782 kcal/mol) and Z260816155(-61.79 kcal/mol), which had the best binding energies according to MD analyses [1], were confirmed to bind to the C-SH2 domain of Syk enzyme in vitro by Differential Scanning Fluorimetry (DSF) and Intracellular FRET-based Syk Biosensor. Biological activities in AML cells, THP-1 and HL-60, showing high Syk expression were evaluated by cell viability using MTT assay,apoptosis and cell cycle effects by Flow Cytometry, modulation of survival pathways (NF-κB-related genes and Bcl-2 family proteins) by R-T PCR and Western Blotting.

Results: DSF results indicated that both compounds cause a significant increase in the protein's thermal stability, which provides clear evidence of a protein-ligand interaction. Both inhibitors strongly bound to the R175 region of the SH2 domain of the biosensor, inhibiting the FRET response.The candidates used decreased cell viability at low micromolar concentrations (1-25 μM) in both cells and, in a correlated manner, induced apoptosis and caused cell cycle arrest cells compared to control cells. Furthermore, they modulated the gene expression of TNF-α and IL-1β and effectively suppressed the anti-apoptotic proteins Bcl-2 and Bcl-xL.

Conclusions: Both candidates successfully target and inhibit the Syk-C-SH2 domain, effectively disrupting critical AML survival pathways, validating SH2 targeting as a novel AML strategy.This thesis was funded by TUBITAK (124Z119) and TUSEB (22905).

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Promoter-level regulation of ADAMTS8 by TGF-β1: evidence for multi-pathway control in colon cancer cells

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Rationale: In colon cancer (CC), the transforming growth factor-β (TGF-β) signalling pathway has a well established biphasic role: tumour-suppressive in early stages and tumour promoting in advanced disease. The tumour suppressive metalloproteinase ADAMTS8 is frequently downregulated in cancer, often through promoter methylation. This study aimed to investigate how TGF-β1 regulates ADAMTS8 gene expression at the promoter level in CC and to determine the intracellular signalling pathways contributing to this regulation.

Methods: SW480 cells were transiently transfected with luciferase reporter constructs ([-854/+323], [-665/+323], [-413/+323], [-226/+323], [-223/+9]) using the calcium phosphate method. After serum depletion, cells were treated with 20 ng/mL TGF-β1 for 24 h, and promoter activity was compared with basal levels. For inhibition studies, cells were pre-treated for 1 h with p38/MAPK, MEK, JNK, PI3K, and SMAD3 inhibitors, followed by TGF-β1 stimulation for 6 h. ADAMTS8 promoter activity was measured by luciferase and SEAP assays.

Results: TGF-β1 stimulation significantly increased ADAMTS8 promoter activity in the [-223/+323], [-665/+323], and [-854/+323] constructs compared to basal levels indicating that TGF-β1 responsive regulatory elements are located within these regions. Inhibition of p38/MAPK, MEK, JNK, PI3K, or SMAD3 signalling markedly reduced TGF-β1 induced promoter activation, demonstrating that multiple intracellular pathways contribute to TGF-β1 mediated transcriptional regulation of ADAMTS8.

Conclusions: This study demonstrates that TGF-β1 enhances ADAMTS8 transcription by activating its promoter in CC cells. The findings indicate that this regulation occurs via coordinated activity of the p38/MAPK, MEK, JNK, PI3K, and SMAD3 pathways. Overall, the results reveal a complex, multi-pathway TGF-β1 mediated mechanism controlling ADAMTS8 expression in CC.

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Metabolic remodeling in the evolution of multicellularity

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Rationale: The evolution of multicellularity was a major step in the rise of complex life on Earth, yet our understanding of this transition is incomplete. Particularly, it remains largely unknown whether changes in metabolism have facilitated early multicellular evolution.

Methods: To investigate whether metabolism reorganizes during the transition to multicellularity, we employed the yeast long-term evolution experiment. Over 1,000 daily rounds of selection for larger size, yeast evolved macroscopic group sizes, with clusters containing hundreds of thousands of clonally related cells. Using steady-state metabolomics, metabolic flux analysis, and transcriptomics, we mapped metabolic changes across this evolutionary trajectory to macroscopic multicellularity.

Results: Our analysis indicates a system-wide metabolic reallocation during the transition to macroscopic multicellularity, marked by declines in amino acids, lipids, nucleotides, and metabolites from glycolysis and the Krebs cycle. This pattern was corroborated by flux analysis, which showed slower incorporation of $^{13}C_6$-glucose through central carbon pathways. This global metabolic downshift correlated with a reduced growth rate in the macroscopic multicellular state. Interestingly, we found that trehalose, a storage sugar and chemical chaperone, rose sharply during the transition. Our prior work found chaperones, particularly Hsp90, declined, thereby facilitating the transition to macroscopic multicellularity, indicating that trehalose upregulation may serve as a compensatory mechanism for proteostasis.

Conclusions: Together, our results show that metabolism is broadly reoriented from growth toward maintenance and structural reinforcement in evolved macroscopic clusters, supporting the progression of an evolutionary transition in individuality from small to macroscopic group sizes. Our results also suggest that trehalose – potentially by stabilizing protein folding and modulating cytoplasmic viscosity – is a major driver of this shift.

Therapeutic modulation of metabolic dysfunction in Alzheimer's disease: cognitive rescue via semaglutide in 5xFAD mice

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Rationale: This work investigates how metabolic dysfunction, including dysregulated lipid and glucose metabolism, contributes to Alzheimer's disease progression. By using high-fat diet and pharmacological interventions in 5xFAD and wild-type mouse models, we aim to elucidate mechanisms linking peripheral metabolism to neurodegeneration and identify potential therapeutic targets.

Methods: 5xFAD and WT mice were fed either standard chow or high-fat diet (HFD) and treated with Semaglutide (SMGL) for 13 weeks. Body weight and glucose tolerance (OGTT) were monitored. Cognitive function was assessed using Y-maze spontaneous alternation. Brain and visceral adipose tissue were collected for histological, biochemical, and molecular analyses, including β-amyloid immunostaining, ELISA for Aβ, adipokines, GLP-1R, and synaptic markers (SYP, Tubb3), as well as neuroinflammation markers (Iba1, CD68) and lipid transporters (CD36). Data were analyzed with GraphPad Prism and R using t-tests and ANOVA.

Results: HFD induced weight gain, glucose intolerance, dyslipidemia, adipocyte hypertrophy, and cognitive deficits, exacerbated in 5xFAD mice. SMGL selectively reduced body weight, improved glucose tolerance, normalized total and LDL cholesterol, restored adipokine balance, and attenuated adipose inflammation in 5xFAD-HFD mice. Cognitive performance improved in 5xFAD-HFD mice. In the brain, SMGL reduced amyloid plaques and soluble Aβ, restored GLP-1R expression, improved synaptic and neuronal markers, and reduced microglial activation. WT-RD mice showed minimal metabolic or cognitive changes with SMGL.

Conclusions: Semaglutide exerts metabolic and neuroprotective effects in 5xFAD mice in a context-dependent manner. Benefits were strongest under metabolic challenge, linking peripheral metabolic correction to improved amyloid pathology, synaptic integrity, and cognition. These results support targeting GLP-1 pathways in metabolically compromised individuals for coordinated systemic and neural neuroprotection.

Targeting stromal-neural interactions: aspirin-treated PSCs suppress PDAC-schwann cell migration

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Rationale: Neural invasion is a major driver of pancreatic cancer (PDAC) progression and arises from interactions among cancer cells, Schwann cells, and pancreatic stellate cells (PSCs). Although PSCs promote tumor aggressiveness and neural tropism, strategies to reprogram them remain largely unexplored. This study examines whether aspirin can shift PSCs toward a tumor-restraining phenotype capable of limiting PSC-driven tumor–nerve crosstalk.

Methods: PSCs were treated with non-cytotoxic ASA doses, and conditioned media (CM) were collected from untreated or ASA-treated PSCs. PANC-1 cells and SCs were exposed to these CM conditions to assess proliferation, migration, invasion, and colony formation. EMT markers in PDAC cells and SC dedifferentiation markers (GFAP, S100) were analyzed by western blot. Stromal–neural dynamics were modeled using 3D co-culture assays quantifying mutual PDAC–SC migration, and directed migration toward dorsal root ganglia (DRGs) was evaluated with fluorescently labelled cells under NT versus ASA-treated PSC-CM.

Results: Untreated PSC-CM markedly enhanced PDAC and SC motility, invasion, and pro-aggressive signaling. In contrast, CM from ASA-treated PSCs significantly reduced these behaviors, indicating functional stromal reprogramming. In 3D co-cultures, NT PSC-CM promoted reciprocal PDAC–SC migration, whereas this effect was diminished under ASAtreated PSC-CM. Likewise, ASA-treated PSC-CM impaired the directional migration of both PDAC cells and SCs toward DRGs, demonstrating disruption of PSC-driven neural tropism.

Conclusions: This study identifies PSCs as central regulators of PDAC–nerve communication and reveals aspirin as an effective modulator of their pro-neural activity. ASA shifts PSCs toward a tumor-restraining state, reducing PDAC and SC migration and weakening cancer–nerve crosstalk. These findings position aspirin as a simple and promising stromal targeting strategy to limit neural invasion in PDAC.

Enhancing diagnostic accuracy for leukodystrophies through functional testing of mitochondrial aminoacyl-tRNA synthetases

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Rationale: Leukodystrophies caused by variants in mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are increasingly recognized, yet their diagnosis remains challenging due to the prevalence of variants of unknown significance (VUS) and the lack of functional validation assays. Establishing a robust method to measure mt-aaRS aminoacylation activity is essential for accurate variant interpretation and improved diagnostic outcomes.

Methods: We aim to develop an LC-MS–based enzymatic assay to quantify the aminoacylation activity of all 19 mt-aaRSs [1]. To support this, we compare several mitochondrial isolation and lysis strategies—including commercial kits, ball-bearing cellcracker homogenization, needle disruption, and CHAPS-based buffers—and assess their performance using western blots to monitor mitochondrial purity and protein integrity [2]. We also assess different tRNA sources from human cells and Brewer's yeast to determine enzyme-specific substrate preferences. Finally, we will apply chemical disruption approaches combined with differential centrifugation and western blotting to determine mt-aaRS localizations, increasing functional analysis efficiency.

Results: Preliminary optimization of mitochondrial isolation and lysis conditions is underway, with early western blot analyses revealing some differences between the tested approaches. Different tRNA sources are currently being tested using the exsisting LC-MS based aminoacylation assay.

Conclusions: This project aims to establish a robust LC-MS–based assay for quantifying mt-aaRS aminoacylation activity. By systematically optimizing mitochondrial preparation, enzyme extraction, and tRNA substrate selection, we expect to generate a reliable platform for the functional assessment of mt-aaRS variants. Ultimately, this work will support more accurate interpretation of VUS in patients with suspected mt-aaRS-related leukodystrophies.

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The molecular mechanisms by which the chaperone αB-crystallin inhibits amyloid-β aggregation

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Rationale: The chaperone protein αB-crystallin (αBc) can inhibit the amyloid formation of the peptide amyloid-β, but the inhibition mechanism at a molecular level remains unknown. Elucidating the interactions underlying this inhibition is an important step in understanding how chaperones can be utilised in the treatment of amyloid diseases such as Alzheimer's disease.

Methods: Monomeric recombinant amyloid-β40 (the most abundant form of the peptide) was isolated with size exclusion chromatography and then combined with αBc and pre-formed fibrils (seeds) at different concentrations. All samples were created with a constant concentration of Thioflavin T, which gives a strong fluorescent signal when bound to amyloid fibrils. By measuring fluorescence over time at 37 °C, we could track the amyloid formation. Using these kinetic traces, we fitted different kinetic models, which take into account the molecular mechanisms of amyloid formation. For amyloid-β, the most important ones are primary nucleation, elongation and secondary nucleation. Additionally, samples from different time points of the aggregation were imaged using cryo-transmission electron microscopy (cryo-TEM).

Results: Across all experiments, the fluorescence intensity at the plateau decreased with increasing αBc concentration. For unseeded experiments, samples with higher αBc concentration showed a longer lag-time before the exponential growth phase began. Some seeded experiments showed a biphasic aggregation curve, where a second phase of exponential growth was observed after an initial plateau had been reached. The slopes and timings of the growth phases depended on both the seed concentration and the αBc concentration. Cryo-TEM images revealed co-aggregates of amyloid-β and αBc.

Conclusions: We conclude that αBc strongly inhibits the amyloid formation of amyloid-β40 and forms co-aggregates with the amyloid fibrils. Furthermore, αBc seems to have an impact on all three steps in the aggregation mechanism of amyloid-β.

Phytanoyl-CoA dioxygenase domain containing 1 (PHYHD1), a 2-oxoglutarate/Fe(II)-dependent dioxygenase found in humans, demethylates 2'-O-methylated nucleosides *in vitro* and *in vivo*

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Rationale: 2'-*O*-methylated nucleosides are a widespread RNA modification found in all domains of life. However, the fate of the ribose-methylated RNA components has not been studied in sufficient detail, as the enzymatic pathways responsible for the catabolism of free 2'-*O*-methylated nucleosides remain largely unexplored.

Methods: Using the auxotrophic *Escherichia coli* DH10B Δ*pyrFEC* strain, we performed a functional screening of metagenomic libraries to identify novel enzymes active toward 2'-*O*-methyl cytidine. A mutant zebrafish (*Danio rerio*) line was generated using CRISPR/Cas9 editing, employing synthetic nCas9n mRNA and two gRNAs flanking the PHYHD1 active-site-coding region.

Results: One of the enzymes identified in our metagenomic screening is a novel bacterial dioxygenase, which we named FJS. The enzyme catalyzes the 2-oxoglutarate and Fe(II)-dependent demethylation of 2'-*O*-methylated nucleosides. We subsequently found that FJS-related enzymes - including the human phytanoyl-CoA dioxygenase domain-containing protein 1 (PHYHD1), for which no primary substrate had previously been identified [1] – also demethylate a broad range of ribose-methylated nucleosides but do not act on modified nucleotides or RNA *in vitro*. To determine whether loss of PHYHD1 function affects 2'-*O*-methylation levels of RNA-incorporated or free nucleosides *in vivo*, we generated a *phyhd1* knockout zebrafish line. Although no visible phenotype changes were observed and RNA modification levels remained unchanged, the levels of free 2'-*O*-methylated inosine, guanosine, cytidine, and uridine were significantly elevated in *phyhd1*-KO embryos and adult zebrafish.

Conclusions: Our study indicates that PHYHD1 functions in the turnover of free 2'-*O*-methylated nucleosides and does not directly demethylate RNA. These findings reveal a previously unrecognized metabolic pathway for 2'-*O*-methylated nucleosides, providing a new insight into the catabolism of modified nucleosides.

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Tracking kinase signaling in mitophagy deregulation using conformational biosensors

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Rationale: Over 500 protein kinases have been identified, each acting as a dynamic molecular switch that regulates essential cellular processes and signaling pathways. Mutations in these kinases disrupt signaling and are often linked to diseases such as cancer and neurodegenerative disorders. Because of their central role in disease mechanisms, kinases have become prime therapeutic targets, with 100 FDA-approved small-molecule inhibitors [1] developed to date. However, our understanding of how inhibitors influence both phospho-transferase function and conformational flexibility remains incomplete, posing challenges for both functional studies and drug development.

Methods: The Kinase Conformation (KinCon) reporter system, developed to monitor kinase activity and drug responses in living cells [2, 3], is a versatile toolbox for studying kinase conformations in diverse settings. It has recently been extended to track conformational changes in E3 ubiquitin ligases.

Results: Building on the versatility of the KinCon system, we first demonstrated that PINK1 regulates the activity of the E3 ubiquitin ligase Parkin and that these dynamics can be tracked with our conformation-based reporter system. Next, we tested small molecules such as CCCP and are currently extending this work to additional PINK1-activating compounds. Third, we established the basis for systematic analyses beyond mitophagy, using this approach to explore additional pathways that regulate mitochondrial quality control and protein homeostasis.

Conclusions: By integrating conformational biosensing with disease-relevant mutations and drug profiling, this approach enables discovery of novel mechanisms and validation of therapeutic targets in PD and related neurodegenerative disorders.

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Modelling chronic-like inflammation in a human in vitro intestinal barrier model

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Rationale: Chronic inflammatory bowel diseases involve repeated inflammatory episodes that impair intestinal barrier function. Current *in vitro* models rarely reproduce these cycles, which limits the study of long-term changes in barrier function. We aimed to develop a controlled model that captures long-term barrier disruption and regeneration and can be used for testing candidate therapeutic compounds.

Methods: A differentiated human intestinal epithelial monolayer containing mucus-producing cells was cultured for 21 days. The model was exposed to three rounds of lipopolysaccharide (LPS)–induced inflammation at four-day intervals, with peripheral blood mononuclear cells (PBMCs) present during each stimulation. The system was monitored for 20 days from the first stimulation. Barrier integrity was assessed by transepithelial electrical resistance (TEER), and cytokine levels (IL-6, IL-1β, IFN-γ) were quantified by ELISA.

Results: Repeated LPS stimulations produced a cumulative decline in TEER, with the most pronounced loss of barrier integrity after the third exposure. During the following ten days, the model showed spontaneous partial recovery. Cytokine measurements confirmed sustained inflammatory activity throughout the stimulation cycles.

Conclusions: This *in vitro* model captures key features of chronic intestinal inflammation, including repeated barrier injury and partial spontaneous recovery. It provides a standardized setting for studying inflammation-driven barrier dysfunction and for evaluating anti-inflammatory or barrier-restoring compounds under repeated inflammatory stress.

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The role of nuclear factor NF-Y in nutrient-mediated tissue turnover

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Rationale: Cellular turnover is the process of replacing old or damaged cells with new ones in the intestinal epithelium. The intestinal turnover relies on dietary cues, mediated by nutrient-sensing pathways, to regulate the balance between cell division and cell death. Several signaling pathways, including the target of rapamycin (mTOR), are known to play a role in this process. However, specific mechanisms of how nutrient-sensing pathways adjust intestinal turnover remain unclear.

Methods: In the current project, I aim to elucidate the role of the highly conserved heterotrimeric transcription factor Nuclear Factor Y (NF-Y) in regulating intestinal turnover. I have employed the fly line expressing the NF-YA (DNA-binding subunit of the NF-Y complex) RNAi (KD) construct. Flies harbouring the KD construct for NF-YA were crossed with an intestinal stem cell (ISC) specific driver line containing a temperature-sensitive transcriptional repressor. To study the role of NF-Y, I employed EDU and antibody staining, followed by confocal microscopy, electron microscopy, and RNA profiling in NF-YA KD in ISCs.

Results: My unpublished results show that silencing of NF-Y in ISCs disrupts cellular turnover, cell adhesion, and cell communication, leading to excessive cell delamination into the intestinal lumen and the development of dysplastic changes in the intestinal epithelium. mRNA sequencing from ISCs shows that NF-Y regulates several genes related to the activity of the nutrient-sensing mTOR pathway. As a result, silencing NF-Y in ISCs leads to the activation of the mTOR pathway and the emergence of enlarged immature cells. Inhibition of the mTOR pathway genetically, through calorie restriction or through rapamycin feeding, prevents the NF-Y phenotype.

Conclusions: These data show that NF-Y is a novel regulator of intestinal turnover in response to nutrients. The results from this project can enhance our understanding of how nutrient-sensing pathways are regulated in pathologies related to epithelial turnover.

Antimicrobial and anticancer properties of the *Galleria mellonella* serine protease inhibitor dipetalogastin-like – preliminary studies

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Rationale: Antimicrobial resistance and cancer refractoriness remain major global health challenges, prompting the search for new therapeutic molecules. Insects are an emerging source of bioactive peptides, and *Galleria mellonella* represents a valuable model for discovering novel antimicrobial and anticancer agents.

Methods: Low-molecular-weight proteomes of control and *Pseudomonas entomophila*-infected *G. mellonella* larvae were analysed using reverse-phase HPLC. Fractions showing infection-dependent changes were isolated and identified by Edman degradation. A newly identified peptide was purified from hemolymph, its activity tested in enzymatic inhibition and antimicrobial assays, and subsequently produced recombinantly in Expi293F cells for further characterization.

Results: A novel serine protease inhibitor, Galleria mellonella serine protease inhibitor dipetalogastin-like (GmSPID), was identified. Native GmSPID inhibited trypsin and elastase and exhibited antimicrobial activity against *P. entomophila*, *P. aeruginosa* and *Candida albicans* [1]. Recombinant GmSPID also demonstrated cytotoxic and antiproliferative effects against A549 lung adenocarcinoma cells.

Conclusions: GmSPID is a newly characterized bioactive peptide with antimicrobial and anticancer properties. These results support its potential relevance as a candidate molecule for future therapeutic strategies targeting multidrug-resistant pathogens and cancer cells.

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Uracil-DNA in early embryonic development of zebrafish

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Rationale: Embryonic development is a highly studied yet still incompletely understood field, and it is commonly investigated using model organisms such as the zebrafish. Our results indicate that elevated dUTP and genomic uracil levels play a functionally important role during the early stages of zebrafish embryogenesis.

Methods: To assess the role of genomic uracilation, we microinjected a dUTP-degrading enzyme (dUTPase), as well as enzymes that remove (active UNG) or just recognize (inactive UNG) uracil-DNA, into fertilized zebrafish eggs and monitored the resulting phenotypic changes. We also injected a fluorescent U-DNA sensor (DsRed-UNG) and followed its localization by confocal microscopy. Furthermore, using our U-DNA-Seq method, we characterized the genomic uracilation pattern in 2.5-hour zebrafish embryos.

Results: Microinjection of dUTPase and both active and inactive UNG led to pronounced phenotypic abnormalities and elevated mortality, indicating that interfering with uracil recognition or processing perturbs early development. The DsRed-UNG sensor colocalized with the DNA signal at 2 and 3 hours post-fertilization (hpf). In contrast, the sensor became excluded from nuclei by 4 hpf, which was consistent with our earlier results showing a decline in genomic uracil levels by this developmental stage. The genomic uracilation pattern determined in 2.5-hour zebrafish embryos by U-DNA-Seq showed extensive colocalization with specific satellite sequences, which may contribute to early genome organization.

Conclusions: Our findings support that genomic uracil is present and functionally relevant during early zebrafish embryogenesis. We propose that uracil incorporation and its scheduled processing contribute to early cell division dynamics and chromatin reorganization necessary for the zygotic genome activation. Our results shed light on novel aspects of early embryonic development involving genome uracilation, which may be a general feature of embryogenesis in other vertebrates, including humans.

AI-driven design of protein therapeutics to block a clinically relevant pathway in triple negative breast cancer (TNBC)

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Rationale: AI-based protein design provides a powerful platform for generating therapeutic proteins with high specificity, an approach of particular relevance for TNBC, where targeted treatment options remain limited. The aim is to design and produce novel proteins capable of blocking a signaling pathway that drives tumor progression and proliferation.

Methods: De novo computational design strategies, combined with paratope-grafting approaches, were used to generate candidate binding proteins. Molecular dynamics (MD) simulations were performed to prioritise designs based on predicted stability and interface behaviour. Selected proteins were expressed in Escherichia (E.) coli and purified by affinity and size-exclusion chromatography. Biochemical characterisation included dynamic light scattering, differential scanning fluorimetry, and circular dichroism. Binding studies were carried out by surface plasmon resonance (SPR). Cell-based assays in MDA-MB-231 cells were employed to assess functional activity.

Results: 36 proteins met the defined computational thresholds, including promising confidence metrics in protein-protein complex structure predictions and favourable MD-derived stability metrics. The proteins were successfully produced in E. coli with high yields. Biophysical analysis showed that the proteins are expected to exhibit the computationally predicted secondary structure and displayed high thermal stability. SPR experiments confirmed binding of the proteins to the target. Cell-based assays in TNBC cell lines revealed that the protein designs exhibited functional activity, reducing cell viability.

Conclusions: Together, these results highlight designed proteins as a promising strategy for selective TNBC targeting. Future studies will involve detailed mechanistic validation, optimization of design candidates, and evaluation of their efficacy in advanced in vitro and in vivo models to establish their therapeutic potential.

Modulation of autophagy: a key to overcoming resistance to colchicine in 2D and 3D models of lung cancer cells

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Rationale: Inherent and acquired resistance to chemotherapy, driven in part by cytoprotective autophagy, remains the main obstacle in lung adenocarcinoma treatment and also limits the antitumor potential of colchicine. We therefore investigate whether inhibiting the cytoprotective mechanism or, conversely, hyperactivating it can enhance colchicine's efficacy and drive cells into cytotoxic autophagic cell death.

Methods: The cytotoxicity of colchicine combined with autophagy modulators was evaluated by WST-1 metabolic activity assay to calculate combination index in both 2D and 3D models (spheroids) of inherently colchicine-resistant lung adenocarcinoma cells (A549) and in the in-house-developed A549-R cells with acquired increased resistance to colchicine. The mechanisms underlying synergistic cytotoxic effect of these combinations was investigated using fluorescent autophagosome labeling, stimulated emission depletion microscopy, confocal imaging of stained spheroids, RNA sequencing, and cell-cycle profiling.

Results: We identified combinations of colchicine with both autophagy inhibitors and inducers that synergistically reduced viability of A549 and A549-R cells in cell monolayers, as well as in cell spheroids. This synergy was associated with impaired autophagic flux, cytoskeletal disruption including altered microtubule organization, mitochondrial fission, and deregulated cell-cycle progression.

Conclusions: Our findings demonstrate that modulating autophagy, either by inhibition or hyperactivation, can re-sensitize resistant lung adenocarcinoma cells to colchicine. This approach offers a promising strategy to overcome both inherent and acquired resistance and expand the clinical potential of colchicine in lung cancer treatment.

Influence of depression-associated factors TNFα and miR-16-5p on the effects of transcranial magnetic stimulation in the SH-SY5Y cell model

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Rationale: Treatment-resistant depression (TRD) involves disruptions in neuroinflammatory and neuroplasticity-related pathways, which may influence responsiveness to repetitive transcranial magnetic stimulation (rTMS), including intermittent and continuous theta-burst stimulation (iTBS, cTBS). Among the molecular factors implicated in this variability, Tumor Necrosis Factor alpha (TNFα) and the microRNA miR-16-5p are known modulators of synaptic and trophic signalling.

Methods: Neuro-differentiated SH-SY5Y cells were exposed to TNFα (10 ng·mL^{-1}) and/or transfected with a 10 nM miR-16-5p inhibitor or mimic (mirVana) prior to TBS-like stimulation using a Cool-B65 figure of 8 coil (Magventure). TBS parameters were: iTBS (3-pulse bursts at 50 Hz, repeated at 5 Hz), cTBS (continuous 50 Hz bursts at 5 Hz). Gene expression was assessed by RT-qPCR, protein levels by Western blot, and metabolic activity by Seahorse oxygen-consumption assays. Statistical analysis used ANOVA with post-hoc correction.

Results: iTBS enhanced the expression of neuroplasticity-associated genes such as *BDNF, VEGFA* and *SYN1*, most notably under conditions of increased miR-16-5p activity (mimic). The strongest gene induction was observed in response to cTBS when miR-16-5p was inhibited. Protein analysis showed that miR-16-5p inhibition diminished the ability of both TBS protocols to increase VEGFA and SYN1 levels. In addition, the Seahorse Mito Stress test assay revealed the TNFα and miR-16-5p-dependent effects of TBS on cell bioenergetics.

Conclusions: These results show that TNFα and miR-16-5p significantly shape cellular responses to TBS-like stimulation, influencing the relative efficacy of iTBS versus cTBS in activating neuroplasticity-related pathways. The findings provide mechanistic insight into molecular states that may contribute to inter-individual variability in rTMS outcomes in TRD.

Cerium oxide nanoparticles modulate the transcriptomic response to halogenated boroxine in osteosarcoma cells

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Rationale: Halogenated boroxine (HB) and dextran-coated cerium oxide nanoparticles (Dex-CeNPs) have demonstrated antitumor potential in osteosarcoma cell lines. However, the molecular consequences of combining HB with Dex-CeNPs on osteosarcoma cells remain unclear. This study investigated how HB alone and in combination with Dex-CeNPs reshape the transcriptome of MG-63 cells over time.

Methods: MG-63 cells were treated for 24 h and 72 h with HB alone at its IC_{50} concentration, an HB-rich combination and an Dex-CeNPs-rich combination. Total RNA was analyzed using the QIAseq Targeted RNA Human Molecular Toxicology Transcriptome panel. Reads were processed in QIAGEN CLC Genomics Workbench, and the identified differentially expressed genes were imported into Ingenuity Pathway Analysis (using relaxed cutoffs to preserve pathway context) to explore canonical pathways and functional networks.

Results: HB alone predominantly activated stress-response and protein-homeostasis pathways, with stronger effects at 72 h. The HB-rich combination maintained this signature and additionally modulated several metabolic programs. In contrast, the Dex-CeNP–rich combination showed a more heterogeneous profile, with generally weaker stress responses and broader effects on signaling and cell-death–related pathways, including several with predicted inhibition.

Conclusions: HB induces a strong, time-dependent stress response in MG-63 osteosarcoma cells, while co-treatment with Dex-CeNPs reshapes this transcriptional program. HB-rich mixtures largely preserve the HB-driven profile, whereas Dex-CeNP–rich mixtures dampen and redirect it toward alternative metabolic responses. The results of this study suggest that Dex-CeNPs can modulate the molecular mechanism of HB in osteosarcoma and propose that combining an potential antitumor agent such as HB with nanoparticles may represent a strategy of interest for developing new osteosarcoma therapies.

Phytochemicals as novel epigenetic modulators in skin cancer therapeutics

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Rationale: This study addresses the urgent need for more effective and selective melanoma therapies by examining whether a natural PEITC-enriched watercress extract (PhEF) can enhance the anticancer activity of established epigenetic drugs. Understanding this interaction is key to developing novel combinatorial treatments that leverage both phytochemical and epigenetic mechanisms.

Methods: Watercress was chemically characterized using a UPLC-MS/MS to confirm its phytochemical composition, revealing increased PEITC content. A PEITC-enriched fraction was produced and its cytotoxicity was evaluated in an *in vitro* model of human malignant melanoma using the Alamar Blue assay. Apoptosis induction was assessed via a qRT-PCR and Western blotting, focusing on 8 apoptosis-related genes and their corresponding proteins. Three epigenetic drugs, Zebularine, Tazemetostat and Entinostat were tested alone or combined with PhEF. Gene expression changes under combinatorial treatments were analysed using ANOVA, and selective inhibitors were applied to validate protein-level effects.

Results: UPLC-MS/MS analysis revealed a diverse phytochemical profile dominated by PEITC. PhEF induced strong time- and dose-dependent cytotoxicity in all melanoma cell lines, while non-melanoma cells were largely unaffected. Apoptosis assays showed activation of the intrinsic apoptotic pathway with significant changes in eight apoptosis-related genes. Epigenetic drugs showed similar cytotoxic trends, and their combination with PhEF further altered gene expression. Notably, BCL2L1 and CASP9 were significantly affected under combined treatments. Inhibition studies confirmed that apoptosis was mainly driven by reduced expression of the anti-apoptotic BCL-XL protein.

Conclusions: PhEF exhibits potent selective cytotoxicity against melanoma cells and enhances the anticancer effects of established epigenetic drugs. These findings support its potential as a complementary anticancer agent capable of modulating both the epigenome and apoptosis pathways.

Structural and functional insights into the modulation of the Sin3L transcriptional regulator

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Rationale: Transcription in eukaryotes is regulated by histone deacetylases (HDACs) operating as macromolecular assemblies, such as the evolutionarily conserved yeast Sin3L HDAC complex. While multiple structures have been characterized by single-particle cryogenic electron microscopy (cryo-EM), existing structures still lack information on transiently interacting subunits, and structural information on the impact of HDAC inhibitors in a multimeric protein complex environment remains elusive.

Methods: In this work, we used a one-step tandem affinity purification (TAP) procedure to isolate the Sin3L HDAC complex via the TAP-tagged Rxt3 subunit. To confirm that the isolated complex is active, we tested its disruption with established HDAC enzymatic inhibitors such as Trichostatin A and Trapoxin A. The interactions of purified proteins with the Sin3L complex were elucidated by cross-linking mass spectrometry (XL-MS). Using cryo-EM, we studied the binding of different molecules including a substrate mimic and HDAC enzymatic inhibitors to the HDAC active sites of the complex.

Results: The Rxt3-TAP purification resulted in remarkable yield of a highly pure Sin3L complex, which showed consistent enzymatic activity in the context of multiple HDAC inhibitors. XL-MS resulted in additional spatial information compared to published studies [1, 2], which was used in a recently published manuscript [3]. Cryo-EM structures demonstrated a catalytically active Rpd3/HDAC subunit, while the other remained blocked by the Rxt2 subunit, consistent with published structures. Remarkably, the binding of Trapoxin A and Trichostatin A to the active Rpd3/HDAC catalytic site of the complex could be structurally characterized, while the other catalytic site remained blocked by Rxt2 despite the presence of these strong HDAC inhibitors.

Conclusions: Overall, our work provides novel insights on the characterization of the conserved Sin3L HDAC complex assembly and the larger network with which it interacts, while substantiating necessary alternative inhibition strategies to potentially target both catalytic sites.

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Plant seed mucilage mimics the function of salivary MUC5B

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Rationale: Currently there is no intervention to relief a dry mouth that offers a long-lasting solution, despite 20% of the population globally suffering from a dry mouth and its affiliated complaints. Plant seeds have mucus forming properties through the presence of polysaccharides – similar to the protein MUC5B in saliva which plays a major role in moistening the oral cavity – raising the question whether plant mucilage could potentially be used for effective dry mouth treatment.

Methods: Mucilage was extracted from commercially obtained seeds of the plants *Salvia hispanica* (Chia), *Ocimum basilicum* (Basil) and *Linum usitatissimum* (Flax). The mucilage of these plant seeds were tested for their pH, buffer capacity, extensional rheology (spinnbarkeit), viscosity and protein quantification. These results were compared to the pH, buffer capacity, spinnbarkeit and viscosity results of unstimulated whole saliva collected from healthy individuals. The spinnbarkeit of unstimulated saliva mixed *in vitro* with the different types of plant seed mucilage was also evaluated. All measurements were conducted in triplicate.

Results: The mucilage of all plant seeds had a pH between 7.0-8.0 and showed no buffer capacity. As for the extensional rheology, the chia seed mucilage showed the highest spinnbarkeit, followed by basil seed and then by flax seed mucilage. Upon mixing unstimulated saliva with plant seed mucilage, the spinnbarkeit of the saliva often changed, going up or down depending on the initial spinnbarkeit of an individuals saliva.

Conclusions: All three plant seed mucilage extracts show promise for potential use as a dry mouth intervention or as an intervention component. Characteristics can still be altered by adjusting the respective concentrations and through the addition of buffering solutions. To evaluate which plant seed mucilage would be the most promising, the adhesion of the mucilage to the mucosal tissue in the oral cavity should still be investigated to ensure prolonged effectivity.

Uncovering the spatial and temporal flow of proteins through the quality control system

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Rationale: The quality control (QC) system carries out the crucial tasks of safeguarding protein biogenesis, curating protein function, and ensuring timely degradation, which together underpin cellular proteostasis. When this system is overloaded – such as by mutations that alter protein function or age-related pressures – loss-of-function (LoF) as well as gain-of-toxicity (GoT) diseases can take hold. While substantial knowledge exists on the individual QC components, a holistic understanding of protein trajectories from their synthesis to degradation is still lacking.

Methods: Here, we aim to visualize the flow of client proteins through the QC system to uncover key molecular triage decisions that determine protein-specific fates. To obtain an unbiased systems-level view of the proteostasis network, we employ cutting edge proteomic technology to map interactions of the QC system with its client proteins from their cradle to the grave.

Results: Our fous lies on cystic fibrosis transmembrane conductance regulator (CFTR) and alpha-synuclein, whose defect can lead to cystic fibrosis (LoF) and Parkinson's Disease (GoT), respectively. These clients are dissimilar in their structure and function, but share all possible fates of misfolding, aggregation, and premature degradation. At present, we are building contextualized and time-resolved interaction networks for CFTR and alpha-synuclein, focused on molecular chaperones and the ubiquitin-proteasome system.

Conclusions: We expect this approach to reveal when and why certain biophysical alterations in protein clients disrupt their proteostatic balance and lead to disease. Moreover, we hope these insights uncover previously unforeseen avenues to steer protein fates back to health.

Structural and functional impact of the K279S mutation on SHMT1 enzyme unveils the protein's conformational plasticity

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Rationale: SHMT1 is a central enzyme in one-carbon metabolism, catalyzing the reversible conversion of serine and tetrahydrofolate into glycine and 5,10-methylenetetrahydrofolate [1]. It also acts as a "moonlighting protein" [2] by binding to the 5'UTR of SHMT2. This RNA-binding property also affects SHMT1's catalytic activity by inhibiting the conversion of serine into glycine, while the reverse reaction remains unaffected, a phenomenon that is called riboregulation [3]. The K279S mutant lacks this RNA-binding ability, and prior analysis suggested altered structural dynamics due to increased thermal stability upon serine binding.

Methods: The experimental design is centered on comparing the wild-type SHMT1 enzyme with the K279S mutant. To assess potential structural differences, Far-UV Circular Dichroism (CD) was performed. The relative stability of the enzymes in the presence and absence of substrates was probed using thermal melting experiments. Additionally, cryo-EM datasets for SHMT1 K279S and wild-type are currently being processed to obtain high-resolution 3D structures for a direct comparison.

Results: The Far-UV CD results confirmed that the wild-type and K279S mutant are structurally similar. Importantly, thermal melting experiments revealed that the K279S mutant is slightly more stable than the wild-type enzyme when its substrate, serine, is present, as confirmed by the thermal melting curve. Structurally, model building on SHMT1 WT in the presence of serine revealed the presence of the external aldimine adduct (PLP-SER). In the mutant, this adduct appears to be absent, while the internal aldimine (PLP-LYS) remains visible.

Conclusions: The results demonstrate that a single amino acid mutation can significantly alter the protein's behavior, specifically by increasing stability and serine binding behavior (as shown with Cryo-EM data). The next step is to further structurally compare the mutant and wild-type protein in order to look at their dynamics and subunit cooperativity in the presence of serine.

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Single session hemodialysis-induced oxidative stress in end-stage renal disease patients

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Rationale: Oxidative stress (OS) has emerged as a novel risk factor for chronic kidney disease. End-stage kidney disease patients on hemodialysis (HD) exhibit high levels of OS, attributed to both progressive decline in kidney function and the dialysis procedure. To date, the impact of HD on OS remains controversial due to heterogeneity in study design and the specificity of OS markers used. The present study aims to investigate whether one session of HD can cause OS, assessed by more representative markers. These are: (i) the OS-inducing reactive oxygen species markers superoxide (O2•−) and hydroxyl radical (•OH), and (ii) the OS-damage markers lipid hydroperoxides (LOOH), protein bound-malondialdehyde (PrMDA), protein bound-thiobarbituric acid reactive substances (PrTBARS) and protein carbonyls (PrCO).

Methods: In this observational study, blood from 68 hemodialysis patients was collected before and after a single dialysis session, and plasma levels of OS markers were evaluated. Associations with dialysis vintage, dialysis modality, comorbidities and medication were also examined.

Results: LOOH levels increased significantly by 50% post-dialysis, whereas PrMDA and PrTBARS levels decreased modestly by 10%. •OH decreased by 24% after dialysis. No significant changes were observed in O2•− and PrCO levels. Dialysis vintage correlated positively with •OH, LOOH, PrMDA and PrTBARS OS markers, but not with O2•− and PrCO. No significant associations were found between OS markers and dialysis modality, comorbidities or medication.

Conclusions: OS increases during dialysis as shown by the rise in LOOH, an early lipid peroxidation marker. The increasing tendency of several OS markers with dialysis vintage further supports the cumulative OS burden and damage over time. Protein damage remains constant regardless of dialysis session or vintage. Interventions with natural antioxidants may help modulate dialysis-induced OS and provide clinical benefits for patients.

Test system for studying viral protease substrate specificity

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Rationale: Viruses lack the machinery necessary for protein synthesis. Nevertheless, they exploit eukaryotic cells to produce viral polyproteins. These polyproteins are then processed by virus-encoded proteases, resulting in mature proteins from which viral particles are formed. Thus, identifying the cleavage site specificity of viral proteases helps us to understand the dynamics of the viral replication cycle.

Methods: Our study focuses on determining the substrate specificity of the 3C cysteine protease of the Cricket Paralysis Virus (CrPV). To achieve this, an *in vitro* test system containing a recombinant protease and a model substrate was designed. Firstly, a recombinant CrPV protease with a GST tag was constructed. Additionally, an inactive protease variant with an alanine instead of a cysteine in its catalytic center was created. The model substrates were designed by taking advantage of the heat shock protein DnaK from *Pseudomonas putida*. Based on an *in silico* study of CrPV polyprotein cleavage sites, a sequence used to process the CrPV structural polyprotein was inserted into the unstructured linker region of DnaK. Mutant substrates with either scrambled or single-residue alterations in the polyprotein cleavage site were created. All the proteins were expressed in and purified from *Escherichia coli* cells.

Results: The reliability of the test system was assessed by conducting an *in vitro* cleavage assay. If the protease was active, cleavage products should accumulate. The results revealed that the recombinant CrPV 3C protease was indeed active because it could cleave a substrate with a CrPV polyprotein processing site. A mutant substrate with a scrambled site remained resistant to cleavage, indicating that the recombinant protease cleaves specifically. Any alteration to the substrate due to a single-residue change lowered the efficacy of the cleavage reaction.

Conclusions: The established functional test system could be used in biotechnology to test sequences that have been designed artificially.

Conformational landscape and TARP-2 Modulation of GluA4 AMPA receptors

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Rationale: AMPA receptors (AMPARs) mediate fast excitatory neurotransmission in the central nervous system and are essential for synaptic plasticity. Among AMPAR subtypes (GluA1-GluA4), GluA4-containing receptors are relatively unexplored, and their structural and functional properties remain poorly understood.

Methods: GluA4-containing AMPA receptors were produced by infecting mammalian suspension cells with baculovirus constructs encoding the receptor, either in isolation or in complex with the auxiliary subunit TARP-γ2. The expressed membrane proteins were purified and used for cryo-electron microscopy (cryo-EM) to capture multiple conformational states along the gating cycle in the presence of different ligands. Electrophysiology and cross-linking assays were performed to assess receptor gating and subunit-dependent modulation, providing complementary functional insights.

Results: Under resting conditions, GluA4-containing AMPA receptors adopt a Y-shaped dimer-of-dimers arrangement, similar to GluA2-containing AMPARs [1]. However, GluA4 exhibits greater conformational flexibility, undergoing substantial structural rearrangements throughout the gating cycle. Transient disruption of dimer interfaces within the ligand-binding domain (LBD) was observed in the desensitized state [1], similar to GluA1 and GluA3. This disruption is markedly stronger in the absence of TARP-γ2, accelerating GluA4 entry into desensitization. Furthermore, a previously unrecognized regulatory site was identified, mediating TARP-γ2-dependent modulation of receptor function [1].

Conclusions: These findings provide a structural and functional framework to understand the architecture of GluA4 receptors and the mechanisms underlying their gating, as well as the role of auxiliary subunits in modulating AMPA receptor behavior.

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When hedgehog meets the stroma: IL-8 as GLI2's voice in ovarian cancer

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Rationale: Given that 3D spheroids more accurately recapitulate the ovarian cancer microenvironment and underlie tumor survival, dissemination, and drug resistance, their biology requires further clarification. This study investigates spheroid-specific mechanisms to advance more effective therapeutic strategies.

Methods: RNA-seq identified IL-8 as a gene upregulated in 3D spheroids versus 2D cultures. In silico analysis revealed putative GLI2 binding sites in the IL8 promoter, and cytokine profiling of GLI2-transfected spheroids confirmed IL-8 among the most induced factors. qPCR validated elevated IL8 expression in 3D cultures, and co-culture experiments showed tumor–stromal interactions modulate IL-8 via GLI2 signaling.

Results: RNA-seq analysis revealed several genes significantly upregulated in a culture-condition–dependent manner, with IL-8 emerging as a prominent candidate. In silico promoter analysis identified putative GLI2 binding motifs within the IL8 promoter region. Cytokine Protein Array analysis of GLI2-transfected 3D spheroids showed a distinct cytokine secretion profile, with IL-8 among the most strongly induced factors. qPCR validation confirmed that IL8 expression was markedly elevated in 3D cultures compared with 2D cultures across all cell lines, despite identical cultivation conditions. Co-culture experiments demonstrated that interactions between tumor and stromal cells influenced IL-8 expression, supporting a role for GLI2-mediated signaling in modulating the cytokine milieu within the tumor microenvironment.

Conclusions: Collectively, our findings suggest that IL-8 represents a previously unrecognized GLI2-regulated cytokine in ovarian cancer and may contribute to tumor–fibroblast crosstalk and invasive behavior within the 3D microenvironment.

Hydration properties, antimicrobial and antiviral activity of nicotine-based ionic liquids with different length alkyl side-chains

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Rationale: Nicotine is a secondary plant metabolite that functions as a natural pesticide, historically used in agriculture before being banned due to its broad, non-selective toxicity. By alkylating nicotine, it is possible to generate ionic liquids containing a quaternary ammonium group and an extended alkyl side-chain, modifications expected to enhance its antimicrobial and antiviral properties. This study aimed to synthesise and characterise new nicotine-based ionic liquids to determine whether these structural modifications increase their antimicrobial and antiviral activity.

Methods: Four nicotine-based ionic liquids with varying alkyl chain lengths (N-octyl-, N-decyl-, N-dodecyl-, and N-tetradecylnicotinium bromide) were synthesised. Their hydration properties were assessed using density, viscosity, and acoustic measurements across concentrations of 0.02–0.12 mol/kg and temperatures of 293.15–313.15 K. Conductometry was used to determine critical micellar concentrations. Antimicrobial activity was evaluated against three Gram-positive and three Gram-negative bacterial strains, two yeasts, and four molds, and antiviral properties were tested using four bacteriophage types.

Results: All four nicotine-based ionic liquids exhibited strong antimicrobial effects against the tested microorganisms and demonstrated antiviral activity against the examined bacteriophages. Their biological activity was significantly higher than that of unmodified nicotine. Physicochemical measurements revealed hydration and micellarization behaviours consistent with surfactant ionic liquids, including identifiable critical micellar concentrations.

Conclusions: The synthesised nicotine-based ionic liquids displayed substantially enhanced antimicrobial and antiviral activity compared to nicotine itself. These findings suggest that combining alkyl chain modification with ionic liquid structures effectively increases the bioactivity of nicotine-derived compounds, supporting their potential as potent antimicrobial and antiviral agents.

Human plasma microRNAs as Alzheimer's biomarkers: cross-species validation

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Rationale: Alzheimer's disease (AD) progresses for years before clinical presentation, creating a critical need for minimally invasive biomarkers that capture early and evolving pathology. Circulating microRNAs (miRNAs) are promising candidates due to their stability in blood and central role in regulating AD-relevant pathways.

Methods: Plasma miRNA expression was quantified by RT-qPCR in triple-transgenic (3xTg-AD) and wild-type mice, and compared with previously patented human plasma miRNA biomarkers from our lab (International Patent EP3449009, 2021). Cross-sectional longitudinal assessment was performed at 4, 6 and 16-20 months old mice group (n = 17/group).

Results: All analyzed miRNAs exhibited altered expression in both mouse and human AD samples. MiR-486 exhibited consistent cross-species dysregulation, confirming its translational robustness. MiR-483 demonstrated stronger human-specific effects, while miR-29b displayed species-dependent regulation. Longitudinal profiling revealed three temporal patterns: (1) early-onset biphasic trajectory with mid-stage normalization and late-stage decline; (2) initial alteration progressing to sustained dysregulation; and (3) early-stage elevation attenuating at advanced stages. These stage-specific trajectories represent miRNA signatures with diagnostic potential.

Conclusions: These findings identify plasma miRNAs as promising blood-based biomarkers for early AD detection and longitudinal disease monitoring. The cross-species agreement and stage-dependent regulation underscore their clinical relevance and validate the 3xTg-AD model as a suitable platform for biomarker development.

Unveiling the membrane insertion mechanisms of tail-anchored membrane proteins in *Escherichia coli*

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Rationale: Tail-anchored (TA) proteins rely on post-translational insertion into the membrane; however, the pathway mediating their membrane integration in Escherichia coli remains unclear. Defining this mechanism is essential for understanding how cells maintain membrane protein homeostasis.

Methods: We combined biochemical insertion assays, CRISPRi-mediated depletion of insertion factors, nanodisc pull-downs, and lipid modulation to define the membrane topology and insertion requirements of selected TA proteins. Depleting YidC and SecY enabled us to assess their contributions, while nanodisc-based pull-downs were used to test for direct or proximal interactions with the insertion machinery. Finally, altering lipid composition enabled us to evaluate how sequence–lipid interplay influences TA protein biogenesis.

Results: Our findings show that neither YidC nor the Sec translocon is the primary pathway for TA protein insertion. TA proteins with hydrophilic C-terminal tails displayed a mild dependence on YidC, and their insertion decreased when both YidC and Sec were depleted, suggesting they can use either pathway. In contrast, other TA proteins are inserted independently of both factors, implying the involvement of an unknown machinery or spontaneous insertion directly to the lipid bilayer. Indeed, these proteins depended on the lipid composition and were substantially decreased in a lipid-altered *E. coli* strain.

Conclusions: Our study advances the understanding of tail-anchored protein insertion in bacteria by highlighting the roles of sequence hydrophilicity and membrane lipid composition. We found that some TA proteins may insert spontaneously under specific lipid conditions - an adaptive strategy when conventional insertion pathways are limited.

Unveiling the role of oxygen in meningioma progression

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Rationale: Meningiomas, the most common intracranial tumors seen in adults, lack effective therapies due to diverse molecular alterations. Meningiomas have been classified into distinct subgroups and there has been a renewed focus on the role of the tumor microenvironment (TME) in their development. Hypoxia in the TME is known to shape tumorigenesis in many cancer types including glioblastoma. We wanted to unveil the role of a hypoxic TME in meningioma pathology.

Methods: To that end, we modeled the disease *in vitro* using cell lines, IOMM Lee, CH157-MN and AC599, overall reflecting each grade of meningioma pathology. We evaluated their responses to hypoxia (1% O2) and extreme hypoxia (1% O2 in degassed medium). We assessed viability by Trypan Blue assay. To further delineate the energetics of the different grades of meningiomas, we exploited the oxygen dependence of our cell lines using inhibitors.

Results: We found out that benign cell line could endure hypoxia and extreme hypoxia as well as normal brain cells. However, malignant cell lines could not withstand extreme hypoxia even for short duration while they could survive in normal hypoxic conditions for prolonged durations. To address HIF dynamics and hypoxic adaptation, we extended our work to pseudohypoxia and observed that only the malignant cells showed a drastically decreased viability with hypoxia but were not affected by pseudohypoxia. Using inhibitors, we discovered that malignant cells are significantly more sensitive to electron transport chain (ETC) impairment explaining their oxygen dependence. Currently, we are investigating the relationship between metabolic rewiring and hypoxia signaling using *in vitro* meningioma models.

Conclusions: We foresee that our results will unveil how benign and malignant cells differentially adapt to hypoxic TME and how it shapes metabolic rewiring. Our work holds promise to identify specific drug targets to treat benign and malignant meningiomas.

ApoA-I mimetic nanodiscs preferentially associate with high-density lipoprotein and enhance its functionality in human plasma

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Rationale: High-density lipoproteins (HDLs) are endogenous nanoparticles crucial for cholesterol transport and vascular protection. Under pathological conditions, HDL can become structurally and functionally impaired, prompting the development of synthetic HDL (sHDL) nanodiscs to mimic and enhance its beneficial roles. Despite increasing interest in sHDL as therapeutics, their fate in human plasma, particularly interactions with native lipoproteins, remains poorly understood.

Methods: In this study, we examined sHDL nanodiscs composed of a proline-linked dimeric ApoA-I mimetic peptide (4F-P-4F) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC). Fluorescent labeling of both the peptide and lipid components allowed independent monitoring of their plasma behavior.

Results: We observed that both rapidly associate with native lipoproteins, with a clear preference for HDL over LDL or VLDL. Association with HDL involves a concentration-dependent displacement of native apoA-I, generating lipid-poor pre-β HDL, a cardioprotective species highly effective at cholesterol efflux. Simultaneously, both the 4F-P-4F peptide and DMPC lipid integrate into the apoA-I-depleted HDL, forming what we term a remodeling product, which we isolated for functional testing. In U937 monocyte cells, this remodeling product demonstrated superior anti-inflammatory activity relative to native HDL after LPS stimulation. In vitro, it also bound LPS much more strongly than native HDL, suggesting that its enhanced anti-inflammatory properties may result from more effective LPS sequestration.

Conclusions: These findings reveal that 4F-P-4F-DMPC sHDL nanodiscs do not persist as independent entities in plasma but instead rapidly engage endogenous HDL to generate a remodeling product with enhanced functionality over native HDL. Given the promising preclinical data for diverse sHDL formulations and their clinical promise, understanding how sHDL interacts with native lipoproteins will be critical for predicting and optimizing therapeutic outcomes.


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