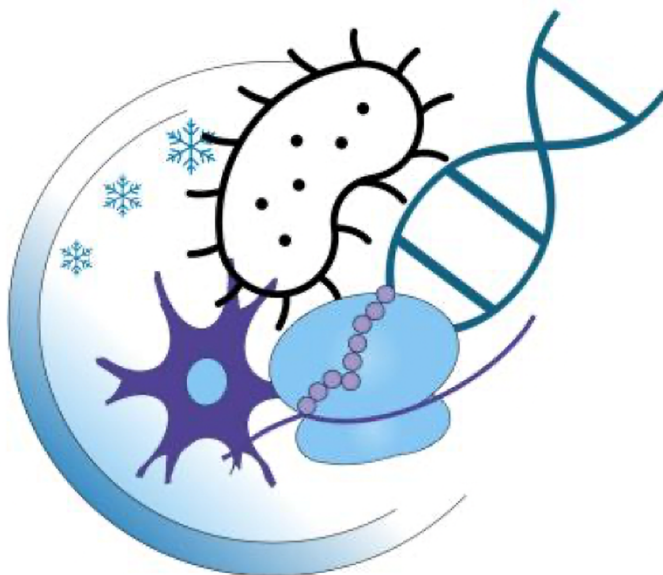


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BOOK OF ABSTRACTS

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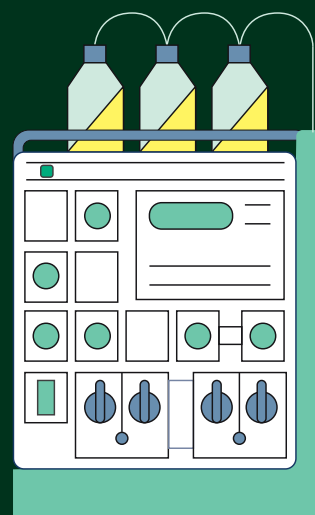
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ORAL PRESENTATIONS

SESSION 1 – Part 1: Molecular Conversations

Decoding the molecular language of life – from structure to systems

Wednesday, February 18, 2026

14:15-16:00

The strength of weak interactions in the control of gene expression

Andrzej Górecki

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In the classical view of biochemistry, protein function is closely associated with a stable three-dimensional structure. However, many proteins operate through intrinsically disordered regions (IDRs) that lack persistent structural organization. In regulators of gene expression, these regions enable dynamic, multivalent interaction networks in which individual contacts are weak, transient, and highly sensitive to the cellular context. Within such systems, regulatory function emerges not from single interactions but from their collective behavior.

IDRs allow transcription factors to integrate signals, modulate binding affinities, and dynamically reorganize local regulatory assemblies on DNA. As a result, transcriptional regulation becomes a distributed and adaptive process, shaped by cooperation between multiple factors rather than rigid, predefined complexes.

The transcription factors YY1 and its paralog YY2 serve as examples of how weak interactions with DNA and molecular partners contribute to flexible control of gene expression. Particular attention is given to the role of intrinsically disordered regions in DNA recognition, partner selection, and context-dependent self-association, highlighting similarities and functional divergences between these two proteins.

Understanding such regulatory mechanisms requires experimental approaches capable of capturing molecular dynamics rather than static structures. Together, biochemical and biophysical studies reveal that biological order can arise from dynamic heterogeneity and that robust gene regulation may emerge from networks of weak, cooperative interactions rather than from stable molecular architectures.

Curcumin–lipid interactions in PEGylated vs. conventional liposomes

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Curcumin, a natural polyphenol derived from *Curcuma longa*, is widely recognized for its therapeutic properties. However, its clinical utility is limited because of poor solubility, rapid degradation and hence low bioavailability. To overcome these issues, nanoformulation approaches, especially PEGylated liposomes, have been explored as advanced delivery systems. PEGylation, which involves attaching polyethylene glycol (PEG) to the liposomal surface enhances circulation time by creating a steric shield that reduces protein interactions and clearance by the mononuclear phagocyte system (MPS). However, PEG can alter lipid membrane properties, which may in turn affect curcumin's solubility and distribution within the liposomal bilayer, ultimately reducing its loading efficiency. To ensure that PEG-modified liposomes can be effectively loaded with curcumin, we investigated curcumin–membrane interactions in saturated (DMPC) and unsaturated (POPC) liposomes, both in the presence and absence of PEG. Based on dissociation constants (K_d) obtained from fluorescence spectroscopy measurements, we found that PEGylated DMPC liposomes exhibit the strongest binding affinity for curcumin. Fluorescence quenching experiments showed that curcumin adopts a transbilayer orientation in all membranes examined. Curcumin's location within PEGylated and non-PEGylated liposomal membranes was further confirmed by examining its effects on membrane properties, including fluidity, polarity, and oxygen transport. These effects were investigated using electron paramagnetic resonance (EPR) spectroscopy with spin labels. The results indicate that PEG does not impose major changes on membrane properties. Curcumin, however, was found to reinforce the liposomal membranes, increase their polarity, and reduce oxygen availability. Overall, the findings suggest that liposomes, particularly those composed of PEGylated DMPC, are effective vehicles for curcumin delivery.

Exploring the structural features of N4BP1 as an RNase associated with mRNA decapping complex

Mateusz Wilamowski¹, Adrianna Graca^{1,2}, Paweł Piłat^{1,2}, Kinga Rapacz¹, Jolanta Jura¹

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²*Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

Exonuclease-mediated degradation of uncapped RNA plays a key role in immune defense against foreign RNA and helps maintaining cell equilibrium by regulating gene expression via RNA surveillance. In eukaryotic cells, mRNA decapping occurs within cytoplasmic processing bodies (P-bodies) - primarily manifested by the EDC4-centered decapping complex and XRN1-driven RNA degradation. The decapping complex includes proteins such as EDC3, DCP1A/B, and the cap-hydrolase DCP2.

Our study investigates the mechanism of human RNase N4BP1 in mRNA decay. Using immunoprecipitation followed by mass spectrometry, we identified N4BP1 as a nuclease associated with the macromolecular complex of decapping machinery. Structural and functional analysis revealed that N4BP1 contains tandem KH (K-homology) RNA-binding domains and a PIN nuclease domain, resembling Regnase1 active site. The KH domains allows RNA recognition and interaction with decapping proteins, particularly EDC4 that acts as decapping scaffold. To reveal N4BP1's structural features and interactions, we analyzed its crystal structure of tandem KH domain. Using AlphaFold2-predicted models we mapped N4BP1 binding interface with EDC4—a central scaffold protein with a core WD40 domain containing 7 blade β -propeller. We employed high-throughput cloning approaches from the structural genomic platform to generate over 200 expression constructs of N4BP1, EDC4 and DCP2 fragments. The soluble proteins were obtained for approx. 30% constructs and are being used for biochemical and structural analyses to define mechanism of N4BP1 as the complex.

To perform structural analysis of the complete N4BP1–EDC4 complex, we expressed N4BP1 and decapping components in high-yield mammalian cell systems (Expi293 and ExpiCHO), purified them via affinity chromatography, and subjected them to analysis using cryo-electron microscopy. Preliminary cryoEM revealed that N4BP1 forms disc shaped complex. These efforts aim to define the mechanistic role of N4BP1 in mRNA turnover and its potential contributions to RNA metabolism and innate immune regulation including responses to exogenous RNA originated from retroviruses.

Acknowledgements: This study was supported by the National Science Centre, grant number 2023/51/D/NZ1/00952 (awarded to M.Wilamowski).

From titin unfolding to muscle contraction: the effect of mechanical stimulation on sarcomere length and generation of force by cardiomyocytes

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Titin is the largest protein of the human body composed of more than 30,000 amino acids, exceeding 1 μm in length. The protein stretches from the “Z-disks” to the “M-lines” of the basic functional units (sarcomeres) and is comprised of over 300 modular domains: immunoglobulin-like (Ig) and fibronectin type III (Fn3), providing structural stability, binding sites for sarcomeric proteins and myofibril elasticity. Titin plays a crucial role in striated muscle cells allowing the functional units to return to their resting position after initiating contraction. Experiments employing single molecule force spectroscopy (SMFS) technique provided evidence that titin can unfold and refold, thus regulating sarcomere length. It is now widely accepted that the sarcomere length-contraction force relationship determines muscle performance. Although titin unfolding has been demonstrated *in situ*, its effect on sarcomere length and shortening as well as generation of force in functional cells has been sought after. This is mostly due to technical limitations and finding a proper cell model for such experiments. Recently, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been extensively studied as functional heart muscle cells for disease modeling and drug screening purposes. Here, we present a cell stretcher device allowing controlled mechanical stimulation of cells under standard *in vitro* conditions. Taking advantage of spontaneous contractility of hiPSC-CMs and their immature phenotype, we employ advanced imaging techniques such as atomic force microscopy (AFM) and structured illumination to show that mechanical stimulation of hiPSC-CMs leads to increased sarcomere length, sarcomere shortening and generation of higher contraction force. Moreover, we show how titin mutations can affect the response of cells subjected to stretching. The platform presented by us for mechanical stimulation, as well as imaging, provides a unique opportunity to study the response of hiPSC-CMs to stretching force in different disease models under controlled conditions.

Direct measurements of singlet oxygen – from simple solvents to cell cultures

Grzegorz Szewczyk¹, Krystian Mokrzyński¹, Tadeusz Sarna¹

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How to find new photosensitizers? Although many molecules with potential as photosensitizers are already known, we continue to search for new candidates for applications in PDT (photodynamic therapy) or aPDI (antimicrobial photodynamic inactivation). Singlet oxygen is considered the most important reactive species in photodynamic therapy; however, determining quantum yield in a simple solvent system can often be misleading. The path toward increasing the resemblance of experimental conditions to those in living organisms is challenging and demanding, so we are exploring new approaches to extract as much information as possible from experimental data.

Materials and Methods: Direct, time-resolved singlet oxygen phosphorescence detection, nanosecond flash photolysis, and EPR spin trapping were employed. Experiments were carried out in simple solutions, liposomal models and in cell suspensions. Cell viability and mitochondrial activity were assessed using the MTT assay, protein oxidation was measured by CBA, and lipid oxidation was evaluated with the iodometric test. Localization of photosensitizers was determined by autofluorescence microscopy.

Results and Discussion: The effectiveness of a photosensitizer can vary significantly when conditions are shifted from a simple solution to a more biologically relevant environment. Analysis of singlet oxygen phosphorescence lifetimes in cell suspensions revealed a correlation between double-exponential decay kinetics and the outcome of photodynamic therapy, most likely reflecting the microenvironment in which singlet oxygen is generated. Expanding the pool of tested photosensitizers could further strengthen this hypothesis.

Conclusions: Observing singlet oxygen production under biologically relevant conditions remains a considerable challenge, yet it holds great promise for accelerating the screening of potential photosensitizers. Advances in detection techniques, together with strategies to enhance tissue oxygenation and optimize delivery of photosensitizers to target sites, are essential steps toward improving photodynamic therapy outcomes.

Mapping of the triplet excited states of melanins using time-resolved singlet oxygen phosphorescence detection

Krystian Mokrzyński¹, Grzegorz Szewczyk¹, Tadeusz Sarna¹

¹*Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland*

Melanins are central to human photobiology as they both protect pigmented tissues by dissipating absorbed UV–visible energy and, under some conditions, participate in oxygen-dependent photochemistry leading to oxidative stress in skin, hair, and the retinal pigment epithelium (RPE). Long-lived triplet excited states are implicated in type II photochemistry and singlet oxygen ($^1\text{O}_2$) formation, the effects that were previously demonstrated for both synthetic and natural pigments [1-3]. Yet, direct detection of melanins' triplet excited states using well-established methods (e.g., laser flash photolysis) is virtually impossible due to melanins' unfavorable optical properties and low apparent triplet yields.

Here we “map” quenchable triplet subpopulations in synthetic eumelanin (DOPA-melanin) and pheomelanin (5-SCD-melanin) by using time-resolved singlet oxygen phosphorescence detection as an indirect readout of melanin triplet formation and quenching. Melanin samples in D_2O were excited with nanosecond laser pulses (320 - 420 nm) under controlled O_2 concentrations. We applied a set of chemically related diene triplet probes to selectively quench triplet excited states, while minimally affecting $^1\text{O}_2$ itself. Probe charge strongly affected apparent quenching efficiency, with the cationic probe showing the highest reactivity, consistent with electrostatic interactions with negatively charged melanin. Wavelength-dependent probing further suggested the presence of heterogeneous triplet pools in melanins, with a larger highly energetic fraction populated at shorter excitation wavelengths, particularly in synthetic eumelanin model.

This framework provides a biologically relevant kinetic–thermodynamic portrait of triplet reactivity in melanin - parameters that may help predict when melanin acts predominantly as a photoprotectant versus when it can become a photosensitizer in pigmented tissues.

Acknowledgements: The research was supported by grant Opus-2021/43/B/ST4/03255 from the National Science Centre of Poland (NCN).

[1] Szewczyk G., Żądło A., Sarna M., Ito S., Wakamatsu K., Sarna T. (2016) *Aerobic photoreactivity of synthetic eumelanins and pheomelanins: generation of singlet oxygen and superoxide anion*. *Pigment Cell Melanoma Res.* 29:669-678; [2] Mokrzyński K., Sarna M., Sarna T. (2023) *Photoreactivity and phototoxicity of experimentally photodegraded hair melanosomes from individuals of different skin phototypes*. *J Photochem Photobiol B.* 243:112704; [3] Mokrzyński K., Wojtala M., Sulkowski M., Ito S., Wakamatsu K., Żądło A., Majka M., Sarna T., Sarna, M. Photoreactive Properties of Melanin Obtained from Human Induced Pluripotent Stem Cell-Derived Melanocytes. *Int. J. Mol. Sci.* (2025) 26,4119.

SESSION 1 – Part 2: Molecular Conversations

Decoding the molecular language of life – from structure to systems

Wednesday, February 18, 2026

16:30-17:40

The TGF-beta pathway as a target to improve immunotherapy in hepatocellular carcinoma (HCC)

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Liver cancer incidence has increased markedly in recent years, with high mortality rates and limited therapeutic options. Transforming Growth Factor- β (TGF- β) has emerged as a promising therapeutic target, particularly in combination with immune checkpoint inhibitors. However, TGF- β is a pleiotropic cytokine that can act as both a tumor suppressor and a tumor promoter, depending on tumor context and the microenvironment. Here, we developed novel in vitro and in vivo models to better characterize the role of TGF- β signaling in liver tumor and stromal cells and to assess whether TGF- β inhibition could represent an effective therapeutic strategy. We established new mouse liver cancer cell lines from tumors induced by hydrodynamic tail vein injection of a transposon vector (MYC-LucOS, CTNNB1, SB13). A syngeneic orthotopic model was generated by injecting tumor cells into the liver parenchyma of C57BL/6J mice. Galunisertib (a TGF- β receptor inhibitor, 150 mg/kg orally), anti-PD-L1 (5 mg/kg), and their combination were evaluated for their effects on tumor progression. Cells displaying an epithelial phenotype responded to TGF- β with growth inhibition, apoptosis, and induction of epithelial-mesenchymal transition (EMT). Chronic exposure to TGF- β (2 ng/mL for four weeks) generated a mesenchymal cell line resistant to the suppressive effects of TGF- β . Upon orthotopic implantation, mesenchymal cells exhibited more aggressive tumor progression than epithelial cells. Galunisertib monotherapy had no effect or exacerbated tumor growth in both models, whereas immunotherapy was particularly effective in epithelial-derived tumors. Notably, combined treatment with galunisertib and anti-PD-L1 significantly improved therapeutic efficacy in both models, leading to reduced tumor burden. These findings indicate that TGF- β inhibition alone may be ineffective or even detrimental. In contrast, its combination with immunotherapy provides therapeutic benefit in hepatocellular carcinoma (HCC), including tumors in which TGF- β retains tumor-suppressive functions.

Acknowledgements: This research was supported by the Spanish Association for Cancer Research (AECC), grant #PRYGN211279FABR.

When a toxic gas becomes protective: hydrogen sulfide in Duchenne muscular dystrophy

Anna Nalepa^{1,2}, Kalina Hajok¹, Małgorzata Myszka¹, Ewa Jakubczak¹, Urszula Waśniowska¹, Olga Mucha¹, Urszula Florczyk-Soluch¹, Józef Dulak¹, Agnieszka Łoboda^{1*}

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Duchenne muscular dystrophy (DMD) is a severe, incurable genetic disorder caused by mutations in the DMD gene, leading to a deficiency of the muscle structural protein, dystrophin, which results in damage to skeletal and cardiac muscles. Hydrogen sulfide (H₂S), traditionally regarded as a toxic gas, has recently emerged as a biologically active gasotransmitter with cytoprotective properties. We previously identified altered expression of H₂S-producing enzymes in dystrophic muscles, suggesting a potential role for H₂S deficiency in DMD pathogenesis¹.

To explore the impact of H₂S signaling in dystrophic muscles, we employed two murine models of DMD, the *mdx* strain, which exhibits a milder phenotype, and the D2.*mdx* mice, known for more severe disease progression. We evaluated the effects of pharmacological H₂S supplementation using a fast-releasing donor (NaHS) and a slow-releasing donor (GYY4137) as well as genetic overexpression of CTH, an H₂S-generating enzyme. NaHS modulated DMD-related gene and protein expression but failed to improve muscle function², while GYY4137 significantly enhanced exercise capacity, attenuated key pathological features, including inflammation, fibrosis, oxidative stress, apoptosis, and necrosis³, and exerted cardioprotective effects. CTH overexpression also affected major hallmarks of DMD.

Collectively, these findings demonstrate that increasing H₂S level, a molecule traditionally viewed as a toxic gas, can exert protective effects in DMD. This work highlights the therapeutic potential of H₂S-based strategies in slowing DMD progression and opens new avenues for the development of novel, mechanism-based interventions targeting muscle degeneration and dysfunction.

Acknowledgements: This work was supported by grant #2019/35/B/NZ3/02817 (to AŁ) from the National Science Centre.

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The interplay of NRF2 and miR-34a in vascular pathophysiology

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Cardiovascular diseases (CVDs) are frequently associated with impaired cellular defense against oxidative stress. The transcription factor NRF2 (*NFE2L2*), a master regulator of cytoprotective and stress-response pathways repressed by KEAP1, has emerged as a critical modulator of vascular homeostasis and endothelial cell (EC) aging. We previously showed that silencing of NRF2 induces premature EC senescence, which is associated with increased protein S-nitrosation. Interestingly, both effects rely on mechanisms independent of NRF2 transcriptional activity and are linked to KEAP1-mediated interactions with alternative binding partners.

In the present study, we investigated the role of NRF2 in aortic structure and abdominal aortic aneurysm (AAA) pathology, an age-related vascular disease. NRF2 deficiency in mice resulted in pronounced ultrastructural alterations of the aorta and premature vascular aging, with a significantly higher susceptibility to AAA formation compared to wild-type controls. Elevated levels of miRNA-34a (miR-34a) were detected in the EC layer of NRF2-deficient aortas, as well as in serum and ECs following stimulation with angiotensin II (Ang II), a hypertensive peptide. Endothelial cell-specific deletion of miR-34a effectively rescued NRF2-dependent premature aging and AAA development, highlighting the critical interplay between NRF2, miR-34a, and the intimal layer in aneurysm susceptibility.

Mechanistically, the protective effect of miR-34a deletion was associated with enhanced EC proliferation, despite the presence of endothelial dysfunction. We identified metastasis-associated protein 2 (MTA2), but not SIRT1, as a functional miR-34a target that suppresses Ang II-induced EC proliferation. In parallel, pharmacological intervention with simvastatin prevented AAA formation in NRF2-deficient mice by promoting EC layer regeneration independently of cholesterol modulation.

Together, these findings establish NRF2 as a key regulator of endothelial aging and aortic integrity and identify the NRF2–miR-34a axis as a critical determinant of AAA development. Strategies aimed at restoring endothelial regenerative capacity may represent a promising therapeutic avenue for aneurysm prevention and treatment.

SESSION 2 – Part 1: Cell-to-Cell Interactions

From single cells to collective behavior-communication and cooperation

Thursday, February 19, 2026

09:00-11:00

Induced pluripotent stem cells and gene editing in disease modeling and therapy

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Induced pluripotent stem cells (iPSCs), combined with modern gene editing technologies, have become transformative tools for modeling human disease and developing targeted therapies. These approaches are applied extensively in research of our Department.

In Duchenne muscular dystrophy (DMD), and its milder form, known as Becker MD, hiPSC derived cardiomyocytes (hiPSC CMs) generated through CRISPR/Cas9 engineering revealed unknown pathogenic mechanisms, including impaired iron homeostasis, mitochondrial dysfunction, and oxidative stress, which were reversible upon CRISPR mediated correction of the DMD mutation or pharmacological modulation of iron handling pathways. Complementary strategies employing CRISPR dCas9 mediated transcriptional activation demonstrated that upregulation of utrophin, a dystrophin paralogue, effectively improved electrophysiological properties, calcium cycling, and structural stability of dystrophin deficient cardiomyocytes, offering a mutation agnostic therapeutic approach for DMD cardiomyopathy.

Beyond DMD, iPSC-based modeling provides mechanistic insight into other disorders. In LAMA2 related muscular dystrophy, hiPSC derived myogenic precursors carrying LAMA2 loss of function mutations display intrinsic defects in proliferation, extracellular matrix remodeling, and regulation of cell cycle genes, driven by loss of laminin $\alpha 2$ secretion. These findings implicate stem cell dysfunction as a central contributor to disease progression and identify new therapeutic targets within the stem cell niche.

Importantly, iPSC derived cells also demonstrate promise in regenerative medicine. Our research in a murine model of myocardial infarction demonstrates that transplanted hiPSC CMs—whether unmodified or engineered to express cytoprotective factors—improve heart function, reduce fibrosis, survive long term in host myocardium, and functionally integrate with native cardiomyocytes. In direct comparison, adipose derived stromal cells confer no benefit, highlighting the unique reparative capacity of hiPSC derived cardiomyocytes for cardiac regeneration.

Together, these advances underscore the power of iPSCs and gene editing for (1) mechanistic dissection of human disease, (2) precision correction or modulation of pathogenic pathways, and (3) research on development of clinically relevant cell-based therapies.

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The immunogenic and paracrine roles of senescent endothelial cells in vascular disease

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Background: Endothelial cell senescence is a key contributor to vascular dysfunction and chronic inflammation, playing a central role in the progression of age-related and metabolic vascular diseases. Senescent endothelial cells exhibit phenotypic and functional changes, including acquisition of a senescence-associated secretory phenotype (SASP), thereby reshaping the tissue microenvironment. The mechanisms by which senescent endothelial cells influence vascular function and immune interactions remain incompletely understood.

Material and methods: Replicative senescence in human umbilical vein endothelial cells (HUVEC) was achieved *via* their extended passages. Human monocyte-derived macrophages (MDM) were obtained *via* differentiation of peripheral blood mononuclear cells. Control and senescent endothelial cells (cEC and sEC, respectively) were subjected to mass spectrometry-based proteomic profiling to identify molecular changes. Cytokine levels and cell-surface molecule expression were evaluated by ELISA and flow cytometry, respectively. Angiogenic responses were assessed using a 3D spheroid sprouting assay.

Results: Proteomic analysis revealed upregulation of pro-inflammatory SASP components and enrichment of pathways related to **antigen processing and presentation, glycosaminoglycan catabolism, endoplasmic reticulum calcium ion homeostasis, and cellular lipid catabolic processes** in sECs. Angiogenesis-related proteins were both up- and downregulated suggesting a dual effect of sEC on vascular development. Upon induction of apoptosis, sEC expressed higher levels of CD47—the “don’t eat me” signal—and were cleared less efficiently by macrophages than apoptotic cEC. In 3D spheroid sprouting assay, sEC formed significantly less and shorter sprouts than cEC, confirming their reduced intrinsic angiogenic capacity. On the other hand, cEC exposed to conditioned media from sEC developed significantly more and longer sprouts compared to exposure to cEC-derived conditioned media, indicating a paracrine stimulation of sprouting angiogenesis by sEC.

Conclusions: Senescent endothelial cells are characterized by proteomic and transcriptomic signatures of immune activation and functionally exhibit impaired clearance by phagocytes alongside a dual role in angiogenesis.

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Recombinant G-CSF uncouples neutrophil maturity from canonical progenitor marker expression

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Recombinant granulocyte colony-stimulating factor (G-CSF) is widely used to treat neutropenia but is often ineffective and mobilizes neutrophils with reduced maturity. In contrast, cobalt protoporphyrin (CoPP), which induces endogenous G-CSF together with additional cytokines, mobilizes neutrophils with higher granularity and higher expression of Ly6G marker. We therefore aimed to more comprehensively characterize the phenotype and functional properties of neutrophils mobilized by G-CSF and CoPP.

Supervised clustering of flow cytometry data identified multiple neutrophil populations, including a distinct subset defined by c-kit expression. Notably, c-kit-expressing neutrophils were present only in mice treated with recombinant G-CSF and were absent in CoPP-treated animals. Because c-kit is classically associated with hematopoietic stem and progenitor cells and is downregulated during granulopoiesis, we hypothesized that these cells might represent an immature population.

Unexpectedly, morphological assessment demonstrated that both c-kit⁺ and c-kit⁻ neutrophils displayed fully segmented nuclei. Quantitative morphometric analysis revealed no differences in nuclear size or complexity between subsets. Furthermore, phagocytic activity was similar between populations as measured using pHrodo-labeled *E. coli* particles. These findings indicate that c-kit expression does not reflect developmental immaturity.

Importantly, c-kit⁺Ly6G⁺CXCR2⁺ neutrophils were also detected in the bone marrow of G-CSF-treated mice but not in controls, suggesting that this population represents a G-CSF-induced, non-physiological neutrophil state rather than a normal developmental intermediate. Collectively, our data demonstrate that recombinant G-CSF uncouples canonical progenitor marker expression from neutrophil maturation, revealing previously unrecognized heterogeneity induced by exogenous cytokine signaling.

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Recent advances in preclinical pO₂ imaging

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Recent advancements in EPR technology and spin probe development have enabled rapid and precise acquisition of three-dimensional oxygen maps in small animal models. Solid-state oximetric probes, such as LiPc or OxyChip, allow longitudinal monitoring of oxygenation within selected regions of the tumor volume. Alternatively, soluble probes (e.g., OXO71) provide comprehensive 3D imaging and visualization of tissue pO₂ distribution. The aim of this study was to characterize oxygenation across diverse tumor types and assess changes induced by therapeutic interventions.

Tumor oxygenation was measured using EPR systems (Jiva-25, O₂M Technology; Bruker E540L, Bruker Biospin). Tumor anatomy and vascular architecture were evaluated by ultrasound and Doppler imaging (Vevo2100 or Vevo F2, FujiFilm VisualSonics). Syngeneic tumor models were established either ectopically (murine glioma GL261, human glioma LN229, human uveal melanoma Mel270) or orthotopically (4T1 breast carcinoma).

Small tumors (<50 µL) exhibited relatively high oxygen levels, with pO₂ ranging from 10 to 50 mmHg. In contrast, larger and older tumors (>250 µL) were markedly more hypoxic, with pO₂ values between 1 and 20 mmHg. The most severe hypoxia was observed in orthotopic gliomas, where pO₂ dropped to as low as 3–5 mmHg.

Tumor oxygenation undergoes substantial changes during growth and following treatment with chemotherapy or hypoxia alleviation treatments. Rapid and accurate assessment of tumor oxygen levels represents a critical tool for therapy monitoring and for elucidating mechanisms of tumor hypoxia. When combined with anatomical ultrasound and Doppler imaging of vascular structures, EPRI offers valuable insights into dynamic alterations within the tumor microenvironment.

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Codes of life and codes of biological information

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The term “code of life” or “language of life” has recently become popular but, at the same time, overused from the point of view of linguistics, semiotics, and information theory. When analyzing various aspects of the expression of genetic information, and more generally, biological information, many types of codes can be distinguished, and compared with one another. Some of them reveal so called “language universals” or “linguistic universals” characteristic of natural language. Since they are important to understand the idea of life as a state, a phenomenon, and a process, as well as for applying informatics, it is crucial to define, characterize, and systematize various types of codes in order to use them effectively when exploring the phenomena of life in research. This presentation attempts to achieve this aim and to organize the main linguistic concepts.

SESSION 2 – Part 2: Cell-to-Cell Interactions

From single cells to collective behavior-communication and cooperation

Thursday, February 19, 2026

11:30-13:00

Stem cells as a source of mediators in cell-to-cell communication - role of extracellular vesicles in tissue repair

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Growing evidence indicates that extracellular vesicles (EVs) released by mammalian stem cells (SCs) of various origin may play a role as important mediators of cell-to-cell communication by transferring their bioactive components such as mRNAs, miRNAs, proteins and lipids from parental cells to target cells, which may be utilized in tissue repair. Various SC populations have been already considered as valuable donor cells for therapeutic EVs, including developmentally early induced pluripotent SCs (iPSCs) and mesenchymal SCs (MSCs).

Several studies including previously published data of our team indicate iPSC- and MSC- derived EVs (iPSC-EVs and MSC-EVs, respectively) to be enriched in several mRNAs, miRNAs and proteins, supporting regeneration of injured tissues. It has been demonstrated that such EVs transfer their bioactive cargo to mature somatic target cells and greatly impact on phenotype and several functions of such cells including proliferation, survival, metabolic status and differentiation potential *in vitro*. Moreover, *in vivo* administrations of SC- derived-EVs in animal models, including ischemic tissue injuries, strongly indicate their safety and beneficial role in tissue repair by stimulating angiogenesis and increasing cell survival and proliferation in place of injury, with no side effects related to the administration of entire cells.

Importantly, the bioactive and pro-regenerative potential of EVs may be enhanced by genetic modifications enriching their bioactive cargo, which approach opens new perspectives for future practical applications of EV- based products in biomedical sciences and in clinic, including in therapy of ischemic tissue injuries.

Better understanding EV mechanism of action also leads to development of stem cell EV- based advanced therapy medicinal products (ATMPs), which may open new perspectives for their use in treatment of patients.

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MesoCellA-Ortho as an advanced therapy medicinal product for use in orthopedics - from research to clinical trial

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Cell therapies and novel biological medicinal products based on human cells and their bioactive derivatives are part of the rapidly developing field of advanced therapy medicinal products (ATMPs). Mesenchymal stem/stromal cells (MSCs), due to their unique properties, including (i) differentiation capacity and (ii) paracrine activity involving the secretion of bioactive molecules and extracellular vesicles (EVs), represent a promising tool for tissue repair as the active substance of ATMPs. The major goal of the project was to develop a novel ATMP based on adipose tissue-derived MSCs (AT-MSCs) intended for the treatment of osteoarthritis (OA), representing a civilization disease.

AT-MSCs isolation and culture processes were fully standardized in accordance with Good Manufacturing Practice (GMP) requirements and the recommendations of the European Medicines Agency (EMA). The AT-MSCs were subsequently used to prepare the formulation of an ATMP named MesoCellA-Ortho, which was classified by the EMA as a tissue-engineered product. Both AT-MSCs and MesoCellA-Ortho underwent extensive biological characterization *in vitro* and *in vivo* in a porcine model of cartilage and bone injury. In pigs treated with MesoCellA-Ortho, cartilage and bone defects were observed to be almost completely healed and predominantly filled with hyaline cartilage, representing the native cartilage type present on joint surfaces.

The safety and efficiency of MesoCellA-Ortho was subsequently evaluated in a phase I/II clinical trial (NCT05081921). The obtained results confirmed a high safety profile of the developed MesoCellA-Ortho medicinal product, along with its significant efficacy in reducing knee pain and improving patients' daily functioning. Moreover, administration of MesoCellA-Ortho demonstrated a beneficial effect on the integrity of joint cartilage.

MesoCellA-Ortho and optimized therapeutic procedure of its application in patients with OA, may revolutionize the treatment of cartilage and bone damage along with pain release in these patients, which may eliminate or delay the need for an invasive and expensive procedure of replacing the knee joint with endoprosthesis.

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Polyelectrolytes as effective cryoprotectants for stem cell-derived extracellular vesicles

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Extracellular vesicles (EVs) are currently recognized as potential new-generation cell-free therapeutic factors in the field of tissue regeneration. However, clinical application of EVs requires the development of standardized procedures of their long-term storage without the loss of structural integrity and biological activity following cryopreservation. Thus, the aim of this study was to develop a method of EV cryoprotection based on their coating with bilayer of biocompatible polyelectrolytes.

EVs secreted by human mesenchymal stem/stromal cells (MSCs) were isolated using ultracentrifugation method and examined according to their size distribution and presence of selected surface markers. Subsequently, EVs were coated with ultrathin bilayer of designed polyelectrolytes, including: 1) cationic block copolymer (PEG46-b-PMAPTAC52; P1) and 2) anionic polymer (PAMPS18; P2), using layer-by-layer technique. Based on the nanoparticle tracking analysis (NTA), high-resolution flow cytometry, as well as mass spectrometry, we analyzed vesicle integrity following single or multiple freezing-thawing cycles, as well as long-term storage. Additionally, we evaluated the effect of cryopreservation on the functional activity of EVs *in vitro*, using human osteoblasts as exemplary target cells.

Obtained data indicate, that coating with P1 and P2 polymers stabilizes structural integrity of EVs following deep freezing and thawing, preserving their biological activity *in vitro*. Additionally, proteomic analysis has confirmed an effect of particle stabilization, as well as an enrichment in EV- and MSCs-related proteins in samples cryopreserved in the presence of tested polymers, indicating protective effect on EV protein cargo.

Taking together, our study indicates that the use of P1/P2 polyelectrolyte bilayer may be a novel, effective way of long-term storage of EV preparations for the purpose of basic research and their clinical applications.

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Investigating the effects of HSPC mobilizing factors on the development of GVHD in a haploidentical murine model

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Allogeneic hematopoietic cell transplantation (HCT) remains the only curative therapy for many hematologic malignancies, yet a subset of healthy donors fails to mobilize sufficient hematopoietic stem cells with standard G-CSF treatment. Cobalt protoporphyrin IX (CoPP) has emerged as an alternative mobilizing agent acting through the induction of endogenous G-CSF and additional cytokines while mobilizing fewer lymphocytes than exogenous G-CSF, raising the possibility of altered transplant immunogenicity and graft-versus-host disease (GVHD) outcomes. We sought to investigate whether transplantation of hematopoietic stem cells mobilized by CoPP or G-CSF influences GVHD development using a haploidentical GVHD mouse model. As expected, CoPP mobilized fewer lymphocytes compared to the G-CSF group. Importantly, CoPP significantly enriched KLS⁺ hematopoietic stem and progenitor cells by proportion compared to G-CSF. Despite these compositional differences, recipients of CoPP-mobilized PBMC showed comparable myeloid, lymphoid, and stem cell and progenitor reconstitution at days 15 and 60 post-transplant, as well as similar thymopoiesis at both timepoints, compared to the G-CSF group. Systemic cytokine profiles, including IL-6, IFN γ , TNF α , and CXCL10, were also similar between groups and consistent with allogeneic immune activation. An increased BAFF/B-cell ratio at day 60 correlated with worse clinical presentation in both haploidentical cohorts. Clinical GVHD-related mortality occurred in both cohorts, with higher mortality in the CoPP group despite more severe clinical observation scores in the G-CSF cohort. Together, these data demonstrate that although CoPP and G-CSF mobilize different cell populations, they result in comparable hematopoietic reconstitution and systemic inflammatory responses in haploidentical transplantation.

Acknowledgements: The study was supported by the Grant from DKMS Foundation and by the Faculty of Biochemistry, Biophysics and Biotechnology (Initiative of Excellence - Research University, Research Support Module 2025).

Brain lipids and cholesterol homeostasis: physiological interactions and relevance in antipsychotic treatment

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Lipid homeostasis is essential for proper brain function, and its dysregulation has been implicated in various neuropsychiatric disorders. Cholesterol, an essential component of cellular membranes, in the human brain not only regulates membrane fluidity and permeability but also contributes to myelin sheath formation, synaptogenesis, neurotransmission, and participates in numerous key biochemical pathways. In the human brain, the maintenance of cholesterol homeostasis relies on molecular interactions between neurons and astrocytes.

Antipsychotic drugs (APDs), primarily used for the treatment of schizophrenia, among other effects, alter lipid metabolism – particularly cholesterol. To further investigate the molecular mechanisms underlying the impact of APDs, three antipsychotic drugs (clozapine, risperidone, and haloperidol) were studied in vitro using cell culture models. Mass spectrometry-based proteomics indicate that antipsychotics significantly affect the levels of proteins implicated in cholesterol metabolism, such as lipid transporters, enzymes involved in cholesterol biosynthesis, and transcription factors regulating their expression. Using a luminescence assay and fluorescence staining, it was demonstrated that the tested drugs change intra- and extracellular cholesterol levels and influence lipid droplet formation. To further characterize morphological and biochemical alterations, particularly lipidomic changes, in cells treated with antipsychotics, Raman imaging was employed, enabling the identification of cellular components most affected by the treatment. The results obtained in this study indicate that clozapine exerts the strongest effect on lipid metabolism compared with the other tested drugs, potentially providing insight into its mechanism of action, which has not yet been fully understood.

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SESSION 3 – Part 1: Host–Microbe Interactions

Conflict, coexistence, and communication across biological boundaries

Thursday, February 19, 2026

14:00-15:40

Keratinocyte-neuro-immune crosstalk in skin pathophysiology

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The skin is one of the largest and most exposed barrier organs. Here, we review current knowledge and concepts—including our own work—on how the epidermis, the immune system, and the sensory nervous system function independently and in concert to maintain skin homeostasis in close proximity to benign or pathogenic microbes, and how these interactions become dysregulated in chronic cutaneous diseases. We also highlight novel technological strategies, including integrated single-cell RNA sequencing (scRNA-seq) and spatial proteomic approaches, that are instrumental in advancing this field.

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Gingival fibroblasts in periodontitis: passive responders or imprinted aggressors?

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Gingival fibroblasts (GFs) are essential components of the periodontium and are responsible for the maintenance of tissue structure and integrity. However, the physiological role of GFs is not restricted to the production and remodeling of the extracellular matrix. GFs also act as sentinel cells that modulate immune responses to oral pathogens invading gingival tissues. As important “nonclassical” components of the innate immune system, GFs respond to bacteria and damage-associated signals by producing cytokines, chemokines, and other inflammatory mediators.

Although GF activation supports the elimination of invading bacteria and the resolution of inflammation, uncontrolled or excessive activation may instead promote inflammation and bone destruction. This occurs in periodontitis, a chronic inflammatory disease of the periodontium initiated and sustained by microbial dysbiosis. In our recent studies, we show that GFs undergo synergistic inflammatory activation in response to oral pathogens and inflammatory cytokines. This enhanced production of inflammatory mediators by GFs may contribute to excessive activation of macrophages and neutrophils and thus represents an important new mechanism of stromal–immune cross-talk in periodontitis [1].

On the other hand, within inflamed gingival tissues, GFs acquire imprinted proinflammatory phenotypes that stimulate osteoclastogenesis and contribute to the chronicity of inflammation. For example, our recent work demonstrated that a sustained reduction in the ability of GFs from periodontitis patients to produce osteoprotegerin underlies their impaired capacity to suppress osteoclastogenesis [2]. These imprinted pathogenic GF phenotypes may be driven by prolonged exposure to oral pathogens, leading to a form of “transcriptional memory” that alters GF biological functions and converts them into “imprinted aggressors” actively contributing to periodontitis pathogenesis.

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Moonlighting proteins of *Candida* fungi in host interactions and contact system binding and activation

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The virulence of *Candida* fungi is primarily mediated by cell wall proteins that govern specific interactions with host molecules. Over the past two decades, work from our group has provided compelling evidence that both classical fungal adhesins and moonlighting proteins, defined as proteins performing two or more distinct functions depending on cellular localization, play critical roles in these host–pathogen interactions. An important consequence investigated extensively in our studies, is the activation of the plasma contact system at the fungal cell surface, leading to the generation of vasoactive and proinflammatory kinins.

Initial research identified *C. albicans* cell wall proteins, including typical adhesin Als3 and moonlighting proteins: enolase, triosephosphate isomerase, phosphoglycerate mutase, and glucose-6-phosphate isomerase, as direct partners of contact system components: high-molecular-weight kininogen, prekallikrein, and coagulation factor XII. Extending these analyses to *C. tropicalis* and *C. parapsilosis* revealed additional adhesins and moonlighting proteins capable of binding human kininogen, thereby identifying activation of the plasma contact system at the fungal cell surface as a critical mechanism in the pathogenesis of infections caused by these fungi.

We further demonstrated broad presence of moonlighting proteins on the surface of *Candida* cells across diverse growth conditions, with enhanced exposure under host-mimicking environments, underscoring the importance of moonlighting proteins in host–pathogen interactions and fungal physiology. We identified both the mechanism of their extracellular transport and a specific pathway for anchoring to the fungal cell wall. Notably, enolase, a major moonlighting protein, binds to the cell surface via direct interactions with the adhesin Als3. Both proteins, along with other key fungal molecules, including proteinases, are co-transported within extracellular vesicles (EVs), which modulate host–pathogen interactions as central mediators of fungal virulence, extending also the functional reach of cell wall proteins beyond the immediate cell surface.

Overall, our long-term research underscores the multifunctional role of *Candida* cell wall proteins in virulence, host colonization, immune evasion, and stress adaptation, while revealing novel targets for antifungal intervention.

Natural diversity of staphylococcal lysostaphins: a foundation for novel antimicrobial drug development

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Antimicrobial resistance contributes to millions of deaths globally each year and represents a critical challenge to healthcare. Staphylococci, particularly the World Health Organization priority pathogen *Staphylococcus aureus* and emerging non-aureus species, remain major drivers of this crisis. As resistance outpaces conventional drug development, peptidoglycan hydrolases are currently gaining prominence as promising enzybiotic alternatives. Among these are lysostaphins, glycylglycine endopeptidases secreted by specific staphylococcal strains to eliminate other competing staphylococci. However, only a few lysostaphins have been fully characterised to date. Our research aims to explore the natural diversity of these enzymes and evaluate their potential as viable alternatives to antibiotics.

Bioinformatic analysis of nearly 100,000 staphylococcal genomes identified more than 300 sequences potentially coding for enzymes with a lysostaphin domain architecture. These included 29 unique lysostaphin mature form sequences that formed 5 distinctive similarity clusters. Functional characterization of 5 representative enzymes, using fluorogenic substrates and a panel of Gram-positive bacteria, revealed diverse enzymatic profiles and species preferences. Notably, we identified Lys-Ssim, representing a previously unknown cluster, which exhibits activity extending beyond the *Staphylococcus* genus, an unprecedented feature for this enzyme class. Furthermore, we demonstrate that susceptibility to the analyzed lysostaphins varies among species and is sensitive to specific alterations within the peptidoglycan pentaglycine bridge, which is targeted by lysostaphins.

Our findings significantly expand the known diversity of lysostaphins and highlight their potential for the development of specific antimicrobials. The discovery of novel variants, including the atypical case of Lys-Ssim, provides a foundation for the design of enzybiotics capable of overcoming staphylococcal resistance.

Acknowledgements: This research was supported by funds granted by the National Science Centre (NCN, Poland) based on decision No. DEC-2021/43/B/NZ6/02413 (to BW).

Zuzalysin: a giant virus-like proteolytic cage produced by the periodontal pathogen *Porphyromonas gingivalis*

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Zuzalysin (ZUZ) is a ~95-kDa metallopeptidase secreted by the keystone periodontal pathogen *Porphyromonas gingivalis*, which is exposed on the bacterial outer membrane and enhances virulence in a murine subcutaneous chamber infection model. ZUZ is produced as a latent, calcium-dependent zymogen that becomes proteolytically active only upon binding Ca²⁺. Activation is coupled to self-assembly into a higher-order proteolytic structure, suggesting that supramolecular machineries may contribute to host tissue damage and immune modulation.

Using biochemical assays, biophysical methods and high-resolution structural biology, ZUZ was shown to undergo Ca²⁺-triggered autolytic removal of a cysteine-switch propeptide and to assemble hierarchically from monomers into ~0.5-MDa pentamers, higher oligomers, and a ~5.6-MDa, ~355-Å dodecahedral particle (DdhZUZ) with icosahedral symmetry and 60 lumen-facing active sites. This proteolytic cage is comparable in size to small viral capsids, yet remains catalytically competent, placing it among the largest known active protein assemblies. X-ray crystallography and single-particle cryo-EM (1.8–3.6 Å) reveal a protomer with an N-terminal oligomerization subdomain, a central catalytic moiety, and a C-terminal regulatory subdomain containing a calcium site positioned ~12 Å from the catalytic Zn²⁺ center, linking metal binding to stability, oligomerization and activity.

Functionally, ZUZ is a broad-specificity endopeptidase with preference for hydrophobic residues at P1', capable of degrading host proteins such as fibrinogen, fibronectin and caseins, as well as diverse fluorogenic peptides, and is sensitive to inhibition by metal chelators. In DdhZUZ, 20 main entry pores provide access to an enclosed lumen, where 60 active sites form a size-selective proteolytic environment that favors small proteins and peptides and excludes larger substrates. Zuzalysin thus defines a virus-like proteolytic nanocage in bacteria and suggests new directions for studying *P. gingivalis* pathogenesis and for exploiting self-assembled cages in bioinspired nanotechnology.

Acknowledgements: This study was supported by a Polish National Science Centre grant no. 2023/51/D/NZ1/02675. We gratefully acknowledge National Synchrotron Radiation Centre SOLARIS which is supported by the Ministry of Science and Higher Education, Poland, under contract no. 1/SOL/2021/2 (project no: 233068) as well as Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and support within computational grant no. PLG/2025/018356.

SESSION 3 – Part 2: Host–Microbe Interactions

Conflict, coexistence, and communication across biological boundaries

Thursday, February 19, 2026

14:00-15:40

The role of the cell wall-binding domain in the activity and specificity of novel lysostaphins

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The widespread and significant impact of bacterial infections on human health and the economy, particularly those caused by multidrug-resistant pathogens, necessitates novel therapeutic approaches. Peptidoglycan hydrolases (PGHs), enzymes that cleave cell wall peptidoglycan (PG), are promising candidates for this purpose. PGHs typically possess a modular molecular architecture comprising an enzymatically active domain (EAD), which cleaves specific bonds in PG, and a cell wall-binding domain (CBD), which recognizes and binds specific ligands. This architecture is characteristic of lysostaphins – a group of PGHs secreted by staphylococci to compete with other strains. However, while most lysostaphin studies have focused on catalytic properties and EADs, the role of CBDs remains underexplored.

This study aimed to characterize the specificity, binding capacity, and physicochemical properties of novel lysostaphin CBDs to determine their potential for bioengineering.

Using a combination of classical and restriction-free cloning, we fused the CBDs of seven lysostaphins (LSs, LSp222, Saur, Sfel, Shom, Smus, and Ssim) with fluorescent proteins (GFP, CFP, and mCh). After expression and purification, we studied the binding of these chimeric proteins to *Staphylococcus aureus*. The results showed that CBDs of LSp222, Saur, and Smus exhibited the highest binding capacity, while Shom showed the lowest. Similar results were observed for binding to *Staphylococcus epidermidis* and *Micrococcus luteus*. Interestingly, CBD binding was found to be dependent on pH, salt, and buffer composition. The introduction of reciprocal point mutations of carefully selected residues in GFP-Smus(ES)-CBD (ES residues in CBD from Shom) and GFP-Shom(KA)-CBD (KA residues in CBD from Smus) resulted in a two-fold decrease and three-fold increase in binding to *S. aureus*, respectively, highlighting the importance of positively charged residues in binding. However, introducing these substitutions into full-length lysostaphins did not affect their enzymatic activity. Moreover, domain shuffling did not affect the activity and/or specificity of the analyzed enzymes, as demonstrated for the chimeric lysostaphin Ssim(EAD)-Smus(CBD).

Acknowledgments: This research was supported by funds granted by the National Science Centre (NCN, Poland) based on decision No. DEC-2021/43/B/NZ6/02413 (to BW).

Yin-Yang – the two-component system of lysostaphin resistance in *Staphylococcus simulans*

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Lysostaphin is one of the most potent antistaphylococcal enzymes. Resistance to this enzyme is mediated by the lysostaphin immunity factor (Lif), which modifies peptidoglycan structure to protect cells from lysis. Lif is encoded within the same operon as the lysostaphin gene; therefore, it was believed that the development or spread of resistance to other susceptible strains is highly unlikely. In addition to the long-known canonical lysostaphin (LSs) produced by strain 20723 of *Staphylococcus simulans*, we recently discovered a novel lysostaphin (LSs01) from another strain of the same species. LSs01 is also encoded along with a gene for the Lif protein, but the corresponding operon has a different architecture.

In this study, we identified and characterized the lysostaphin immunity factor Lif and the FmhA protein from the LSs01 producer strain. We compared their activity to that of known Lif_Ss against LSs and LSs01 in lysostaphin-susceptible *S. aureus*.

The LSs producer strain is resistant to its own lysostaphin (LSs) and shows moderate resistance to LSs01. The *S. simulans* strain that produces LSs01 is moderately lysed by LSs and, surprisingly, efficiently by LSs01, despite carrying the gene for a specific Lif_Ss01. Expressing Lif in *S. aureus* mirrors the protective effect seen in its native hosts. Additionally, *S. simulans* strains carry a gene encoding the FmhA protein, which, when overexpressed in *S. aureus*, provides protection against both lysostaphins. Notably, Lif-driven resistance to lysostaphins imposes a significant burden on cells, impairing their growth. We suggest that Lif-modified peptidoglycan cannot be efficiently remodeled or turned over by endogenous host autolysins during cell growth and division.

In conclusion, resistance to lysostaphin in lysostaphin-producing *S. simulans* strains is driven by a two-component system. It consists of a strain-specific Lif protein, genetically linked to lysostaphin, and the FmhA protein, widely encoded in *S. simulans*. However, their contributions to protection differ between the strains.

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Puzzling contradictions in research on immunomodulatory activity of bacteriocin BacSp222

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Bacteriocins are proteins or peptides produced by bacteria to kill or inhibit the growth of closely related bacterial strains. Unlike classical antibiotics, bacteriocins are ribosomally synthesized polypeptides that act at lower doses and exhibit a narrower spectrum of activity. Bacteriocin BacSp222, investigated by our team, is a member of this group of compounds; it is a peptide produced by *Staphylococcus pseudintermedius* 222, a strain of commensal Gram-positive bacteria residing in domestic animals. However, in addition to bactericidal activity, our research also focuses on the immunomodulatory potential of BacSp222 and its interaction with Toll-like receptors [1].

Our results demonstrated that BacSp222 displays proinflammatory effects toward immune cells, mediated by activation of the human TLR2/TLR6 heterodimer. Interestingly, BacSp222 also interacts with another proinflammatory compound, lipopolysaccharide (LPS), a structurally and functionally distinct molecule produced by Gram-negative bacteria, whose proinflammatory activity is mediated by TLR4 signaling. We demonstrated that the two proinflammatory molecules (BacSp222 and LPS) do not exhibit synergistic effects; instead, they mutually inhibit their respective proinflammatory activities [1].

[1] Śmiałek-Bartyzel J., Bzowska M., Frączek A., Wojda I., i in. (2025) Interactions between Lipopolysaccharide and Peptide Bacteriocin BacSp222 Influence Their Biological Activities. *ACS Infectious Diseases* 11:2116–2130

Oral pathogens induce trained-like immunity in gingival fibroblasts: A novel mechanism of innate immune memory in periodontal disease

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Gingival fibroblasts (GFs), traditionally recognized for maintaining connective tissue integrity, also act as active participants in innate immune responses. While trained immunity has been extensively characterized in myeloid cells, its presence and functional relevance in fibroblasts remain poorly understood. This study investigated whether oral bacterial pathogens induce long-term inflammatory reprogramming in human GFs.

Primary human GFs were infected with the periodontal pathogens *Porphyromonas gingivalis* or *Fusobacterium nucleatum* for 24 h, followed by antibiotic-mediated bacterial clearance and a resting period prior to secondary stimulation. Inflammatory memory was assessed by re-exposure to TNF- α or bacteria.

GFs previously exposed to bacteria exhibited significantly enhanced cytokines production at both protein and mRNA levels upon secondary TNF- α stimulation or *P. gingivalis* re-infection compared to un-infected controls. This primed-like response persisted for up to one week after primary infection and was associated with increased metabolic activity rather than elevated cell proliferation. Importantly, inflammatory reprogramming required infection with live bacteria and could not be reproduced using bacterial components alone.

Transcriptomic analysis revealed altered expression of genes involved in tissue remodeling and intracellular signaling, including matrix metalloproteinase 3 and regulator of G-protein signaling 5. In addition, *P. gingivalis* infection promoted fibroblast-to-myofibroblast transition, indicating a potential link between inflammatory memory and pathological tissue remodeling. Despite pronounced functional changes, no significant global or locus-specific differences in DNA methylation profiles were detected. Instead, enhanced TNF-induced phosphorylation of NF- κ B p65 observed 7 days after infection suggests persistent alterations in inflammatory signalling pathways and raises the possibility of alternative regulatory mechanisms, including histone-mediated chromatin remodelling. These findings reveal a novel mechanism by which bacterial infection induces a persistent pro-inflammatory state in GFs, expanding the concept of trained immunity beyond myeloid cells. This discovery also highlights GF inflammatory activation as potential therapeutic target host modulation therapeutic strategies in periodontal disease.

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Can I eat this? Algae as a valuable component of the human diet

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As global interest in sustainable and nutrientdense food sources grows, algae are increasingly recognized as promising candidates for human consumption. Yet, many terrestrial and freshwater species remain poorly characterized, and tested. In this study, we assessed the antioxidant potential, pigment composition, mineralrelease profile, and basic safety of three understudied green microalgae: *Auxenochlorella protothecoides*, *Bracteacoccus glacialis*, and *Jenufa aeroterrestica*. Antioxidant activity quantified via DPPH• assays revealed strong radicalscavenging properties in methanolic extracts of *B. glacialis* (56% reduction) and *A. protothecoides* (45%), while *J. aeroterrestica* showed minimal activity. Pigment profiling further highlighted *A. protothecoides* as the most metabolically active species, accumulating the highest levels of chlorophylls and carotenoids, key compounds contributing both to cellular photoprotection and dietary antioxidant capacity.

To evaluate suitability for dietary applications, **acute toxicity tests using *Thamnocephalus platyurus*** were performed. **None of the algal extracts nor cell suspensions exhibited toxicity**, confirming a favorable safety profile. To explore their nutritional mineral contribution, we conducted **ionrelease analyses** following complete cell lysis. All species released physiologically relevant ions, though with distinct profiles: *J. aeroterrestica* liberated the most potassium ($>17.5 \text{ mg} \cdot \text{g}^{-1} \text{ d.w.}$), *B. glacialis* released the highest magnesium ($7.2 \text{ mg} \cdot \text{g}^{-1} \text{ d.w.}$), while *A. protothecoides* showed the greatest calcium ($1.6 \text{ mg} \cdot \text{g}^{-1} \text{ d.w.}$) and phosphate ($\sim 5.8 \text{ mg} \cdot \text{g}^{-1} \text{ d.w.}$) release. Levels of potentially harmful ions - nitrite, nitrate, and fluoride - remained low across all species, supporting their safety for nutritional use.

Together, these findings position *A. protothecoides* and *B. glacialis* as particularly valuable candidates for functional foods, nutraceuticals, and supplementation. Their strong antioxidant capacity, high pigment content, safe toxicological profile, and measurable release of essential dietary minerals underscore the potential of freshwater microalgae as promising, sustainable components of the human diet.

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Uncovering the role of FEN1 phosphorylation in *Arabidopsis thaliana* by the atypical cyclin-dependent kinase CDKF;1

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Cell cycle control is a key mechanism regulating eukaryotic cell function. Plants are unusual organisms: not only are they autotrophic and capable of continuous, lifelong growth, but their sessile lifestyle has also driven the evolution of distinct adaptive strategies. Consequently, the mechanisms governing cell cycle regulation in plants differ in several respects from those described in animals and fungi.

The cyclin-dependent kinase F;1 (CDKF;1)—the only subtype identified to date—in the model organism *Arabidopsis thaliana* represents a unique component of plant cell cycle control¹. Although CDKF;1 belongs to the family of cyclin-dependent kinases (CDKs), owing to its conserved kinase domain, its activity is not dependent on cyclins, contrary to what its name suggests. CDKF;1 is also classified as a CDK-activating kinase (CAK), placing it hierarchically above other CDKs, as it is capable of phosphorylating both CDKs and other CAKs². In addition to its CAK activity, CDKF;1 undergoes autophosphorylation, phosphorylates the C-terminal domain of the large subunit of RNA polymerase II, and is thought to play an important role in regulating cell division and cell elongation³. This role may be further supported by its ability to phosphorylate Flap endonuclease 1 (FEN1), a protein involved in DNA metabolism, particularly in DNA replication and repair.

FEN1 exhibits three distinct enzymatic activities: flap endonuclease (FEN), gap-dependent endonuclease (GEN), and nick-specific exonuclease (EXO)⁴. FEN1 is a crucial interacting partner of proliferating cell nuclear antigen (PCNA) within the DNA replication machinery. Therefore, investigating the functional relationship between CDKF;1 and FEN1—particularly CDKF;1-mediated phosphorylation of FEN1 in the presence of PCNA—constitutes the main objective of this study.

Acknowledgements: This work was financially supported by NCN grant OPUS 27/ UMO-2024/53/B/NZ3/00981 to WS. [1] J.G. Tank, V.S. Thaker (2011) doi.org/10.1007/s10535-011-0031-9; [2] A. Shimotohno et.al (2004) doi: 10.1105/tpc.104.025601; [3] M. Hajheidari et al. (2012) doi: 10.1105/tpc.112.096834; [4] Zheng et al. (2005) doi.org/10.1038/sj.embor.7400313.

Cryo-EM of bioenergetic complexes in native membranes

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Single-particle electron cryomicroscopy (cryo-EM) is a powerful technique that has revolutionized the field of structural biology over the past decade. However, in the case of membrane proteins and complexes, especially obtained from native sources, structure determination is still considered a hard task, even with the use of cryo-EM. Moreover, the usage of detergents to extract membrane proteins from their lipid environment is sometimes considered a harsh interference with their structural features.

Herein, in the search for a method to determine the structures of large respiratory supercomplexes of the thermoacidophilic archaeon, *Sulfolobus acidocaldarius*, we developed a protocol for direct, single-particle cryo-EM imaging of proteins embedded in membrane vesicles. For tests, we picked a well-known system of bacterial chromatophores, which comprises an entire photosynthetic apparatus of purple bacteria, such as *Rhodobacter* or *Allochromatium*. The developed method allowed us to gain unprecedented insight into the structure of specific components of the bacterial photosynthetic system and to determine how they interact with their natural lipid environment. Moreover, we have uncovered the existence of an over 1.5 megadalton cytochrome *bc*₁-centered, dimeric megacomplex in chromatophores of *Rhodobacter sphaeroides*. This megacomplex, which we call ‘photosynthesome’, comprises a central cytochrome *bc*₁ dimer, two dimeric reaction centers, and two light-harvesting II complexes. This discovery sheds new light not only on the structural interactions between particular elements of the photosynthetic apparatus but also explains interactions of the photosynthetic enzymes with the quinone pool and resolves multiple issues related to the kinetic models.

Acknowledgements: This research was supported by the National Science Centre in Poland under the SONATA17 grant: Extremophilic archaea bioenergetics: respiratory oxidoreductases structure and function in *Sulfolobus acidocaldarius* (to S. Pintscher) 2021/43/D/NZ1/02280, WBBiB Faculty minigrant ID.UJ (to S. Pintscher), UK Research and Innovation funds BB/X003035/1 (to J. N. Blaza), European Research Council (ERC) no. 854126 (to N. C. Hunter), Royal Society URF\R\241006 (to A. Hitchcock), and PLGrid computational grant no. PLG/2025/018653 (S. Pintscher).

Hyperforin-mediated mitochondrial uncoupling sensitizes A549 lung cancer cells to everolimus in 2D and 3D models

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One of the most recent directions in research on chemotherapeutic agents focuses on their impact on cellular metabolism. Cancer cells adapt to oxidative stress and hypoxia, as well as their ability to switch between oxidative phosphorylation and glycolysis, constitutes a key survival mechanism and may contribute to the development of drug resistance. Hyperforin (HPF), the major constituent of *Hypericum perforatum* L., is currently being intensively investigated for its properties as a mitochondrial uncoupler. This study examined HPF as a sensitizer of everolimus (EV) in A549 lung cancer cells using 2D monolayers and 3D spheroids, assessing viability, bioenergetics, metabolic adaptation, redox balance, migration, and selected metabolic and stress response proteins.

In 2D cultures, HPF strongly reduced cell viability, while EV induced time-dependent metabolic adaptation and showed lower toxicity to normal cells. Co-treatment with 0.75 μ M HPF lowered the effective EV dose to 10 μ M, indicating an additive effect. Both compounds inhibited proliferation; HPF induced apoptosis, and their combination markedly decreased cell viability. In contrast, no significant changes in viability were observed in 3D spheroids. In the 2D model, HPF rapidly uncoupled mitochondria, reducing membrane potential, depleting ATP, and shifting metabolism toward glycolysis. EV slightly increased mitochondrial membrane potential, reflecting compensation. Combined treatment intensified bioenergetic stress. In 3D spheroids, prolonged HPF–EV exposure reduced ATP levels despite unchanged viability. HPF and EV also differentially regulated redox and hypoxia-related pathways. In 2D cultures, both increased reactive oxygen species; HPF strongly activated Nrf2 signaling as well as decreased HIF-1 α and selected metabolic enzymes. In contrast, 3D spheroids exhibited an opposing protein pattern with reduced Nrf2 activity, and HPF enhanced HIF-1 α expression under hypoxic conditions. Additionally, HPF inhibited cell migration in 2D, while EV strongly suppressed migration from spheroids.

In conclusion, HPF acts as a mitochondrial-targeting sensitizer that enhances EV efficacy by disrupting cellular bioenergetics. Differences between 2D and 3D models underscore the critical role of the tumor microenvironment, particularly hypoxia, in shaping metabolic and therapeutic responses.

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Application and comparison of composites coated with TiO₂ – does it have an impact on cyanotoxins and cyanobacterial blooms?

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Freshwater ecosystems are crucial for aquatic fauna and flora, but they are vulnerable to harmful algal blooms caused by cyanobacteria (cyano-HAB). These cyanotoxins pose a threat to public health through skin contact or consumption of contaminated drinking water or food. To reduce the occurrence of cyanobacterial blooms, effective physical, chemical, technological or biological procedures are needed to remove extracellular toxins dissolved in water. Therefore, in search of an innovative approach that rapidly and effectively eliminates cyanotoxins in a relatively short time - Advanced oxidation processes (AOPs) with activated carbon cores and polymer composites are a promising tool. Titanium dioxide (TiO₂) photocatalysis is an effective technique for degrading pollutants, a relatively inexpensive and simple approach. The study aims to find an effective and inexpensive method for the photocatalytic-chemical remediation of cyanotoxins and the impact on CyanoHabs. In this study, the efficacy of adsorption and photocatalytic degradation of microcystin-LR and anatoxin-a were compared between three TiO₂-coated composites. Results demonstrated that for McLR, the composite, RFM-15520PT35-850, RF-520PT36-850 achieved upto 80% adsorption in dark conditions and 100% degradation under UV-a within 60 minutes of irradiation. For ATX-a, the NLP composite proved most effective, achieving 100% degradation within 60 minutes of irradiation, whereas RF showed limited efficacy. Furthermore, the RF composites successfully degraded 100% of extracellular and 60% of intracellular toxins in live *Microcystis aeruginosa* cultures. These composites show promise in the photocatalytic-chemical remediation of cyanotoxins, and further studies with different cyanotoxins will allow comparison of how this remediation can be achieved.

Acknowledgements: We would like to thank Prof. dr hab. Piotr Kuśtrowski and the Department of Chemical Technology of the Faculty of Chemistry at the Jagiellonian University for their cooperation in formulating the composites for carrying out this research work.

POSTER SESSION

A

Wednesday, February 18, 2026

20:00-21:00

A1: Chromosome-level genome assembly of *Periplaneta lateral* Walker, 1868 (Blattodea, Blattidae)

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The Turkestan cockroach, *Periplaneta lateral* Walker, 1868 (Blattodea, Blattidae), is a broadly distributed synanthropic species that has become increasingly relevant as a model for studying cockroach evolution, pest biology, physiology, and host–pathogen interactions within Blattodea. Its ease of laboratory maintenance, ecological adaptability, and phylogenetic position within Blattidae make it particularly suitable for comparative and functional studies. Despite these advantages, no reference genome has previously been available for *P. lateral*. Consequently, research addressing many aspects of its biology has remained limited, largely due to the absence of a high-quality genomic framework to support integrative omics approaches.

Here, we present a high-quality *de novo* genome assembly of *P. lateral* generated using a combination of 90 Gb of PacBio High-Fidelity (HiFi) long-reads and 120 Gb of data from chromatin conformation capture (Hi-C). Integrating data from these complementary methods resulted in a draft assembly of 3.04 Gb across 290 contigs. We performed decontamination of contigs prior to scaffolding, ensuring that only host-derived sequences were used in the chromosome reconstruction driven by Hi-C reads.

The resulting assembly displayed high contiguity, completeness, and structural accuracy, with the majority of the genome organized into chromosome-scale scaffolds. The structural integrity of the assembly is strongly supported by BUSCO completeness assessments, providing a reliable genomic resource for future evolutionary, ecological, and functional studies in this species.

A2: The role of oxidative stress in the development of pancreatic β -cells

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Understanding the molecular mechanisms that guide cell fate decisions remains not completely understood, despite extensive efforts across developmental biology and stem-cell research. Previous studies have suggested that GPX2, the principal antioxidant enzyme in the gastrointestinal tract, may also influence pancreas development in both mouse and human systems. Building on this premise, we examined the consequences of GPX2 loss- and gain-of-function using spheroid models derived from pluripotent stem cells of wild-type (WT), GPX2 knockout (GPX2 KO), and GPX2 overexpression (GPX2 OE) cell lines.

Our analyses reveal that GPX2 plays a critical role in maintaining the balance between pancreatic and hepatic lineage identity. Both spontaneous and directed differentiation toward β -cell fates show that GPX2 KO cells exhibit early and robust upregulation of hepatic markers at the definitive endoderm stage, indicating a shift toward a hepatic-like phenotype. Conversely, GPX2 OE cells display reduced expression of hepatic marker proteins, supporting a protective role for GPX2 in preserving pancreatic identity. Beyond transcriptional changes, GPX2 deletion also induces pronounced alterations in extracellular matrix (ECM) organization, suggesting broader effects on the niche.

Importantly, we demonstrate that these phenotypic deviations are reversible. Modulation of oxidative stress levels and targeted regulation of BMP and TGF β signaling pathways restores pancreatic-biased differentiation in GPX2-deficient cells.

Together, these findings identify GPX2-mediated antioxidant activity as an essential regulator of redox homeostasis that safeguards proper pancreatic lineage development. Disruption of GPX2 function destabilizes ECM architecture, elevates BMP/TGF β pathway activation, and ultimately promotes hepatic fate. Our work positions GPX2 as a key molecular determinant ensuring fidelity of pancreas-directed differentiation. The data presented here constitute part of a study published in Nature Communications [1].

[1] Szpotkowska, J., Szlachcic, W.J., Blaszczyk, K. et al. Oxidative stress and GPX2 control pancreatic vs. non-pancreatic cell fate in human endoderm. Nat Commun (2026).

A3: Well tempered metadynamics reveals helix bending transition pathways in metavinculin-tempered -bending transition pathways in

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Metavinculin is a muscle-specific variant of vinculin that plays an important role in force-dependent cell adhesion through its interaction with the actin cytoskeleton. Cryogenic electron microscopy studies have revealed that its F-actin-binding domain can adopt both parallel and kinked α -helical conformations [1], yet the relative stability and transition pathways of these states remain unclear. Here, we use molecular dynamics simulations with atomic resolution complemented by well-tempered metadynamics to explore the conformational landscape associated with helix bending in the metavinculin cytoskeleton binding domain. Unbiased simulations indicate that the parallel helix conformation is stable on accessible simulation timescales, with no spontaneous shift toward a kinked state, whereas simulations initiated from the kinked conformation transition toward the parallel state within tens of nanoseconds. Well-tempered metadynamics was applied to the kinked conformation to estimate the free-energy profile along collective variables to explore possible alternative conformations. Our results suggest that the parallel conformation corresponds to a pronounced free-energy minimum, while kinked conformations are less readily accessible and separated by finite energetic barriers. These findings provide an initial energetic characterization of helix bending in metavinculin and establish a computational framework for future studies addressing force, mutations, or actin binding.

[1] Rangarajan, E.S.; Izard, T. The Cryogenic Electron Microscopy Structure of the Cell Adhesion Regulator Metavinculin Reveals an Isoform-Specific Kinked Helix in Its Cytoskeleton Binding Domain. *Int. J. Mol. Sci.* 2021, 22, 645. <https://doi.org/10.3390/ijms22020645>

A4: Loss of cell-autonomously secreted laminin- α 2 drives muscle stem cell dysfunction in LAMA2-related muscular dystrophy

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LAMA2-related muscular dystrophy (LAMA2 MD) is a severe congenital muscle disorder caused by mutations in the *LAMA2* gene, which in its most severe forms leads to the absence of laminin- α 2, a key component of the muscle stem cell (MuSC) niche. Loss of laminin- α 2 compromises muscle fiber integrity, resulting in progressive muscle degeneration for which no curative treatments are currently available, while this protein's cell-autonomous role in human MuSCs remains poorly understood.

In this study, we show that human MuSCs express *LAMA2*, as evidenced by snRNA-seq data from healthy muscle and independent datasets from the Genotype-Tissue Expression (GTEx) Portal, suggesting that *LAMA2* mutations may intrinsically impair MuSC function in LAMA2 MD. To model the impact of LAMA2 loss independently of patient muscle environment, we generated two isogenic *LAMA2* knockout human induced pluripotent stem cell (hiPSC) lines by editing healthy donor hiPSC and creating an exon 3 deletion (*LAMA2* Ex3 KO) abolishing laminin- α 2 expression and an exon 7 frameshift (*LAMA2* Ex7 KO) introducing a premature stop codon and loss of *LAMA2* mRNA. Following differentiation into myogenic precursor cells, the deletion of *LAMA2* significantly reduced EdU incorporation, indicating slowed cell-cycle progression, while myotube formation was preserved. Transcriptomic profiling revealed robust separation of control and *LAMA2*-deficient cells, with differential gene expression consistent with impaired proliferation. Gene set enrichment analysis showed activation of the p53 pathway and downregulation of G2/M checkpoint, E2F target genes, and mitotic spindle assembly programs.

Together, these findings demonstrate that loss of *LAMA2* intrinsically impairs human MuSC cell-cycle progression through conserved transcriptional programs, supporting a direct, cell-autonomous contribution of MuSC dysfunction to the pathophysiology of LAMA2-related muscular dystrophy, as we additionally evidenced in murine models [1].

Acknowledgements: Grants support: National Science Centre: MAESTRO 2018/30/A/NZ3/00412. Jesús Dante Ramírez López is acknowledged for technical support.

[1] McGowan TJ, Reinhard JR, Lewerenz N, Białobrzęska M, Lin S, Stępniewski J, Szade K, Dulak J, Rüegg MA. (2025) *Loss of cell-autonomously secreted laminin- α 2 drives muscle stem cell dysfunction in LAMA2-related muscular dystrophy*. Nat Commun 16(1):10674.

A5: Structure-function studies of the respiratory supercomplexes of the thermoacidophilic archaeon, *Sulfolobus acidocaldarius*

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Sulfolobus acidocaldarius is a thermoacidophilic archaeon that utilizes an unusual set of respiratory complexes, characterized by a surprising redundancy in the type and function of these enzymes. Within the *S. acidocaldarius* genome, three alternative terminal oxidases (SoxB, SoxM, and DoxB) and three cytochrome-ISP complexes (SoxCL₂, SoxNL, and SoxGF) have been identified. It was proposed that SoxB and SoxM form obligatory supercomplexes with SoxCL₂ and SoxGF, respectively, while DoxB and SoxNL are standalone enzymes. However, it was never explained why this organism uses three distinct sets of respiratory enzymes, nor has structural data on them been available.

Herein, using the native organism as a source, we performed the extraction and partial purification of SoxM and SoxB comprising complexes. Using cryo-EM, we determined their structures, and then we used novel computational tools, such as AlphaFold and ModelAngelo, to identify previously unknown subunits present in these assemblies. We show that the SoxABCDL₂ supercomplex can comprise at least two additional cytochromes *a*, both with an exotic type of heme A. Moreover, we found that the blue copper protein, SoxE, is a core subunit of SoxEFGHIM, which may function as an electron transfer protein in a way similar to the Rieske protein known from regular cytochromes *bc*. Based on the atypical, dimeric architecture of SoxM, we also hypothesize that the SoxEFGHIM may form a multimeric megacomplex in native membranes.

To gain insight into the function of these enzymes, we designed a simple setup for kinetic studies on the terminal oxidase complexes, harnessing a soluble version of SoxE and isolated native caldariellaquinone-6. We developed a system for heterologous production of soluble SoxE in *E. coli*, which we are currently testing. We hope that our contribution will help to fill the existing gaps in knowledge of the long-neglected archaeal respiratory enzymes.

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A6: Protease-driven NETs promote breast cancer aggressiveness

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Neutrophils are innate immune effector cells identified within tumors, which influence tumor growth and progression. One mechanism by which neutrophils contribute to tumorigenesis is through the generation of neutrophil extracellular traps (NETs). These structures, composed of DNA-histone complexes and azurophilic granule proteins, were originally described as antimicrobial defense mechanisms. However, recent studies highlight their significant role in carcinogenesis. Therefore, understanding the molecular mechanisms regulating NET formation within the tumor microenvironment is of great importance. In this study, we focus on proteolytic enzymes, hypothesizing that proteases highly expressed and accumulated in the tumor microenvironment act as key mediators promoting and/or modulating NET formation. Our research focuses on breast cancer, a malignancy characterized by high protease activity and where neutrophil influx correlates with poor prognosis. We demonstrate that host-derived proteases accumulated in the breast tumor microenvironment induce NET formation via activation of Protease-Activated Receptor (PAR)-dependent signaling pathways. Furthermore, we show that PAR-mediated NETs enhance proliferation and migration of MDA-MB-231 breast cancer cells through activation of the β -catenin signaling pathway and induction of epithelial-to-mesenchymal transition (EMT). Notably, these effects are more pronounced when NETs directly associate with MDA-MB-231 cells and form clustered structures. Taken together, our findings show for the first time that proteases in the tumor microenvironment contribute to cancer progression by promoting NET formation and consequently enhancing tumor cell aggressiveness.

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A7: Loss of endothelial LYVE-1 enhances leukocyte adhesion to endothelial cells

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Hyaluronic acid (HA), an important component of extracellular matrix, influences endothelial cell behavior, including migration, permeability and immune cell adhesion. Our preliminary data indicate increased expression of two HA receptors, CD44 and LYVE-1, in senescent endothelial cells. As the role of these receptors in ageing endothelium is not well defined, the aim of this study was to investigate the contribution of CD44 and LYVE-1 to endothelial-immune cell interactions under basal and senescent conditions.

Human umbilical vein endothelial cells (HUVEC) were driven into replicative senescence. Expression of CD44 and LYVE-1 was silenced in early-passage (control) and late-passage (senescent) cells using siRNA. Gene expression was analyzed by RT-qPCR. Functional consequences were evaluated by measuring adhesion of MCP-1-activated THP-1 monocytes to the endothelial monolayers.

siRNA-mediated silencing substantially reduced expression of CD44 and LYVE-1 receptors in control as well as in senescent HUVECs without loss of cell viability. Knockdown of LYVE-1 led to a significant increase in adhesion of MCP-1-activated THP-1 monocytes to both control and senescent HUVECs. This was associated with significantly increased ICAM-1 expression in senescent endothelial cells, with a similar trend observed in control HUVECs. Knockdown of CD44 affected neither THP-1 adhesion, nor ICAM-1 expression. Interestingly, concomitant knockdown of LYVE-1 and CD44 normalized monocyte adhesion to the levels observed in untreated and scrambled siRNA-treated cells.

Together, these findings suggest a functional interplay between CD44 and LYVE-1 in regulating endothelial-leukocyte communication, potentially mediated by HA availability, and identify endothelial LYVE-1 as a modulator of leukocyte adhesion under both basal and senescent conditions.

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A8: Beyond neurons: clozapine modulates migration of human astrocytes

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Clozapine (CLO) is an atypical antipsychotic widely used in the treatment of treatment-resistant schizophrenia, although its precise mechanism of action remains incompletely understood. Growing evidence indicates that astrocytes—glial cells essential for neuronal support and function—contribute to the pathophysiology of schizophrenia. Previous proteomic studies of human astrocytes derived from the NTERA-2 cell line have suggested that clozapine may influence actin cytoskeleton dynamics.

In this study, we investigated the effects of CLO and risperidone (RIS) on the migratory behaviour of NTERA-2-derived human astrocytes. Astrocyte migration was assessed using a wound-healing assay. The results demonstrated that CLO significantly inhibited astrocyte migration, whereas RIS did not produce a comparable effect. MTT assays confirmed that this reduction in migration was not attributable to changes in cellular metabolism.

Immunocytochemical analysis of the actin cytoskeleton did not reveal statistically significant alterations in cytoskeletal structure; however, CLO treatment was associated with a subtle tendency toward changes in cytoskeletal fibre organization.

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A9: Effects of antipsychotic drugs on calcium signaling in human astrocytes

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Astrocytes are essential components of the central nervous system and play a crucial role in supporting and regulating neuronal function. Calcium signaling is a key cellular process in astrocytes, as it underlies their ability to sense neuronal activity, regulate gliotransmitter release, control synaptic transmission, and maintain metabolic and ionic homeostasis in the brain. Schizophrenia is associated with dysregulated calcium signaling involving altered calcium channel function and impaired calcium-dependent synaptic and glial processes. In our proteomic analyses, we demonstrated that clozapine affects the levels of cellular calcium sensors and proteins involved in calcium transport. Therefore, the aim of this study was to investigate the functional effects of antipsychotic drugs: clozapine and risperidone on calcium signaling in astrocytes derived from the human pluripotent NT2 cells. Intracellular calcium dynamics in response to ATP stimulation were monitored by confocal microscopy using a fluorescent calcium indicator, with quantitative analysis of calcium wave frequency and spatial propagation. The obtained data were analyzed using advanced analytical software, which was additionally modified and expanded to incorporate dedicated statistical analysis workflows. We observed consistent trends indicating differential effects of clozapine and risperidone on calcium signaling relative to control cells. Given the limited knowledge of calcium signaling in human astrocytes, these findings add important insight into the effects of antipsychotic drugs on astrocytic calcium regulation.

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A10: KEAP1-driven dysfunction of aging endothelial cells

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Cardiovascular diseases (CVDs) are the leading cause of death worldwide. Endothelial cells (ECs), which line blood and lymphatic vessels, are central to vascular homeostasis, although their function deteriorates with age, increasing CVD risk. Our data indicate that NRF2 and KEAP1 are likely contributors to this age-related decline.

KEAP1 (Kelch-like ECH-associated protein 1) is a redox-sensitive inhibitor of NRF2 (NFE2L2), a transcription factor that coordinates antioxidant and cytoprotective responses. Beyond this canonical role, we have proposed that KEAP1 also functions independently of NRF2. Together with nitric oxide synthase (NOS) and the transnitrosating protein GAPDH, KEAP1 may contribute to S-nitrosothiol formation, leading to altered protein function. In NRF2-deficient ECs, this pathway drives protein aggregation, loss of cellular function, and premature senescence.

We demonstrate that NRF2 levels decline with age in ECs, suggesting that similar dysfunctional phenotype may occur during physiological aging. To address this, we are investigating the relationship between protein aggregation and endothelial function in primary human ECs from young and aged donors, focusing on the KEAP1/NOS/GAPDH S-nitrosation (SNO) complex. Our results demonstrate an age-dependent increase in protein aggregates accompanied by impaired endothelial function. Importantly, these effects can be reversed by modulating NRF2/KEAP1 activity.

A11: pRb1 tumor suppressor protein is dispensable for *TGF-β2* expression induced by inhibition of BRAF^{V600E} kinase

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Metastatic dissemination is the leading cause of cancer-related mortality and can, in some contexts, be promoted by molecular responses elicited by anticancer therapies. In melanoma, inhibition of oncogenic BRAF^{V600E} signaling has been linked to increased expression of pro-metastatic mediators, including transforming growth factor-β2 (TGF-β2), although the regulatory mechanisms underlying this response remain incompletely understood.

Here, we investigated the relationship between BRAF^{V600E} inhibition, cell cycle regulation, and *TGF-β2* expression, with particular focus on the potential involvement of the retinoblastoma protein pRb1. Using BRAF^{V600E}-mutant human melanoma cell lines, we examined the effects of pharmacological BRAF^{V600E} blockade on cell proliferation, cell cycle progression, pRb1 expression, and transcriptional regulation of *TGF-β2*.

Treatment with the selective BRAF^{V600E} inhibitor PLX4720 potently suppressed cell proliferation without compromising cell viability and induced a marked accumulation of cells in the G1 phase of the cell cycle. This growth arrest was associated with increased *TGF-β2* transcription in a cell line-dependent manner. Unexpectedly, BRAF^{V600E} inhibition resulted in a substantial reduction in pRb1 protein levels despite elevated *pRb1* mRNA expression. A similar decrease in pRb1 protein abundance was observed following inhibition of CDK2 or CDK4/6, indicating that pRb1 stability is regulated by downstream cell cycle kinase activity. Notably, depletion of pRb1 did not impair *TGF-β2* induction in response to BRAF^{V600E} inhibition.

Collectively, these findings demonstrate that pRb1 is dispensable for *TGF-β2* upregulation following BRAF^{V600E} blockade. Our data contribute to the current knowledge of therapy-induced pro-metastatic signaling in melanoma.

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A12: ModelExplorer: A PyMOL plugin for assessing the quality of macromolecular models

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Protein structure models are an indispensable resource for a wide range of research objectives in basic science, medicine, and industrial applications. However, not all macromolecular models are created equal, structural data vary in quality, affecting the reliability of downstream analyses. To aid researchers in assessing the quality of models available for their protein of interest, we introduce the ModelExplorer (PyMEx) PyMOL plugin. The aim of the PyMEx project is to provide an integrated interface between the popular molecular graphics viewer PyMOL and authoritative resources, including RCSB PDB, PDBe, PDB-REDO, UniProt, EMDB, and AlphaFoldDB. PyMEx also guides visual model exploration by providing streamlined workflows that combine functional annotations with residue-level properties not captured by global validation metrics and offers features tailored to models derived from crystallography, cryo-EM, and computational prediction.

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A13: Oxygen response to radiotherapy in uveal melanoma murine tumors

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Uveal melanoma (UM) is the most common primary intraocular malignancy in adults and represents a major clinical challenge due to its high metastatic potential and limited therapeutic options. Although radiotherapy is widely used in UM, its impact on tumor oxygenation remains poorly understood *in vivo*. To date, reliable *in vivo* models that closely mimic the human disease have been lacking; here we present a robust and physiologically relevant stabilized model subjected to radiotherapy, which offers significant value for advancing translational research in tumor oxygenation and treatment response. Understanding tumor behavior and therapeutic response requires precise and reliable methods. Electron Paramagnetic Resonance (EPR) imaging has recently become an indispensable non-invasive technique for assessing oxygenation levels within tumors. This method leverages paramagnetic probes to provide quantitative measurements of tumor oxygenation dynamics. These advancements enable a more comprehensive characterization of tumor physiology, particularly in the context of radiotherapy, facilitating improved diagnostic and therapeutic strategies. We developed a novel *in vivo* model of uveal melanoma by establishing Mel270 tumors in the intrascapular fat pad of scid mice, providing a stable and physiologically relevant site for tumor growth. Tumor oxygenation was monitored using the trityl-based OX071 probe with pulse EPR imaging. Animals received a single 12 Gy dose of radiotherapy, and oxygenation measurements were performed both before and after irradiation to capture dynamic changes in tumor physiology. The use of the OX071 probe with EPR imaging provided detailed oxygenation maps, identifying hypoxic regions correlating with aggressive tumor growth and poor vascularization. Oxygenation of Mel-270 tumors is heterogenous and tumor response to radiotherapy varied between animals.

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A14: Non-destructive DNA extraction enables museomics in Blattodean specimens

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Few animal species have adapted so successfully to urban environments as cockroaches. Around 40 species of cockroaches (out of the >5,000 described cockroach species) have been reported to be able to live in human habitats, but one of the most relevant insect pest is *Blattella germanica*, which is successfully living in cities, towns, and villages around the world, even in stations in Antarctica. What makes this species particularly interesting is that *B. germanica* has evolved to live exclusively within human habitats and has never been reported in wild environments.

To have a more clear picture of the evolutionary origin of this species and understand the genetic basis of its astonishing adaptation to human habitats and pesticides, we will compare genomes from museum collections of circa 100 years ago - before the widespread usage of synthetic pesticides - with genomes of current populations of cockroaches collected at the same or similar locations all over the world.

The initial results of our study demonstrates that is possible to extract >50ng of DNA from *B. germanica* samples preserved in museum collections using a non-destructive method, without the need to amputate any part of the specimen. To our knowledge, non-destructive DNA extraction methods have not been previously used in Blattodea specimens; therefore, these results contribute to improve museomics practices by preventing the destruction of this valuable and irreplaceable material.

A15: How does sorafenib resistance affect hepatocellular cancer cells?

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Tyrosine kinase inhibitors (TKIs) are the most commonly used targeted therapeutics in hepatocellular carcinoma (HCC). Among them, sorafenib primarily inhibits VEGFR 1-3, PDGFR and Raf kinase, exerting its effects mainly through anti-proliferative and anti-angiogenic mechanisms. Despite an initial period of disease stabilization, most patients eventually develop resistance to sorafenib, which leads to tumor progression and relapse.

The main aim of this study was to investigate the molecular basis of sorafenib resistance in HCC. We used two cell lines HUH7 (epithelial phenotype) and SNU449 (mesenchymal phenotype) continuously treated with sorafenib. We observed that sorafenib resistance led to decreased proliferation of both cell lines. Western blot analysis revealed increased levels of mesenchymal markers vimentin, fibronectin and N-cadherin in sorafenib-resistant HUH7 cells. These cells were also characterized by higher mRNA levels of fibronectin, Twist and PTK2. Immunofluorescence staining showed that resistant HUH7 cells acquired mesenchymal phenotype. In contrast, sorafenib resistance in SNU449 cells led to lower expression of epithelial-to-mesenchymal transition (EMT) markers. Immunofluorescence staining revealed that both, wild type and sorafenib-resistant SNU449 cells were characterized by spindle shape and a mesenchymal phenotype.

Our study demonstrates that sorafenib resistance affects HCC cells differently, depending on their initial phenotype. In HUH7 cells resistance is associated with EMT and cytoskeletal remodeling, whereas in SNU449 sorafenib treatment led to proliferation inhibition and decreased expression of EMT markers. These findings highlight the heterogeneity of resistance mechanisms in HCC and underscore the need for phenotype-specific therapeutic strategies to overcome sorafenib resistance.

A16: Decoding the CK2–YY1–Zn²⁺ axis in prostate cancer malignancy

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Protein kinase CK2, transcription factor YY1, and zinc ions are well-established contributors to prostate cancer progression, yet their functional crosstalk has remained elusive. Here, we investigate how these three factors converge to shape malignant phenotypes in prostate cancer cells.

CK2 is frequently overexpressed in prostate cancer and phosphorylates a wide range of substrates essential for tumor progression, including proteins involved in zinc homeostasis and key regulatory factors such as the androgen receptor, NF-κB, and YY1. Through these phosphorylation events, CK2 modulates protein stability, signaling dynamics and cellular stress responses, thereby enhancing the ability of cancer cells to adapt to oncogenic and metabolic changes. YY1 is likewise overexpressed in prostate cancer and regulates processes central to malignancy, including proliferation, metastasis, and metabolic reprogramming, through modulation of chromatin structure and oncogene expression. Notably, elevated Zn²⁺ levels induce YY1 dimerization, altering its DNA-binding preferences and transcriptional output.

Zinc itself plays a pivotal role in prostate cell metabolism. Normal prostate epithelial cells accumulate exceptionally high intracellular Zn²⁺ concentrations, which suppress mitochondrial respiration and promote citrate production. In contrast, malignant prostate cells exhibit markedly reduced zinc levels, a change that correlates with disease progression and serves as a prognostic indicator.

While pharmacological inhibition of CK2 has been shown to impair cancer cell growth, the interpretation of these effects has been complicated by limited selectivity of available compounds and broad interference with cellular kinase networks. Here, we introduce newly developed CK2 inhibitors with improved selectivity profile and use them as molecular probes to dissect CK2-dependent regulation of YY1 transcriptional activity, and intracellular zinc homeostasis. Our findings reveal a coordinated regulatory network involving CK2, YY1, and Zn²⁺, that sustains malignant phenotype in prostate cancer cells and provide a new framework for understanding how their interplay contributes to disease progression.

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A17: Cellular and genetic mechanisms of limb regeneration in insects

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Limb regeneration after damage or amputation is a fascinating feature that not all animals possess. While insects have been extensively used as models in genetic studies, they also provide a good model system to study how animals regenerate complex appendages, for which many of the cellular and genetic mechanisms remain unknown.

This project aims to characterize the cellular and genetic mechanisms involved in wound healing and limb regeneration in insects, using as models the cricket *Gryllus bimaculatus* and the cockroach *Blattella germanica*.

Towards this aim, we will apply a standardized limb amputation protocol and track survival and regenerative outcomes in juvenile stages, in which they regenerate limbs, and adults, in which there is no limb regeneration. To characterize the cellular and molecular response to amputation and regeneration, we will perform single-nuclei RNA-seq (snRNA-seq) of regenerating limbs at three different time-points, covering early wound response, early regrowth, and completed regrowth. We also sample one adult timepoint per species during scab formation or early repair. This design will provide eight transcriptional snapshots of regeneration at a single cell level, which we can leverage to identify cell types involved in regeneration at the different time points and their transcriptional profiles. Furthermore, we will reconstruct transcriptional trajectories and identify candidate regulators.

By integrating morphological staging with snRNA-seq, this project will unveil the necessary cell types and expression profiles necessary to regenerate an amputated leg in these insect models.

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A18: Impact of melatonin on cell migration in RIPK4-deficient human melanoma model

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Despite significant advances in targeted therapies and immunotherapy, melanoma frequently develops resistance, leading to the relapse of the disease. This highlights the need to identify new therapeutic targets that may be less harmful than conventional chemotherapeutic agents.

Melatonin, a pineal gland hormone, that regulates circadian rhythm, has been shown to exert oncostatic effects and to modulate cancer cell motility *in vitro*. RIPK4, a kinase involved in the regulation of multiple signalling pathways in cancer cells, may also influence melanoma cell migration and invasiveness. The aim of this study was to assess how the interaction between melatonin treatment and RIPK4 modulation affects melanoma cell migration *in vitro* and *in vivo*, using the *Danio rerio* model.

For this purpose, A375 melanoma cells were pretreated with melatonin (1 mM) for 48 h. *In vitro* migratory activity was assessed using scratch (wound-healing) assays and time-lapse microscopy, where quantitative analysis of cell movement and morphology was used. For *in vivo* experiments, A375 cells were injected into the yolk sac of 48-hour old *Danio rerio* larvae, and tumour cell migration and colonization of distant tissues (tail metastasis) were monitored for up to 96 h post-injection.

Our data show that *in vitro* melatonin treatment inhibited melanoma cell migration and this effect was further enhanced in RIPK4 knock-out cells. This was shown by delayed wound closure and reduced migration speed and distance. In contrast, no significant inhibition of metastatic spread was observed *in vivo*.

These results demonstrate notable differences between the effects of melatonin observed *in vitro* and *in vivo* and highlight the critical role of the microenvironment in regulating cancer cell migration.

A19: Targeting inefficient angiogenesis and hypoxia to improve cancer immunotherapy

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Inefficient angiogenesis and tumor hypoxia are hallmarks of cancer and contribute to reduced efficacy of immunotherapy in cancer patients. The transcription factor STAT3 (Signal Transducer and Activator of Transcription 3) has been implicated in the promotion of aberrant angiogenesis and hypoxia within the tumor microenvironment. This PhD project aims to investigate the effects of the STAT3 inhibitor WP1066 on cancer immunotherapy using in vivo breast cancer models. In this study, we present the response of the 4T1 breast cancer cell line to treatment with the STAT3 inhibitor WP1066.

A total of 1,000 4T1 cells per well were seeded into 96-well plates and treated with the STAT3 inhibitor WP1066 in RPMI medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. The proliferative capacity of 4T1 cells following WP1066 treatment was evaluated using MTT and crystal violet assays, with absorbance measured using a CLARIOstar microplate reader (BMG LABTECH, Ortenberg, Germany).

In vitro treatment with WP1066 for 24 hours at concentrations of 8 μ M and 10 μ M resulted in approximately 60% and 75% reductions in 4T1 cell proliferation, respectively, as assessed on day 5.

WP1066 concentration below 5 μ M did not affect the proliferation, suggesting a possible application as an adjuvant in further studies. The 4T1 breast cancer cell line implanted in BALB/c mice represents a useful preclinical model for investigating the tumor microenvironment, including hypoxia, aberrant angiogenesis, and responses to immunotherapy. This model holds potential to enhance the understanding of factors influencing immunotherapy efficacy in breast cancer.

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A20: Targeting LL-37–driven inflammation in psoriasis with apolipoprotein A-1

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Psoriasis is a chronic inflammatory skin disease driven by dysregulated innate immune responses. The human cathelicidin LL-37 plays a dual role in skin immunity: while protective under physiological conditions, its overexpression in psoriatic skin promotes inflammation. Elevated LL-37 forms complexes with self-derived nucleic acids released from damaged cells, facilitating their uptake by dendritic cells and keratinocytes, enhancing Toll-like receptor activation, and inducing robust type I interferon production thereby amplifying and sustaining cutaneous inflammation. Mild psoriasis is usually treated with topical agents, such as glucocorticosteroids or vitamin D analogues, whereas moderate to severe disease requires systemic therapies, including biologics targeting TNF- α , IL-17, and IL-23. However, long-term immunosuppression associated with these treatments increases the risk of infections and malignancies, underscoring the need for novel therapeutic strategies. In this context, we propose that controlling elevated levels of LL-37 in psoriasis may limit self-perpetuating inflammation that is detrimental to the host. Apolipoprotein A-1 (ApoA-1), a major plasma protein, has been shown to bind LL-37. Here, we demonstrate that ApoA-1 acts as a natural scavenger of LL-37 by reducing LL-37:nucleic acid complexes, thereby inhibiting LL-37-induced type I interferon production in human dendritic cells and epidermal keratinocytes. Moreover, ApoA-1 exhibits therapeutic efficacy in psoriasis using an imiquimod-induced murine skin inflammation model and an organotypic psoriatic tissue model. These findings identify ApoA-1 as a previously unrecognized regulator of LL-37-driven inflammation and highlight its potential as a novel therapeutic strategy for psoriasis and other inflammatory diseases.

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A21: Integrative high-throughput approaches to elucidate DCP2 structure and function in RNA decapping

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mRNA decapping is crucial in post-transcriptional gene regulation, as removal of the 5' cap commits mRNA to degradation by 5'→3' exonucleases. The catalytic core of the eukaryotic decapping machinery is DCP2, a conserved Nudix domain-containing hydrolase whose activity is regulated within a multiprotein decapping complex. DCP2 hydrolyzes the 7-methylguanosine cap through a preserved Nudix motif that coordinates divalent metal ions and catalyzes cleavage of the triphosphate bridge, producing m⁷GDP and a 5'-monophosphorylated RNA that are exposed for degradation by exonucleases, like XRN1.

Efficient decapping depends on proper assembly of the decapping complex. The essential cofactor DCP1A binds DCP2 and helps stabilize its active conformation, while the scaffold protein EDC4 organizes the DCP1A–DCP2 module and connects it with additional regulatory factors. Components of the decapping machinery localize to cytoplasmic processing bodies (P-bodies), which are involved in mRNA storage, translational repression, and decay. N4BP1 has also been identified as a regulatory factor that associates with the decapping complex and may influence its assembly or activity. Together, these interactions link translational repression with decapping-dependent mRNA degradation.

To support biochemical and structural studies of DCP2 and its interacting partners, a high-throughput molecular cloning approach in *E. coli* was employed. A total of 48 constructs, including full-length and domain-specific fragments, were designed, resulting in 47 plasmids verified by restriction analysis. Recombinant DCP2 expression was obtained for 89.58% of constructs, and 47.92% yielded soluble protein. These expression and solubility rates were essential for identifying constructs of DCP2 suitable for structural studies by X-ray crystallography and for biochemical assays. The high-throughput strategy enabled rapid screening of multiple polypeptide sequences, which is particularly advantageous for the analysis of multiprotein complexes. Together, this approach provides a foundation for further investigating the molecular mechanisms of the decapping complex and drug development interfering activity of DCP2.

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A22: Antibody-dependent cellular cytotoxicity (ADCC) in immunology

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Antibody-dependent cellular cytotoxicity (ADCC) is a key immune mechanism linking innate immunity with antibody-based cancer therapies. Natural killer (NK) cells are the main effectors of ADCC and mediate cytotoxicity without prior sensitization through the Fc receptor CD16, which recognizes IgG-coated target cells and triggers “immune synapse” formation and cell lysis. Trastuzumab, a humanized IgG1 monoclonal antibody targeting HER2, effectively induces ADCC in HER2-overexpressing breast and gastric cancers by engaging NK cells alongside blocking oncogenic signaling. The efficiency of trastuzumab-mediated ADCC depends on HER2 expression levels, antibody concentration, and NK-cell functional status. Emerging NK-cell-based immunotherapies aim to enhance ADCC. Therefore, standardized NK-cell isolation methods and reliable in vitro ADCC assays are essential for evaluating therapeutic potential and optimizing immuno-oncology strategies.

The presented study describes ADCC assay establishment at Ryvu Therapeutics, starting from the analysis of HER2 protein expression levels in selected cancer cell lines, which were subsequently classified based on their HER2 surface expression. The characterized cell lines were then evaluated in functional assays for both direct cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) using the therapeutic antibody Trastuzumab. Based on the obtained results, two cancer cell lines exhibiting distinct susceptibility to Trastuzumab-mediated ADCC were selected for further in-depth analyses.

The study was performed as part of a project investigating the effects of the CDK8/CDK19 (cyclin-dependent kinase 8/19) inhibitor (RVU120) on NK cells' activity.

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A23: Ancestral insect sex chromosome dosage compensation mechanism in *Gryllus bimaculatus* crickets

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In many species, males carry a single X chromosome, while females have two, which can create an imbalance in X-linked gene expression between sexes. This is often corrected by dosage compensation (DC)—regulatory mechanisms that equalize X-linked expression despite different X copy number. Insects have been central for understanding how sex chromosomes and their regulation evolve, yet the specific factors that implement DC are known in only a few model systems.

Evidence suggests that an ancestral insect X chromosome arose early in insect evolution and is retained (and dosage compensated) in several lineages, pointing to an ancient and potentially conserved DC strategy. In two species, this ancient X is compensated by an orthologue of Painting of fourth (POF), a protein implicated in chromosome-specific gene expression regulation.

My project aims to identify the DC mechanism in *Gryllus bimaculatus*, an emerging model for studying early-branching insect lineages. Using a new chromosome-level genome assembly and publicly available transcriptomic datasets, I show that the *Gryllus* X chromosome is of ancestral origin and that DC varies across tissues. I also identify genes that are consistently male biased across tissues and describe an alternative isoform of *pof* that may affect its regulation or function. Taken together, these results form the basis for my future investigation of the dosage compensation mechanism in hemimetabolous insects.

In my upcoming work, I plan to characterize the sex-specific expression in early embryos. I will also investigate the epigenetic landscape of the dosage-compensated X chromosome using a novel chromatin profiling method, CUT&Tag. Finally, I will describe the effects of *pof* depletion on the X-chromosome expression in males and females.

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A24: Multilevel analysis of coding sequences: nucleotide, amino acid, and structural perspectives

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Homologous protein sequences exhibit varying degrees of conservation depending on the position of an amino acid within the sequence. With the current availability of protein structure databases and coding sequence repositories, it is possible to perform codon-level analyses to verify their frequencies within different elements of local protein structure.

Coding sequences are subject to evolutionary pressure and optimization according to multiple criteria. These include, in particular, the conservation of the encoded amino acid sequence, the variability of amino acid substitutions that preserve selected physicochemical properties within the protein structure, and the optimization of protein expression through the utilization of differential tRNA availability, ranging from frequently used codons to so-called rare codons. The formation of transcript structure also significantly influences the translation process, as do the transcript's resistance to mutations and its resistance to adverse RNA interference effects, such as target region inaccessibility resulting from transcript secondary structure.

In this study, experimentally determined protein structures deposited in the RCSB database were used. Some criteria determining coding sequences were proposed and evaluated. Protein classification was adopted from the SCOPe2 database, in which hierarchical taxonomy is based on elements of local protein structure. Sequence comparisons were performed at three steps: nucleotide, amino acid, and structural (protein) levels. Additionally, the results were compared with intrinsically disordered protein regions obtained from the DisProt database.

The obtained results indicate the presence of common features among protein structures, while simultaneously revealing certain deviations within the analyzed groups, particularly in the case of arginine.

A25: Functional impact of mesenchymal stem cell-derived extracellular vesicles on model cardiac cells in vitro

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Cardiovascular diseases (CVD) remain one of the leading causes of death worldwide, largely due to the limited regenerative capacity of the heart following ischemic injury. In recent years, regenerative approaches involving stem cells (SCs) and their derivatives such as extracellular vesicles (EVs) have gained attention as promising strategies for cardiac tissue repair.

The aim of this study was to investigate the functional potential of mesenchymal SC-derived EVs (MSC-EVs) on model human cardiomyocytes obtained from induced pluripotent SCs (iPSC-CMs) *in vitro*, with a particular focus on their impact on cell viability.

Optimized differentiation protocols were established to generate functional iPSC-CMs, which was confirmed with morphological and molecular analyses. Apoptosis was induced in iPSC-CMs using both hypoxic and cytotoxic conditions. EVs were isolated from MSCs, characterized by nanoparticle tracking analysis and applied to iPSC-CMs under stress conditions. The results showed that MSC-EV treatment significantly reduced caspase-3/7 activity, indicating their cytoprotective effect against programmed cell death. Moreover, apoptosis inhibition was dose-dependent, with stronger effects observed at higher EV concentrations.

These findings highlight the potential of MSC-EVs as a cell-free therapeutic alternative for cardiac repair. By reducing apoptosis in stressed cardiomyocytes, EVs may support myocardial repair while avoiding risks associated with direct stem cell transplantation. This work contributes to the growing evidence supporting EV-based therapies in regenerative medicine and may aid the development of safer and scalable treatments for heart disease.

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A26: Two ways to see one lncRNA: head-to-head live cell imaging of *NORAD* with sm-LiveFISH and MS2-HaloTag

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Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that do not undergo translation. One such example is the long non-coding RNA activated by DNA damage (*NORAD*), which is characterized by high conservation and expression in mammalian cells. The function of *NORAD* is to maintain genomic stability by sequestering PUMILIO family proteins, which are responsible for accelerating transcript degradation. It has been proven that in adverse conditions *NORAD* localizes to stress granules – membraneless organelles consisting of various proteins and RNAs. However, the dynamics and localization of the transcript in living cells were previously not explored. To achieve that we will use two distinctive methods: single molecule LiveFISH and MS2 RNA-labeling system.

Sm-LiveFISH allows for imaging of endogenous transcripts, at a single molecule resolution, by employing CRISPR-Csm system. The system comprises of sgRNAs specific to the transcript and Csm proteins fused with GFP. Complementary sgRNAs bind to target RNA, which triggers the assembly of Csm complex. Similarly to smFISH many sgRNAs bind to a single transcript and increase signal to noise ratio.

MS2 RNA-labeling system is based on the bacteriophage MS2 coat protein (MCP), which exhibits high-affinity to RNA hairpin binding site (MSB). For the system to work we must label the transcript with MSB which usually requires introduction of an exogenous transcript labelled with multiple MSB copies into cells of interest, and express MCP with a fluorescent tag in the same cells. In our current approach we utilize MCP tagged with HaloTag – a non-fluorescent tag that can be easily labeled with a fluorescent Halo-tag ligand through covalent bonding which allows maximum imaging flexibility.

Both imaging systems will be used to analyse and compare the subcellular localization of *NORAD* and its dynamics in cells challenged with hyperthermia and DNA damage.

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A27: Large-scale small RNA-seq analysis reveals widespread non-gonadal piRNA expression in arthropods

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PIWI-interacting RNAs (piRNAs) were discovered in the early 2000s and are a class of small non-coding RNAs known for protecting the germline genome from mobile genetic elements. Initially, piRNAs were thought to be expressed exclusively in germ cells, and their disruption was shown to increase transposon activity and cause infertility.

More recently, contrary to this long-standing assumption, published studies have suggested that piRNAs may also be expressed in non-gonadal tissues in certain arthropod species. In this project, we aimed to determine whether non-gonadal piRNA expression is restricted to a limited number of species or represents a general feature of arthropods.

Hence, we developed a bioinformatic pipeline that leverages publicly available small RNA-seq datasets to detect evidence of piRNA expression in individual samples. Using this pipeline, we analyzed 168 datasets derived from six tissue types across 17 arthropod species. Contrary to previous assumptions, our results indicate that piRNAs are commonly expressed in non-gonadal tissues across multiple arthropod lineages. Moreover, these non-gonadal piRNAs display the potential to regulate mobile genetic elements as well as endogenous mRNAs.

A28: Differential equations and machine learning methods in creating tumor growth model

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The project aims at developing an integrated description of tumor growth that combines quantitative analysis of microscopic images, machine learning methods, and differential equation models. Experimental data (cell counts over time and microscopy images) contain three human uveal melanoma cell lines: OMM1.3, derived from a hepatic uveal melanoma metastasis, Mel270, and 92.1, derived from primary uveal melanoma tumors. A convolutional neural network was designed to segment and count cells, partially automating image analysis, although its accuracy decreased at very high cell densities. Additionally, a system of differential equations was adapted to better capture the dynamics of the studied cancer cell populations. Comparing both approaches is intended to yield a more robust and biologically meaningful *in silico* description of tumor growth.

A29: Interference of wheat flour biocompounds with the diapedesis of thyroid cancer cells

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Background: Thyroid cancer has shown a significant increase in incidence worldwide over recent decades. Impairment of thyroid peroxidase (TPO) correlates with its progression; therefore, a diet rich in TPO activators may help to delay this process. To verify this notion, we estimated the effects of wheat flour extracts (WF; a rich source of TPO activators) on the metastatic potential of thyroid cancer cells. In particular, we concentrated on thyroid cancer cell-induced endothelial activation, as milestone of cancer cell diapedesis and metastatic cascade.

Methods: For this purpose, we used an experimental approach based on the monitoring of human umbilical cord endothelial cells' (HUVECs) motility and cytoskeletal architecture in the proximity of benign (TPO⁺ B-CPAP) and anaplastic (TPO⁻ 8505C) thyroid cancer cells. The effects of WF extract on these parameters were further interpreted in terms of their influence on connexin(Cx)43 status and metalloproteinase activity in co-cultures and cancer cell populations, respectively.

Results: Whereas 8505C exerted more pronounced activating effects on endothelial motility than their B-CPAP counterparts, the effects of both thyroid cell lines on HUVEC cytoskeleton were less intense. Application of WF extract attenuated endothelial activation in the proximity of TPO⁻ 8505C thyroid cancer cells. Less pronounced effect of WF extract on the endothelial activation in the proximity of B-CPAP cells correlated with attenuated increase of endothelial Cx43 levels in WF extract-treated co-cultures and was accompanied by enhanced activity of MMPs in WF extract-treated cancer cell populations.

Conclusion: These findings indicate multifactorial effects of WF extract on thyroid cancer progression. Whereas the role of Cx43 and MMPs in this process requires further study, retained activity of WF extract in TPO^{neg} 8505C/HUVEC co-cultures suggests a negligible role of TPO activators in the inhibition of thyroid cancer cell diapedesis.

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A30: Investigating hematopoietic memory in long-term hematopoietic stem cells

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Hematopoietic stem cells (HSCs) ensure lifelong blood cell production through their capacity for self-renewal and multilineage differentiation. Beyond this classical role, emerging concepts suggest that HSCs may retain information about previously encountered stimuli, thereby contributing to a form of long-term hematopoietic memory. The mechanisms by which such memory is established, maintained, and functionally recalled at the stem cell level remain largely unexplored.

The aim of this study is to investigate whether repeated exposure to the same mobilizing stimulus induces persistent, HSC-intrinsic functional adaptations that influence subsequent hematopoietic output. Using a murine model, animals were treated with granulocyte colony-stimulating factor (G-CSF) to mobilize myelopoiesis. One month after this primary stimulation, long-term HSCs (LT-HSCs) were isolated and analyzed either in single-cell colony-forming unit (CFU) assays or following transplantation into lethally irradiated recipients. After hematopoietic reconstitution, recipients were subjected to a secondary mobilization, enabling assessment of HSC responses upon re-exposure to the same stimulus.

In parallel, individually sorted LT-HSCs were cultured *in vitro* in medium supplemented with G-CSF, and the colonies formed from single cells were analyzed using a multimodal workflow combining morphometric imaging (colony size and shape classification), cytological staining, multiparameter flow cytometry, and index sorting. This integrative approach allows high-resolution characterization of colony diversity and lineage output while capturing functional heterogeneity within a phenotypically defined HSC population.

Together, this multilevel strategy provides a robust framework for studying functional adaptations of HSCs following repeated stimulation and establishes a strong foundation for future investigations into the epigenetic regulation of hematopoietic memory.

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A31: Regnase-2-dependent changes in epiregulin expression in glioblastoma cells

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Epiregulin (EREG) is a ligand of the epidermal growth factor receptor (EGFR) and is involved in the regulation of cancer cell proliferation, migration, and survival. In glioblastoma multiforme (GBM), increased EREG expression has been reported and shown to correlate with tumor aggressiveness. Activation of the EREG-EGFR axis triggers intracellular signaling pathways, including the MAPK/ERK cascade, thereby promoting tumor progression.

Regnase-2 (Reg-2) is an RNase involved in post-transcriptional regulation of gene expression through modulation of mRNA stability. Transcriptomic analyses indicate decreased Reg-2 expression in GBM, suggesting its potential role in the pathogenesis of this tumor. Notably, in our mass spectrometry-based proteomic analysis of human glioblastoma cells U87-MG with Reg-2 overexpression, EREG was identified as a potential Reg-2 substrate. The aim of this study was to investigate the relationship between Reg-2 levels and EREG expression in glioblastoma cells.

To assess Reg-2-dependent changes in EREG expression, RT-qPCR and ELISA were applied. The obtained results suggest that Reg-2 may participate in the regulation of EREG expression in glioblastoma cells, potentially influencing the EREG-EGFR-ERK axis. Further studies are required to elucidate the molecular mechanism underlying this regulation.

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A32: Immune profiling of gut from keratinocyte-specific regnase-1 deficient mice: challenges and optimization of cell isolation

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Barrier tissues such as the gut and skin rely on tightly coordinated interactions between epithelial and immune systems to maintain tissue homeostasis. Regnase-1 (Reg1/MCPIP), encoded by *Zc3h12a*, is a post-transcriptional regulator that limits inflammation through degradation of pro-inflammatory mRNAs. While Reg1 is well established as a key regulator of skin barrier integrity and immune homeostasis, its contribution to gut immune homeostasis remains incompletely understood.

Primary observations indicate that both colon-specific and keratinocyte-specific *Zc3h12a* knockout mice (Reg1-cKO) exhibit marked gut enlargement accompanied by lymphocytic infiltration, prompting further study of Reg1 in intestinal immune homeostasis. In this context, we are establishing and optimizing protocols for isolation of intestinal immune cells from whole mouse gut for multiparameter flow cytometric analysis to characterize immune alternations.

Initial enzymatic digestion-based approaches revealed technical challenges related to digestion efficiency, cell recovery, and cell viability. Notably, gut tissue from Reg1-cKO mice appears macroscopically and histologically enlarged and thickened, which may influence tissue dissociation efficiency and immune cell yield. Consistent with this, preliminary flow cytometric analyses indicated under-representation of certain immune cell populations compared with wild-type controls.

Given the variability, time consumption, and reported cell-type bias associated with enzymatic digestion methods, we evaluated a recently described mechanical dissociation protocol as a potentially standardized and simplified approach for global immune profiling of the mouse colon. Initial application of this method in both wild-type and Reg1-cKO mice demonstrated improved feasibility and immune cell recovery relative to enzymatic digestion. Further optimization is ongoing to improve cell viability, enabling assessment of immune cell composition across intestinal segments and compartments.

Together, this work aims to establish a robust experimental framework for intestinal immune cell isolation, enabling future studies to dissect how Regnase-1 regulates immune cell composition and function in the gut and to gain insight into mechanisms underlying chronic inflammation at barrier tissues.

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A33: PIN-containing RNases - role in inflammation related processes, including skin cancer

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The amount of mRNA in a cell is regulated at both the transcriptional and post-transcriptional levels. Post-transcriptional regulation is a critical determinant of cellular homeostasis and is largely governed by RNA stability mechanisms mediated by exo- and endonucleases. The Monocyte Chemotactic Protein Induced Protein (MCPIP), also called Regnases is the one of the protein family involved in transcript regulation [1]. The four proteins belonging to this family contain a characteristic PiIT N-terminus (PIN) domain, which is responsible for the ribonucleolytic activity, a C3H1-type zinc finger domain responsible for RNA binding, and two ubiquitin-binding domains: a ubiquitin associated (UBA) domain and a coupling of ubiquitin conjugation to ER degradation (CUE) domain. CUE domain is responsible for the negative regulation of the NF- κ B pathway, whose dysregulation contributes to the development and progression of various human malignancies through impaired expression of hundreds of genes. MCPIP1, participates in the degradation of mRNAs encoding pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α , and takes part in processes essential for the initiation and development of cancer, such as differentiation, cell cycle and cell division, angiogenesis, and apoptosis. In addition, MCPIP1 also degrades pre-miRNAs, which ultimately affects the levels of mature miRNAs and modulating epigenetic regulation of gene expression. Moreover, downregulation of MCPIP1 enhances inflammation and promotes the proliferative capacity of cancer cells.

The aim of this project is to determine which domain PIN or CUE plays a more prominent role in cancer development. By molecular profiling of samples collected from *in vitro* and *in vivo* models we will establish a domain-resolved understanding of MCPIP1-mediated transcriptome remodeling. By establishing a domain-resolved understanding of MCPIP1 function, this study will provide critical insight into the role of PIN-containing RNases in cancer and inflammation.

[1] Mizgalska D, et al. (2009) Interleukin-1-inducible MCPIP protein has structural and functional properties of RNase and participates in degradation of IL-1 β mRNA. FEBS J. 276(24):7386-99.

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A34: Social media posts as a source of ecological information over time: using Twitter (X) as a proof of principle

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Acquiring data about ecology over long periods of time and large geographical areas is often difficult, expensive, and takes a long time. Here, we explore whether, through millions of daily posts on social networks, we could be constantly and unconsciously accumulating data regarding important biological patterns over the years.

Data analysis from generalist social networks has been successfully applied in different research fields, most notably in political science and epidemiology. Here, we evaluate their potential and drawbacks for studying biological patterns of other organisms over time. To that end, we used millions of posts on Twitter (currently X), over 11 years, on four different insect groups: cockroaches, crickets, Monarch butterflies, and mosquitoes.

Our results show that through millions of daily tweets, we could identify temporal periodic patterns that reflect their unique known phenology of the four insect taxa studied. Given the 11-year span of the dataset, we could also track changes in their patterns over the years that might be related to environmental factors. Using a sentiment analysis, we could also characterize the emotions of people towards these animals, which is important for the design of awareness campaigns. We also discuss the limitations of these data, such as the potential that social media data might have for spatial tracking of migrations.

In summary, we show compelling evidence for the use of social network data for biologists and provide a theoretical and practical framework for exploring these sources of data.

A35: Analysis of changes in roughness and nanomechanical properties of melanocytes irradiated with a solar simulator

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Melanocytes are pigment-producing cells of neuroectodermal origin, located mainly in the basal layer of the epidermis. The main function of the cells is synthesis of melanin and its subsequent transfer to neighboring keratinocytes. One of the key elements regulating the maturation and transfer of melanosomes is the cellular cytoskeleton, particularly microtubules and actin filaments. In addition, the cytoskeleton determines cell rheology, i.e. roughness and nanomechanical properties such as elasticity. Although the effect of light on cytoskeletal reorganization during melanin transfer is well recognized, it remains unknown how such changes affect the rheological properties of melanocytes. The aim of this study was therefore to investigate the effect of solar irradiation on the rheological properties, such as roughness and elasticity of primary human epidermal melanocytes obtained from donors of three skin phototypes: I, III and V. Cell viability after irradiation was assessed using MTT assay and propidium iodide staining. Melanin pigmentation was investigated using electron paramagnetic resonance spectroscopy. Rheological properties as well as cytoskeleton organization were examined using combined atomic force and structured illumination microscopy. The obtained results showed that after irradiation, the cells exhibited increased roughness and elevated elastic modulus. The effect was more pronounced in cells with a higher skin phototype. The observed changes in roughness and nanomechanical properties correlated with cytoskeletal reorganization, in particular with an increased number of actin filaments in the cortical region of the cells. The results indicate an adaptive response of melanocytes to light irradiation, leading to the optimization of melanin transport. Increased cell surface roughness may promote more effective anchoring of melanocyte dendrites to keratinocytes, while increased stiffness may facilitate the process of cytophagocytosis.

A36: Cryo-EM structure of a novel type of complex I (NDH) from *Sulfolobus acidocaldarius*

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In mitochondrial and bacterial respiratory electron transport systems, complex I is a key component that delivers electrons to the quinone pool by coupling NADH oxidation to quinone reduction. However, the oxygen-respiring Archaea remain a persistent puzzle, as still no complex I counterpart has been isolated or characterized in their membranes. The general conclusion was that Archaea lack the complex I analog.

In this work, we challenge this notion by presenting a 2.9 Å cryo-EM structure of the complex I analog (NDH) that we isolated from native membranes of a thermoacidophilic archaeon, *Sulfolobus acidocaldarius*. Our structure reveals structural motifs specific to Archaea and also *Sulfolobus*-specific adaptations for survival in harsh environments. Importantly, a gentle protocol we used for the protein isolation allowed us to retain supernumerary subunits and unique ligands bound to the complex. The extra subunits, not present in the core operon, were identified as indole-3-glycerol phosphate synthase and a dimeric 2-oxoacid:ferredoxin oxidoreductase. Both enzymes were previously shown to have catalytic activity, and we now propose that they are coupled to the NDH via redox interactions with a small redox carrier. We have also isolated and identified Zn-containing ferredoxin (Zn-Fdx) from *S. acidocaldarius* cells, and now we speculate that this small redox protein may interact with the NDH.

We have proposed a mechanism of action for this newly discovered NDH. However, as this hypothesis needs to be tested, we have planned several experimental approaches involving the Zn-Fdx, oxoacids, and native caldariellaquinone-6. If the proposed mechanism is correct, it will suggest that *Sulfolobus* utilizes a rewired electron transport system by coupling the Krebs cycle to complex I analog instead of succinate dehydrogenase. This will mark a major step in understanding the archaeal respiratory systems.

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A37: Concept and implementation of cell segmentation algorithm in a low-density cell culture

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This paper presents segmentation algorithm of microphotographs cells of line UM565/B16. The purpose of its creation is to improve computing parameters such as area, perimeter, bounding box, roundness, circularity, solidity, bounding ellipse, and elliptic smoothness, which calculation allows analysis of physical parameters of cells including examination of biological mechanisms, as well as correlation with cell culture conditions. The vision system was designed for image analysis: an algorithm for instance segmentation of cancer cells, as a result the manual process of marking cells in microscopic photos of low-density cultures was partially automated, which contributes to improving the accuracy and efficiency of the analysis. The results of the work can be used for further research in the field of cell biology and cancer biology.

A38: Clozapine regulates neuronal activity-dependent pathways by decreasing AP-1 activity in human neurons

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Clozapine remains one of the most effective antipsychotic drugs and is uniquely approved for treatment-resistant schizophrenia. However, its clinical use is limited by the risk of severe side effects. Despite extensive research, the mechanism of action responsible for clozapine's unique therapeutic efficacy is still not fully understood. In this study, ATAC-seq assay was employed to investigate genome-wide chromatin accessibility changes induced by antipsychotic drugs. The effect of clozapine was compared to that of the commonly used antipsychotic risperidone. Differential accessibility analysis revealed that clozapine caused a widespread reduction in chromatin accessibility across most analyzed genomic regions, whereas risperidone induced only minimal changes, indicating a major difference in the mechanisms of action of these two drugs. Transcription factor footprinting showed a significant decrease in the activity of basic leucine zipper transcription factors from the FOS and JUN families following clozapine treatment, with an opposite effect observed for risperidone. These results indicate a potentially important role of these transcription factors in clozapine's mechanism of action, especially since basic leucine zipper transcription factors are involved in regulation of neuronal activity and plasticity.

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A39: Intermolecular interactions and first electronic excited states as determinants of eumelanin–pheomelanin differences

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Melanin pigments are a key component of photoprotection in human tissues, and their physicochemical properties strongly depend on redox chemistry and supramolecular organization. The two major animal melanin types are eumelanin and pheomelanin. Eumelanin arises from indole-based units derived from DHI and DHICA, whereas pheomelanin originates from sulfur-containing benzothiazine (BT) and benzothiazole (BZ) motifs, leading to distinct aggregate architectures and excited-state properties. The aim of this study was to characterize and compare key differences between eumelanin and pheomelanin at the level of functional groups, aggregate organization, and the energetics of the first electronic excited states. Fourier-transform infrared (FTIR) spectroscopy was applied to synthetic pigments to identify pigment-specific structural signatures and markers of intermolecular interactions, with emphasis on hydrogen-bond-related bands attributed to carbonyl dimer motifs. In parallel, first electronic excited states were computed for representative eumelanin and pheomelanin models across distinct redox states and increasing structural complexity. Vertical and adiabatic transitions were analyzed, and ETS-NOCV energy and charge decomposition was used to characterize the noncovalent interactions stabilizing the aggregation motifs. FTIR spectra highlighted clear differences: pheomelanin showed more pronounced intermolecular-interaction bands, while the OH/NH stretching maximum in eumelanin shifted to lower wavenumbers, consistent with a different hydrogen-bond network geometry. Benzothiazole-like motifs exhibited interaction patterns distinct from benzothiazines and eumelanin-like structures, including diminished carbonyl dimer-type signatures. The computational analysis further indicated that the propensity of pheomelanin building blocks to form hydrogen bonds changes with the degree of oxidation: oxo-benzothiazine forms show enhanced hydrogen-bonding capacity relative to the reference benzothiazine motif, whereas more oxidized benzothiazole-like motifs do not retain the same hydrogen-bond-dominated interaction pattern. Consistent with these trends, eumelanin aggregation involved a combined contribution of π – π stacking and hydrogen bonding, whereas pheomelanin aggregation was dominated by hydrogen-bonding interactions. Excited-state calculations indicated two clearly separated energetic classes of the first electronic excitations in eumelanin that depend on redox state, while pheomelanin displayed a more heterogeneous excited-state energy landscape.

**POSTER SESSION
B**

Thursday, February 19, 2026

16:50-17:50

B1: Gingipain-derived NETs as a source of novel epitopes linking periodontitis and rheumatoid arthritis

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Porphyromonas gingivalis is a keystone periodontal pathogen whose cysteine proteases, gingipains, play a central role in the pathogenesis of chronic periodontitis (PD). Beyond its local effects, *P. gingivalis* infection has been consistently associated with systemic inflammatory diseases, including rheumatoid arthritis (RA). Despite strong clinical and epidemiological evidence supporting this association, the molecular mechanisms linking PD to the development and progression of RA remain poorly understood. Our previous work demonstrated that *P. gingivalis* induces gingipain-dependent neutrophil extracellular trap (NET) formation via cleavage of the PAR-2 receptor on neutrophils, while concomitantly modifying NET components in a manner that impairs their antibacterial activity. Importantly, persistent inflammation characterized by neutrophil accumulation, sustained NET formation, and defective NET clearance, features common to both PD and RA, is increasingly recognized as a source of autoantigens. Moreover, post-translational modifications (PTMs), including citrullination occurring during NETosis, may generate neoepitopes capable of triggering autoimmune responses. Based on these observations, we investigated the hypothesis that NETs induced by the arginine-specific gingipain RgpA generate neoepitopes capable of activating autoimmune responses, thereby contributing to the development and/or progression of RA and mechanistically linking the two diseases. To address this hypothesis, we performed a comparative proteomic and citrullinomic analysis of RgpA-induced NETs. Our results demonstrated that RgpA-derived NETs are a rich source of citrullinated peptide sequences, including vimentin, a well-established RA biomarker previously identified in the synovial fluid of RA patients. Furthermore, *in vitro* analyses revealed that sera from RA patients exhibited an intensified antibody response against RgpA-induced NETs (ANETA). In addition, we showed that modification of recombinant vimentin by RgpA enhances its citrullination by PAD enzymes and increases its immunoreactivity with RA patient sera. Collectively, these findings identify gingipain-induced NETs as a novel source of autoantigenic epitopes and suggest a previously unrecognized role for gingipains in immune modulation. This protease- and NET-driven mechanism provides new insight into how periodontal infection may contribute to the initiation and perpetuation of systemic autoimmune diseases such as rheumatoid arthritis.

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B2: Enzymatically inactive but functional: proteolytically inactive gingipains (piGING) from *Porphyromonas gingivalis* in esophageal cancer progression

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Periodontal disease (PD) is a common chronic inflammatory condition characterized by progressive destruction of periodontal tissues and resorption of the alveolar bone supporting the teeth. Beyond its local pathology, PD has been associated with several systemic inflammatory diseases, and strong clinical correlations have been reported between PD and gastrointestinal malignancies, including esophageal cancer. Among the virulence factors of the keystone pathogen of PD - *Porphyromonas gingivalis* - are cysteine proteases known as gingipains. While the contribution of gingipain proteolytic activity to immune subversion is well established, our recent findings indicate that proteolytically inactive gingipains can exert biological effects independent of their enzymatic activity [1]. We have previously shown that enzymatically inhibited RgpA activates the EGFR–PI3K/AKT signaling axis, inducing a strong pro-inflammatory response in gingival keratinocytes and dendritic cells. Gingipains can disseminate systemically, either as soluble proteins or in association with outer membrane vesicles (OMVs), thereby extending their impact beyond the periodontal niche. Because gingipain enzymatic activity is strongly influenced by the tissue microenvironment and can be limited under oxidative conditions, we hypothesized that proteolytically inactive gingipains (piGING) may modulate host responses in distal tissues. Using *in vitro* models, we demonstrated that gingipains diffuse from bacterial biofilms and undergo oxidative inactivation, while *in vivo* studies in a mouse model confirmed their distribution to distal organs, including esophageal tissues. Functional analyses using esophageal cancer cell lines (KYSE410 and OE21) revealed that piGING, particularly RgpB, induce the expression of IL-1 family cytokines and partially activate epithelial–mesenchymal transition (EMT)-associated genes, including *SNAIL* and *TWIST*. Although complete cadherin switching was not observed, exposure to piGING inhibited cell proliferation and enhanced migratory capacity, consistent with features of partial EMT and collective cell migration. While further studies are required to elucidate the precise molecular mechanisms underlying piGING activity, our findings identify a previously unrecognized role for proteolytically inactive bacterial proteases in modulating esophageal cancer cell biology. These results suggest a potential mechanistic link between periodontitis and esophageal cancer and highlight the possible relevance of piGING in patients with coexisting periodontal disease.

Acknowledgements: This work was supported by National Science Center, Poland SONATA19 2023/51/D/NZ6/02982 (to I.C.). [1] Ciastoń I. *et. al* (2022) Proteolytic Activity-Independent Activation of the Immune Response by Gingipains from *Porphyromonas gingivalis*. *mBio*. 2022 Jun 28;13(3):e0378721.

B3: Gingipains of *Porphyromonas gingivalis* modify the virions of *Herpesviridae*

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Periodontitis (PD) is a highly prevalent chronic inflammatory disease of the gingiva. Clinical studies indicate a significant association between PD and the increased incidence of viral infections, including those caused by *Herpesviridae*; however, the molecular mechanisms underlying this phenomenon remain poorly understood. Our previous data demonstrated that key virulence factors of *Porphyromonas gingivalis*, gingipains, degrade host signaling molecules in gingival keratinocytes, leading to suppression of antiviral responses. Nevertheless, the direct physical interactions between herpesviruses and *P. gingivalis* proteases have not been investigated. As such interactions may result in either synergistic or antagonistic effects on viral infectivity, we aimed to determine how gingipains affect herpesvirus virions.

Using atomic force microscopy, we found morphological changes in HSV-1 virions treated with gingipains: RgpA, RgpB and Kgp. Mass spectrometry revealed extensive proteolysis of numerous HSV-1 proteins, including surface glycoproteins as well as tegument and capsid proteins. The degradation of selected proteins was confirmed by Western blot analysis. Moreover, we observed that protein degradation depends on the presence of the hemagglutinin domain of gingipains, suggesting that this domain may mediate gingipain binding to virions. Among the most efficient modified proteins was glycoprotein B (gB) which is essential for viral attachment and entry into host cells. We found that glycoprotein B of HSV-2 is also a target of gingipains. Given that gB is highly conserved across the *Herpesviridae* family, our findings indicate that this mechanism may be broadly applicable to other herpesviruses.

Taken together, our results show that *P. gingivalis* proteases directly modify viral particles, thereby potentially altering their infectivity modifying its binding and internalization. This study provides novel insights into the molecular cross-talk between bacterial periopathogens virulence factors and virions and expands current understanding of bacterial-viral interactions in the context of periodontal disease.

Acknowledgements: This project has been supported by National Science Center grant UMO-2021/43/D/NZ6/01906

B4: Engineering lysostaphins for targeted antimicrobial activity

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Staphylococcus spp. are Gram-positive bacteria commonly inhabiting human skin and mucous membranes, capable of causing severe opportunistic infections. The rise of antibiotic resistance poses a major threat, driving the urgent need for new antimicrobial strategies. Lysostaphins, enzymes that degrade staphylococcal peptidoglycan, represent a promising alternative, yet only a few have been characterised to date. Here, we investigate the impact of selected point mutations on the enzymatic properties and substrate specificity of three lysostaphins: Lys-Ss (*S. simulans* K-6-WI), Lys-Sp222 (*S. pseudintermedius* 222), and the yet uncharacterised Lys-Ssim (*S. simulans* MR1).

Mutations were introduced in the vicinity of the active site, targeting amino acid residues that differ among the analysed lysostaphins and contribute to the architecture of the catalytic cleft. This strategy aimed to modulate the active-site environment and substrate accessibility. The engineered enzymes were subsequently assessed for lytic activity and minimal inhibitory concentration (MIC) against *S. aureus*, *S. epidermidis*, and *Micrococcus luteus*.

The results demonstrate that single amino acid substitutions can significantly modulate enzyme activity, leading to either attenuated or enhanced lytic capacity and altered substrate specificity. Mutations at positions F39 (in LysSs-Ss), G40, and F88 (in Lys-Sp222) specifically attenuated activity against *S. epidermidis* while maintaining efficacy against *S. aureus*. Additionally, the F39G substitution increased activity against *M. luteus*. Notably, the A39F mutation in Lys-Ssim enhanced activity against *M. luteus* without compromising efficacy against staphylococci. Furthermore, multiple other mutations inspired by the Lys-Ssim structure and introduced into LysSs-Ss and Lys-Sp222 successfully conferred lytic activity against *M. luteus*, a species typically resistant to these enzymes.

In conclusion, our findings demonstrate that rationally designed amino acid changes can expand the utility of lysostaphins, paving the way for the development of novel, selectively acting antimicrobial enzymes (enzybiotics) capable of combating antibiotic-resistant infections.

Acknowledgements: This research was supported by funds granted by the National Science Centre (NCN, Poland) based on decision No. DEC-2021/43/B/NZ6/02413 (to BW).

B5: Application of XANES spectroscopy to assess the plant physiology state under drought-induced stress - preliminary study

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XANES (X-ray Absorption Near Edge Structure) spectroscopy is a sophisticated tool for determining the oxidation state and local chemical environment of an element, including ligand type and coordination geometry. For the first time, XANES spectra of plant tissue were measured at the ASTRA beamline at the SOLARIS National Synchrotron Radiation Centre. The aim of this study was to investigate the effects of drought-induced oxidative stress on sulfur (S) chemical speciation in *Euphorbia milii* Des. Moul leaf tissue culture treated with PEG 6000. *E. milii* is an important medicinal plant with significant biotechnological potential. Sulfur is involved in redox homeostasis and can serve as an indicator of oxidative stress in plant tissue. XANES spectra were measured at the sulfur K-edge for two types of plant samples, namely lyophilized leaves as well as lyophilized and then powdered samples pressed into pellets. The XANES spectra of plant material were compared to spectra of various sulfur compounds, such as MgSO₄, Na₂SO₃ and L-cysteine. Analysis of the spectra revealed multiple sulfur oxidation states in the studied material, providing insight into the effects of PEG-induced drought stress. Both sample types appeared suitable for analysis; however, the results favoured the pellet form. Our results demonstrate the potential of the ASTRA beamline at the SOLARIS Centre to conduct exploratory research in plant science. Plants represent more than 80% of the living biomass on Earth, and it is difficult to imagine life in its current form without them. Plants synthesize sulfur-containing amino acids, such as methionine and cysteine, which constitute an essential dietary source of sulfur for humans. Moreover, sulfur is a constituent of many biologically important plant secondary metabolites with potential medical application. Therefore, this method can be successfully applied in the analysis of economically important crop plants.

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B6: Modulation of fosmanogepix antifungal activity by *Candida albicans* extracellular vesicles

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Current antifungal therapies are constrained by increasing antifungal resistance, restricted activity against rare and emerging fungal pathogens, and significant adverse effects, such as nephrotoxicity associated with amphotericin B or hepatotoxicity exerted by azoles. Therefore, the necessity for the development of broad-spectrum therapeutics with improved safety remains in high demand. Fosmanogepix (FGX) is a novel, first-in-class antifungal compound, a phosphate prodrug of manogepix (MGX), currently undergoing phase II clinical trials. MGX targets the highly conserved fungal enzyme Gwt1, which is essential for the biosynthesis of glycosylphosphatidylinositol (GPI)-anchored cell wall mannoproteins. Inhibition of Gwt1 disrupts maturation of cell surface proteins, thereby impairing fungal growth and adhesion, which underlies the promising broad-spectrum activity of this compound.

The molecular microenvironment of targeted fungal pathogens is crucial and should be taken into consideration when evaluating effectiveness of a drug. Therefore, the aim of our study was to investigate the potential impact of extracellular vesicles (EVs) secreted by *Candida albicans* on the efficacy of FGX. Our analysis focused on the impact of EVs on FGX during *C. albicans* biofilm development and invasion into human epithelial cell monolayer. Furthermore, we examined whether released EVs previously exposed to subinhibitory concentrations of FGX could transmit signals affecting recipient fungal cells.

Preliminary evidence suggests that the presence of EVs enhances the antifungal activity of FGX, resulting in reduced fungal biofilm development and decreased adhesion of *C. albicans* yeasts to host epithelial cells. Furthermore, the presence of EVs was associated with a decrease in the effective FGX concentration required to inhibit fungal growth.

These results highlight the important role of fungal EVs in modulating antifungal drug activity but further thorough investigation is still required.

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B7: Zc3h12b destabilizes mRNAs encoding transcriptional regulators of neural development

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The ZC3H12 family of proteins (ZC3H12A–D), also known as MCPIP/Regnase proteins, functions as endoribonucleases that regulate mRNA stability by selectively degrading transcripts through their PIN-like catalytic domains. ZC3H12B (MCPIP2/Regnase-2) exhibits predominant expression in the central nervous system (CNS) and is involved in guarding glioblastoma development. However, its role in *in vivo* neural development remains unexplored. To address this gap, we employed zebrafish as model organism to investigate the biological function of Zc3h12b during early embryogenesis. Our findings demonstrate that ectopic overexpression of Zc3h12b led to profound developmental defects. We performed RNA-seq, RT-qPCR, and 3'UTR luciferase assays to elucidate the underlying mechanisms.

RNA-seq data from Zc3h12b-overexpressing zebrafish embryos revealed downregulation of transcripts encoding transcriptional regulators as the most significantly affected targets. Among the top differentially expressed genes between embryos overexpressing wild-type Zc3h12b and those expressing the catalytically inactive Zc3h12b-D/A variant, we identified key neural development regulators, including *bhlhe22*, *sox11b*, *olig2* and *notch3*. 3'UTR luciferase assays confirmed that Zc3h12b destabilizes these mRNAs through their 3'UTRs. These findings provide evidence that Zc3h12b influences neural development through 3'UTR-mediated mRNA decay.

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B8: Modification of herpesviruses by gingipains limits their replication

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Periodontitis (PD) is a highly prevalent chronic inflammatory disease of the gingiva, and clinical evidence indicates that patients with PD are frequently co-infected with herpesviruses, including HSV-1. A proposed molecular basis for this association is the ability of gingipains, cysteine proteases of *Porphyromonas gingivalis*, a key etiological agent of PD, to impair host antiviral defenses. Since the direct influence of gingipains on HSV-1 virion infectivity remains unknown, we investigated their functional impact across the viral replication cycle. Our data demonstrate that although gingipains alter virion structure, they do not impair herpesvirus entry into human epithelial cells. Instead, gingipain treatment leads to a significant reduction in viral replication at later stages of infection, resulting from limited penetration of the virion into the cell nucleus. Moreover, we show that virions pre-treated with Kgp or RgpA elicit a stronger host interferon response, consistent with more efficient viral clearance and a reduced viral load. The molecular basis of this phenomenon is the degradation of ICP0, UL41, and UL46 in virion particles, proteins that normally restrict the expression of IFI16, a key host nuclear sensor of viral DNA. These data demonstrate that gingipains accumulating in gingival crevicular fluid directly modify herpesvirus virions, thereby limiting their infectivity. Collectively, our findings reveal a previously unrecognized complexity in *P. gingivalis*-herpesvirus interactions and highlight the need for further investigation.

Acknowledgements: This project has been supported by National Science Center grant UMO-2021/43/D/NZ6/01906.

B9: The effect of *Candida albicans* aspartyl protease (Sap6) on the function of HL-60 neutrophil-like cells

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Candida albicans is an opportunistic yeast-like fungus that can cause severe systemic infections in immunocompromised individuals, characterized by high mortality rates and increasing resistance to antifungal therapy. Virulence factors play a crucial role in the pathogenesis of these infections, including secreted aspartyl proteases (Saps), which can modulate the host immune response. One such protease, Sap6, has been suggested to impair neutrophil function.

The aim of this study was to evaluate the effects of the *Candida albicans* protease Sap6 on neutrophil-like cells derived from the differentiated HL-60 cell line, with particular emphasis on cell viability, proliferation, and effector functions. Experiments were performed using flow cytometry, viability and proliferation assays, chemotaxis analyses, measurements of reactive oxygen species (ROS) production, and fluorescence microscopy.

The results demonstrate that Sap6 inhibits the differentiation of HL-60 cells toward neutrophils in a concentration-dependent manner, reduces cell viability, and limits replicative capacity. Moreover, rHL-60 cells exposed to Sap6 exhibited altered functional responses, both in terms of reactive oxygen species generation and cell migration. Collectively, these findings indicate that Sap6 contributes to immunosuppression, thereby promoting the survival and dissemination of *Candida albicans* within the host.

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B10: New variants of microcystins synthesized by *Trichormus variabilis*

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Trichormus variabilis is a recently described cyanobacterial species that exhibits toxicity, although the mechanisms underlying its toxicity remain unclear. Cyanobacteria synthesize a wide array of toxic secondary metabolites across multiple chemical classes; in this case, the toxin profile of the strain suggested the presence of microcystins. However, earlier analyses failed to detect the most common microcystin variants.

Toxicity assessment using bioassays with the crustacean *Thamnocephalus platyurus* confirmed the presence of multiple toxic compounds in the samples. Subsequent mass spectrometric analysis identified nine novel microcystin variants featuring atypical -amino acid- substitutions. Each variant incorporated glycine at position 7 (Gly⁷) – a substitution not previously recorded in the CyanoMetDB database – along with glycine at position 1 (Gly¹). Many of the variants contain D-Asp³ residue, a relatively common modification. All microcystins, except one, carry an aromatic amino acid (Hph or Hty) at one variable position and arginine (R) at the other; however, three of the variants harbor a novel residue, isopentyl-R, which has never been reported in microcystin structures.

Further studies examined the stability of selected compounds and found them highly resistant to environmental stressors. Stability assays spanned a broad pH range (pH 4–10), included boiling temperatures, and involved exposures to UV-A, UV-C, and UV-A in combination with TiO₂. The results demonstrate that these microcystins remain chemically stable across this pH spectrum and resist degradation even at high temperature and upon exposure to UV radiation.

B11: When bacteria talk to fungi. The role of *P. gingivalis* extracellular vesicles in *Candida albicans* biofilms

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Extracellular vesicles (EVs) released by *Porphyromonas gingivalis* contain a broad range of molecules derived from bacterial cells. These EVs represent an important virulence factor, as they allow bacteria to effectively deliver components involved in infection. During the progression of periodontitis, *P. gingivalis* employs EVs to interact and collaborate with other pathogenic microorganisms. It is well documented that EVs play a crucial role in the coaggregation of *P. gingivalis* with other bacteria, thereby promoting the development of mixed-species biofilms.

In addition to bacteria, the human oral cavity is also inhabited by *Candida albicans*. As an opportunistic pathogen, *C. albicans* is capable of causing infections in humans, and its ability to form biofilms is essential for disease progression. Interactions between bacteria and *C. albicans* have been reported in multiple diseases, and bacterial modulation of *C. albicans* biofilm formation contributes significantly to the course of many co-infections.

This poster presents findings from a study investigating the effects of *P. gingivalis* EVs, produced by three different bacterial strains, on *C. albicans* biofilm development. Increased *C. albicans* growth following exposure to bacterial EVs was demonstrated using OD600 measurements, crystal violet staining, and Colony-forming unit enumeration, along with enhanced hyphal formation observed by fluorescence microscopy and confirmed by electron microscopy. In addition, fluorescently labeled EVs were visualized to examine their interaction with fungal cells. Furthermore, EVs were tested in combination with amphotericin B to evaluate their influence on the activity of this antifungal agent. The findings indicate that bacterial EVs may provide a protective effect for *C. albicans* against amphotericin B.

B12: Structural characterization of the inhibitory mechanism of α 2-macroglobulins from human periodontopathogens

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Proteolysis is irreversible; hence, it must be tightly controlled to prevent cell or whole organism damage. One way of controlling proteolytic activity, especially in multicellular organisms, is synthesis of proteinaceous protease inhibitors. By stark contrast to all known protease inhibitors, members of the large multidomain α 2-macroglobulin (A2M) inhibitor family inhibit a broad spectrum of endopeptidases of all known catalytic classes without disturbing their active sites. It is achieved through a unique trapping mechanism similar to the hunting of Venus flytraps on insects. Although A2Ms are mainly present in mammals, A2Ms are also expressed in *Porphyromonas gingivalis* (Pg) and *Tannerella forsythia* (Tf), two bacterial species and significant etiological factors of periodontal diseases that affect severe forms up to 15% of adults worldwide.

PgA2M and TfA2M, on contrary to all known bacterial and/or mammalian A2Ms, are inhibitors possessing unique properties such as impressive inhibitory repertoire, extreme inhibitory efficacy in monomeric form (PgA2M) and being active only as a dimer (TfA2M). Thus, it is tempting to speculate that possessing A2Ms enables the survival of *P. gingivalis* and *T. forsythia* in hostile environment of dental pockets full of bacterial and host proteases¹. Therefore, we aim to characterize the inhibition process exerted by PgA2M and TfA2M structurally. 3D reconstruction of the map for the TfA2M complex with the Karilysin protease (Kly-18) was obtained.

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B13: A modular genome engineering toolkit to dissect Regnase-1/2 contributions to temozolomide resistance in glioblastoma

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Glioblastoma (GBM) rapidly acquires resistance to temozolomide (TMZ), limiting treatment efficacy. Regnase-1 (Reg-1) and Regnase-2 (Reg-2) are RNA-binding endoribonucleases that regulate mRNA stability and decay, yet their role in TMZ response remains unclear. In GBM, Reg-1 is reported to be upregulated, and Reg-2 downregulated, and both changes correlate with poor prognosis. Since MGMT activity and mismatch repair (MMR) status are key determinants of TMZ sensitivity, we will track both to interpret Reg-1/2-dependent effects.

We are building a modular genome engineering toolkit to test the contribution of Reg-1/2 to TMZ adaptation and define underlying regulatory programs. Preliminary data in U87 cells show that increased TMZ resistance correlates with elevated Reg-1, while Reg-2 levels remain unchanged. To establish causality, we will combine CRISPR interference (CRISPRi) with Sleeping Beauty-based gain-of-function and rescue constructs. Mechanistic profiling will include CUT&RUN, complemented by single-cell visualization of protein and transcript distribution using immunofluorescence with single-molecule RNA fluorescence in situ hybridization (IF–smRNA FISH). This integrated approach will link Reg-1/2 perturbations to regulatory changes and phenotypic TMZ resistance in GBM.

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B14: *Tannerella forsythia* a2-macroglobulin (TfA2M) is more similar to the human one than other bacterial A2Ms

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Inhibition of numerous proteases by the large multidomain A2M inhibitor family is achieved through a unique, irreversible trap mechanism resembling carnivorous plants. Cleavage of the A2M bait region by a prey protease triggers major conformational arrangements resulting in encapsulation of protease. In stark contrast to mammalian ones, bacterial A2Ms, such as ECAM from *Escherichia coli*, are inefficient monomeric protease inhibitors believed to protect cell envelope proteins due to their presence in the periplasm. Surprisingly, A2M, present in the genome of *Tannerella forsythia* (TfA2M), has a different signal peptide than other known bacterial A2Ms.

Contrary to bacterial A2Ms, TfA2M was utterly inactive as a monomer, and dimerization led to inhibitory activity. TfA2M inhibited a *Tf* metalloprotease, karilysin and subtilisin Carlsberg with the stoichiometry of inhibition (SI) of approximately 9 and 6, respectively, and an association rate constant (k_{ass}) of 0.2 and $1.7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, respectively. Inhibition of both proteases was achieved through the formation of covalent inhibitory complexes. Surprisingly, the inhibition was also observed on peptide substrates (~3 kDa). Removing the hyperreactive thioester bond by site-directed mutagenesis did not affect the inhibitory properties of TFA2M and only prevented the formation of covalent complexes. The formation of inhibitory complexes with karilysin by outer membrane vesicles (OMVs) isolated from wild-type (wt) *Tf* and not from the A2M deletion mutant confirms that TFA2M is a secretory lipoprotein attached to the cell surface.

To conclude, TfA2M is more similar to human A2M than to A2Ms present in other bacteria inhabiting the human body.

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B15: Exploring a conserved outer-membrane system involved in fimbriae biogenesis and surface lipoprotein homeostasis in Bacteroidota

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Bacteria belonging to the Bacteroidota phylum are characterized by an unusually high abundance of surface lipoproteins (SLPs), which often function in multi-protein complexes with integral outer-membrane (OM) β -barrels. In the oral pathogen *Porphyromonas gingivalis*, SLPs include proteins involved in nutrient acquisition as well as prefimbrilins, the precursors of fimbriae that are one of the key virulence factors of this bacterium. Despite their importance, the mechanisms governing SLP secretion and maturation remain poorly understood.

Here we describe the discovery of a previously uncharacterized system involved in fimbriae biogenesis in *P. gingivalis*. This system consists of two homologous protein pairs, each composed of an OM β -barrel and an associated periplasmic protein. Deletion of either pair results in defects in prefimbrilin secretion and maturation, leading to disrupted fimbriae assembly. In addition, loss of these components alters the levels of other SLPs, suggesting a broader role in surface protein homeostasis. Biochemical analyses show that both protein pairs co-purify with fully polymerized fimbriae and, to a lesser extent, with other SLPs. Although our efforts to obtain high-resolution cryo-electron microscopy structures were thus far unsuccessful, purification of the complexes from multiple mutant backgrounds proved highly informative. Comparative analysis of these preparations revealed differences in associated proteins and assembly states, providing valuable insights into the maturation pathway of fimbriae.

Together, our findings identify a novel system required for prefimbrilin secretion and fimbriae biogenesis in *P. gingivalis* and suggest a more general role in SLP biogenesis in Bacteroidota. This work lays the groundwork for future structural and mechanistic studies of surface protein assembly in these bacteria.

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B16: Searching for the function of novel M97 protease from *P. gingivalis*

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We have recently identified a large number of previously uncharacterized M97 proteases within among others the phylum *Bacteroidota*. In *Porphyromonas gingivalis*, we identified a single periplasmic ortholog, which we named Zuzalysin. The gene was cloned and characterized both biochemically and structurally, revealing a calcium-dependent enzyme that very slowly degrades folded proteins. Moreover, structural analysis of recombinant Zuzalysin demonstrated a remarkable ability to form oligomeric and polymeric assemblies. However, despite these intriguing *in vitro* properties, the biological significance of Zuzalysin in *P. gingivalis* remains unclear.

In the present study, we aimed to address this knowledge gap. We generated an isogenic *zuzalysin* deletion mutant, as well as double mutant strains lacking Zuzalysin in combination with either the HtrA protease or a functional type IX secretion system (T9SS). These strains were subjected to temperature stress, acidic stress, and nutrient-limitation conditions. In addition, the role of Zuzalysin in the degradation of bactericidal peptides was investigated. Finally, the effect of temperature shock on *zuzalysin* gene expression was examined in the wild-type strain.

B17: Subcellular localisation and role in virulence of bacterial α 2-macroglobulins

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Periodontitis is a chronic inflammatory disease affecting 20-50% of the population worldwide. This condition has a microbiological origin and, if left untreated, can lead to the development and/or progression of severe systemic conditions such as Alzheimer's disease, diabetes and cardiovascular disease. The main bacteria associated with periodontitis form the so-called red complex, which includes *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*.

α 2-Macroglobulins (A2M) are a family of inhibitors that inhibit a broad spectrum of endopeptidases without destroying their active sites. Protease inhibition occurs when the prey protease hydrolyses the bait region, leading to significant structural rearrangements in the inhibitor and to the entrapment of the enzyme. A2Ms are also found in the genomes of bacteria that cause periodontal disease, including *P. gingivalis* (PgA2M) and *T. forsythia* (TfA2M).

Bioinformatic analyses indicated that the PgA2M protein is localised in the periplasm, while TfA2M from *Tannerella forsythia* is a secretory outer-membrane lipoprotein. To validate these predictions, fractions obtained from whole bacterial cultures were analysed by Western blot using polyclonal antibodies specific to PgA2M and TfA2M. PgA2M was identified inside the cell, while TfA2M was attached to the cell surface and present in outer membrane vesicles (OMVs). OMVs from wild-type *T. forsythia*, but not from the TfA2M deletion mutant (Δ TfA2M), were able to bind covalently to the target proteases.

To investigate the role of A2M in the virulence of *T. forsythia*, the zebrafish larvae model was employed. Lack of TfA2M resulted in a decrease in the severity of larval infection.

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B18: Development of a single-cell transcriptomic workflow to study hepatocellular carcinoma initiation in a murine model of *Zc3h12a* deficiency

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Hepatocellular carcinoma (HCC) incidence and mortality continue to rise worldwide, yet the mechanisms driving cancer initiation and shaping its early genomic heterogeneity remain poorly understood. Our preliminary data indicate that Monocyte Chemoattractant Protein-1 Induced Protein (MCPIP1), which regulates inflammatory responses and possesses RNase activity, plays a crucial role in maintaining liver homeostasis and suppressing tumor development.

We observed that lack of MCPIP1 in liver-specific conditional knockout (KO) mice significantly increased tumorigenesis after diethylnitrosamine (DEN) induction, particularly in females. To identify an optimal time point for single-cell profiling of early carcinogenic events, we performed bulk RNA-seq at 0, 3, 6, and 9 months post-DEN and selected 4.5 months post-DEN as the most suitable window for capturing molecular changes preceding overt tumor formation.

To precisely characterize the impact of *Zc3h12a* deletion in the liver during early stages of hepatocarcinogenesis, we undertook the development of an optimized single-cell isolation workflow. The protocol was designed to enable rapid and efficient recovery of all major liver cell populations while preserving high cell viability and minimizing transcriptional artifacts induced by the isolation procedure itself.

Liver dissociation was performed using the gentleMACS™ system, followed by cell purification based on Nycodenz density-gradient centrifugation. This approach enabled the simultaneous processing of up to four mice under identical conditions. Importantly, the use of the 10x Genomics Flex workflow allowed fixation of isolated cells, facilitating coordinated library preparation across all experimental groups and substantially reducing batch effects. Using this strategy, we generated four single-cell libraries representing male and female wild-type and KO animals, each yielding minimum 10,000 high-quality cells. Sequencing confirmed the presence of all major hepatic cell populations.

We anticipate that downstream analysis of these datasets will provide new insights into the role of *Zc3h12a* in shaping the early cellular and molecular landscape of liver tumorigenesis.

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B19: Impact of cystathionine γ -lyase overexpression in the *mdx* mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD), an X-linked neuromuscular disorder, is caused by the absence of functional dystrophin protein, resulting from frameshift mutations in the dystrophin-encoding gene. Dystrophin is a main structural component of the dystrophin-associated protein complex (DAPC), which stabilizes the sarcolemma and protects myofibers from contraction-induced damage. Loss of dystrophin leads to chronic muscle inflammation, excessive fibrosis, and progressive myofiber degeneration. Consequently, patients develop severe cardiomyopathy and respiratory insufficiency, culminating in premature death during the second or third decade of life.

Recently, using proteome-based analysis, we demonstrated reduced levels of hydrogen sulfide (H₂S)-generating enzymes in diaphragm tissue from *mdx* mice, a widely used model of DMD [1]. In the present study, we examined whether enhancing endogenous H₂S synthesis through overexpression of cystathionine γ -lyase (CTH), a key H₂S-generating enzyme, on muscle pathology and function in *mdx* mice. Muscle-specific CTH overexpression was achieved by myotropic adeno-associated virus (AAV)-mediated gene delivery. Quantitative PCR and Western blot analyses confirmed increased CTH mRNA and protein levels in both diaphragm and gastrocnemius muscles of treated animals. Histological analysis of haematoxylin and eosin-stained gastrocnemius sections demonstrated reduced immune cell infiltration, myofiber swelling, and rhabdomyolysis in CTH-overexpressing *mdx* mice. Furthermore, Masson's trichrome staining revealed a significant attenuation of muscle fibrosis. Notably, these therapeutic effects were not recapitulated in the diaphragm, where CTH overexpression failed to elicit measurable improvements in tissue pathology. Nevertheless, these findings suggest that enhancing endogenous H₂S production through CTH overexpression may mitigate hallmarks of DMD and represent a promising therapeutic strategy for slowing disease progression.

Acknowledgments: This work was supported by grant #2019/35/B/NZ3/02817 (to AŁ) from the National Science Centre.

[1] Mucha O, Myszk M, Podkalicka P, Świdarska B, Malinowska A, Dulak J, Łoboda A. Proteome Profiling of the Dystrophic *mdx* Mice Diaphragm. (2023) *Biomolecules*. 13;13(11):1648.

B20: Do the toxin–antitoxin systems control antibiotic susceptibility and virulence in *Staphylococcus aureus*?

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Type II toxin–antitoxin (TA) systems are increasingly recognized as modulators of bacterial stress responses, persistence, and antimicrobial susceptibility, yet their mechanistic contribution to resistance and virulence in *Staphylococcus aureus* remains poorly defined. A recent study reported a negative correlation between the presence of the YefM/YoeB TA system and macrolide/trimethoprim resistance in sequence type (ST) 398 lineages. The same study described a non-canonical TA arrangement in which a gene coding for Fic-domain toxin (FicDoc) is co-localized with the one for MazE-like antitoxin. TA systems such as YefM/YoeB are known to influence global gene expression by antitoxin-driven regulation of transcription and/or ribosome-dependent RNase activity of the toxin. In *S. aureus*, TA modules have been implicated in stress tolerance and virulence regulation; however, whether TA loss, retention, or rewiring causally affects antibiotic susceptibility and pathogenic phenotypes across distinct STs remain unresolved. The central gap addressed in this study is whether the observed absence of YefM/YoeB in erythromycin- and trimethoprim-resistant ST398 strains reflects true genetic loss and whether this loss functionally affects resistance and virulence. We further address whether the MazE/FicDoc locus constitutes a bona fide functional TA system. Comparative genomics and phylogenetics are used to assess TA distribution, integrity, and association with antibiotic resistance across *S. aureus* lineages. Functional analyses include construction of Δ YefM/YoeB single and double mutants; minimum inhibitory concentration (MIC) and biofilm assays; single-copy chromosomal *lacZ* transcriptional reporters; EMSA and DNase I footprinting of TA operator palindromes; RNase activity assays and mapping of stress-induced RNA cleavage sites. The MazE/FicDoc interaction is evaluated using heterologous co-expression, toxin neutralization assays, and structure-guided insilico docking. Preliminary broth microdilution data indicate altered MIC trends in Δ YefM/YoeB mutants relative to wild-type strains. Collectively, this work establishes a mechanistic framework linking TA system presence or loss to susceptibility to antibiotics and virulence regulation in *S. aureus*.

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B21: When fibroblasts age too soon: pathogen-induced senescence in periodontitis

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Background: Periodontitis progression is driven by chronic inflammation rather than microbial burden alone. Gingival fibroblasts are essential for connective tissue integrity, and exhibit features of cellular senescence in diseased tissues. Senescent fibroblasts remain metabolically active and secrete pro-inflammatory mediators through a senescence-associated secretory phenotype (SASP), promoting immune dysregulation, osteoclast activation, and impaired tissue regeneration. Major periodontal pathogens have been implicated in triggering senescence, but the underlying mechanisms are not well elucidated. Defining these mechanisms is critical to understanding persistent inflammation and tissue destruction in periodontitis.

Hypothesis: Periodontitis-associated oral pathogens induce senescence in gingival fibroblasts through pathogen-driven molecular pathways, thereby promoting chronic inflammation.

Objectives: This study aims to develop a chemically induced senescent gingival fibroblast model. Secondly, it seeks to compare the effects of chronic exposure to periodontitis-associated pathogens on gingival fibroblast senescence in cells from young and aged donors. Lastly, it aims to identify senolytic agents capable of eliminating senescent fibroblasts and reducing SASP.

Methods and preliminary results: We will develop a chemically induced senescent gingival fibroblast model using a panel of senescence inducers, confirming senescence through established markers and SASP factors. Fibroblasts from young and aged donors will undergo long-term exposure to *Porphyromonas gingivalis* and *Fusobacterium nucleatum* to compare effects on senescence induction, SASP secretion, and underlying regulatory pathways. Preliminary data from the aphidicolin induced senescence model demonstrate modulation of senescence markers and SASP factors, accompanied by changes in ECM dynamics.

Anticipated Results & Significance: We anticipate identifying specific pathogen-driven molecular mechanisms responsible for inducing fibroblast senescence and sustaining the inflammatory SASP and could lead to the identification of potential senolytic agents as novel therapeutic targets.

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B22: Alternative approaches for miRNAs inhibition in mesenchymal stem cells when CRISPR/Cas is not applicable

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Mesenchymal/stromal stem cells (MSCs) represent an important population of stem cells used in regenerative medicine. To improve their biological functions or to better understand their mechanisms of action, safe methods for gene expression modification are sought. One such method is microRNA (miRNA) silencing. These are short, non-coding RNA molecules, 20-25 nucleotides in length, which regulate gene expression by binding complementarily to target mRNAs, inhibiting their translation or directing them for degradation. miRNAs with similar sequences—particularly in the target recognition region known as the "seed" region—and similar functions are called families (e.g., Let-7 family). Currently, the most popular method for silencing miRNA families is the CRISPR/Cas technique. However, this method is applicable mainly when family members are located close to each other in the genome as miRNA clusters, transcribed together as a single primary transcript (e.g., miRNA 17-92). When family members are distributed across different chromosomes, alternative silencing approaches are needed. One such approach involves the use of anti-miRNA sequences, known as antagomirs, which bind permanently to mature miRNAs, blocking their biological activity and directing them toward degradation.

In the presented study, we aimed to optimize the process of transient silencing of the miRNA Let-7 family in an immortalized human MSC line (SCRC4000) using specially designed FAM-labeled anti-miRNA molecules. Different concentrations of the anti-miRNA mixture and transfection reagents were tested. Cytometric analysis was used to evaluate the viability of transfected cells and the percentage of FAM-positive cells at various time points. Subsequently, to assess the effectiveness of the silencing, expression levels of selected target genes were analyzed at different time points after transfection. Based on the conducted experiments, the most optimal conditions for transient silencing of the miRNA Let-7 family were identified, providing the best parameters for effective temporary suppression of these miRNAs.

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B23: Effects of RIPK4 knockout on redox response to cisplatin and vitamin D3 in melanoma cells

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Cisplatin (CisPt) is not routinely used in melanoma therapy due to its limited efficacy, partly resulting from resistance mechanisms and redox adaptation in tumor cells. We previously demonstrated that RIPK4 knockout increases melanoma cell sensitivity to CisPt. In this study, we investigated whether RIPK4 knockout modulates redox homeostasis and the antioxidant response, which shape cellular responses to CisPt and to combined treatment with 1,25-dihydroxyvitamin D3 (1,25-D3).

To address this, we compared A375 and WM266.4 wild-type (WT) melanoma cells with RIPK4 knockout (RIPK4.KO) cells treated with CisPt either as monotherapy or in combination with 1,25-D3. GPX4 activity analysis revealed that CisPt exposure significantly increased GPX4 activity, with the highest levels observed under combination treatment and in RIPK4.KO cells. ROS measurements using the H2DCFDA probe showed increased oxidative stress following CisPt treatment, which was further enhanced in combination therapy, with the highest levels in RIPK4.KO cells. Treatment also increased nuclear NRF2, more prominently in RIPK4.KO cells. HO-1 levels were higher in WT cells, with maximal induction after 1,25-D3+ CisPt, while FTH1 was lower in RIPK4.KO, although 1,25-D3 increased its levels in both cell types.

In summary, these findings indicate that RIPK4 knockout amplifies oxidative stress and reshapes the antioxidant response in melanoma cells exposed to CisPt. The strongest effects were observed with combined 1,25-D3 and CisPt treatment, suggesting that this regimen has the greatest therapeutic potential and represents a promising strategy to sensitize melanoma cells to CisPt, particularly in the context of RIPK4 knockout.

B24: Properties of erythrocytes of omnivorous and vegetarian human - case study

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Erythrocytes of omnivorous and plant-feeding animals differ in contents of lipids which are targets for lysenin, earthworm pore-forming peptide. The main lipid which is target for lysenin is sphingomyeline. The erythrocytes from sheep and the cows contain more than 90% of sphingomyeline, measured in the pool of both phosphatidylcholine and sphingomyeline. The content of sphingomyeline in erythrocytes of omnivorous animals is several times smaller. The question arises whether a food used by humans might cause a difference resembling the difference between omnivorous plant-feeding animals. The blood samples were taken from omnivorous person and from vegetarian. The erythrocytes were isolated by standard procedures removing leukocytes and serum from the samples. The erythrocytes from both donors were compared from the point of view of resistance to osmotic stress and to the susceptibility to damage caused by earthworm celomatic fluid derived from *Eisenia fetida*. All the measurements used the standard method using spectrophotometric measurement of heme released from erythrocytes under strictly defined experimental conditions.

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B25: Exploring immunomodulatory properties of mesenchymal stem cell-derived extracellular vesicles – role in cardiac tissue repair

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Extracellular vesicles (EVs) represent a group of membrane enclosed particles secreted by all cell types, including stem cells (SCs). They are heterogeneous in dimension, density and, most importantly, in their molecular cargo. EVs carry several factors on their surface and in their cytoplasmic compartment, including proteins, mRNAs, non-coding RNA and lipids, that reflect the characteristics of the cell of their origin. These bioactive molecules can be internalized by recipient cells and alter their functional properties, both in physiological and pathological conditions. EVs represent an assessed element of intercellular communication and, for their characteristics, can be also exploited as therapeutic tool.

Previous *in vitro* and *in vivo* studies indicated the regenerative properties of EVs derived from mesenchymal stem cell (MSC-EVs) on injured tissue, including after acute myocardial infarction (AMI). The injection of MSC-EVs into the myocardium was recently proposed as an alternative therapeutic approach to stimulate pro-reparative effects in the cardiac tissue. In addition, recent studies reported the impact of SC-derived EVs on the functional properties of immune cells, mainly macrophages and T cells, but the involved mechanisms are still under investigation.

The main aim of this study was to explore the immunomodulatory properties of MSC-EVs in the context of ischemic tissue treatment, including heart repair. For this purpose, MSC-EVs were isolated and characterized in number and dimension according to ISEV guidelines. Additionally, two monocytic cell lines, THP1 and U937, were characterized as model cells used for macrophage specification. The study of the MSC-EV cargo and its impact on immune cells will contribute to better understanding the mechanisms of MSC-EV activity in the context of AMI treatment.

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B26: Identification of new components of the lipoprotein secretion system in *Porphyromonas gingivalis*

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Porphyromonas gingivalis is involved in development of periodontitis – a chronic inflammation of the gingiva. Many of the proteins known to be major contributors to the pathogen's virulence are surface lipoproteins (SLPs). Even though SLPs are indispensable in *P. gingivalis* pathogenesis, molecular mechanism of their secretion remains elusive. Some of the most important SLP are the proteins forming fimbriae – proteinaceous filaments allowing for the association of bacteria with the host cells and biofilm formation. Fimbrial subunits are encoded in two operonic gene clusters: *mfa1-5* and *fimA-E*. Moreover, *fim* operon includes genes (*pgmA* and *fimX*) of unknown function. Analysis of *P. gingivalis* genome revealed homological genes to *pgmA* and *fimX*, named accordingly *pgmB* and *fimY*. All four proteins, encoded by *pgmA/fimX* and *pgmB/fimY* genes, are proposed to be directly involved in lipoprotein secretion. However, these components are not sufficient to carry out the full range of functions required for protein secretion, given the complexity of secretion systems machinery. Hence the primary aim of this study is to identify and characterize proteins associated with the lipoprotein secretion system (LSS) in order to gain insight into the molecular mechanisms of the secretion.

The experimental approach includes isolating his-tagged LSS in the presence of membrane-permeable cross-linker (DTSSP) and using mass spectrometry to identify potential new components of LSS. Selected proteins will be characterized by constructing a deletion mutant strain and then by analyzing their influence on lipoprotein secretion and *P. gingivalis* proliferation. This approach will elucidate protein–protein interactions and connect molecular findings to a broader understanding of the LSS, potentially enabling development of targeted therapeutic strategies.

B27: KH domains of N4BP1 determine P-body localization without being required for decay of selected mRNA

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N4BP1 (NEDD4 binding protein 1) is a multidomain ribonuclease that has been implicated in immune signaling, NFκB regulation, and viral mRNA turnover. The protein features two tandem K homology (KH) domains, commonly involved in RNA binding, and a NYN endonuclease domain flanked by two ubiquitin-binding motifs.

In this work, we demonstrate that N4BP1 associates with EDC4, the central scaffold protein of the mRNA decapping machinery, as well as with additional 5' cap-associated factors localizing to processing (P) bodies, including DCP1A, DCP2, and XRN1. Using mutational analysis and domain deletion approaches, we identified the tandem KH 1 and KH 2 domains of N4BP1 as crucial for binding to EDC4 and demonstrated that the KH region alone is sufficient to maintain interaction with EDC4. Disruption of the N4BP1's KH domains, or mutations in their GxxG motifs, markedly weakened N4BP1-EDC4 association. Interestingly, benzonase treatment did not destabilize the complex, indicating that the core interaction is resilient to mild condition nuclease treatment. However, N4BP1-EDC4 interaction is sensitive to RNase A treatment, suggesting an RNA-dependent mechanism of association. Furthermore, EDC4 is required for efficient recruitment of N4BP1 to other decapping factors. Transcriptome profiling of human keratinocyte cell line HaCaT revealed that N4BP1 regulates set of mRNAs involved in blood vessel development, cell adhesion and differentiation. Further validation indicated that selected N4BP1 responsive transcripts are regulated independently of P-bodies. Similarly, viral transcripts previously shown to be direct substrates of N4BP1 were still efficiently degraded by N4BP1 mutants that failed to colocalize with EDC4.

This study provides valuable insight into N4BP1's new function as a component of a multiprotein assembly related to mRNA turnover. Our current aim is to refine N4BP1's role within RNA-protein assemblies and its contribution to mRNA decay pathways.

B28: Structural elucidation of the neutrophilic azurosome complex involved in innate immune response to microorganisms

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Neutrophils, the most abundant leukocytes in the circulation, serve as the vanguard of the innate immune system. As first-line defenders, neutrophils are equipped with Azurophilic granules, which are packed with key serine proteases and antimicrobial proteins such as Myeloperoxidase (MPO), Neutrophil Elastase (NE), Proteinase 3 (PR3), Azurocidin (AZU), Lysozyme (LYZ), and Cathepsin G (CG) - these proteins, responsible for the destruction and digestion of microorganisms, form the Azurosome complex. The complex is involved in the formation of neutrophil extracellular traps (NETs), which are a weblike structure composed of decondensed chromatin laden with antimicrobial proteins. Neutrophils release NETs in response to microbial stress to ensnare and neutralize pathogens. The process of NETosis begins with the production of reactive oxygen species (ROS) by the neutrophil's NADPH oxidase complex. By means of ligand-protein interaction of ROS to MPO, the NE and other proteases are transported into the cytoplasm for F-actin, following which the NE is then transported to the nucleus, where the NE degrades histones and facilitates the chromatin decondensation and the establishment of NETs [1]. While the individual components of the azurosome are documented, the functioning of the quaternary complex or its structure remains unknown. The lack of 3D structure creates a knowledge gap and prevents understanding of the functional mechanism, assembly, and regulation of the complex. The overarching goal of this project is to provide the first 3D structural reconstruction of the azurosome. First, we aim to establish a robust methodology to enrich the native complex in a pure, stable, and biochemically active form. This is an essential prerequisite for the second step: high-resolution structural analysis using Cryo-EM.

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[1] Metzler, K. D., Goosmann, C., Lubojemska, A., Zychlinsky, A., & Papayannopoulos, V., (2014) A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. *Cell Rep.*;8(3):883-96.

B29: The regulation of staphyloxanthin production by SrrAB in *Staphylococcus aureus*

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Background and Aims: *Staphylococcus aureus* is a major human pathogen capable of surviving diverse host environments, including the bloodstream. An important virulence-associated trait of *S. aureus* is the production of the yellow carotenoid pigment staphyloxanthin, which protects the bacterium from oxidative stress generated by host immune cells. While staphyloxanthin biosynthesis is known to be environmentally regulated, how these signals are integrated by bacterial regulatory systems, remains poorly understood. The two-component regulatory system SrrAB has been implicated in metabolic and redox regulation, suggesting a potential role in controlling pigment production. This study aims to directly compare wild-type and $\Delta srrAB$ strains to define if and how SrrAB modulates staphyloxanthin production in different Columbia agar formulations.

Methods: Wild-type *S. aureus* USA300 LAC and a $\Delta srrAB$ mutant were grown on commercial and homemade Columbia agar variants supplemented or depleted with sheep blood, individual blood components, peptides, or nutrient sources. Following incubation, staphyloxanthin was extracted using a methanol-based protocol and quantified spectrophotometrically. Pigment levels were normalized to bacterial biomass, and statistical comparisons were performed to assess the impact of *srrAB* deletion under each condition.

Results and conclusion: Across nearly all tested media, the $\Delta srrAB$ mutant showed significantly elevated pigment production compared to the wild-type strain. This increase was particularly pronounced under blood- and serum-containing conditions, as well as in nutritionally depleted agar formulations, which are most similar to the conditions encountered in real bloodstream during infection. This illustrates that SrrAB acts as a broad and dominant repressor of staphyloxanthin pigment production across various environmental contexts. The results support a model in which SrrAB functions as a global metabolic checkpoint, where it limits energetically costly pigment synthesis when *S. aureus* encounters host-associated or nutrient-poor environments.

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B30: Pharmacological modulation of TRPA1 and its impact on calcium signalling in phenotypically distinct human pancreatic stellate cells

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Pancreatic stellate cells (PSCs) play a crucial role in maintaining pancreatic homeostasis and responding to tissue injury. However, their excessive activation, often associated with chronic inflammation and cancer, contributes to the development of pancreatic fibrosis. This study aimed to investigate the involvement of the TRPA1 channel in regulating calcium signalling in human PSCs (hPSCs) representing three distinct phenotypes: quiescent (qhPSC), spontaneously activated (shPSC), and TGF- β -activated (ahPSC). Pharmacological modulation of TRPA1 was achieved using a selective agonist (ASP7663) and antagonist (A-967079), while calcium responses were recorded in real time using live-cell fluorescence microscopy. The most prominent calcium responses were observed in qhPSCs, whereas activated phenotypes exhibited significantly weaker responses, which correlated with reduced TRPA1 expression at both mRNA and protein levels, as assessed by qPCR and immunofluorescence. Administration of the antagonist following prior activation did not fully suppress the calcium response, whereas preincubation with A-967079 effectively blocked agonist-induced responses. These results indicate a phenotype-dependent sensitivity of hPSCs to TRPA1 activation and suggest that ASP7663 and A-967079 may interact with the channel through distinct, conformation-dependent binding mechanisms. Together with accumulating evidence supporting a role for TRPA1 in fibrogenesis in other tissues, these findings provide a rationale for further exploration of TRPA1 modulation in pancreatic fibrosis.

B31: Decoding memory in hematopoietic stem cells: a multi-omic and clonal tracing approach

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The adaptive immune system utilizes T and B cells to confer long-term memory of pathogens. Under "trained immunity" immune cells exhibit memory-like properties via epigenetic reprogramming. Whether hematopoietic stem cells (HSCs) remember prior challenges and subsequently use it to shape future responses remains a mystery. Despite emerging evidence suggesting that long-term functional changes can be acquired by HSCs, key mechanistic gaps remain. It is uncertain whether memory is restricted to specific clones or represents uniform change across all HSCs, and whether it is stimulus-specific or agnostic.

We hypothesize that HSCs function as a central memory source for the hematopoietic system, where distinct clones, defined by stable epigenetic states, provide a framework for accelerated responses to recurrent challenges. To test this, we propose an integrated experimental strategy combining single-cell technologies with robust lineage tracing.

Our primary objective is to establish a lentiviral barcoding platform coupled with the framework of sister-clone splitting, through this genetically identical HSCs will be split into treatment wells, allowing us to analyze memory clones by subjecting them to priming and challenge regimens. We will target both mouse and human HSCs during ex vivo expansion and stimulation, linking clones to molecular function and state. This objective will be integrated with two parallel strategies:

- utilizing already established single cell-multiomic atlases of mouse hematopoiesis, we will perform secondary analysis to define the molecular landscape of HSCs serving as a framework for downstream barcoding experiments
- for in vivo validation, we will employ the DRAG mouse model, tracking the fate of HSC clones in response to repetitive hematopoietic challenges to correlate our ex vivo findings with clonal dynamics.

The findings are expected to advance fundamental knowledge of stem cell regulation and contribute to the development of improved strategies for enhancing hematopoietic regeneration and stem cell-based therapies.

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B32: Applying the WGCNA method to investigate the molecular mechanisms of drug action in nerve cells

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Despite their widespread clinical use, the molecular effects of antipsychotic drugs in neuronal cells are not fully understood. This study aimed to investigate molecular responses to selected antipsychotic drugs in human neuronal cells using a systems-level proteomic approach. LUHMES neurons were treated with clozapine, risperidone, or solvent control, followed by quantitative proteomic analysis. To identify drug-associated molecular patterns, Weighted Gene Co-expression Network Analysis (WGCNA) was applied. This unsupervised method enables the detection of co-expressed protein modules and represents a non-conventional analytical strategy that may reveal biologically relevant associations not accessible through standard statistical analyses.

Additionally, two protein quantification normalization methods were compared: the widely used MaxLFQ algorithm implemented in MaxQuant software and the recently developed directLFQ approach. According to current literature, directLFQ offers significantly reduced computation time while maintaining comparable or improved quantitative performance^[1]. The impact of both normalization strategies on downstream network analysis was evaluated.

The study was further extended by functional enrichment analysis using Gene Ontology (GO) terms and KEGG pathways to characterize the biological significance of identified protein modules. The results highlighted several proteins potentially involved in the molecular mechanisms of antipsychotic drug action and revealed enrichment of specific GO categories and KEGG pathways associated with neuronal function and cellular signaling.

Overall, this integrative analytical framework combining advanced normalization techniques, network-based analysis, and functional enrichment provides novel insights into antipsychotic drug responses at the molecular level and demonstrates the utility of WGCNA in neuropharmacological research.

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[1] Ammar C., et al. (2023) *Accurate Label-Free Quantification by directLFQ to Compare Unlimited Numbers of Proteomes*. *Molecular & Cellular Proteomics* 22:100581.

B33: Multifunctional roles of *Nakaseomyces glabratus* yapsins – Yps3 and Yps9 – in host defense evasion and epithelial barrier disruption

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Nakaseomyces glabratus (formerly *Candida glabrata*) is a leading etiological agent of invasive candidiasis. In contrast to *Candida albicans*, which relies on secreted aspartic proteases (Saps), *N. glabratus* produces a separate set of aspartic proteases – yapsins (Yps) – that are associated with the cell surface and remain far less characterised in the context of host–pathogen interactions. In this study, we combined biochemical profiling with cellular and in vivo models to define the activities and host-relevant effects of two representative yapsins – Yps3 and Yps9 – purified from RPMI-grown cultures.

Using high-performance liquid chromatography coupled to mass spectrometry, we demonstrated efficient cleavage of host-derived antimicrobial peptides, including NAT26 (kininogen-derived), LL-37, and histatin 5, under mildly acidic and neutral conditions (pH 5.5 and 7.0), consistent with activity at host-relevant microenvironments. Given this capacity to neutralise peptide-based defences, we next examined whether yapsins also affect multicellular fungal structures and host barriers. In mixed-species biofilm assays, both proteases promoted detachment of *C. albicans* biofilms, indicating a contribution to biofilm remodeling. Moreover, fluorescently or biotin-labelled Yps3 and Yps9 showed binding to epithelial cell surfaces (A431 and Cal-27), and exposure to purified enzymes compromised epithelial monolayer integrity, resulting in increased permeability in vitro. Immunologically, yapsins triggered only modest changes in IL-1 β and IL-8 release in THP-1 monocytes, pointing to a limited but detectable impact on inflammatory signalling. Notably, pre-treatment of *Galleria mellonella* larvae with Yps3 or Yps9 improved survival following *N. glabratus* infection, supporting an immune-priming effect.

Together, these findings identify Yps3 and Yps9 as multifunctional factors that undermine peptide-based host defences, perturb epithelial barrier properties, reshape biofilm architecture, and modulate host responses, supporting their further evaluation as targets for therapeutic intervention and immunoprophylaxis.

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B34: Novel endocytic and phagocytic functions of bone marrow sinusoidal endothelial cells

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Bone marrow endothelial cells (BMECs) form arteriolar and sinusoidal vessels that constitute the hematopoietic niche, but their ability to take up cargo by endocytosis or phagocytosis remains poorly characterized. In our previous experiments we demonstrated that sinusoidal BMECs express the phagocytosis-linked receptors FcγRIIb and Stabilin-2 [1]. Given these observations, we asked whether these cells internalize ligands across a broad size range and whether they can ingest damaged red blood cells (RBCs) *in vivo*. To this end, we intravenously administered fluorescently labeled 10-kDa dextran or 2-μm latex beads to mice. Flow cytometry and microscopy demonstrated uptake of both 10-kDa dextran and 2-μm beads by FcγRIIb-positive BMECs, indicating capacity for fluid-phase and particulate internalization. To test RBC engulfment by BMECs, we transfused mice with heat-damaged RBCs that exhibit features of senescence such as phosphatidylserine exposure. Flow cytometry revealed RBC-derived signal within bone marrow endothelium, indicating uptake of whole or fragmented RBCs *in vivo*. Bulk RNA-seq of RBC-positive BMECs revealed transcriptional activation of endocytic pathways and genes linked to heme metabolism.

Together, these results suggest that sinusoidal BMECs help remove damaged or senescent RBCs and activate programs to process heme, adding a functional role beyond supporting hematopoiesis. They also point to FcγRIIb and Stabilin-2 as candidate receptors for recognizing RBC-associated ligands, and provide a straightforward framework to further test endothelial contributions to RBC clearance and iron recycling

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B35: MCPIP1 as a gatekeeper of tumour suppression in clear cell renal cell carcinoma

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Clear cell renal cell carcinoma (ccRCC) is the most prevalent and lethal histological subtype of renal cancer, characterized by a poor 5-year survival rate of approximately 12% in advanced cases. Monocyte Chemotactic Protein-1 Induced Protein (MCPIP1), encoded by the ZC3H12A gene, is a critical negative regulator of the inflammatory response, capable of degrading transcripts of major pro-inflammatory cytokines such as IL-6 and IL-1 β , modulating microRNA (miRNA) biogenesis, angiogenesis, and hypoxia signaling. In ccRCC, MCPIP1 is often downregulated, which allows pro-tumor signals to increase, promoting tumor growth, blood vessel formation, and metastasis.

Despite the success of immune checkpoint blockade (ICB) and targeted therapies, drug resistance occurs almost inevitably within 6–15 months of treatment. Currently, no genetic biomarker has been implemented as a reliable predictor of ccRCC prognostic or therapeutic outcomes. While inflammation is recognized as a hallmark of cancer, the specific mechanisms by which it triggers the transformation of normal renal epithelial cells into neoplastic cells remain poorly understood.

This study aims to comprehensively analyze neoplastic initiation and progression using a unique tissue-specific knockout mouse model in which the Zc3h12a gene is deleted specifically in proximal tubule kidney epithelial cells under the control of the Slc34a1 promoter. Renal carcinomas will be induced with streptozotocin to examine early tumorigenesis, with longitudinal monitoring over 12 months of kidney morphology, DNA repair efficiency (using STRIDE technology), and immune cell infiltration. By combining genetically modified mice with advanced molecular profiling, the project seeks to define how MCPIP1 regulates the tumor microenvironment and immune response, ultimately identifying new biomarkers and therapeutic targets to improve outcomes for patients who do not respond to current treatments.

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B36: Revealing KHNYN as a new player in stress granule regulation

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Cells are constantly exposed to stress, which threatens their survival and demands rapid, precisely coordinated adaptive responses. One prominent consequence of stress is a global reorganization of mRNA metabolism, which includes the transient sequestration of transcripts into stress granules (SGs). In tumors, this protective mechanism is commonly hijacked to sustain cell survival, proliferation, evasion of therapy, and cancer progression.

Our studies of stress granule dynamics uncovered a previously unrecognized role for KHNYN, a largely uncharacterized RNase. We found that KHNYN suppresses stress granule assembly under oxidative stress. Overexpression of wild-type KHNYN reduced the proportion of HeLa cells forming sodium arsenite-induced SGs by approximately 50%, whereas deletion of the NYN nuclease domain completely abolished this effect. Deletion of the KH domain did not impair KHNYN-mediated SG suppression, while removal of the C-terminal CUBAN domain enhanced this activity. Importantly, these mutations also produced distinct changes in KHNYN subcellular localization, indicating that individual domains differentially regulate both its intracellular distribution and functional activity.

These pronounced domain-dependent effects highlight the need for a systematic analysis of the protein interaction networks through which KHNYN acts. To address this, we employ TurboID-based proximity labeling to identify protein interaction partners of KHNYN. These analyses are performed using both wild-type KHNYN and a panel of domain-altered variants to determine how individual structural elements contribute to protein partner engagement. Together, this work will provide fundamental insight into KHNYN biology and establish a molecular basis for its role in the cellular stress response.

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B37: Analysis of Mcpip1 deletion in murine cholangiocytes on liver function

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Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by chronic inflammation and progressive damage to intrahepatic bile ducts, potentially leading to cirrhosis, liver failure, and cancer. A model that closely mimics PBC are Mcpip1^{fl/fl}Alb^{Cre} mice with a deletion of the *Zc3h12a* gene, encoding the Mcpip1 protein, in the liver epithelial cells. Importantly, cholangiocytes, which are biliary epithelial cells, exhibit the highest level of Mcpip1 expression. The aim of this study was to test whether PBC development depends on Mcpip1 function in these cells.

Cholangiocyte-specific Mcpip1 deletion was achieved using the Cre-loxP system in Mcpip1^{fl/fl}Krt19^{CreERT} mice, with Cre recombinase induced by tamoxifen administration at 6 weeks of age. Liver tissue was collected 6 and 12 weeks post-induction from male and female mice and subjected to histological and gene expression analysis. In the next step, another cohort of mice was subjected to chemically induced cholestasis one week after tamoxifen administration. Two models of cholestasis were employed: DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine); 4 weeks of a diet containing 0.1% DDC or ANIT (alpha-naphthylisothiocyanate); a single intragastric administration of 75 mg/kg of ANIT.

Histological analysis demonstrated no morphological abnormalities in livers from both male and female Mcpip1^{fl/fl} Krt19^{CreERT} mice. Additionally, there were no changes in the expression of genes involved in the immune response (*Lcn2*, *S100a8*) or fibrosis (*Colla1*, *Mmp9*) between control and Mcpip1^{fl/fl} Krt19^{CreERT} mice. Both cholestatic models significantly induced total bile acids concentrations and liver fibrosis regardless of mouse genotype. However, Mcpip1 deletion in cholangiocytes did not affect liver pathology. Cholangiocyte-specific deletion of Mcpip1 in adult mice was asymptomatic suggesting an important role of Mcpip1 at the stage of liver development.

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B38: A CRISPRi-based platform for multiplex repression of PUMILIO proteins

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The Cas9 protein is an RNA-guided endonuclease that introduces double-strand breaks at specific target site. Engineered variants of Cas9 include a catalytically inactive form, dead Cas9 (dCas9), which lacks nuclease activity. When fused to transcriptional effector domains, dCas9 can serve as a programmable tool for targeted gene regulation.

In this project, we employ a two-component CRISPR interference (CRISPRi) system for gene repression based on dCas9 fused to a bipartite repressor domain composed of KRAB and MeCP2. Target specificity is provided by single guide RNAs (sgRNAs) delivered using plasmid vectors. The sgRNA platform was designed to be compatible with Golden Gate Assembly, enabling efficient cloning of up to three independent sgRNA expression cassettes into a single vector and allowing multiplex gene repression.

Using this system, we achieved targeted silencing of the RNA-binding proteins PUMILIO 1 (PUM1) and PUMILIO 2 (PUM2). PUM proteins are members of the conserved PUF family of post-transcriptional repressors that bind PUMILIO response elements (PREs) within the 3' untranslated regions of target mRNAs. This interaction promotes mRNA deadenylation and decay, resulting in reduced transcript stability and translational repression. Through regulation of a broad set of target genes, PUM1 and PUM2 play essential roles in controlling gene expression programs involved in cell cycle regulation and genome maintenance.

PUM protein activity is tightly regulated by the long non-coding RNA NORAD (Noncoding RNA Activated by DNA Damage), which functions as a multivalent molecular scaffold that sequesters PUM1 and PUM2 and limits their repressive activity. The presented CRISPRi-based approach provides a tool to investigate regulatory interplay with PUM1/2 and NORAD.

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B39: Research on the metabolome and toxicity of the cyanobacterium***Raphidiopsis raciborskii***

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Cyanobacteria produce a wide range of secondary metabolites with diverse biological activities, but the metabolomic diversity and toxic potential of many species remain poorly characterized. *Raphidiopsis raciborskii* is a bloom-forming cyanobacterium of increasing concern due to strain-specific toxin production and expanding distribution. An integrated metabolomic and toxicological approach was applied to characterize metabolites and biological response from different geographical regions.

The studied strains included two cylindrospermopsin-producing isolates from Australia (CS-505, CS-506) and several non-toxic strains from lakes in Poland and Israel. Targeted analysis using solid-phase extraction coupled with HPLC confirmed higher cylindrospermopsin content in CS-505, than in CS-506. Untargeted metabolomics by UHPLC–Orbitrap high-resolution mass spectrometry revealed 881 unique molecular features, of which 124 were annotated using CyanoMetDB and ChemSpider. Multivariate analysis showed clear differentiation among strains, with toxic strains and selected non-toxic Polish isolates displaying higher numbers of unique metabolites, indicating substantial strain-level metabolic variability. Biological activity of those secondary metabolites (extracted with 75% methanol) was evaluated using toxicological assays. Extracts caused acute and sublethal toxicity in invertebrates, disrupted cellular metabolism and gap junctional intercellular communication in mammalian cells, and induced DNA damage in human mononuclear cells. Zebrafish embryo assay revealed lethal and developmental effects, including impaired swim bladder inflation, reduced body length, and axial malformations. Several non-toxic strains exhibited bioactivity comparable to cylindrospermopsin producers, demonstrating that toxicological risk cannot be inferred solely from the presence of cyanotoxins. These results demonstrate that *R. raciborskii* strains produce diverse bioactive metabolites beyond well-known cyanotoxins, highlighting potential ecological and human health risks associated with both toxic and non-toxic strains.

B40: Distinct roles of bacterial and neutrophil-derived proteases in periodontal bone remodelling

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Periodontitis is a chronic inflammatory disease characterized by progressive destruction of tooth-supporting tissues, ultimately leading to alveolar bone loss. Gingival inflammation is driven by the development of subgingival dysbiotic biofilms, in which *Porphyromonas gingivalis* plays a key role. Its major virulence factors are gingipains, arginine- and lysine-specific cysteine proteases, whose enzymatic activity has been extensively studied. However, increasing evidence indicates that proteolytically inactive gingipains (piGING) can also strongly modulate host immune and tissue responses. piGING have been shown to induce pro-inflammatory signaling in epithelial cells and promote Th17 polarization; nevertheless, their role in the regulation of bone homeostasis remains unexplored. Periodontal lesions are also characterized by massive neutrophil infiltration. Activated neutrophils release serine proteases, including neutrophil elastase, cathepsin G, and proteinase 3, which modulate inflammatory signaling and disrupt bone remodeling through degradation of extracellular matrix components and regulatory molecules, such as osteoprotegerin, thereby favoring osteoclastogenesis. The aim of this study was to investigate the contribution of bacterial and host-derived proteases to the osteoblast:osteoclast balance. We analyzed osteoblast biology (Saos-2 cells and adipose-derived stem cells – AD-SCs) and osteoclast precursors (monocytes) in the presence of piGING and neutrophil proteases. TRAP staining demonstrated that piGING, particularly RgpB, significantly enhanced osteoclast differentiation. This effect was accompanied by increased expression of genes and proteins associated with bone resorption and remodeling, including TRAP, CTSK, DCSTAMP, and MMPs, as assessed by qPCR and Western blotting. In parallel, piGING suppressed the expression of osteoblast-associated genes involved in differentiation and extracellular matrix formation (ALPL, OMD, SPP1, SP7), including the key transcription factor RUNX2. Impaired osteogenic potential was further confirmed by reduced mineralization capacity, as shown by Alizarin Red staining. Similarly, neutrophil proteases indirectly inhibited calcium deposition and downregulated the expression of genes essential for bone formation (OCN, SPP1, OMD, SPARC, TGF- β 3). Collectively, these findings demonstrate that both bacterial gingipains and neutrophil proteases independently disrupt bone homeostasis by promoting osteoclastogenesis and impairing osteoblast differentiation, revealing a protease-driven mechanism contributing to periodontal bone loss within the inflammatory microenvironment.

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B41: Phenotype-dependent effects of TRPA1 modulation on migration and viability of human pancreatic stellate cells

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Human pancreatic stellate cells (hPSC) play a central role in extracellular matrix (ECM) homeostasis and pancreatic fibrosis. Under physiological conditions, hPSC remain quiescent (qhPSC), whereas inflammatory or profibrotic stimuli induce their activation, leading to cytoskeletal remodeling, altered migration, and enhanced ECM production. The transient receptor potential ankyrin 1 (TRPA1) ion channel regulates intracellular calcium (Ca^{2+}) signaling and may influence hPSC function in a phenotype-dependent manner.

The aim of this study was to assess the effects of pharmacological modulation of TRPA1, using the agonist ASP7663 and the antagonist A-967079, on cell viability and migration in three different phenotypes of hPSC: quiescent (qhPSC), spontaneously activated (shPSC), and TGF- β -activated (ahPSC). Cell death was monitored by real-time imaging over 15 h, while cell motility was assessed for 6 hours in the presence of TRPA1 modulators. Potential mechanisms of TRPA1-dependent effects on migration were further explored by immunofluorescence analysis of focal adhesions and by bioluminescence-based measurements of intracellular ATP levels.

Pharmacological modulation of TRPA1 had limited effects on cell death in qhPSC and shPSC; however, high concentrations of ASP7663 induced death in ahPSC, despite significantly lower TRPA1 expression in these cells. qhPSC exhibited the highest migration speed and displacement, whereas ahPSC displayed reduced motility but increased directional persistence. Immunofluorescence analysis revealed a higher proportion of elongated focal adhesions in ahPSC compared to qhPSC, which may contribute to their reduced migratory capacity. TRPA1 activation significantly reduced migration exclusively in qhPSCs, without affecting focal adhesion number or length. Notably, TRPA1 activation was associated with a significant decrease in ATP levels in qhPSCs, which may explain diminished migratory properties.

These findings indicate that TRPA1 may indirectly modulate hPSC survival, motility and metabolic state in a phenotype-dependent manner and support further investigation of TRPA1 as a potential pharmacological target in pancreatic diseases associated with fibrosis.

B42: Protease-induced neutrophil extracellular traps in breast cancer progression: insights from proteomic analysis

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Neutrophil extracellular traps (NETs) are extracellular, web-like structures composed of decondensed chromatin fibers decorated with proteins derived from azurophilic granules. They are released by neutrophils in response to infectious and inflammatory stimuli, including proteases that signal through protease-activated receptor-2 (PAR2) [Bryzek, 2025]. Notably, PAR2-derived NETs are unique in that they do not exhibit antimicrobial activity; however, their biological role remains poorly understood. Given that NETs are considered pivotal contributors to tumor metastasis and immune escape, we hypothesized that PAR2-derived NETs may play a role in breast tumor development within a protease-rich tumor microenvironment. Our data reveal that protease-induced NETs, in contrast to classical PMA-induced NETs, display a distinct proteomic signature. Comprehensive proteomic analysis identified 43 proteins that were significantly upregulated in PAR2-derived NETs compared with PMA-derived NETs. Among these, six proteins were classified as having potential oncogenic relevance, four of which have previously been implicated in breast cancer. NET-associated oncogenic proteins may influence cancer cells through multiple receptor-mediated pathways. To validate these proteomic findings, we visualized interactions between NETs and MDA-MB-231 breast cancer cells and assessed whether these interactions preferentially involved PAR2-dependent NETs. We observed a highly specific interaction with protease-derived NETs when compared to PMA-NETs. Based on these findings, future studies will aim to identify the specific NET-associated pro-oncogenic proteins responsible for mediating this interaction. Altogether, our observations support a distinct role for protease-derived NETs in tumor cell interaction and further strengthen the evidence that NETs play a pathological role in carcinogenesis.

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B43: Comparison of the effects of ionizing radiation and full range of solar radiation in the Martian gaseous atmosphere on the content of low-molecular-weight antioxidants in the thalli of *Cetraria aculeata* and *Diploschistes muscorum*

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Lichens can inhabit extreme environments thanks to various adaptive mechanisms, including biochemical ones involving the production of a wide range of compounds with high antioxidant activity. Both ionizing radiation and ultraviolet radiation, which is part of the full range of solar radiation reaching the surface of Mars, can be a source of strong oxidative stress for cells, particularly during metabolic activity.

The aim of this study was to compare the antioxidant response of two terricolous lichen species: *Cetraria aculeata* and *Diploschistes muscorum*, exposed in their metabolically active state to ionizing radiation (50 Gy) or the full spectrum of solar radiation (200-2500 nm) under the conditions of the Martian gaseous atmosphere (95% CO₂) and reduced pressure (6 mbar). The total antioxidant capacity and the content of non-enzymatic low-molecular-weight antioxidants were determined using spectrophotometric and LC-MS/MS methods.

Of the two species studied, *D. muscorum* was more effective in activating defense mechanisms, including significantly increase in the total phenol and flavonoid content, ascorbic acid, ribitol, arabitol, ergosterol, and secondary metabolites – orsellinic acid and lecanoric acid – in response to X-ray radiation. With respect to solar radiation, most of the parameters studied remained unchanged; only the content of glutathione and ergosterol increased significantly, while the content of ribitol and arabitol decreased compared to the control. The particularly pronounced increase in ergosterol level under both experimental conditions indicates remodeling of cell membranes as a result of the harmful effects of ionizing and ultraviolet radiation. The fruticose lichen, *C. aculeata* demonstrated a limited biochemical response, with most parameters unchanged or decreased, suggesting impaired antioxidant mechanisms and weaker adaptive capacity, particularly with respect to X-rays.

The differences in the response of both lichen species may be attributed to their morphological differences – the crustose *D. muscorum* seems to be less sensitive to both types of harmful radiation.

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