

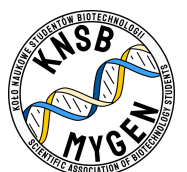
10TH STUDENT
GENETICS & BIOTECHNOLOGY
CONFERENCE "GENOMICA"

15-17 MAY, CRACOW

BOOK OF ABSTRACTS



UNIwersytet JAGIELLOŃSKI
W KRAKOWIE



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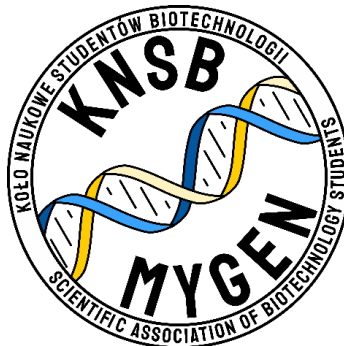
The event organizer, Scientific Association of Biotechnology Students “Mygen”, is not responsible for spelling, grammar errors, content in these abstracts, or for any inaccuracies or ambiguity in the identification or affiliation of their authors. This book contains 80 abstracts presented as oral presentations or posters at the 10th Edition of the Student Genetics & Biotechnology Conference “Genomica”, held on 15–17 May 2026 at the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Kraków.

About the conference

The Student Genetics & Biotechnology Conference "Genomica" is an annual event gathering students, specialists, and outstanding scientists interested in biotechnology and genetics. This year, we are celebrating the 10th anniversary of "Genomica".

About the event organizer

The Student Scientific Association of Biotechnology "Mygen", operating at the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University, was founded in 1997. Since then, the Association has continuously developed its activities through organizing and participating in numerous scientific initiatives. The main aim of the Association is to support students' self-education by exploring the latest achievements in biology and biotechnology, as well as the research methods used in these fields. Members of the Association also participate in research projects carried out at the Faculty of Biochemistry, Biophysics and Biotechnology, as well as in national and international student scientific conferences and biotechnology-related events



In collaboration with

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**Thank you to all the people
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CONFERENCE SCHEDULE

FRIDAY 15.05.2026

13:00-14:30 Participants registration

14:30-14:45 Conference opening

14:45-15:45 Inaugural lecture: Ondřej Štěpánek, PhD: "Revisiting the importance of LCK in T-cell responses"

15:45-16:00 Genomed presentation: "NGS sequencing in microbiome analysis - comparison of available sequencing approaches using the example of V3-V4 16S rDNA metabarcoding service at Genomed S.A."

16:20-17:40 First oral session

18:00-19:20 First poster session

SATURDAY 16.05.2026

10:00-11:00 Special lecture: Mostafa Bakhti, PhD: "Decoding β -Cell development & function: Paving the way for novel diabetes treatments"

11:00-11:20 Bioceltix presentation: "Veterinary Biotechnology - Stem cell-based therapies at a turning point"

11:30-13:00 Second poster session

13:00-15:00 Lunch break

15:00-16:20 Second oral session

16:40-18:00 Third poster session

19:00 Social event at Bracka 4

SUNDAY 17.05.2026

10:00-11:00 Special lecture: Žaneta Matuszek, PhD: "Targeted Base Editing Strategies for Neurological Disease"

11:20-12:40 Third oral session

13:00-14:15 Fourth poster session

14:15-15:30 Lunch break

15:30-16:00 Conference conclusion, award ceremony

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SPECIAL LECTURES

Revisiting the importance of LCK in T-cell responses

Ondřej Štěpánek¹

¹*Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czechia*

LCK is a central SRC-family kinase that initiates T-cell receptor signaling and is essential for positive and negative selection during thymocyte development. However, its role in mature peripheral T cells remains less well defined, partly because conventional LCK deficiency profoundly disrupts T-cell development. To overcome this limitation, we used whole-body and tamoxifen-inducible conditional LCK knockout mouse models, enabling acute deletion of LCK in mature TCR-transgenic OT-I T cells. Using this system, we compared wild-type and LCK-deficient T cells in vivo in two infection models and an autoimmune diabetes model.

Unexpectedly, acute loss of LCK uncoupled two major components of the T-cell response. Although LCK-deficient T cells displayed reduced antigen-driven proliferation, they showed enhanced effector differentiation in vivo. This phenotype was associated with selective disruption of downstream TCR signaling, with LCK being more important for ERK and NFAT activation than for AKT/mTOR signaling. T cells lacking the related SRC-family kinase FYN showed a similar, albeit weaker, bias toward effector differentiation, suggesting that LCK and FYN jointly regulate this process rather than acting through strictly non-overlapping functions.

Together, these findings identify LCK as a dual intrinsic regulator of peripheral T-cell responses: it promotes clonal expansion while restraining effector differentiation. Our study refines the current view of proximal TCR signaling in vivo and has implications for understanding immune dysregulation in human LCK deficiency, as well as for the design of adoptive T-cell therapies.

Decoding β -Cell development & function: Paving the way for novel diabetes treatments

Mostafa Bakhti¹

¹Helmholtz Munich, Helmholtz Diabetes Center, Institute of Diabetes and Regeneration Research

Pancreatic β -cells are essential for glucose homeostasis, and their loss of function contributes to diabetes. In this talk, I will discuss emerging mechanisms involved in β -cell differentiation and islet formation, with a focus on feedback signaling from pancreatic hormones in regulating pancreatic lineage formation. I will also present our recent findings on novel factors controlling β -cell function, particularly how vesicle-trafficking proteins coordinate precise insulin secretion to maintain optimal β -cell functionality

Targeted Base Editing Strategies for Neurological Disease

*Żaneta Matuszek*¹

¹Harvard University, Department of Stem Cell and Regenerative Biology

Precision genome editing technologies are transforming therapeutic development by enabling targeted and durable correction of disease-causing mutations. Here, I highlight the application of base editing to neurological disorders, with examples from spinal muscular atrophy (SMA), Huntington's disease (HD), and Friedreich's ataxia (FRDA).

Base editing enables precise single-nucleotide changes directly in genomic DNA. In SMA, where SMN1 is lost or mutated, the nearly identical paralog SMN2 differs by a single nucleotide at position 8 of exon 7, leading to exon exclusion and reduced SMN protein levels. Base editing of this nucleotide restores exon inclusion and SMN expression from SMN2 in vitro and in vivo, improving motor function and extending survival in $\Delta 7$ SMA mice.

In HD and FRDA, disease pathology is driven by expanded CAG or GAA repeat tracts that undergo somatic repeat instability, particularly in the brain. To address this, we applied targeted base editing to introduce benign interruptions within expanded repeat tracts, reducing somatic instability in patient-derived cells and in animal models.

Together, these studies demonstrate how precision genome editing can be tailored to distinct disease mechanisms, while highlighting both the promise and the challenges of editing disease-relevant genomic loci.

FIRST ORAL SESSION

***In Silico* and *In Vitro* Analysis of the *aga* Operon: Linking GalNAc Metabolism to *Proteus mirabilis* Virulence**

Leon Petruńko^{1,2,*}, Dawid Gmter^{1,3}

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*Field of Study: Microbiology, 1st year PhD student

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Proteus mirabilis is a leading opportunistic pathogen responsible for catheter-associated urinary tract infections (CAUTIs). Unlike many other uropathogens it causes complicated infections, thanks to its wide range of virulence factors such as swarming motility, urease, crystalline biofilm formation and many other. These infections often lead to kidney stone formation, pyelonephritis and even death. The host-pathogen interactions during infections depends on ability to metabolite multiple compounds, including amino sugars. The urinary tract provides abundant N-acetylgalactosamine (GalNAc), an amino sugar. GalNAc is metabolized via the *aga* operon, which role in *P. mirabilis* remains unknown. This study aimed to investigate the conservation of the *aga* operon among *P. mirabilis* strains and to assess the impact of GalNAc utilization on the physiology of *P. mirabilis* strain HI4320.

Structural and functional predictions of Aga proteins were determined utilizing the Phyre2 server. *In silico* comparative genomics revealed high structural conservation of the *aga* operon across multiple clinical isolates. *In vitro* growth analysis in minimal media (M9) demonstrated that while GalNAc delays the logarithmic growth phase compared to glucose, it significantly enhances biofilm production. Furthermore, RT-qPCR analysis showed a massive, up to 500-fold upregulation of *aga* operon genes during growth on GalNAc.

These findings indicate that GalNAc serves not only as a nutritional source but acts as a molecular signal triggering biofilm formation, potentially impacting strains virulence. Targeting this highly conserved metabolic pathway could provide novel anti-virulence strategies to overcome multidrug resistance in *P. mirabilis* infections.

This work was co-financed by the Minister of Science (Poland) under the "Regional Excellence Initiative" program (project no.: RID/SP/0015/2024/01).

Anticancer properties of graphene oxide aerosol. *In vitro* studies on pancreatic ductal adenocarcinoma cell lines.

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**Field of study: Animal Bioengineering, 2nd year MEng student*

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Because of its unique properties, graphene oxide (GO) can be considered a potential candidate for cancer therapies. It is precisely the strength and unique structure of its sharp-tipped petals that enable this nanomaterial to mechanically damage cancer cells, which can lead to the breakdown of their membranes and ultimately to their death. Because this nanomaterial does not form a uniformly flat surface and its flakes tend to fold and stack, GO does not provide a surface conducive to adhesion, which is crucial for cells to adhere to the extracellular matrix (ECM). The aim of the study was to evaluate the effect of a graphene oxide aerosol nanofilm on the morphological and functional state of pancreatic ductal adenocarcinoma (PDAC) cells in *in vitro* experiments using the BxPC-3 and AsPC-1 cell lines

The biological model used to assess the effect of graphene oxide on *in vitro* cultures was PDAC cell lines (AsPC-1 and BxPC-3).

The study was based on a physicochemical analysis of the graphene oxide aerosol at a concentration of 4.5 g/L, utilizing zeta potential and DLS analysis to assess the stability and particle size of the nanomaterial. The nanomaterial was also examined for its chemical composition using laser-induced breakdown spectroscopy (LIBS), energy-dispersive X-ray analysis (EDX), and ATR-FTIR spectroscopy. To visualize the surface ultrastructure of the aerosol, STEM analysis was used.

To analyze the effect of GO aerosol on the biological model, morphological analysis was performed using scanning electron microscopy (SEM), an XTT metabolic activity assay, and migration analysis using wound-healing assay.

It has been shown that GO significantly reduces the viability of the pancreatic cancer cell lines studied, which may have been primarily due to impaired adhesion to the bottom of the culture vessels caused by disruptions in mechanotransduction signaling. Reduced migration and changes in the morphology of cancer cells indicate a stressful environment for the biological model under investigation.

Targeted disruption of *SREBF1a* gene using CRISPR/Cas9 method in a porcine model of adipogenesis

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Adipogenesis is the process by which mesenchymal stem cells (MSCs) differentiate into adipocytes. During this process, a cascade of gene expression is activated, including genes from the *SREBF* family, which encode transcription factors that bind to sterol response element (SRE) sequences. The *SREBF1* gene has two isoforms, *SREBF1a* and *SREBF1c*. To date, the role of the *SREBF1c* isoform in adipogenesis has been well characterized. In contrast, the *SREBF1a* is primarily known as a transcriptional activator of genes involved in cholesterol, fatty acid, and triglyceride synthesis, but its role in adipogenesis has not been extensively studied. The aim of this study was to generate modified MSCs carrying a deletion in the 5' UTR region of the *SREBF1a* gene using the CRISPR/Cas9 method.

The study material consisted of MSCs isolated from the adipose tissue of domestic pigs. Designed gRNA oligonucleotides were cloned into the pX330 vector and introduced into competent *Escherichia coli* cells by transformation. Plasmid DNA was isolated and used to transfect MSCs via nucleofection. Transfection efficiency was assessed using a control plasmid carrying GFP. After 24 hours, cells were subjected to puromycin selection for 48 hours and then cultured to obtain single-cell colonies. PCR and Sanger sequencing were used to analyze the genotypes of the resulting clones. The impact of the introduced genomic modifications on adipogenesis was assessed by quantifying lipid droplet accumulation using BODIPY staining.

The applied methodology enabled efficient genome editing and successful isolation of modified MSC clones, with a transfection efficiency of approximately 40%. Genetically modified MSCs were obtained, including clones carrying a 549 bp deletion in both homozygous and heterozygous states, as well as clones with a 130 bp heterozygous deletion. Among the 49 analyzed clones, *SREBF1a* gene modifications were identified in 7 cases, corresponding to an editing efficiency of 14.3%.

In conclusion, the CRISPR/Cas9-based approach enabled efficient and targeted introduction of deletions within the 5' UTR region of the *SREBF1a* gene in porcine mesenchymal stem cells. The resulting modified MSCs constitute a valuable model for future studies aimed at elucidating the impact of these genomic alterations on the regulation of genes involved in adipogenesis.

A proxyphylline derivative as a new tool for controlling the cytokine storm in SARS-CoV-2 infection

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SARS-CoV-2 is a viral infection associated with cytokine storm which is involved by elevated levels of IL-6, IL-1 β , and TNF- α responsible for severe COVID-19. A new drug, PB-WUT-01, which was developed using *in silico* techniques was tested as a compound intended to block the pathway of cytokine synthesis by inhibiting calcineurin. We compared the immunomodulatory potential of our drug with classical calcineurin inhibitors: tacrolimus (FK506) and cyclosporine A (CsA).

We tested the drug using an *in vitro* model that simulates the immunological microenvironment of the respiratory tract. Human mast cells (HMCs) and peripheral blood mononuclear cells (PBMCs) were exposed to three SARS-CoV-2 variants of concern: Alpha, Delta, and Omicron^{1,2}. Viral stimulation triggered a strong pro-inflammatory response, particularly involving increased secretion of IL-1 α , IL-6, and MCP-1². Our new drug significantly reduced the expression of key inflammatory mediators, including IL-6, TNF- α , and IL-17F, in both cell types³. Additionally, it modulated the expression of the Ace2 receptor⁴, which may suggest a potential impact on the mechanisms of viral entry into cells.

Compared to classical calcineurin inhibitors, PB-WUT-01 demonstrated more selective immunomodulatory properties - it effectively inhibited excessive cytokine production while preserving basic immune system activity. In contrast, FK506 showed a weaker suppressive effect under some of the analyzed conditions.

The structural features of PB-WUT-01 enabled improved interaction with the active site of calcineurin, potentially enhancing its ability to regulate the signaling pathway. Therefore, the developed compound represents a promising therapeutic candidate for controlling excessive inflammatory responses associated with viral infections. These results suggest PB-WUT-01 may represent a next-generation calcineurin inhibitor, capable of modulating the immune response during SARS-CoV-2 infection.

SECOND ORAL SESSION

Analysis of the copy number of the *RSPO1*, *DAX1*, *SOX3* and *WWOX* genes in dogs diagnosed with Disorder of Sex Development (DSD)

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Disorders of Sex Development (DSD) are rising health issues in humans and domestic animals, leading to sterility, increased risk of gonadal carcinogenesis and associated behavioral problems. DSD are congenital conditions characterized by a differences between phenotypic/gonadal and chromosomal sex. They can be divided into three different groups. While sex chromosome abnormalities are well described in other species, they are rare in dogs. On the other hand, the molecular basis of XX and XY DSD remains poorly understood. In human XX DSD phenotype has been molecularly linked to several genes critical for gonadal development, for example *SOX3*, *RSPO1*, *WWOX*, and *DAX1*. It has been shown that *SOX3* overexpression and mutations in *RSPO1* lead to increased *SOX9* expression, promoting the male phenotype in XX individuals. Regarding *DAX1* and *WWOX*, copy number variations (CNV) and structural variants are associated with both XX and XY DSD. The aim of this study was to analyze the number of copies of the *RSPO1*, *DAX1*, *SOX3*, and *WWOX* genes in dogs diagnosed with XX DSD (*SRY*-negative).

The study material comprised peripheral blood samples collected from dogs from 28 different breeds, including 85 XX DSD cases and 79 control females. Copy number variation was assessed using droplet digital PCR (ddPCR), with specific assays for four analyzed studied genes. For accurate quantification, the *C7orf28B3* and *HSD17B7* genes, which are present in two copies in the dog genome, were used as reference genes. Probes for studied and references genes were labeled with FAM and HEX fluorescent dyes, respectively, while genomic DNA was digested with either *HaeIII* or *MspI* restriction enzymes, depending on the studied gene. Following droplet analysis on a QX200 reader was performed and gene copy numbers were determined using Quantasoft software.

Regarding the *SOX3*, *RSPO1*, and *DAX1* genes, two copies were identified in all analyzed animals. In contrast, for the *WWOX* gene, we observed copy number variation in XX DSD dogs: 68 analyzed individuals carried two copies, 13 individuals had three copies, and one individual had four copies of *WWOX* gene. In the control group, all 79 analyzed individuals consistently showed two copies.

Abnormal copy numbers of *DAX1*, *RSPO1*, and *SOX3* genes are not the cause of disorder of sex development in dogs. However, the observed increased copy number in *WWOX* gene may be associated with the abnormal XX DSD phenotype.

When the mean fails – creating a story vs looking at facts

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Hymenoscyphus fraxineus is a pathogenic fungus responsible for the rapid dying out of the tree *Fraxinus excelsior* (common ash) in Europe. Because of this, Researchers are trying to find a way to combat the pathogen by e.g. in-vitro dual cultures with other unharmed microorganisms. Our research was an attempt at verifying the results of a previous experiment that indicated the existence of a link between the available area for growth and the growth rate of *H. fraxineus* in a Petri dish. A deeper understanding of the pathogen's general growth mechanisms could help in fighting or preventing ash dieback and be crucial for performing e.g. dual-culture tests.

In the experiment, Petri dishes with agar substrate were inoculated asymmetrically along their diameter in order to create two distinct directions for growth differing in the total available area. The growth was measured with a ruler by noting the distance between the centre of the inoculum and the growth front in both directions.

To verify that no micro-hyphae invisible to the naked eye exist beyond the macroscopically identified front, fragments of agar just beyond the visible mycelium were sampled. After a DNA extraction procedure, a PCR reaction was carried out using starters targeted at *H. fraxineus* specific sequences of genes coding E2 ubiquitin-conjugating enzymes and α -tubulin. The positive control was a fragment of agar containing visible mycelium, while the negative controls were fragments containing mycelium of a different fungal species and fragments of non-inoculated agar. The results showed that fungal DNA was absent beyond the visible mycelium, which solidified macroscopic observation as a viable method for identifying the growth front.

The original experiment indicated that the mycelium was slowing down its growth rate when nearing the edge of the dish. This observation was further reinforced by the statistically significant difference between the mean values of mycelium growth between the two directions at a point where it hadn't yet reached the edge. Our results, however, showed no such slowdown for any of the samples when looked at individually. This showcases the dangers of indiscriminate application of even the most routine methods of data processing, which may serve as a warning of possibly drawing false conclusions from such processed data.

Identification of Subtype-Specific Transcriptomic Signatures in Soft Tissue Sarcomas Using Negative Binomial Modeling of RNA-Seq Data

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Soft tissue sarcomas (STS) are rare, heterogeneous mesenchymal malignancies. Morphological overlap between subtypes complicates routine histopathological diagnosis, necessitating precise molecular signatures for accurate patient stratification and targeted therapy.

This study aimed to identify robust transcriptomic biomarkers for four major STS subtypes: Synovial Sarcoma, Dedifferentiated Liposarcoma, Myxofibrosarcoma, and Myxoid Liposarcoma.

To ensure maximum statistical rigor, analysis was performed exclusively on raw RNA-seq count data using the DESeq2 framework (negative binomial distribution). Median of Ratios normalization and Empirical Bayes dispersion shrinkage were applied to minimize compositional bias and false positives. Subtype-specific markers were extracted using "One-vs-Rest" contrast matrices. Significance was defined by $\log_2\text{FoldChange} > 0.5$ and a False Discovery Rate (FDR) < 0.05 via Benjamini-Hochberg correction.

The pipeline revealed distinct molecular signatures for each STS subtype. The model's clinical validity was confirmed by the unbiased identification of *MDM2* and *CDK4* as the top significant markers for Dedifferentiated Liposarcoma, perfectly aligning with established diagnostic gold standards. These signatures successfully segregated patients into non-overlapping clusters consistent with their primary histopathological diagnosis.

By leveraging raw count modeling and strict multiple testing correction, we identified specific, noise-free transcriptomic panels for STS classification. These signatures offer a powerful tool to complement classical pathology, paving the way for precision diagnostics.

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Genomic Guardian Under Pressure: Tracking the Stress-Induced Localization of lncRNA *NORAD* in Living Cells

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Long non-coding RNAs (lncRNAs) are defined as transcripts exceeding 200 nucleotides that do not undergo translation. Among these, the non-coding RNA activated by DNA damage (*NORAD*) stands out due to its high evolutionary conservation and robust expression levels across mammalian cells. *NORAD* serves as a critical guardian of genomic stability by sequestering PUMILIO family proteins, which prevents PUMILIO from prematurely accelerating the degradation of essential stability-related transcripts. While it has been established that under adverse conditions *NORAD* localizes into stress granules, membraneless organelles composed of ribonucleoprotein complexes, the precise spatiotemporal dynamics and localization of this transcript within living cells have yet to be fully elucidated. This study addresses this knowledge gap by investigating the real-time behavior of *NORAD* *in vivo* by employing MS2 RNA-labelling system.

The MS2 RNA-labeling system leverages the high-affinity interaction between the bacteriophage MS2 coat protein (MCP) and RNA stem-loop binding site (MBS). Implementation of this platform requires the genetic engineering of an exogenous transcript containing MBS repeats, as well as the expression of MCP with a reporter tag. In this study, we utilize an MCP-HaloTag protein to visualize RNA dynamics. Unlike conventional fluorophores, the HaloTag facilitates the covalent binding of cell-permeable Halo-tag ligands, offering superior signal-to-noise ratios and maximum experimental flexibility for high-resolution live-cell imaging.

Using the MS2 labeling system, we characterized the subcellular distribution of *NORAD* in HeLa cells under both homeostatic conditions and environmental stress. To induce stress response, cells were subjected to various stimuli, including sodium arsenate treatment, osmotic shock via sorbitol, and thermal stress. Simultaneously, the localization of the G3BP1 protein was monitored to serve as a marker for stress granule formation. This dual-labeling approach allowed for the precise spatial correlation of *NORAD* transcripts with ribonucleoprotein condensates during the cellular stress response.

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THIRD ORAL SESSION

Phenotypic variability and microbial community structure in *Philaenus spumarius* spittlebugs

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Auchenorrhyncha is a diverse clade of insects that feed exclusively on plant sap - a diet deficient in essential amino acids. To compensate, these insects maintain obligate mutualistic relationships with bacteria that provide essential nutrients. This symbiosis is estimated to have originated approximately 300 million years ago when their last common ancestor was colonized by a bacterium from the phylum Bacteroidetes, now known as *Sulcia*, which was later supplemented by a betasymbiont, known as *Zinderia*, *Vidania* or *Nasuia*, depending on the Auchenorrhyncha clade. While *Sulcia* infection has remained remarkably stable and is retained in most of the extant members of this clade, association with the betasymbiont is more dynamic, as in some lineages, betasymbiont appears to have been replaced by other bacteria e.g., *Zinderia* by *Sodalis*.

Such a replacement has occurred in a polymorphic spittlebug species *Philaenus spumarius* - the focus of this study. We analysed 679 individuals from 93 populations across 18 countries in Europe. To assess host genetic diversity and microbial community structure, multitarget amplicon sequencing was performed on all samples. Additionally, specimens were assigned to dorsal colour morphotypes to investigate potential associations between morphotype variation and microbiome structure. Analysis of 16S rRNA data revealed three main bacterial symbionts in *Philaenus spumarius*: *Sulcia*, *Sodalis*, and *Wolbachia*. *Sulcia* was detected in all individuals and represented by a single zOTU, which confirmed the evolutionary stability of this association. In contrast, *Sodalis* exhibited a high strain-level diversity, with one dominant zOTU and multiple low-abundant zOTUs suggesting multiple *Sodalis* infections. *Wolbachia* showed lower diversity and less consistent distribution than *Sodalis*. It was present in the majority of northern populations but absent in southern ones. Both *Sodalis* and *Wolbachia* displayed population-level structuring, with distinct variants associated with different geographic regions; however, no correlation with colour morphotype was detected.

This study highlights the evolutionary complexity of *Philaenus spumarius* microbial communities and provides new insights into the roles of phenotype and geography in shaping host-microbiome interactions.

Sf1 is translationally controlled through Igf2bp2 to regulate hematopoiesis

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RNA splicing consists of removal of introns and ligation of exons to form mature mRNA, expanding the coding capacity of the genome. This process is carried out by the spliceosome. One of the initial steps during splicing is the recognition of the branchpoint adenosine within introns, facilitated by binding of splicing factor 1 (Sf1). Defects in splicing are implicated in many diseases i.e. cancer. However, the role of splicing in homeostasis, particularly in stem cell differentiation, remains unclear.

Here, we analysed how the levels of Sf1 are regulated during activation of hematopoietic stem cells (HSCs). HSCs are a paradigmatic stem cell system, with important translational value. We observed that Sf1 is dynamically regulated at the protein level during HSCs differentiation, in the absence of associated changes in mRNA, suggesting posttranscriptional mechanism of regulation. Strikingly, Sf1 is essential for the loss of stemness and differentiation. Therefore, our aim was to understand how Sf1 is regulated during blood differentiation.

To address this question, we analysed the genomic locus of Sf1, revealing evolutionary conserved and highly structured 5' UTR. To assess the *cis*-acting factors, we generated the reporter system with Firefly luciferase controlled by deletion and substitution mutants of murine Sf1 5' UTR and measured luciferase activity. Regions crucial for efficient translation were identified (stem loops 2 and 3). Subsequently, to discover *trans*-acting factors, we performed RNA immunoprecipitation experiments with full-length or mutant, translationally deficient variant of Sf1 5' UTR. Coupled mass spectrometry analysis identified Igf2bp2, a well-studied 5' UTR binding translational activator, to be enriched in the full-length variant. We propose the new model of HSCs activation through upregulation of splicing factors translation, leading to spliceosome remodelling, resulting in global alternative splicing changes promoting differentiation.

Development of the transcription factor-based biosensor for efficient erythromycin overproducers screening

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Erythromycin is a broad-spectrum macrolide antibiotic widely used in medicine. Despite the availability of industrial producer strains, further optimization of erythromycin biosynthesis remains important. Spontaneous mutagenesis is commonly applied to enhance antibiotic production; however, screening large mutant libraries is often inefficient and resource-intensive. Transcription factor-based biosensors offer a promising alternative, as the target metabolite acts as an allosteric effector, modulating gene expression and enabling selective detection of high-producing strains.

In this study, the MphR protein was evaluated as a regulatory component of such a biosensor. MphR is a repressor of the *mphA* gene, which encodes a phosphotransferase responsible for macrolide resistance in *Aeromonas hydrophila*. Binding of erythromycin induces conformational changes in MphR, causing its dissociation from DNA and activation of transcription. Previously, we constructed a genetic cassette containing the apramycin resistance gene under the control of MphR, cloned into an integrative vector and integrated into the streptomycete genome. The system demonstrated functionality, as erythromycin-dependent activation of apramycin resistance was observed. The obtained construct was then transferred to *S. erythraea* via intergeneric conjugation. Subsequent UV mutagenesis and cultivation on medium containing apramycin led to the isolation of mutants with increased erythromycin production.

These results confirm that MphR functions effectively in actinomycetes and can be applied as a tool for the selective screening of strains with enhanced erythromycin production.

Design and characterization of bioinks derived from photocrosslinkable chitosan and polydopamine for tissue engineering applications

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Tissue engineering increasingly incorporates advanced biofabrication approaches to generate biomimetic constructs capable of supporting tissue regeneration and functional restoration. Among these approaches, 3D and 4D printing have attracted considerable attention due to their ability to provide precise control over scaffold architecture, spatial organization, and material responsiveness.

Nevertheless, the development of bioinks that combine adequate printability, structural fidelity, mechanical robustness, and favorable biological properties remains a significant challenge. In this study, multifunctional bioinks derived from photocrosslinkable chitosan and polydopamine were designed and comprehensively characterized for potential application in tissue engineering.

The developed formulations exhibited enhanced printability, improved structural stability following photocrosslinking, and superior biological performance in comparison with conventional chitosan-based systems. Polydopamine nanoparticles were synthesized via the auto-oxidation of dopamine hydrochloride, whereas chitosan was chemically functionalized with methacrylic anhydride to obtain a photocrosslinkable derivative. The resulting materials were subsequently photocrosslinked using various photoinitiators.

A comprehensive physicochemical characterization was performed, including UV–Vis and FT-IR spectroscopy, as well as the assessment of mechanical and sorption properties. In parallel, the biological performance of the developed bioinks was evaluated through cytotoxicity, genotoxicity, antibacterial activity, biodegradation, and cell viability studies.

The obtained findings demonstrate that bioinks based on photocrosslinkable chitosan and polydopamine constitute promising candidates for advanced biofabrication strategies and may offer substantial potential for future tissue engineering applications.

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FIRST POSTER SESSION

Effects of surface mutations on interactions between the cytochrome c_1 subunit and the iron-sulfur protein in bacterial cytochrome bc_1 complex

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The mitochondrial electron transport chain (ETC) couples the exergonic flow of electrons between membranous protein complexes with generation of the proton-motive force (PMF) which powers the production of ATP necessary for many biochemical processes in cells. Cytochrome bc_1 plays a crucial role in ETC by translocating protons through the membrane and connecting two different pools of electron carriers: in-membrane quinone and water-soluble cytochrome c .

The bacterial cytochrome bc_1 is the model for the study of the family of bc_1 complexes. It occurs naturally as a homodimer in which each monomer is made up of three distinct and redox active subunits: cytochrome c_1 , cytochrome b and the iron-sulfur protein (ISP). It operates through the modified Q cycle in which the quinol is oxidized at the Q_0 site during bifurcation reaction splitting electrons to two pathways: a low potential hem b chain and a high potential chain of Rieske cluster of ISP and c type hems of cytochrome c_1 subunit and cytochrome c_2 .

Movement of the head-domain of ISP from near the Q_0 site to a position on the surface of cytochrome c_1 facilitates the reduction of hem c_1 and consequently the reduction of free cytochrome c_2 . Thus, surface interactions of the iron-sulfur protein and cytochrome c_1 play an important role in the functioning of the bc_1 complex.

Single amino acid substitutions removing protonable residues from the cytochrome c_1 - ISP interface were introduced in cytochrome c_1 . Resulting muteins R182Q and D94N were examined with spectroscopic (UV-Vis, EPR) and electrochemical methods (potentiometry). The enzymatic activity of mutated proteins was measured both in chromatophores (bacterial membranes) and in an isolated form. Superoxide production of these muteins was also estimated. Experimental data shows a difference in enzymatic activity of mutated proteins with respect to the wild-type of bc_1 . This result may indicate a relevance of this region for electron transfer from ISP to cytochrome c_1 which may also affect the generation of ROS during bc_1 catalytic cycle.

Expression and Affinity Chromatography Purification of the Human Rio2 Protein using Nonadherent Expi293 Cells

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Rio2 is an atypical serine kinase with ATPase activity that participates in the cytoplasmic maturation stages of pre-40S ribosomal subunit. To enable functional analyses of the polymorphism Ser96Cys identified in *RIOK2*, a mammalian expression and purification system was established for recombinant Rio2 carrying this substitution.

The coding sequence of *RIOK2* was amplified from HEK293 cDNA and cloned into a pcDNA3.0 vector containing C-terminal Fc tag to facilitate detection and affinity-based isolation. Expression was performed in non-adherent Expi293 cells supplemented with translation enhancers, which supported high-density cultures reaching 7.8×10^6 cells/mL with approximately 95% viability. Following transient transfection, cells were harvested on day 4 after transfection. Cell lysates were subjected to affinity purification on an ÄKTA Pure system using a HiTrap Protein G column. Chromatographic analysis revealed a distinct elution peak corresponding to Fc-tagged Rio2. Fractions collected from this peak displayed a clear band at the expected molecular mass on SDS-PAGE, confirming that the elution profile reflected the presence of purified Rio2-96Cys.

The developed system yields Rio2-96Cys of purity and stability sufficient for downstream biochemical and structural studies, including particles imaging on cryoEM. This material enables examination of the structural consequences of the Ser96Cys substitution and supports investigations aimed at uncovering mechanism of Rio2 interactions within the pre-40S maturation pathway.

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

Previously, using proteome-based analysis, we demonstrated reduced levels of hydrogen sulfide (H₂S)–generating enzymes, including cystathionine γ -lyase (CTH), in diaphragm tissue from *mdx* mice, a widely used model of DMD. In the present study, we examined whether enhancing endogenous H₂S synthesis through CTH overexpression can improve muscle pathology and function in dystrophic 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 results support our previous findings obtained using H₂S donors and suggest that enhancing endogenous H₂S production through CTH overexpression may mitigate key hallmarks of DMD.

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Cellular Stress-Related Proteins in the Cerebral Cortex of Novel Model of ASD - *Trap1* Mutant Mice

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Background: TNF Receptor Associated Protein 1 (TRAP1) is a mitochondrial-specific chaperone from the HSP90 family. It functions as an ATP-dependent dimer, ensuring the correct folding and stability of numerous mitochondrial substrate proteins. The mutation in TRAP1 gene p.Q639* was identified recently in two unrelated male ASD patients. In order to research the functional consequences of this specific TRAP1 variant, a knock-in mouse model with identical mutation that was identified in ASD patients was developed (*Trap1* p.Q641*). *Trap1* mutation resulted in decreased *Trap1* mRNA levels and absence of detectable *Trap1* protein levels in the brain of *Trap1* mutant mice.

Aim: This study aims to determine whether the mutation on *Trap1* p.Q641* affects the expression of other heat shock proteins (HSP) and mitochondrial protein *Nlr1* in the mouse cerebral cortex.

Methods: Cortical tissue was obtained from 8-week-old wild-type (WT) and *Trap1*-mutant mice. Protein expression was quantified via Western Blotting for a comprehensive panel including *Nlr1*, mitochondrial chaperones (*Grp75*), endoplasmic reticulum stress markers (*Grp78*, *Grp94*), and cytoplasmic chaperones (*Hsp27*, *70*, *105*). *Tubulin* and *Gapdh* were used as a loading control for densitometric normalization.

Results: Verification of the model confirmed the total absence of *Trap1* protein in *Trap1*-mutant mice. The quantitative assessment of mitochondrial, cytoplasmic, and ER-resident proteins provided a comprehensive overview of the cellular stress response machinery in the *Trap1* mutants. This analysis revealed differences in the levels of GRPs and HSPs in WT mice and those with a mutation in the *Trap1* coding gene. Additionally, *Trap1*-mutant mice exhibited lower levels of *Nlr1* in cortices, compared to wild-type controls.

mRNA and protein expression of GLUT4, ADRP and ATGL in porcine ovary during the estrous cycle

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Introduction: Ovarian lipid metabolism acts as an important energy source, regulator of oocyte and follicle maturation and plays a crucial role in steroidogenesis, i.e. sex hormones production. Hormone profile of ovary is precisely related to the estrous cycle and the level of steroid hormones depends on current physiological demand. Not only is the lipid metabolism controlled by gonadotropins but also by a myriad of proteins related to lipid droplets, including adipocyte triglyceride lipase (ATGL) and adipose differentiation-related protein (ADRP), as well as by insulin-dependent glucose uptake via GLUT4 transporter. ATGL is an antagonistic protein to ADRP; while the former one has an active role in lipid catabolism, the latter one reduces this process. In the ovary GLUT4 is considered as a facultative glucose transporter in case of large energy demand. Currently, there is a lot of studies researching the expression of those proteins in mammals' reproduction system (e.g. cows, sheep or rats), and their role in lipid processing but there is little to no research on this topic in porcine ovaries. Therefore, the aim of this study was to investigate the mRNA and protein expression of ATGL, ADRP and GLUT4 in the ovary during the pig's estrous cycle.

Materials & methods: Ovarian follicles and corpora lutea at different stages of the estrous cycle (days 10-12, 14-16 and 17-19) were isolated and then mRNA and protein expression were analyzed using RT-qPCR and Western blot, respectively. GraphPad Prism 8.0. and ImageJ were used to conduct statistical analyzes (one-way ANOVA and post-hoc Tuckey tests) (n=3, p<0,05).

Results: Our results showed that the mRNA and protein levels of all analyzed molecules were expressed in every stage of estrous cycle, and both transcript and protein levels were correlated in every case. ATGL and ADRP expression declined along with the follicle development, while it increased during the luteal phase. As for the GLUT4, we observed an opposite tendency.

Conclusion: Those results confirm ATGL, ADRP and GLUT4 expression in ovary both in gene and protein levels, and consequently the intensity and nature of metabolic processes, are dependent on the stage of the estrous cycle.

Design and characterization of Stimuli-Responsive Hybrid Bioinks for 4D bioprinting of Soft Tissues

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Tissue engineering demands high biofunctionality and structural precision from scaffold materials. 4D bioprinting, using stimuli-responsive materials, enables the fabrication of structures capable of controlled morphological evolution over time. This is crucial for neural tissue regeneration, where mimicking natural biological dynamics determines therapeutic success.

This study focused on the development of a hybrid bioink based on chitosan, chosen for its biocompatibility and mucoadhesive properties. In the first stage, the chitosan backbone was chemically modified with a diverse set of amino acids: acidic (L-glutamic acid, L-aspartic acid), polar (glycine, L-serine), and non-polar (L-leucine). This modification aimed to tune hydrophilicity, functional group density, and specific cellular interactions. The product was purified via membrane dialysis and subsequently lyophilized, with the synthesis confirmed by FTIR spectroscopy through the identification of newly formed amide bonds.

The next stage involved functionalization with methacrylic anhydride. The introduction of methacrylate groups enabled photochemical crosslinking under UV or visible light, ensuring high printing resolution and hydrogel stability under physiological conditions. Due to the potential toxicity of methacrylating agents, a rigorous purification process was employed to ensure neural cell viability.

The final stage involved the incorporation of growth factors to create a bioactive environment that promoted neuronal adhesion, migration, and differentiation. Comprehensive characterization included rheological studies, in vitro degradation kinetics, and cytotoxicity assays. This approach combined 3D printing precision with fourth-dimension functional responsiveness to stimuli, offering new perspectives for the treatment of nervous system injuries.

The research presented in this study was funded under the project entitled "*A novel range of innovative, stimuli-responsive bioinks obtained from waste biomass as a new era in 4D bioprinting*", implemented as part of the "*Student Research Clubs Create Innovations*" program and financed by the Ministry of Science and Higher Education (project no. SKN/SP/631435/2025).

RPSA on the night shift: what does the ribosomal protein lose when its C-terminal domain is removed?

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Ribosomal protein SA (RPSA) is traditionally recognized as a component of the 40S ribosomal subunit. Interestingly, in higher organisms, this protein contains a C-terminal intrinsically disordered region (IDR; aa 210–295) that may be responsible for non-ribosomal moonlighting functions. We hypothesize that RPSA's IDR could facilitate binding to gelsolin (GSN), an actin-binding protein, as our previous studies have shown interactions between RPSA and GSN. Our group found that GSN's activity recruits ribosomes to the submembranous region of skin melanoma cells and regulates local translation, which is relevant for the invasive behavior of cancer cells. The aim of this project (conducted as part of a Master's thesis) is to examine whether a truncated version of RPSA, lacking the C-terminal IDR, influences its subcellular localization in melanoma cells and its interaction with GSN. Human cutaneous melanoma cells were transfected with plasmids encoding either the truncated or full-length, HA-tagged RPSA. Subsequently, RPSA localization was analyzed using confocal immunofluorescence microscopy, and its proximity (<40 nm) to GSN, indicating a direct interaction, was assessed using the Proximity Ligation Assay (PLA). To further investigate the RPSA and GSN direct interaction, a NanoBiT[®] assay was conducted. By fusing the truncated RPSA and GSN with complementary luciferase fragments, we were able to quantitatively analyze the impact of RPSA's IDR on the interaction of those proteins in living cells.

Muscle-specific anti-apoptotic effects of hydrogen sulfide donor GYY4137 in a severe Duchenne muscular dystrophy mouse model

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Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder that affects 1:5000 boys. It is caused by a point mutation in the *DMD* gene, which encodes dystrophin, a protein that ensures the integrity of the sarcolemma during muscle contraction. The lack of dystrophin causes chronic inflammation, fibrosis, and increased apoptosis in muscle tissue. This results in the premature death caused by decreased respiratory function and cardiomyopathy [1].

Hydrogen sulfide (H₂S) is one of the most important gasotransmitters naturally produced by human cells and is known for its cytoprotective effects, including anti-inflammatory, anti-fibrotic, anti-oxidative and anti-apoptotic properties [2].

In our recently published study, treatment with GYY4137 (a slow-releasing hydrogen sulfide donor) attenuated DMD-related pathological features, including decreased apoptosis, in *mdx* mice, the most commonly used DMD model [3]. Given the relatively mild phenotype of these dystrophic mice, in the present study, we tested the effects of GYY4137 on D2.*mdx* mice, which better recapitulate the human DMD condition. Using Western Blot analyses and immunofluorescent stainings, we detected decreased levels of total caspase 3, cleaved caspase 3, caspase 8, and caspase 9, as well as an increase in the pAKT/AKT ratio in *gastrocnemius* tissues of D2.*mdx* mice treated with H₂S donor. In diaphragm samples, however, neither in the previous nor in the current study were differences detected in any of the measured caspase levels or in the pAKT/AKT ratio. Moreover, no anti-apoptotic effect was found after administration of GYY4137 in the hearts of D2.*mdx* mice. Collectively, these results indicate muscle-specific anti-apoptotic properties of hydrogen sulfide and provided grounds for further studies in using H₂S as a therapeutic agent for DMD.

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Multiplex PCR-based detection of *vanA*, *vanB* and *vanC1* genes in newly isolated *Enterococcus* strains

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Antibiotic resistance within the human gastrointestinal microbiota constitutes a major public health concern, as it serves as a reservoir of antimicrobial resistance genes. Enterococci are of particular importance due to their ability to acquire and accumulate resistance determinants, including those conferring resistance to glycopeptides such as vancomycin.

While *Enterococcus faecalis* and *Enterococcus faecium* are among the most common enterococcal species in the human gut, other species, including *E. gallinarum*, *E. casseliflavus*, and *E. durans*, may also be present. Notably, strains of this genus are increasingly investigated for potential probiotic applications, which highlights the importance of excluding isolates harboring clinically relevant resistance determinants, particularly vancomycin resistance genes.

Among the best-characterized vancomycin resistance determinants in enterococci are *vanA*, *vanB*, and *vanC*-type genes, which mediate the synthesis of altered peptidoglycan precursors with reduced affinity for glycopeptide antibiotics. The *vanA* gene confers high-level resistance to both vancomycin and teicoplanin, *vanB* mediates inducible and variable resistance to vancomycin, while *vanC1* is associated with intrinsic low-level resistance in specific species.

The aim of this study was the molecular detection of *vanA*, *vanB*, and *vanC1* genes in newly isolated *Enterococcus* strains using multiplex PCR. Genomic DNA extracted from pure cultures of 12 isolates obtained from infants and children under three years of age was analyzed. The analysis included an internal control targeting the 16S rRNA gene and species-specific primers for selected *Enterococcus* species, enabling the simultaneous detection of resistance determinants. Different profiles of *vanA*, *vanB*, and *vanC1* were detected among the tested isolates. Multiplex PCR proved useful as a preliminary screening method for the detection of selected vancomycin resistance genes in *Enterococcus* isolates. However, further studies are required to confirm species identity and determine the genetic context and biological significance of the detected genes.

Limitations of Short-Read NGS in Friedreich Ataxia: Assessing (GAA)_n Repeat Detection in the FXN Gene

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Friedreich ataxia (FRDA) is the most common autosomal recessive inherited ataxia in Caucasian populations. The disease is caused by reduced levels of frataxin, a mitochondrial protein essential for iron homeostasis and cellular energy production. As a result, FRDA represents a multisystem neurodegenerative disorder rather than a condition limited to the nervous system. Frataxin deficiency is most commonly (approximately 98% of cases) associated with expansion of (GAA)_n repeats in intron 1 of the *FXN* gene, representing a dynamic mutation. The disease typically manifests during adolescence and is characterized by progressive gait ataxia and loss of tendon reflexes.

Next-generation sequencing (NGS) based on short reads is widely applied in molecular diagnostics. The aim of this study was to evaluate whether whole genome sequencing (WGS), combined with different bioinformatic pipelines, enables accurate determination of the (GAA)_n repeat number in the *FXN* gene.

The study was performed on WGS data from 54 gDNA samples obtained from individuals referred to the Institute of Mother and Child for indications unrelated to FRDA. Sequencing data were analyzed using a dedicated institutional bioinformatic pipeline based on short-read alignment followed by analysis with ExpansionHunter, a tool designed for detection of short tandem repeat (STR) expansions. The analysis was additionally supported by PCR and Sanger sequencing of the repeat region.

Short-read NGS approaches have inherent limitations, mainly due to limited read length and challenges in accurate alignment within repetitive regions, leading to imprecise repeat estimation. Although tools such as ExpansionHunter partially address these issues, their accuracy remains limited.

In conclusion, short-read NGS approaches, including WGS, are not sufficiently reliable for precise assessment of repeat expansions such as the (GAA)_n region in the *FXN* gene. Despite the use of dedicated bioinformatic tools, the inherent technical limitations of short-read sequencing hinder accurate determination of repeat length. Further development and broader implementation of long-read sequencing technologies are needed for accurate analysis in clinical diagnostics.

Kinetic changes in the bone marrow after G-CSF or PHZ stimulation

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Hematopoietic stem cells (HSCs) reside at the apex of the blood cell hierarchy, giving rise to all types of mature blood cells. Under the influence of various factors and stressors, HSCs can alter their behavior, adjusting their lineage commitment to meet the body's demands. The administration of certain substances may lead to changes in peripheral blood cell counts and induce modifications in HSC colony morphology.

Granulocyte colony-stimulating factor (G-CSF) and phenylhydrazine (PHZ) are widely used modulators of hematopoiesis: G-CSF promotes the mobilization of hematopoietic stem cells (HSCs) and drives their differentiation toward the myeloid lineage, whereas PHZ induces acute hemolytic anemia, leading to enhanced erythropoiesis. Both provide excellent models to study HSC responses and adaptations.

Aim: We aimed to evaluate the dynamics of the bone marrow response to physiological stimulation (G-CSF) and acute hematopoietic stress (PHZ). By tracking isolated HSCs over a 21-day recovery period, we determined how these distinct challenges influence *in vitro* colony-forming capacity.

Methods: Mice were treated with NaCl (control), G-CSF, or PHZ. Independent cohorts were evaluated at days 0, 7, 14, and 21 post-treatment. We analyzed peripheral blood parameters alongside HSC colony formation efficiency (success rate), morphology and composition of the colonies.

Results: While peripheral blood analysis confirmed distinct systemic kinetic changes following treatments, the *in vitro* colony-forming capacity of isolated HSCs remained notably stable across all cohorts. Detailed assessment revealed no significant treatment-induced deviations in colony morphology or internal cell composition compared to controls.

Conclusions: Our findings highlight the extraordinary robustness of HSC intrinsic developmental programs. Although pharmacological stimulation and severe hemolytic stress successfully drive systemic hematopoiesis adjusted to new environment, they do not perturb the fundamental multilineage differentiation capacity or heterogeneity of individual HSCs. HSCs maintain their integrity even during extreme physiological demands.

Design of siRNA Targeting Conserved Regions of the ToBRFV Genome (*Tomato brown rugose fruit virus*)

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Tomato brown rugose fruit virus (ToBRFV) has emerged as a major challenge for modern tomato cultivation due to its high infectivity, environmental persistence and the absence of effective resistance in *Solanum lycopersicum* L. This study explores a targeted RNA interference approach based on the rational design of small interfering RNA (siRNA) molecules directed against conserved regions of the viral genome.

The core of the work is a multi-stage design pipeline integrating comparative genomics, sequence conservation profiling and RNA structural evaluation. The proposed protocol introduces a refined selection strategy combining thermodynamic optimization, structural accessibility and assessment of potential off-target interactions within the host transcriptome. This enables identification of highly specific siRNA candidates while minimizing the risk of unintended effects. To support practical application, two implementation strategies are proposed: spray-induced gene silencing (SIGS) using a recombinant pET-21 system with delivery carriers (e.g., LDH, chitosan, carbon nanotubes), and stable genetic transformation via a pCAMBIA-based vector for shRNA expression. Future work will focus on the development of a computational tool for automated and scalable siRNA design, as well as experimental validation of the proposed constructs in collaboration with Adam Mickiewicz University in Poznań.

The presented strategy provides a coherent framework for RNAi-based plant protection and defines clear directions for future research. By linking advanced siRNA design with prospective experimental validation, it opens new possibilities for precise and sustainable antiviral solutions in agriculture.

Genotoxicity assessment of PDA-based hybrid bioinks for 3D/4D bioprinting applications

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This study investigates the properties of bioinks based on chitosan and polydopamine for potential applications in 3D and 4D bioprinting. Methacrylated chitosan derivatives synthesized under different pH conditions were developed and characterized. Their physicochemical behavior was evaluated through swelling studies in aqueous, acidic, and physiological-like environments, as well as through enzymatic degradation assays.

The ability of the materials to undergo photo-cross-linking in the presence of a photoinitiator was confirmed, and FT-IR analysis verified successful chemical modification and cross-linking. In addition, the antibacterial and antioxidant properties of the biomaterials were assessed, highlighting their relevance for biomedical applications.

The biological safety of the developed bioinks was assessed using cytotoxicity assays, the Ames test, and DNA degradation analysis.

Overall, the results suggest that chitosan- and polydopamine-based bioinks are promising candidates for further optimization in bioprinting and biomedical applications.

Cytotoxicity assessment of innovative bioinks based on chitosan derivatives for 3D/4D bioprinting applications

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This thesis focuses on the analysis of bioinks based on a chitosan derivative for applications in 3D/4D bioprinting. The analysed derivative is methacrylated chitosan of fungal origin. The study includes the synthesis of the derivative and the evaluation of its cytotoxicity, as well as the analysis of sorption, antioxidant, antibacterial properties and crosslinking ability using various photoinitiators.

The research methodology began with the synthesis of the chitosan derivative obtained as a result of reaction carried out at different pH values. In addition, varying amounts of cerium nanoparticles were added to the samples, using cerium oxide (CeO₂).

The samples were examined for sorption properties, which proved to be extremely absorbent compounds with high potential for use in materials for gauze and medical dressings designed to absorb large volumes of fluids.

Antioxidant properties constituted the next stage of research. A DPPH radical scavenging assay were performed, which showed that sample 1A exhibited a high ability to inhibit free radicals, achieving an 84% inhibition compared to the reference DPPH solution.

The antibacterial properties of bioinks enriched with cerium nanoparticles were also evaluated. Nanocompounds were added to the samples in varying amounts, and a control sample without their addition was prepared. The number of bacterial colonies grown on the culture medium was analysed, and the results were correlated with the amount of added compounds.

Cytotoxicity was also assessed using the XTT assay, an improved version of MTT method as well as by qualitative method. In the final stage, FT-IR analysis was performed to investigate the structure of the obtained bioinks. The results were collected and analyzed.

PARylation in response to DNA double-strand breaks

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Poly-ADP-ribosylation (PARylation) is an ubiquitous post-translational modification that is known to play a pivotal role in DNA single-strand break (SSB) repair. It remains unclear, however, whether it is part of the cellular response to DNA double-strand breaks (DSBs) as well. Although there has been indication of its occurrence *in vitro*, there is no evidence that PARylation occurs in response to DSBs *in vivo*.

The purpose of the conducted research was to determine whether PARylation occurs in response to DSBs in DlvA cells. In this cell line, 4-hydroxy-tamoxifen activates an oestrogen receptor ligand-binding domain that causes relocation of AsiSI restriction enzyme to the nucleus. An eGFP-tagged WWE domain, which binds to poly-ADP-ribose, was used to detect PARylation. Cells were transfected with a plasmid encoding a WWE domain tagged with eGFP, exposed to tamoxifen, and observed under fluorescence microscope.

Following induction of DSBs, an increase in eGFP-WWE concentration was observed in cell nuclei. These results indicate that PARylation may be a component of the DSB damage response. The influence of other stress factors related to transfection and the imaging process is investigated to exclude the possibility of nonspecific cellular stress reaction.

SECOND POSTER SESSION

Expression Analysis of the *umoB* Gene in *Proteus mirabilis* HI4320 under Extracellular Stress Conditions

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Proteus mirabilis is a Gram-negative, facultatively anaerobic rod that has been phylogenetically reclassified from the *Enterobacteriaceae* family to the *Morganellaceae* family. This pathogen is ubiquitous in terrestrial environments, including soil, water, and sewage, and frequently colonizes the gastrointestinal tracts of both humans and animals. A definitive virulence factor of *P. mirabilis* is its high-level production of urease, which catalyzes the hydrolysis of urea, leading to crystalline biofilm formation and complicated catheter-associated urinary tract infections (CAUTIs).

The *umoB* gene has been characterized as a critical regulator of swarming motility which is a specialized multicellular movement characteristic of this species. Recent evidence suggests that *umoB* may function as a pleiotropic regulator, modulating bacterial fitness in response to environmental stressors and potentially linking motility to broader virulence pathways. Despite its significance, the precise molecular mechanisms and regulatory networks involving *umoB* remain largely elusive.

The primary objective of this study was to quantify the transcriptional activity of *umoB* in the *P. mirabilis* HI4320 strain under diverse extracellular stress conditions. Using RT-qPCR, we analyzed gene expression levels following exposure to EDTA, polymyxin B, hydrogen peroxide H₂O₂, magnesium ions Mg²⁺, urea, and sodium chloride (NaCl). Furthermore, the impact of swarming-inductive conditions on *umoB* expression was evaluated.

Our study elucidate the factors governing the pathogenic potential of *P. mirabilis*, thereby providing a foundation for understanding the survival strategies of this pathogen during infection.

This work was supported by the Polish National Science Centre (Grant No. 2019/33/N/NZ6/02406 for D.G.). This work was co-financed by the Minister of Science (Poland) under the "Regional Excellence Initiative" program (project no.: RID/SP/0015/2024/01).

Asprosin regulates proliferation and cell cycle progression in mouse pituitary gonadotroph L β T2 cells

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Introduction: Asprosin is a fasting-induced adipokine involved in glucose metabolism; however, its role in the pituitary remains unknown. Proliferation is crucial for maintaining anterior pituitary plasticity, enabling adaptation of gonadotroph to metabolic and reproductive cues. This study aimed to evaluate the effects of asprosin on proliferation and cell cycle progression in mouse pituitary gonadotroph L β T2 cells.

Methods: This study evaluated the individual and combined effects of asprosin and GnRH on the proliferation and cell cycle of the L β T2 mouse pituitary gonadotroph cells line. Cells were treated with 1, 10, and 100 nM of asprosin, 50 nM of GnRH, or a combination of both for 24 and 48 hours. Cell proliferation was assessed using a BrdU incorporation assay, and cell cycle progression was analyzed with flow cytometry. The gene and protein expression of the proliferation marker PCNA and cyclins (A2, B1, D1, E1) were determined using RT-qPCR and Western blotting. Statistical analysis was performed using Student's t-test and two-way ANOVA ($p < 0.05$, $n \geq 3$).

Results: Our results demonstrate that asprosin modulates the proliferation and cell cycle of pituitary gonadotrophs in a dose- and time-dependent manner. BrdU incorporation was significantly reduced after 24 h of treatment with 10 nM asprosin, as well as GnRH alone, compared to control. Flow cytometry revealed a decreased proportion of cells in the S phase following 10 nM asprosin and GnRH, while the amount of cells in the G2/M phase increased after GnRH alone and in combination with asprosin. At the molecular level, treatment with 10 nM asprosin increased PCNA protein levels despite reduced gene expression. Moreover, gene expression of cyclins A2, B1, and D1 was downregulated at 24 h. Protein analysis showed that cyclins A2, B1, and E1 reflected their gene expression patterns, whereas cyclin D1 protein levels increased after 48 h of incubation.

Conclusion: In conclusion, we provide the first *in vitro* evidence that asprosin directly modulates the cell cycle of pituitary gonadotrophs. Specifically, its predominantly anti-proliferative effect suggests that this adipokine may act as a negative regulator of the reproductive axis.

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Influence of the RNase MCPIP1 on the genes involved in initiation of ccRCC.

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Clear cell renal cell carcinoma (ccRCC) accounts for 75% RCC cases and is characterized by a 5-year survival rate reduction from 90% to 12% after metastasis. An increased risk of ccRCC is observed in individuals with genetic predisposition. Genetically, ccRCC is characterized by a high frequency of mutations, deletions, or methylation within chromosome 3p. The most frequently mutated genes include: *VHL*, *PBRM1*, *SETD2*, *BAP1*, *MTOR*, *TCEB1*, *PIK3CA*, *KDM5C*, *TP53*, and *PTEN*.

MCPIP1 has recently been proven to be a tumor suppressor, regulating angiogenesis and epithelial-mesenchymal transition crucial for ccRCC tumor growth and metastasis. Among the endonucleases, which have a suppressive effect in breast cancer and renal cell carcinoma, is the *ZC3H12a* gene encoding Monocyte Chemoattractant Protein-1 Induced Protein. Levels of this endonuclease are diminished even in early grade ccRCC, compared to healthy renal tissue. In our research, we investigate whether MCPIP1 influences the regulation of genes involved in the initiation of ccRCC. The level of genes encoding subunits of SWI/SNF remodelling complexes and enzymes taking part in histone modifications with different levels of MCPIP1 was assessed.

The analysis was performed from a human renal proximal tubule epithelial cell line (RPTEC-tert1), clear cell renal cell carcinoma (Caki-1) cell line and ccRCC tumors developed in 6 week NON-SCID mice after subcutaneous injection with Caki-1 cells with stable overexpression and mutant form of the MCPIP1. The mRNA levels of SWI/SNF complex genes and kinases involved in ccRCC initiation processes such as proliferation and cell survival were assessed.

In this study, we present that the endonuclease activity of MCPIP1 might influence ccRCC initiation by regulating the level of *SETD2*, *EZH2*, *SMARCA4/A2*, *ARID1A/B*, and *PBRM1*. The results obtained in the *in vivo* model were compared with changes in normal and cancer cell lines. Levels of kinases such as Akt and Stat3, which are regulators of early processes involved in the initiation of carcinogenesis, were also assessed.

The obtained results suggest that MCPIP1 plays a significant role in initiation of ccRCC as a tumor suppressor gene. Further research will enable us to understand the molecular mechanisms of ccRCC initiation in which MCPIP1 is involved.

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Structural and Functional Characterization of PMI1047 and PMI0275 Proteins Involved in Polymyxin B Resistance in *Proteus mirabilis*

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Proteus mirabilis is a Gram-negative opportunistic bacterium and a major cause of urinary tract infections, particularly in hospitalized patients. One of the key mechanisms of its antibiotic resistance is lipopolysaccharide (LPS) modification involving the attachment of 4-amino-4-deoxy-L-arabinose (L-Ara4N) to lipid A, which reduces affinity for polymyxin B, an antibiotic of last resort. However, the mechanism of L-Ara4N attachment in *P. mirabilis* remains incompletely understood.

In the reference strain HI4320, two variants of the *arnT* gene encoding the responsible transferase were identified: one within the *arn* operon and the other an orphan form (PMI0275). Their functions have not been experimentally characterized. This study aimed to structurally and functionally characterize PMI0275 and PMI1047 using bioinformatics approaches.

AlphaFold models showed high confidence and predominantly α -helical structures. Membrane topology analysis confirmed that both proteins are integral membrane proteins with multiple transmembrane domains. Structural similarity analysis (Foldseek) revealed strong alignment with membrane glycosyltransferases involved in bacterial surface modification. Potential ligand-binding pockets and transport tunnels were identified, suggesting functional sites. Evolutionary conservation and in silico mutagenesis indicated key residues important for stability and function.

These findings suggest that PMI0275 and PMI1047 are membrane glycosyltransferases involved in LPS modification via L-Ara4N attachment, potentially contributing to polymyxin B resistance. This study provides a basis for further experimental validation.

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Distribution and Diversity of Type VI Secretion System Operons in *Proteus mirabilis*

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Proteus mirabilis exhibits a swarming motility phenotype, during which it utilizes the Type VI Secretion System (T6SS) to recognize kinship among neighboring cells. To date, seven operons associated with T6SS have been identified, including a main operon encoding the core structural machinery and several auxiliary effector operons. However, the distribution and variability of these operons in *P. mirabilis* have not been fully characterized. Therefore, the aim of this study was to perform an in silico analysis to investigate the genetic conservation of T6SS-related genes in *P. mirabilis*.

Complete genome sequences of *P. mirabilis* were obtained from the NCBI database. Gene identification was performed using local databases composed of nucleotide sequences of T6SS-associated genes. Database construction and analysis were conducted using the ABRicate v1.0.1 software.

The results revealed a high level of conservation of the main T6SS operon, which was present in 95% of the analyzed strains. In contrast, the auxiliary operons were detected at lower frequencies. Additionally, structural variations in operon organization were observed.

The findings provide new insights into the evolution of the Type VI Secretion System in *P. mirabilis* and contribute to a better understanding of the molecular mechanisms underlying kin recognition among strains.

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The effect of asprosin on the levels of proteins involved in lipid and glucose metabolism in adipocytes from control mice and mice with the B6.129-*Fbn1*^{tm2Rmz}/J mutation

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Introduction: Adipose tissue is associated with many lifestyle-related diseases; adipocytes are responsible for maintaining energy balance in the body and also performs endocrine functions. Lipid and glucose metabolism is regulated by proteins associated with lipid droplets in adipocytes, including ATGL (Adipose Triglyceride Lipase), ADRP (Adipocyte Differentiation-Related Protein), PLIN1 (Perilipin1), and GLUT4 (insulin-dependent glucose transporter 4). The hormone involved in adipogenesis is asprosin, an adipokine produced by the cleavage of fibrillin-1 in adipocytes. The aim of the study was to determine the effect of asprosin on the levels of protein involved in lipid (ATGL, ADRP, PLIN1) and glucose (GLUT4) metabolism in mouse adipose tissue.

Material & methods: The experimental model consisted of sexually mature females control mice (C57BL/6J) and mice with the B6.129-*Fbn1*^{tm2Rmz}/J mutation, which is characterized by reduced asprosin expression. The mice were administered asprosin (1µg/g body weight) or saline (0.9% NaCl) for 14 days. After that, the white adipose tissue was isolated and protein levels were examined using Western blot. The obtained data were statistically analyzed using GraphPad Prism with Student's t-test.

Results: Reduced asprosin expression resulted in decreased expression of ADRP and GLUT4, while increased ATGL. Asprosin injection in mutant mice led to an increase in the expression of ADRP and ATGL, decreased PLIN1 in adipocytes. In wild-type mice asprosin increased only GLUT 4 expression.

Conclusion: Asprosin, by modulating lipid and glucose metabolism, appears to be an important regulator of metabolic homeostasis. These findings may contribute to a better understanding of the molecular mechanisms underlying obesity and insulin resistance.

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Rapid intranuclear depletion of NAD⁺ marks the onset of DNA damage response

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Nicotinamide adenine dinucleotide (NAD⁺) is a key molecule in cell biology, essential for the cellular response to DNA damage (DDR). NAD⁺ plays a crucial role in the repair of single-strand DNA breaks (SSBs), where PARP1 and PARP2 (poly(ADP-ribose) polymerases 1/2) detect DNA strand breaks and regulate the repair machinery. NAD⁺ is a substrate for the synthesis of poly(ADP-ribose) (PAR) chains and mono(ADP-ribose) (MAR). These modifications induce relaxation of the chromatin structure and promote the recruitment of subsequent repair factors, such as XRCC1 and ligases.

The purpose of this study was to analyze the kinetics of intranuclear NAD⁺ depletion at the single cell level after DNA damage induced by laser microirradiation with a focused 405 nm beam.

DNA damage was induced in HEK-293T cells that stably expressed the NS-Grapefruit sensor. This ratiometric FRET-based biosensor enables real-time monitoring of intranuclear NAD⁺ levels in a single cell by measuring the ratio of red-to-green fluorescence emission.

A rapid decrease in intranuclear NAD⁺ levels was detected immediately after DNA damage induction (within 0.3 s). PARP1 accumulation and subsequent elongation of the PAR chain occurred. Furthermore, the extent of NAD⁺ loss was correlated with the intensity of the induced DNA damage.

Analysis of the kinetics and scale of NAD⁺ depletion in response to DNA damage may provide further information on the role of PARPs in DNA damage response mechanisms and contribute to future studies on PARP and PARG inhibition in cancer therapy.

Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Cardiac Progenitor Cells for Cell-Based Therapy in Murine Model of Myocardial Infarction

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Myocardial infarction (MI) is a leading global cause of death and results from sudden occlusion of coronary blood flow, leading to massive cardiomyocyte loss. Current therapies focus on reducing ischemia duration and limiting infarct size but do not restore damaged myocardium, which is replaced by non-contractile scar tissue.

Cell-based therapy has emerged as a promising approach for cardiac regeneration, however, the clinical efficacy of such therapies is limited. We aim to investigate the therapeutic potential of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and cardiac progenitor cells (hiPSC-CPCs) and hypothesize that hiPSC-CMs and hiPSC-CPCs may differ in engraftment efficiency due to their distinct proliferative and differentiation potential.

hiPSC-CPCs and hiPSC-CMs were obtained by differentiation of genetically modified hiPSC cell line, with GFP and luciferase expression, enabling detection of transplanted cells by fluorescence and bioluminescence. MI was induced by left anterior descending artery ligation in NOD-SCID mice and cells were transplanted directly thereafter. The animals were observed and investigated for 12 weeks and then euthanized for histological assessment.

Our results indicated more efficient engraftment of hiPSC-CMs than hiPSC-CPCs based on bioluminescence assessment. Moreover, a significant decrease in cardiac functional parameters was observed after MI induction, which improved in animals receiving cell therapy. In vitro phenotypic characterization by flow cytometry showed that ~35% of hiPSC-CPCs were OCT3/4⁺, whereas hiPSC-CMs lacked OCT3/4 expression, and that differentiation from CPCs to CMs was associated with a decrease in GATA4 expression and an increase in cTnT expression. Whereas histological analyses of post-MI cardiac sections showed no significant differences in myocardial fibrosis between groups.

Our results contribute to understanding the regenerative potential of hiPSC-derived cardiac cells for post-infarction repair.

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Overexpression of MCPIP1 changes extracellular lactate levels, cell proliferation and viability in human hepatocellular carcinoma SNU449 cell culture.

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Liver cancer is one of the most common type of cancer worldwide. Hepatocellular carcinoma (HCC) accounts for majority of primary liver cancer cases. However, the cellular mechanisms that lead to cancer initiation and progression are not well understood. *In vitro* models that allow further studies include cell cultures such as human HCC cell lines, e.g., SNU449.

One of the hallmarks of cancer is the Warburg effect. It is a metabolic switch from oxidative phosphorylation (OXPHOS) being main source of energy, to a process known as aerobic glycolysis. Even under normoxic condition and with functioning mitochondria, glycolysis becomes the main source of ATP synthesis despite being less efficient than OXPHOS. This process produces intermediates used in cancer metabolism, that are not produced in the Krebs cycle. The end product of glycolysis, pyruvate, is oxidized to lactate to replenish cellular NAD⁺ levels, creating an acidic environment that further promotes cancer progression.

Monocyte Chemotactic Protein-Induced Protein 1 (MCPIP1) is an RNase, involved in the downregulation of inflammatory response, which is another hallmark of cancer. Previous studies, including RNA sequencing (RNA-seq) of liver tumors from MCPIP1-deficient mice, have shown that this protein regulates many metabolic pathways involved in cancer transformation. Based on these data, lactate secretion (associated with aerobic glycolysis), cell viability and proliferation were studied in SNU449 cell line with MCPIP1 overexpression.

Lactate levels in the culture medium were measured 24 hours after plating using a chemiluminescent enzymatic kit, while cell viability was assessed with alamarBlue reagent over 72 hours. Cell proliferation was measured by cell counting, over the same time period.

Overexpression of MCPIP1 showed a visible trend in reducing cell proliferation and decreasing viability in HCC cells. It also altered the extracellular lactate levels after 24 hours of culture, however, further research is required to fully understand this effect. These results suggest that MCPIP1 negatively regulates the survival of hepatocellular carcinoma cells, but its role in cancer metabolism and its influence on the Warburg effect need to be further determined.

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Influence of Phytohormones on *Portulaca oleracea* L.: In Vitro Callus Induction and Biomass Accumulation

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The *Portulaca oleracea* commonly known as purslane is a widely distributed plant with a long history of use in food and medicine. It is one of the richest terrestrial sources of omega-3-fatty acids and it is abundant in various phytochemicals such as alkaloids, carotenoids, flavonoids, terpenoids and vitamins such as A, C, E and B. Callus derived from *P. oleracea* enables controlled production and study of its bioactive compounds. In this poster, we showcase our study on optimizing callus induction on leaf explants and assessing biomass accumulation using different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (BAP) and evaluate the overall regeneration tendencies across different explant types, growth conditions and phytohormone concentrations.

Callus Induction and Photosynthetic Characterization of *Portulaca oleracea* L: Influence of Explant Type and Light Conditions on In Vitro Development.

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Portulaca oleracea L. is a globally significant medicinal and nutritional plant, distinguished by its high levels of omega-3 fatty acids and bioactive antioxidants. Beyond its biochemical profile, it represents a rare and crucial biological model for studying the C4-CAM photosynthetic transition, a unique mechanism of environmental adaptation. This research investigates callus induction across various explant types under light and dark conditions, paired with photosynthetic characterization. Another objective of the study is to attempt to induce the C4-to-CAM transition within the resulting callus tissues. Understanding these processes is essential for exploring the metabolic plasticity and biotechnological potential of this species.

Analysis of the Effect of Doxorubicin on the Secondary Structure of Human Serum Albumin Using Circular Dichroism Spectroscopy.

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Human serum albumin (HSA) is the most abundant protein in human blood plasma, with a physiological concentration of 35–70 g/L. The protein consists of 585 amino acid residues, including methionine, phenylalanine, cysteine and a single tryptophan residue. HSA is a globular protein with a characteristic heart-like shape and plays a crucial role as the main ligand-binding protein in plasma. Two principal binding regions, known as Sudlow site I and Sudlow site II, are responsible for interactions with numerous endogenous and exogenous ligands. In addition to its transport function, albumin also exhibits antioxidant activity.

Doxorubicin is an anthracycline antibiotic widely used in chemotherapy for the treatment of various cancers. Its antitumor activity is mainly associated with DNA intercalation and inhibition of DNA repair processes through interaction with topoisomerase II, as well as with the generation of reactive oxygen species that lead to oxidative damage of cellular components. Despite its high therapeutic efficiency, the clinical use of doxorubicin is limited by significant adverse effects, particularly cardiotoxicity. Therefore, considerable attention has been devoted to the search for appropriate carriers that could improve drug bioavailability and reduce toxicity toward normal cells. Human serum albumin is considered a promising model carrier due to its natural transport properties.

The aim of this study was to investigate the influence of doxorubicin on the conformation and secondary structure of human serum albumin using circular dichroism spectroscopy. The obtained spectra were analyzed using dedicated computational software for secondary structure estimation. The results indicate that formation of the HSA–doxorubicin complex causes only a slight decrease in α -helix content, which is the dominant structural element of albumin (approximately 60–70%). These findings suggest that the interaction between albumin and doxorubicin does not significantly disturb the native structure of the protein, indicating that HSA may serve as a suitable carrier for this drug.

Comparison of size and membrane fluidity of Giant Plasma Membrane Vesicles from HEK293 and TR33B cell lines.

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Research conducted on membrane proteins often require the usage of specialized methods capable of maintaining the native environment of biological membranes during extraction, purification and analysis to ensure the preservation of proteins' native structure, conformation and functionality. *In vitro* membrane model systems, such as liposomes, bicelles or supported lipid bilayers, are capable of simulating the lipid environment necessary for protein structure upkeep, however synthetically-obtained models may not always provide an accurate representation of membrane environment in cell due to the lack of compositional heterogeneity and complexity present within the native cell membrane.

This issue can be minimized by using *in vitro* lipid bilayer model systems isolated directly from living cells via chemically-induced vesiculation, such as Giant Plasma Membrane Vesicles (GPMVs). While the model is widely used to study membrane proteins, many underlying processes and properties of GPMVs remain unknown.

This study compares size and lipid bilayer fluidity of GPMVs derived from HEK293 and TR33B cell lines. Vesicle size was assessed via confocal microscopy imaging of vesicles. Membrane fluidity data was collected using Fluorescence Lifetime Imaging Microscopy of di-4-ANEPPDHQ-labeled GPMV and calculations of generalized polarization of vesicle membranes labeled with polarity-sensitive laurdan dye.

Differences in GPMV size between TR33B- and HEK293-derived vesicles were accompanied by variability in membrane fluidity at both the single-vesicle level and across both cell lines. These observations indicate heterogeneity within GPMVs' membrane environment, mirroring key features of native cell membranes.

miR-126-enriched exosomes as a tool supporting muscle regeneration

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Duchenne's muscular dystrophy (DMD) is a severe, progressive disease that leads to skeletal muscle degeneration, causing weakness and eventual loss of function. New, innovative therapeutic strategies are being sought. Lately, more and more studies concerning exosomes' vector properties have been conducted, including their role as vectors for regulatory molecules, such as microRNA (miRNA). miRNA can modulate regenerative processes and angiogenesis, and miR-126 seems to play a special role in tissue regeneration.

This study aimed to evaluate the miR-126-enriched exosomes' potential in the regeneration of damaged skeletal muscle in the *mdx* mouse model. Human skeletal muscle cells (hSkM) were transfected with miR-126, and exosomes were extracted. The level of miR-126 expression was measured using the RT-PCR method. Previously obtained exosomes were used both in *in vivo* and *in vitro* studies. Exosomes were injected into the *Tibialis anterior* muscle and isolated at different time points. Isolated and stained muscles were subjected to histological and immunocytochemical analysis. Hematoxylin-Eosin (HE) staining enabled an evaluation of general tissue structure and muscle fibre regeneration, and Masson's trichrome staining enabled the evaluation of morphological changes and the level of fibrosis. Isolectin immunocytochemical staining was also performed to assess the activity of differentiation processes and muscle regeneration.

Preliminary results show that miR-126-enriched exosomes could influence and support muscle regeneration and angiogenesis. The obtained data suggest a potential of exosomes and miRNA in supporting DMD muscle regeneration.

Comparative characterization of human and porcine mesenchymal stem cells

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The pig (*Sus scrofa domesticus*) is a widely known research model which both on anatomical and cellular level resembles humans. Identifying similarities and differences is crucial for the accurate translation of preclinical findings to medicine. This study aims to evaluate proliferative capacity and mitochondrial dynamics in the porcine and human *in vitro* models.

The study utilized primary adipose-derived mesenchymal stem cells from both human and porcine (hAD-MSC and pAD-MSC, respectively). To assess cell proliferation and doubling time, tetrazolium salt reduction assay (MTS) was performed on days 1, 4, 7 and 10 post seeding. Following 24 hours incubation with osteogenic (ascorbic acid, dexamethasone, β -glycerophosphate) or adipogenic (dexamethasone, indomethacin, 3-isobutyl-1-methylxanthine, Insulin-Transferrin-Selenium) differentiation factors, mitochondrial membrane potential was visualized via MitoTracker™ Orange and semi-quantitatively analyzed using confocal laser scanning microscopy (CLSM).

Porcine AD-MSC demonstrated a higher proliferative capacity relative to the hAD-MSC. Regarding early metabolic responses, preliminary CLSM imaging showed that hAD-MSC exhibited mitochondrial hyperpolarization in both osteogenic and adipogenic lineages relative to control groups. Conversely, pAD-MSC displayed depolarization during osteogenesis and hyperpolarization during adipogenesis.

These findings reveal distinct interspecies differences. While pAD-MSC exhibit a superior baseline proliferative capacity, their early metabolic adaptations during osteogenesis differ fundamentally from those of hAD-MSC. The hyperpolarization in hAD-MSC suggests a classical activation of oxidative phosphorylation (OXPHOS). In contrast, the depolarization in pAD-MSC suggests a distinct or delayed metabolic response to osteogenic stimuli. Further studies are required for full validation. Ultimately, understanding these differences is crucial for improving the reliability of the porcine model and ensuring the accurate translation of preclinical data to medicine.

A Missing Piece of the Envelope Puzzle in Bacteroidota: Functional Insights into PGN_1323 of *Porphyromonas gingivalis*

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The integrity of Gram-negative bacteria depends on coordinated interactions between the outer membrane (OM) and the underlying peptidoglycan (PG) layer. While this connection has been well characterized in model organisms such as *Escherichia coli*, where it is mediated by Braun's lipoprotein, the mechanisms responsible for OM–PG attachment in phylogenetically distant bacteria remain largely unexplored.

Porphyromonas gingivalis, a member of the Bacteroidota phylum and a key pathogen associated with periodontitis and systemic diseases, lacks any described OM–PG tethering system. In a previous quantitative proteomic analysis, a highly abundant outer membrane-associated protein of unknown function, PGN_1323, was identified. Bioinformatic predictions indicated that this protein possesses features characteristic of lipoproteins and contains a conserved domain potentially involved in peptidoglycan binding, making it a compelling candidate for mediating OM–PG interactions.

To investigate this possibility, a deletion mutant was constructed and subjected to phenotypic characterization, including analysis of cell morphology, membrane integrity, and outer membrane vesicle production. In parallel, recombinant PGN_1323 was produced to examine its biochemical properties and its potential to interact with isolated peptidoglycan *in vitro*.

This study addresses a fundamental gap in our understanding of cell envelope organization in Bacteroidota and explores whether PGN_1323 could represent a previously unrecognized component involved in OM–PG association. The results of this work contribute to a broader effort to uncover alternative strategies of bacterial envelope stabilization beyond those described in classical model organisms.

Visfatin regulates factors involved in human placental angiogenesis: modulation by folic acid and aspirin

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Visfatin is an adipokine that performs multiple functions in the body, including the regulation of human trophoblast proliferation, apoptosis, and endocrine activity. However, its role in placental angiogenesis and its interactions with pharmacological agents used during pregnancy remain unclear. This study aimed to investigate the effects of visfatin on the angiogenesis-related factors, including vascular endothelial growth factor A (VEGFA) and placental growth factor (PLGF), as well as their receptors, VEGFR1 and VEGFR2. Additionally, we evaluated whether folic acid (FA) and aspirin (ASA) modulate its activity.

JEG-3 trophoblast cells and placental villous explants from term pregnancies were treated with visfatin (1–100 ng/mL) for 48 hours. VEGFA and PLGF levels were assessed *via* ELISA. To investigate the underlying mechanisms, a pharmacological inhibitor of the insulin receptor (INSR) and selected kinase pathways (ERK1/2, AKT, STAT3, AMPK) was applied in JEG-3 cells, and the effects were tested by ELISA. Additionally, the role of FA (2–100 nM) and ASA (0.1 mM), administered alone or in combination with visfatin, on visfatin and INSR expression, as well as on VEGFA, PLGF, VEGFR1, and VEGFR2 levels, were studied by Western blot and ELISA. Statistical analysis was performed using one-way ANOVA followed by Tukey's test ($n \geq 3$; $p < 0.05$).

Visfatin decreased VEGFA and PLGF levels *via* distinct molecular pathways. FA reduced visfatin levels and modulated INSR expression, whereas ASA decreased both visfatin and INSR expression. Co-treatment with FA or ASA altered the effects of visfatin on VEGFA and PLGF levels. Moreover, visfatin and FA reduced VEGFR1 and VEGFR2 levels, while ASA modulated their levels.

In conclusion, visfatin acts as a downregulator of angiogenesis-related factors, while folic acid and aspirin modulate its activity, suggesting potential relevance in pregnancy-related disorders.

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THIRD POSTER SESSION

Impact of vaspin on cell viability and proliferation in human placental BeWo cells

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Vaspin is an adipokine, a hormone produced by adipose tissue cells and has pleiotropic effects in the body, including reducing food intake, lowering inflammation and enhancing insulin sensitivity. It also regulates reproductive system function by stimulation steroidogenesis and proliferation of ovarian cells. It is expressed in the placenta, but its effects on placental cells are not well understood. Therefore, the aim of this study was to investigate the effects of vaspin on the viability and proliferation of human placental cells.

BeWo placental cell lines were used to investigate the dose-dependent (0.1, 1, 10 ng/ml) and time-dependent (24, 48, 72 h) effect of vaspin on cell viability (AlamarBlue), and the dose-dependent (0.1, 1, 10 ng/ml) effect on cell proliferation (BrdU assay). The effect of vaspin (0.1, 1, 10 ng/ml) on mRNA expression of PCNA (a marker of proliferation), was also examined (RT-qPCR). The results from at least three independent experiments ($n \geq 3$; $p < 0.05$) were analyzed in GraphPad Prism 8.0.1 using one-way ANOVA and Tukey's post hoc test.

A decrease in cell viability was observed after 24 h of treatment with vaspin at 1 ng/ml, and after 48 h and 72 h at each of the tested concentrations (0.1, 1, and 10 ng/ml). After 24 h, an increase in viability was observed at vaspin at 10 ng/ml. BeWo proliferation was reduced by 1 ng/ml of vaspin. The antiproliferative effect of vaspin in placental cells was confirmed by reduced PCNA mRNA expression at all tested doses.

The results suggest that vaspin may be involved in placental development and function through its effects on cell viability and proliferation, processes important for the proper progression of pregnancy. However, further research is required to understand the mechanism of action of vaspin on human placental cells.

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Tryptophan hydroxylase 1 gene polymorphism rs211105 in autistic patients

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The serotonergic system and genetic variability of tryptophan hydroxylase 1 (TPH1) - an enzyme that plays a key rate-limiting role in the serotonin biosynthesis pathway - are involved in numerous pathophysiological mechanisms underlying the development of autism spectrum disorder (ASD). Their contribution is associated both with the occurrence of stereotyped behaviors and with learning processes, which are essential for the social adaptation of individuals with ASD. The aim of this study was to evaluate the relationship between the rs211105 polymorphism of the TPH1 gene and serum serotonin level in patients with ASD and in a control group. Allelic discrimination of the rs211105 polymorphism was performed using real-time PCR method. The results demonstrated a significantly higher presence of the T allele and the TT genotype in the rs211105 polymorphic site in ASD group, suggesting its potential role as a risk factor in the etiopathogenesis of autism spectrum disorder.

The effect of silver nanoparticles on *Toxoplasma gondii* tachyzoites

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Toxoplasma gondii is a cosmopolitan protozoan parasite that causes toxoplasmosis in humans, affecting 30–50% of the global population. While typically asymptomatic in healthy individuals, the infection may lead to severe complications in immunocompromised patients and congenitally infected infants. This is largely due to the parasite's ability to evade the host's immune system and cross the body's physiological barriers (e.g. blood-placenta and blood-brain barrier) to migrate to immunologically-privileged sites. The current problem in treating toxoplasmosis is a lack of a successful therapy that would also be relatively safe to use. At present, available treatment options are only partially effective and often associated with severe adverse effects, such as bone marrow suppression. Recent studies have demonstrated the antimicrobial potential of silver nanoparticles (AgNPs), including their enhanced efficiency when synthesised using tea extracts. The purpose of this study was to assess the potential effects that AgNPs and green tea extract could have on *T. gondii* tachyzoites. Parasites were incubated for 48h with the solutions of 1) AgNPs; 2) AgNPs synthesised using green tea extract (GT-AgNPs); and 3) green tea extract (GT); at concentrations of 1,25–10 µg/ml. After the 24h and 48h incubation periods, the parasite's cell count and viability were determined. The results showed that green tea extract alone did not significantly affect the parasite's survival. In contrast, the solution of AgNPs demonstrated the highest antiparasitic effect, reducing viability by 37–73% after 48h. The effect was both dose- and time-dependent, as higher concentrations and longer exposure led to greater reduction in the parasite's viability. Overall, the results suggest that AgNPs may serve as a promising alternative strategy for limiting *T. gondii* proliferation.

DNA single-strand breaks in somatic cells

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Single-strand DNA breaks (SSBs) are continuously generated in cells. They typically arise as a result of errors in the repair of oxidative damage, transcription, or replication. The level of DNA oxidative damage depends on metabolic activity, cell, and tissue type.

The aim of this study was to directly quantify the steady-state levels of SSBs in selected mouse tissues, including the brain, heart, skeletal muscle, lungs, liver, kidneys, and spleen. In contrast to other approaches, the sSTRIDE (SensiTive Recognition of Individual DNA Ends) technique enabled direct detection of free 3' DNA ends in tissue sections, which were subsequently imaged using confocal microscopy.

We demonstrated that the number of SSBs and the range varies significantly both between different tissues and among cells within the same tissue. Statistical analysis further revealed that these tissues can be classified into three groups based on SSB levels. The brain contains the highest level of damage, reaching 10,18 SSBs/Gbp. The heart, skeletal muscle, and lungs form a group with an intermediate level of SSBs, while the remaining tissues belong to the low-damage group, with the spleen showing the lowest value – 0,36 SSBs/Gbp. Brain tissue showed the largest range of SSBs (0 to 50 SSBs/Gbp). Various levels of SSBs most likely reflect a balance between DNA damage caused by metabolic activity and its repair, and the capacity to neutralize oxidative species. The findings contribute to a better understanding of the mechanisms underlying DNA damage and repair.

***BRCA1* promoter polymorphism rs799905 as a potential protective factor against breast cancer in women carrying pathogenic *BRCA1* variants**

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Breast cancer is the most common cancer in women worldwide. Pathogenic variants (PVs) in *BRCA1* are among the most significant risk factors, with carriers having an estimated lifetime risk of 65-75%. However, this risk can be further modified by reproductive and environmental factors, as well as by additional genetic variants. Single nucleotide polymorphisms, such as the promoter polymorphism c.-20+ 101 C/G (rs799905) located in the regulatory region of *BRCA1*, may influence gene expression and potentially modulate susceptibility to breast cancer. Recently, genotype frequencies of this polymorphism have been shown to differ significantly between *BRCA1* PV carriers and non-carriers. Investigating the potential protective effect of rs799905 in women carrying PVs in *BRCA1* can provide valuable insights into personalized risk assessment and prevention strategies.

This study aimed to evaluate the association between the *BRCA1* rs799905 and breast cancer risk in carriers of PVs in *BRCA1*. A total of 675 genomic DNA samples were collected from women, patients of the International Hereditary Cancer Center in Szczecin, along with their clinical and pedigree data. All participants were Polish women, carriers of one of founder variants (c.5266dupC, c.181T>G or c.4035delA). Among them, 127 had developed breast cancer, while 548 were cancer-free. Genotyping was performed using TaqMan real-time PCR on a LightCycler® 480 system. Associations between rs799905 genotypes and breast cancer risk were evaluated using multivariate logistic regression.

The results indicate that the *BRCA1* promoter polymorphism rs799905 may be associated with a reduced risk of breast cancer in women carrying PVs in *BRCA1*. Specifically, carriers of the GC or CC genotypes exhibited an almost two-fold lower risk of developing breast cancer compared to those with the GG genotype (OR = 0.64; $p < 0.046$).

These findings suggest that rs799905 may act as genetic modifier of breast cancer risk, supporting a potential role in the molecular mechanisms underlying tumor development and contributing to the development of more individualized prevention and therapeutic strategies. Further studies with larger cohorts are required to validate and extend these observations.

Impact of resistin on lipid metabolism in porcine oocytes during *in vitro* maturation

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Adipose tissue is an active endocrine organ that secretes peptides called adipokines, which participate in the regulation of the body's energy homeostasis and link metabolic status to the functioning of the reproductive system. One of these adipokines is resistin, which influences lipid metabolism by disrupting lipid storage and utilization in different tissues. Such effects may contribute to the development of obesity-related diseases and, consequently, reduced fertility; however, its role in oocyte maturation and energy metabolism remains unclear. The aim of this study was to determine the effect of resistin on lipid levels in porcine oocytes and to analyze the expression of ATGL (adipose triglyceride lipase; a key enzyme involved in lipolysis) and ADRP (adipose differentiation-related protein; a lipid droplet-associated protein and marker of lipogenesis) in oocytes and cumulus cells after *in vitro* maturation.

Oocytes were isolated from ovaries obtained from sexually immature pigs (4–5 months old) and cultured for 44 h in control medium or medium supplemented with resistin at a concentration of 1 ng/mL, which dose was chosen based on a preliminary study. After culture Nile Red dye was used to visualize and quantify lipids in oocytes. The expression of marker genes for lipolysis (ATGL) and lipogenesis (ADRP) was analyzed using real-time PCR, while protein levels were evaluated by Western blot analysis. Statistical analysis was performed using GraphPad Prism 8.0 with Student's t-test.

It was demonstrated that resistin increased lipid levels in oocytes. It also inhibited the expression of the lipolysis marker ATGL in the oocyte at the gene level and in both oocytes and cumulus cells at the protein level. In contrast, an increase in ADRP expression at both the gene and protein levels was observed in cumulus cells.

In summary, resistin increases lipid accumulation in oocytes, likely through the inhibition of lipolysis, while promoting lipogenesis in cumulus cells, indicating enhanced lipid storage within the cumulus–oocyte complex, which may impair oocyte maturation and developmental competence.

Resistin as a modulator of glucose uptake by oocytes during *in vitro* maturation and GLUT4 expression in porcine cumulus cells

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Proper glucose metabolism is essential for both nuclear and cytoplasmic oocyte maturation, as it ensures an adequate energy supply and supports effective communication between the oocyte and cumulus cells, with glucose uptake mediated by transporters such as GLUT4, whose expression and activity are regulated by metabolic factors, including adipokines. Resistin, an adipokine associated with metabolic disorders, has been shown to influence glucose metabolism in various tissues, but its role in regulating glucose metabolism in the oocyte is still unclear. The aim of this study was to investigate the effect of resistin on glucose levels and on the expression of the GLUT4 transporter in cumulus cells during the *in vitro* maturation of porcine oocytes.

Oocytes were isolated from ovaries obtained from sexually immature pigs (4–5 months old) and cultured for 44 h in control medium or medium supplemented with resistin at a concentration of 1 ng/mL, which dose was chosen based on preliminary study. The effect of resistin on glucose levels in the culture medium was assessed using an ELISA assay. The impact on *GLUT4* mRNA expression was determined by real-time PCR, while GLUT4 protein levels were evaluated by Western blot analysis. The obtained data were statistically analyzed using GraphPad Prism with Student's t-test.

The results showed that resistin decreased glucose uptake by cumulus cells and increased *GLUT4* mRNA and protein expression (n = 3, p < 0.05).

In conclusion, resistin modulates oocyte glucose metabolism by reducing glucose uptake in cumulus cells while increasing both GLUT4 mRNA and protein expression, suggesting a complex regulatory effect on glucose transport at both the transcriptional and translational levels.

Can fullerenes be an efficient elicitors in *in vitro* cultures of *Euphorbia milii* Des Moul and *Taraxacum belorussicum* V.N. Tikhom? – preliminary studies.

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Fullerenes as carbon nanostructures with unique physicochemical properties including antioxidants, are attracting growing interest in plant biotechnology also as a potential elicitors. An elicitor is a molecule that triggers a hypersensitive response in a plant, e.g. it increases the level of secondary metabolites. The aim of this study was to assess the effect of C[60] fullerenes (F19) with +D-glucosamine substituent on modulating secondary metabolite levels in *in vitro* cultures of two species. One of them is *Euphorbia milii*, an ornamental and medicinal plant known for its valuable bioactive compounds, such as latex which is used as an alternative to niclosamide, a medicine against schistosomiasis, and *Taraxacum belorussicum* medicinal and obligatory apomictic species. Plant material was cultured in several media (with/without F19 as well as with/without phytohormones) also under PEG-induced physiological drought. The F19 impact on *in vitro* morphogenesis in several conditions was analyzed as well as biochemical activity using FT-Raman spectroscopy.

The obtained results indicate that the use of F19 can influence both the state of *in vitro* cultures and can modulate the content of secondary metabolites, with this effect dependent on the culture conditions and plant species. In *E. milii* cultures, F19 redirected the widespread rhizogenesis to caulogenesis, while the same concentrations of F19 in *T. belorussicum* cultures intensified degenerative processes. The greatest changes in the biochemical profile in *E. milii* were observed under induced drought conditions and the presence of phytohormones, and F19 in the wavelength range characteristic of tetraterpenes, alkaloids, and lignins. In dandelion cultures, the effect of F19 was more pronounced and encompassed a broader wavelength range. The spectral spectra of explants cultured on individual media differed from those of control explants cultures. The most significant differences were observed for those placed on control medium supplemented with F19 (a strong decrease in biochemical activity) and in the presence of F19 and phytohormones (increased biochemical activity). This suggests that fullerenes can act as effective

elicitors in *in vitro* cultures, but their action requires further optimization and detailed understanding of the molecular mechanisms.

Miropin, a serpin from *Tannerella forsythia*, as a platform for developing novel potent protease inhibitors with potential therapeutic applications

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Background: Serine proteases regulate key cellular processes, while dysregulation of their activity disrupts homeostasis and is associated with many diseases. The activity of proteases is precisely regulated, among other mechanisms, by their synthesis as inactive zymogens and by the production of protease inhibitors. In contrast to almost all known serpins (serine protease inhibitors), the serpin from periodontopathogen *Tannerella forsythia*, miropin, has the unusual ability to efficiently inhibit different serine and cysteine proteases, employing three active sites within the reactive center loop (RCL), which are equivalent in respect to inhibitory power.

Objective: The overarching aim of this project was to determine whether miropin could serve as a scaffold for developing a potent novel inhibitor targeting proteases involved in etiology of human diseases: *Staphylococcus aureus* V8 protease, human furin and thrombin.

Methods: The series of miropin mutants in which amino acids from positions P1-P3 were changed to Arg, Glu or other amino acids, were obtained by site-directed mutagenesis. The obtained genetic constructs were verified by sequencing and later used to produce recombinant proteins using an *Escherichia coli* expression system and affinity and size-exclusion chromatography. The interaction of miropin with target proteases was analyzed in detail by determining the stoichiometry of inhibition (SI) and the association rate constant (k_{ass}).

Results: Different miropin mutants were purified into homogeneity. The mutants were able to inhibit proteases such as human thrombin and furin, as well as the bacterial V8 protease. Of note, wild-type miropin did not inhibit any of these proteases. All proteases, regardless of their specificity, were inhibited with a similar SI that ranged from 1.5 to 3.0, while k_{ass} was around $10^5 \text{ m}^{-1}\text{s}^{-1}$.

Conclusions: Due to its three reactive sites, miropin could be used as a scaffold to design and develop a multispecific protease inhibitor for the treatment of diseases in which uncontrolled proteolytic activity plays a crucial role, such as cystic fibrosis, cancer, coagulation disorders, and infections caused by *S. aureus*.

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Differential hypoxia-driven metabolic adaptation in glioblastoma cell lines: LDH cytotoxicity and HIF pathway dynamics

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Glioblastoma is a highly aggressive brain tumor characterized by metabolic and transcriptional heterogeneity, across varying microenvironments such as normoxia and hypoxia. Cellular adaptation to hypoxia is primarily mediated by hypoxia-inducible factors (HIF-1 α and HIF-2 α), while lactate dehydrogenase (LDH) is a marker of cell damage and metabolic adaptation. The aim of this study is to examine how different glioblastoma cell lines respond to hypoxia in 2D and 3D models by assessing LDH release and HIF-associated gene expression profiles.

Experiments were conducted using human glioblastoma cell lines LN229, U87, U251 and murine GL261 in 2D monolayers (48h) and 3D spheroid models in a hanging drop (5 days) under normoxic (21% O₂) and hypoxic (1% O₂) conditions. Cytotoxicity was estimated using LDH assay, while transcriptional responses of key hypoxia-responsive genes HIF1 α , EPAS1 (HIF-2 α), SLC2A1, PDK1, and CA9 were assessed using quantitative PCR.

No significant differences in LDH release observed between normoxia and hypoxia in 2D cultures. In 3D models most cell lines showed non-significant trends except for the increase in LDH observed in U87 under hypoxia. Results demonstrated heterogeneity in hypoxia-induced gene expression across cell lines and culture conditions in both 2D and 3D models. Moreover, distinct expression profiles observed in 3D models suggest altered transcriptional responses compared to 2D monolayers. Increased CA9 expression confirmed activation of hypoxic signaling in several models (except 3D U87 and U251 cultures). Compared to HIF1A which often showed decreased or minimal expression changes, EPAS1 expression was more consistently upregulated across the different cell lines and models. Classical HIF-1-dependent metabolic target, PDK1 was often decreased, while SLC2A1 changes remained modest.

Differences in HIF1 α and EPAS1 expression suggest that hypoxia-induced responses in glioblastoma may not follow canonical HIF-1-driven metabolic reprogramming and depend on the relative balance between HIF isoforms. Moreover, glioblastoma cells exhibit diverse adaptive strategies under hypoxia, ranging from effective metabolic adaptation to hypoxia-induced cytotoxicity indicating that hypoxia response may strongly dependent on cell line and model system.

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Broken hearts and leaky vessels: The off-target cardiovascular effects of Ibrutinib in a transgenic zebrafish model

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Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, has revolutionized the treatment of hematological malignancies. However, its clinical application is increasingly complicated by severe cardiovascular adverse events. Understanding the mechanisms underlying these toxicities requires robust *in vivo* models. The transparent larval zebrafish (*Danio rerio*) offers a non-invasive real-time imaging of both vascular dynamics and cardiac function. This study aimed to investigate the toxic effects of ibrutinib in zebrafish larvae. We used transgenic reporter line *Tg(kdrl:mTurquoise)* combined with lightsheet fluorescence microscopy (LSFM) to visualize the cardiovascular system. To assess vascular dysfunction, we performed FITC-dextran microangiography to determine potential vascular leakage and vasoconstriction. We also analyzed heart function by measuring ventricular fractional shortening and area change. Additionally, RT-qPCR was performed to analyze endothelial, cardiac, inflammatory and oxidative stress gene markers. Exposure to ibrutinib caused significant cardiovascular damage. LSFM imaging showed severe vascular dysfunction, including enhanced permeability and vasoconstriction in the dorsal aorta. Cardiac function collapsed, evidenced by a decline in ventricular fractional shortening and area change, leading to severe hemodynamic compromise. These physiological defects were confirmed at the molecular level by the upregulation of the stress markers. The exact molecular mechanisms driving these off-target effects are currently under investigation in our laboratory. Our results suggest that combining lightsheet imaging with gene expression analysis in zebrafish provides a highly effective model to study the toxicity of targeted therapies and screen potential protective drugs.

Inhibitor WP1066 — Mediated Suppression of STAT3 in Breast Adenocarcinoma: Insights from 2D and 3D in vitro models

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STAT3 has emerged as a promising therapeutic target in many types of cancer, as it is a central signalling hub that promotes tumour progression, proliferation and metastasis. Furthermore, its expression influences the remodelling of the tumour microenvironment, whilst actively suppressing the immune system. The goal of our study was to check the effects of STAT3 inhibition by WP1066 in murine breast cancer cells in 2D and 3D cultures.

The therapeutic potential of this inhibitor was investigated in three phenotypes of the E0771 mouse adenocarcinoma (WT, 24x2, TGF- β). A wound healing assay was performed for the 2D model with inhibitor added. Spheroids cultured using the hanging drop method, together with an appropriate dose of the inhibitor, were harvested after 5 days. Growth kinetics of the spheroids were observed over a 5–9-day period. qPCR has been conducted for gene analysis typical of epithelial-mesenchymal transition or cellular structural remodelling: MMP9, N-cadherin or SLC2A1. Furthermore, spheroids were harvested, embedded in agarose, dehydrated and embedded in paraffin. Sections were then stained using the haematoxylin and eosin method, which revealed morphological changes. Additionally, Picro-Sirius Red staining was performed.

The scratch assay revealed a reduction in migratory capabilities in the treated cell lines. No significant changes in spheroid surface area were observed between doses, although spheroids formed by the TGF-B phenotype exhibited an increased surface area compared to the wild-type. The 6.75 μ M of WP1066 induced significant morphological changes in all samples tested – loosening of the spheroid structure and a larger cytoplasmic surface area. A significant increase in collagen levels was observed, proportional to the inhibitor concentration. A decrease in expression was observed for: *VIM*, *CDH2*, *SLC2A1* and *SNAIL* in treated cell lines compared to control groups. On the other hand, *MMP9*, *CDH1*, *STAT3* showed an increased expression in the treated lines.

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From Gene to Structure: Design, Expression and Structure of Recombinant Human Polynucleotide Phosphorylase in FreeStyle™ 293-F Cells.

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Mitochondria contain a complex RNA metabolism system that is essential for proper expression of the mitochondrial genome and maintenance of cellular homeostasis. A key component of this system is polynucleotide phosphorylase (PNPase), a conserved exoribonuclease encoded by the *PNPT1* gene and localized in the mitochondrial intermembrane space and matrix in human cells. PNPase is involved in RNA degradation, processing, and putative import of selected RNAs into mitochondria. Defects in PNPase function have been associated with mitochondrial diseases, neurological abnormalities, and immune dysfunction, emphasizing its importance for mitochondrial biology. However, the structural and molecular basis of PNPase activity in human mitochondria remains insufficiently understood.

In this studies, we developed an approach for the recombinant expression and purification of human PNPase in a human cell line system that preserves its mitochondrial context. A construct encoding PNPase fused to a Strep-tag was introduced into human cell line, allowing expression of the protein within a physiological cellular environment. The presence of recombinant PNPase was confirmed by immunodetection, while mitochondrial targeting was validated through subcellular fractionation analysis. The protein was purified using affinity chromatography. Purified PNPase samples were further prepared for structural characterization using cryogenic electron microscopy (cryo-EM). This approach enables high-resolution visualization of macromolecular complexes and provides insights into the structural organization of the enzyme and its potential RNA-binding interfaces.

The strategy provides a new approach for obtaining human PNPase in a state resembling its native mitochondrial form and establishes a platform for integrated genetic, biochemical, and structural studies into its role in mitochondrial function and human diseases.

Effect of limited oxygen availability on the expression of the *arcA* gene in *Proteus mirabilis* cells

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Proteus mirabilis is a Gram-negative bacterium belonging to the *Morganellaceae* family. It is rod-shaped and facultatively anaerobic. Classified as an opportunistic pathogen, it is commonly found in the gastrointestinal tract of warm-blooded animals, including humans. *P. mirabilis* is recognized as a significant uropathogen implicated in catheter-associated urinary tract infections (CAUTIs), one of the most common healthcare-associated infections worldwide. Its urease activity promotes urea hydrolysis and the formation of crystalline biofilms, which contribute to the persistence of infection.

The *arcA* gene is a part of a two-component regulatory system, the anoxic redox control (ArcAB). The Arc system occurs in facultative anaerobes and enables a switch from aerobic respiration to fermentation or anaerobic respiration when oxygen is limited. Oxygen-limited conditions, occurring in the urinary tract, activate *arcA* gene expression via phosphorylation. The activated form of *arcA* is able to regulate other genes directly or indirectly, leading to the repression of genes involved in the Krebs cycle and aerobic respiration.

The main aim of this study was to investigate the expression of the *arcA* gene in *P. mirabilis* HI4320 under low-oxygen conditions. Gene expression levels were analyzed by RT-qPCR under aerobic, hypoxia, and hypoxia conditions with H₂O₂, with *rpoA* serving as the reference gene.

Our study provides insights into the significance of the *arcA* gene under low-oxygen conditions, which are frequently encountered in urinary tract infections.

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Effect of limited oxygen availability on the expression of the *mrpJ* gene, an important regulator of biofilm formation in *Proteus mirabilis*

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Proteus mirabilis is a Gram-negative, facultatively anaerobic bacterium with a rod-shaped morphology, recently assigned to the *Morganellaceae* family. It occurs in soil and aquatic habitats, where it plays an important role in the decomposition of organic matter, and as a commensal in the digestive system of animals and humans. One of the virulence factors of *P. mirabilis* is the synthesis of enzyme urease, which catalyzes the hydrolysis of urea. This results in the formation of a crystalline biofilm characteristic of this bacterium, which is particularly important in catheter-related urinary tract infections (CAUTI).

MrpJ is a regulatory gene belonging to the fimbrial *mrp* operon in *P. mirabilis*, which controls the expression of genes associated with virulence. MrpJ has been shown to directly inhibit the expression of the *flhDC* flagellar operon, limiting bacterial motility and promoting an adhesive phenotype and biofilm formation. The literature suggests that the *mrpJ* gene may function as a global regulator of processes related to pathogenesis, but its role in this regard still requires further research.

The primary objective of our study was to analyze *mrpJ* gene expression in *P. mirabilis* HI4320 under limited oxygen conditions. Using RT-qPCR, we compared gene expressions under normal oxygen concentration, hypoxia, and H₂O₂-supplemented hypoxia conditions, using the *rpoA* gene as a reference.

Our study allows us to understand the role of the *mrpJ* gene in the adaptation of *P. mirabilis* to low-oxygen environments and in the regulation of the transition to the biofilm form.

This work was co-financed by the Minister of Science (Poland) under the "Regional Excellence Initiative" program (project no.: RID/SP/0015/2024/01).

Chorioallantoic Membrane (CAM) as a Model for Tumor Development and Nanocarrier Accumulation Studies

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Pancreatic cancer remains one of the most challenging malignancies to treat, emphasizing the need for accessible models to study its biology and explore new therapeutic directions. This work aimed to develop and use the chick embryo chorioallantoic membrane (CAM) model as an *in vivo* system to study tumor development and assess liposome bioaccumulation within tumor tissues. The studies focused on improving embryo culture protocols, analyzing the formation of tumor microenvironment after inoculation with human pancreatic cancer cells (*BxPC-3*), and assessing the behavior of fluorescently labeled liposomes after direct intratumoral administration. A comparison of *in ovo* and *ex ovo* culture systems revealed that the *in ovo* method yielded significantly higher survival and reproducibility, whereas *ex ovo* culture was associated with higher mortality and greater sensitivity to environmental conditions. Tumor development was strongly dependent on the dynamics of the CAM membrane and the properties of the tumor cells used, and not on the technique or the moment of inoculation, which were uniform throughout the experiment. Their intratumoral administration allowed the observation of fluorescence changes over time, such as changes in the size of the area and signal intensity, which may indicate liposome transformations in the tissue. The presence of fluorescently labelled liposomes within the tumor was confirmed by staining of cryosections. This paper highlights both the advantages and limitations of the CAM model as an ethically sound and low-cost alternative to initial drug carrier and cancer biology research.

FOURTH POSTER SESSION

The structural and protective role of myxoxanthophyll in stabilizing the photosynthetic apparatus of cyanobacteria

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Myxoxanthophyll is an amphipathic carotenoid glycoside unique to cyanobacteria, which is located in both the outer membrane and the inner membrane (thylakoids), where it is dissolved freely in the lipid bilayer. It plays a crucial role both in protecting cells from photooxidation and in the proper organization and stabilization of membranes.

To clarify its physiological role, we analyzed the stress response and characteristics of the $\Delta cruF$ mutant strain of the model cyanobacterium *Synechocystis* sp. PCC 6803, which lacks the first step of myxoxanthophyll biosynthesis. Wild-type and $\Delta cruF$ strains were cultivated under three different conditions: control, high-light, and low-temperature.

Pigment analysis by HPLC revealed that the absence of myxoxanthophyll leads to a distinct shift in carotenoid composition, including pronounced zeaxanthin accumulation, indicating a compensatory photoprotective response.

Transmission electron microscopy (TEM) demonstrated that $\Delta cruF$ cells exhibit disrupted thylakoid organization, and under stress conditions, show early signs of membrane destabilization, supporting the hypothesis that myxoxanthophyll contributes to preventing thylakoid collapse.

We observed increased accumulation of storage granules colocalized with thylakoids, which hints at altered regulation of metabolism. Moreover, TEM observations revealed a diminished S-layer (glycocalyx) in the $\Delta cruF$ mutant, consistent with increased cell clumping that we observed, suggesting that myxoxanthophyll plays a role in anchoring the S-layer in the outer membrane.

Oxidative stress assays for ROS production and protein carbonylation showed altered ROS levels and protein oxidation in the mutant, confirming reduced stress tolerance. EPR spectroscopy revealed changed fluidity in $\Delta cruF$ thylakoids, consistent with the structural role of myxoxanthophyll in stabilizing the bilayer.

Together, these results demonstrate that myxoxanthophyll is essential not only for maintaining thylakoid membrane architecture but also for enabling compensatory carotenoid responses and protecting the photosynthetic apparatus under abiotic stress.

Bioinformatic analysis of zuzalysin metalloprotease homologues from *Porphyromonas gingivalis* within the Bacteroidota phylum

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Zuzalysin from *Porphyromonas gingivalis* is a protein exhibiting unusual structural properties, which makes it an interesting subject for research and justifies the need for further characterisation of its homologues in other prokaryotic organisms. In order to gain a better understanding of this phenomenon, it is necessary to identify and compare homologous proteins found in other prokaryotic organisms.

The aim of the project is to develop a structured genomic database covering prokaryotic organisms possessing zuzalysin homologues, which will serve as a basis for genetic and comparative analyses of this group of proteins. As part of the research, sequences homologous to the reference protein Q7MTD8 are identified and then integrated with genomic data from public repositories. The database being created includes, amongst other things, information on the number of homologue copies in genomes, the quality and completeness of genomic sequences, and sequencing methods. In subsequent stages, it is also planned to analyse the co-occurrence of catalytic domains with other protein domains, the presence of elements associated with secretion systems, and the predicted intracellular localisation of homologues, which will allow for a more comprehensive characterisation of their genetic and functional diversity.

This project is exploratory in nature and serves as a preparatory stage for further evolutionary, phylogenetic and functional analyses. The results obtained will be crucial for a better understanding of the diversity of zuzalysin homologues among prokaryotes and will enable the selection of the most promising organisms for further experimental studies conducted under laboratory conditions.

Optimization of expression, purification, and RNase activity assays for MCPIP2

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MCPIP2 (monocyte chemoattractant protein-1 induced protein 2) is a 94 kDa member of the MCPIP family that contains a highly conserved NYN/PIN ribonuclease domain and a CCCH zinc finger domain. It functions as a negative regulator of inflammation by promoting the degradation of pro-inflammatory transcripts, including *IL6*. Its catalytic center depends on four aspartate residues that coordinate a magnesium ion required for nuclease activity. Although mutation of the catalytic aspartate abolishes endonuclease activity in other MCPIP proteins, cellular assays suggest that the MCPIP2-D196A mutein retains the ability to degrade selected transcripts, such as the lncRNA *NORAD*, while losing activity toward *IL6*. Because these observations are based on cellular overexpression systems, they may be influenced by interactions with endogenous MCPIP proteins, as previously shown for MCPIP1. At present, the *in vitro* RNase activity of MCPIP2 has not been directly characterized.

This project aims to establish a robust workflow for the expression and purification of recombinant wild-type MCPIP2 and the catalytically inactive D196A variant, enabling direct comparison of their *in vitro* RNase activities. Both proteins will be produced in *E. coli* BL21(DE3) using a codon-optimized pET-HALO-MCPIP2 construct encoding N-terminal His, Halo, and Twin-Strep tags. The D196A substitution will be introduced by site-directed mutagenesis. Protein expression will be verified by SDS-PAGE and western blotting. Purification will involve HisTrap affinity chromatography, followed by proteolytic tag removal using HRV 3C or TEV protease and final polishing by size-exclusion chromatography. Purified proteins will then be incubated with *in vitro*-transcribed *NORAD* and *IL6* RNAs, and transcript degradation will be assessed by denaturing agarose gel electrophoresis.

This approach will provide the first direct *in vitro* assessment of MCPIP2 RNase activity and may clarify whether transcript degradation by the D196A mutein depends on its intrinsic catalytic function.

This work was funded by the Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Poland, Student Research Project funding for "Purification and characterization of the RNase activity of the MCPIP2 protein" project.

The impact of aldolase inhibitor (UM0112176) on FBP2 complex formation with transcription factors involved in carcinogenesis

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Fructose 1,6-bisphosphatase 2 (FBP2) is an enzyme involved in carbohydrate metabolism, regulating both gluco- and glyconeogenesis. Beyond its metabolic function, FBP2 plays additional cellular roles, including the suppression of cancer cell proliferation and migration through interactions with multiple protein partners.

HIF1 α and NF κ B are transcription factors playing key roles in carcinogenesis. HIF1 α promotes cancer cell survival under hypoxic conditions by enhancing glycolysis and angiogenesis, whereas NF κ B regulates cell proliferation, migration and apoptosis. Notably, FBP2 can interact with both transcription factors, facilitating their degradation.

In this study, I investigated the effect of the anticancer agent UM0112176 on the formation of FBP2-HIF1 α and FBP2-NF κ B (p65) complexes in KLN-205 lung cancer cells. Protein-protein interactions were detected using the Duolink Proximity Ligation Assay. The number of interactions per cell was quantified using ImageJ/Fiji software.

The results demonstrated a statistically significant reduction in the number of FBP2-NF κ B complexes following UM0112176 treatment, however, the overall number of complexes per cell was low. In contrast, no statistically significant difference was observed in the number of FBP2-HIF1 α complexes between treated and untreated cells; however, a clear downward trend was evident.

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

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Background: Thyroid cancer incidence has increased worldwide in recent decades. Impairment of thyroid peroxidase (TPO) correlates with its progression; therefore, a diet rich in TPO activators may help delay this process. We investigated the effects of wheat flour extracts (WF; a rich source of TPO activators) on the metastatic potential of thyroid cancer cells, focusing on cancer cell-induced endothelial activation as a key step in diapedesis and metastasis.

Methods: Human umbilical vein endothelial cells (HUVECs) were analyzed for motility and cytoskeletal organization in the presence of benign (TPO⁺ B-CPAP) and anaplastic (TPO⁻ 8505C) thyroid cancer cells. The effects of WF extract were further evaluated in relation to connexin(Cx)43 status and metalloproteinase (MMP) activity.

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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Synergistic Anti-Tumor Activity of Temozolomide and the PARP Inhibitor Talazoparib in a Mouse Model of Human Glioblastoma

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Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumour in adults, accounting for ~50% of all gliomas. Annual incidence is 3–4 cases per 100,000, with peak onset in the sixth and seventh decade and slight male predominance. Median overall survival following standard radiochemotherapy with Temozolomide (TMZ) does not exceed 15 months, and five-year survival remains below 5%. A major limitation of classical TMZ therapy is acquired resistance driven by MGMT overexpression and PARP-mediated base excision repair. PARP inhibitors such as talazoparib (BMN-673) may overcome this resistance by trapping PARP on damaged DNA.

Human GBM cells from surgical specimens (Ethics Committee, Medical University of Lodz, no. RNN/23/22/KE) were stereotactically implanted into immunodeficient mice (approval: WAW2/32/2017, WAW2/075/2019, WAW2/006/2022). Animals received TMZ, BMN-673, or TMZ+BMN-673. Brains were sectioned and stained with Hematoxylin and Eosin, tumour volumes were quantified by morphometry. TMZ monotherapy demonstrated significant anti-tumour activity, while BMN-673 alone showed no meaningful efficacy in this model. Strikingly, their combination resulted in complete absence of histologically detectable tumour in both experimental replicates. These findings indicate that concurrent PARP inhibition markedly potentiates TMZ cytotoxicity, positioning TMZ+BMN-673 as a compelling candidate for further pre-clinical and translational investigation in GBM.

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Hemp meal in extruded diets for European perch (*Perca fluviatilis*) aquaculture

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European perch (*Perca fluviatilis*) is considered a promising aquaculture species due to its high environmental plasticity and increasing consumer demand. Rising feed costs drive the search for affordable and readily available alternatives to fishmeal; hemp meal (HM) is a potential plant-protein ingredient. We therefore evaluated HM as an alternative protein source in extruded diets for perch reared in a recirculating aquaculture system (RAS).

Fish (initial body weight 68,1g) were assigned to four dietary treatments (HM0, HM10, HM20, HM30; n = 100 per treatment) and fed for 10 weeks diets containing 0, 10, 20, or 30% HM, respectively. HM inclusion did not adversely affect growth performance, muscle proximate composition, or basic blood biochemistry, and no severe histopathological lesions were observed. Performance indices (SGR, FCR, PER) were highest in the HM20 group, while the HM treatments were associated with predominantly beneficial features of the intestinal mucosa. Moreover, gene-expression profiles in the intestine and liver differed between the higher (HM20–30) and lower/no HM (HM0–10) inclusion levels, and hepatic ALP and GPX activities decreased with increasing HM content.

Overall, HM can partially replace fishmeal without compromising digestive organ homeostasis, with 20% HM appearing the most promising inclusion level under the conditions tested.

This study was a part of the project titled “Diversification of the productive function of earthen ponds based on semi-intensive rearing of *Perca fluviatilis*—PROPERCH” (project no. 00002-6521.1-OR1400004/17/20), funded by the European Union through the Operational Program “Fisheries and Sea (2014–2020)”, The Agency for Restructuring and Modernization of Agriculture (ARMA) of Poland.

The potential epigenetic role of E93 regulating gene expression during insect metamorphosis

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It is estimated that insects account for between 60 and 70 per cent of all animal species living on Earth. They owe this extraordinary success, among other things, to the invention of metamorphosis. One of the key genes in insect development is E93, which controls insect metamorphosis. Its expression varies during ontogenesis between insects possessing different types of metamorphosis.

The aim of my work was to determine the expression of E93-dependent genes during the ontogenesis of the cockroach and the fruit fly, which undergo different types of metamorphosis (partial and complete, respectively). To this end, I analysed data from RNA-seq and ChIP-seq experiments to identify E93-dependent genes in each species. Then, I analyzed which orthologous genes were potentially regulated by E93 in both species and examined their expression profiles throughout ontogenesis in both organisms.

In this study, we identified 23 genes associated with E93 that are orthologous in both species. Among these genes, 21 showed reduced expression following E93 depletion. Their expression profiles were more closely resembling the E93 expression profile of the cockroaches than in fruit flies.

The findings suggest a potential epigenetic role of E93 in commonly regulating the expression of 21 genes in both insect models, leading to different expression profiles of these orthologous genes that could explain differences in their development. Further research is needed, including studies on chromatin accessibility in the identified genes, in order to obtain more definitive results.

Dissecting MCPIP3: How Specific Domains Drive Protein Interactions and Function

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Monocyte chemotactic protein-induced protein 3 (MCPIP3) is a ribonuclease of the MCPIP family, known for degrading specific mRNAs. MCPIP3 was recently shown to be a regulator of biological processes associated with keratinocyte homeostasis and proliferation-differentiation balance. MCPIP3 is a multifunctional protein with multiple domains such as the catalytic PIN domain and the CCCH-type zinc finger for molecular recognition.

Previously, immunoprecipitation followed by mass spectrometry (IP-MS) was performed to identify potential binding partners of MCPIP3. The aim of this research was to map the specific interaction interfaces between MCPIP3 and its key partners, namely Keratin 14 and 14-3-3 σ , to better understand its molecular scaffolding function. To achieve this, we generated a series of deletion constructs, such as **1. C-end (without N-end+PIN+ZnF)**, **2. Δ N-end (C-end+PIN+ZnF)**, **3. Δ C-end (N-end+PIN+ZnF)** and analyzed their behavior in HEK293 and HaCaT cell models.

Beyond mapping protein-protein interactions via co-immunoprecipitation (Co-IP) and Western Blotting, we investigated the functional impact of these domains on cellular physiology using viability and luciferase reporter assays.

Our findings could provide a detailed functional map of MCPIP3, highlighting how its distinct domains coordinate protein interactions and RNA decay to maintain skin cell homeostasis.

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Analysis of the interaction between the PafA protein from *Streptomyces venezuelae* and selected proteins using a bacterial two-hybrid system

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Bacteria, like eukaryotes, use post-translational modifications (PTMs) of proteins to regulate various intracellular processes. A unique PTM found exclusively in actinobacteria is pupylation, which functionally corresponds to eukaryotic ubiquitination. Pupylation involves the covalent attachment of the Pup protein to a target polypeptide, marking it for degradation by the cellular proteasome. The enzyme responsible for attaching Pup is the PafA ligase.

Streptomyces are soil-dwelling bacteria from the actinomycete group, characterized by filamentous growth similar to that of filamentous fungi. They are important model organisms due to their ability to produce numerous secondary metabolites. It has been shown that deletion of the *pafA* gene negatively affects the growth of *Streptomyces venezuelae*, leading to the formation of spores lacking a nucleoid—an effect not observed when other genes of the pupylation system (PPS) are deleted.

This suggests that PafA may have an additional function independent of PPS. In this study, a bacterial two-hybrid system was used to analyze interactions between PafA and the proteins DnaA, PupQ, and PupE; no interactions were detected. Furthermore, screening of the *S. venezuelae* genomic library identified three potential protein partners that interact with PafA.

Characterization of the *nar* Operon in *Proteus mirabilis*: Genomic Conservation, Expression, and Impact on Biofilm Formation

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Proteus mirabilis, a Gram-negative opportunistic pathogen, is responsible for various infections, predominantly urinary tract infections in hospitalized patients and nursing home residents requiring extended catheterization. Nitrate reduction to nitrite, a key component of the nitrogen cycle, is facilitated by nitrate reductase. In *P. mirabilis*, this process may be regulated by proteins encoded by the *narKGHJI* operon, potentially activated by the *narLX* two-component system. The *narK* gene encodes a nitrate/nitrite antiporter, while *narGHJI* genes encode nitrate reductase subunits. These genes have been identified as potential fitness factors during *P. mirabilis* infection, however our knowledge about the role of this gene remains limited. Therefore, this study aimed to characterize the structural and functional features of the *nar* operon in *P. mirabilis*.

In silico comparative genomics using the Clinker tool revealed high structural conservation of the *nar* operon across multiple *P. mirabilis* clinical isolates. Structural and functional predictions of Nar proteins were determined utilizing the Phyre2 server. *In vitro* growth kinetics measured by OD600 in aerobic and anaerobic conditions with or without nitrate supplementation (20 mM NaNO₃) demonstrated that nitrate presence significantly enhances bacterial growth under aerobic conditions, without affecting biofilm production. In contrast, under anaerobic conditions, nitrate supplementation significantly promoted both bacterial growth and biofilm formation, assessed by crystal violet staining. Furthermore, RT-qPCR analysis revealed constitutive expression of *nar* operon genes even in the absence of nitrate, suggesting a basal metabolic activity of this pathway.

These findings indicate that nitrate metabolism plays a condition-dependent role in *P. mirabilis* physiology, influencing growth and biofilm formation particularly under anaerobic conditions. Targeting this highly conserved metabolic pathway could provide novel anti-virulence strategies against multidrug-resistant *P. mirabilis* infections.

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Modulation of the TRPA1 channel affects functional properties of human pancreatic stellate cells in a phenotype-dependent manner

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Human pancreatic stellate cells (hPSC) are key regulators of extracellular matrix (ECM) homeostasis and major contributors to fibrosis in chronic pancreatitis and pancreatic cancer. Under physiological conditions, hPSC remain quiescent, whereas inflammatory and profibrotic stimuli promote their activation, leading to cytoskeletal remodelling, altered motility and increased ECM production. Since the transient receptor potential ankyrin 1 (TRPA1) channel is a regulator of intracellular Ca²⁺ signalling, its modulation may affect these processes.

This study examined the effects of pharmacological TRPA1 modulation using the agonist ASP7663 and the antagonist A-967079 in three hPSC phenotypes: quiescent (qhPSC), spontaneously activated (shPSC), and TGF- β -activated (ahPSC). The analysis included cell migration, focal adhesion organisation, viability, proliferative activity (EdU incorporation, Ki-67) and intracellular ATP levels.

Inhibition/activation of TRPA1 had limited effects on cell death in qhPSC and shPSC, whereas higher concentrations of ASP7663 increased cell death in ahPSC despite lower TRPA1 expression. The three phenotypes also differed in migratory properties: qhPSC exhibited the highest migration speed and displacement, while ahPSC showed reduced motility and greater directional persistence. Consistently, ahPSC displayed a higher proportion of elongated focal adhesions than qhPSC. TRPA1 activation selectively impaired migration in qhPSC, without altering focal adhesion number or length. Notably, this reduction in migration was associated with decreased intracellular ATP levels, suggesting that altered cellular energetics may contribute to TRPA1-dependent regulation of hPSC motility.

These findings indicate that TRPA1 contributes to the regulation of hPSC (patho)physiology. By linking ion channel activity with cellular energetics and migration, TRPA1 may be a relevant target in pancreatic diseases associated with fibrosis.

Pan-genome-wide analysis of coding and intergenic regions in *Klebsiella pneumoniae* population

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Klebsiella pneumoniae is a significant opportunistic pathogen and one of the leading causes of hospital-acquired infections. Its evolutionary success and rapid adaptation stem from high genomic plasticity and a diverse pan-genome. While most studies focus on CDSs, the variability and evolutionary signals present in intergenic regions (IGRs) remain less studied.

This study aimed at comprehensive pan-genomic evaluation of *K. pneumoniae* isolates, specifically comparing the dynamics between coding sequences and IGRs. By employing Heaps's rule, we modeled the pan-genome, while population structure was determined through PCA and IGR-based phylogenetics.

The genome sequences of *K. pneumoniae* strains were obtained from the NCBI database. The following tools were used: REALPHY (phylogenomics), Prokka (Galaxy version 1.14.6+galaxy1), Roary v3.11.2 (annotations and pan-genome), Piggy v1.5 (analysis of IGRs). The data was visualized using FigTree v1.4.4, Phandango v1.3.1, and R packages.

K. pneumoniae possesses an open pan-genome, with a continuous increase in new genes and IGRs as more genomes are added. Interestingly, the accumulation curves for IGRs showed a trajectory similar to coding sequences, indicating significant diversity in the non-coding landscape. Phylogenetic analysis based on Core IGRs provided a higher resolution than traditional multi-locus sequence typing, allowing for more precise differentiation of closely related clones. PCA analysis based on the accessory IGR content effectively clustered isolates according to their evolutionary lineages.

IGRs constitute a crucial, yet understudied, component of the *K. pneumoniae* pan-genome. Their variability reflects the adaptive potential of the species and provides a powerful tool for molecular epidemiology and evolutionary studies, offering insights beyond the coding regions.

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The TRPA1 channel differentially regulates calcium signalling in phenotypically distinct human pancreatic stellate cells

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The transient receptor potential ankyrin 1 (TRPA1) channel is a non-selective cation channel involved in cellular responses to chemical and physical stimuli, including the regulation of intracellular calcium levels. It is expressed in the plasma membrane of pancreatic stellate cells (PSCs). PSCs are key regulators of pancreatic tissue homeostasis, yet their persistent activation contributes to fibrosis under pathological conditions such as chronic inflammation and cancer.

This study examined TRPA1-dependent calcium signalling in human PSCs (hPSCs) representing three phenotypic states: quiescent (qhPSC), spontaneously activated (shPSC), and TGF- β -induced (ahPSC). Channel activity was pharmacologically manipulated using the agonist ASP7663 and the antagonist A-967079, while intracellular calcium dynamics were monitored using live-cell fluorescence imaging.

TRPA1 activation evoked robust calcium responses predominantly in qhPSCs, whereas both activated phenotypes displayed markedly attenuated signalling. This reduced responsiveness was associated with lower TRPA1 expression, as confirmed by qPCR and immunofluorescence analyses. Notably, antagonist application after channel activation did not fully suppress calcium signals, while preincubation with A-967079 effectively prevented their induction. Preliminary binding site modelling analyses suggest that both the agonist and antagonist may potentially interact with the same binding pocket.

Overall, the data identify TRPA1 as a potential modulator of calcium signalling in PSCs and support its further investigation in the context of pancreatic fibrosis.

The effect of zinc ions on the type VI secretion system in *Proteus mirabilis* strain HI4320

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Zinc plays a key role in regulating numerous biological processes, including the response of microorganisms to environmental stress and the modulation of secretion systems such as the Type VI Secretion System (T6SS). The aim of this study was to analyze the effect of zinc ions on the expression of T6SS-related genes in *Proteus mirabilis* HI4320.

Bacterial cultures were grown in LB medium and supplemented with zinc to assess its direct impact on gene expression, while N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), a zinc chelator, was used to evaluate the effects of zinc depletion. A control condition consisted of LB medium without the addition of zinc or TPEN. Gene expression levels were quantified using real-time PCR (RT-qPCR) in three independent biological replicates. Relative expression levels were calculated using the $\Delta\Delta C_t$ method, with the *rpoA* gene serving as the internal reference.

The results demonstrated that the presence of zinc led to a significant downregulation of T6SS-associated genes. In contrast, zinc chelation by TPEN resulted in increased expression of these genes, indicating an inverse relationship between zinc availability and T6SS activity.

These findings suggest that zinc plays a regulatory role in modulating T6SS gene expression in *P. mirabilis* and may influence bacterial adaptation and competitiveness in varying environmental conditions.

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Phytohormones in the Search for Novel Therapies Against Triple Negative Breast Cancer (TNBC): An In Vitro Evaluation

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Triple-negative breast cancer (TNBC) is a highly aggressive disease with a high mortality rate, especially among young women under 40 years old. The lack of key receptors (ER, PR, HER2) leads to ineffectiveness in standard targeted therapies. Patients must rely on heavy, toxic chemotherapy, which often causes drug resistance and early relapses. This creates an urgent need for safer treatments. Phytohormones, natural plant compounds, are emerging as a promising alternative, capable of triggering cancer cell apoptosis or inhibition of proliferation. This study evaluated the anti-cancer potential of five phytohormones against the MDA-MB-231 TNBC cell line.

In vitro cell viability and morphological alterations were assessed using the MTT assay and confocal microscopy. This included differential staining (DAPI/propidium iodide) for live/dead ratios, and specific organelle labeling (LysoTracker/MitoTracker) counterstained with DAPI. Methyl jasmonate (MeJA) exhibited the strongest anticancer properties (IC₅₀ = 1,376 μM), followed by the moderate activity of 24-epibrassinolide (EBL) and jasmonic acid-isoleucine (JA-Ile) (IC₅₀ ~5 μM). Indole-3-acetic acid (IAA) showed a weak effect, while ethephon (ETH) was non-toxic. Confocal imaging and quantitative DAPI/PI analysis strictly corroborated these metabolic results. Treatment with active compounds induced subtle nuclear fragmentation and mitochondrial disruption. These structural changes combined with a substantial viability drop from 81.1% to 37.8% under 9.27 μM MeJA exposure suggest the activation of mitochondrial cell death pathway.

The most important conclusions from the conducted experiments clearly demonstrate that phytohormones, with particular emphasis on jasmonates and brassinosteroids, drastically reduce the viability of TNBC cells and destroy the integrity of their cell membranes. They represent a highly promising group of compounds that may become the foundation for new chemopreventive strategies in the future. Their potential to inhibit tumor growth offers real hope for more effective prevention of the recurrence of this aggressive disease.

Comparative and GO-based analysis of genetic variation in female, hermaphroditic, and male cucumber (*Cucumis sativus* L.) lines

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Cucumber (*Cucumis sativus* L.) is a key model species in genomic research due to its compact genome and short life cycle. Understanding genetic variation at the molecular level is essential for developing modern breeding strategies. In this study, a comprehensive comparative genomic analysis of five lines representing female (2gg, Gy3), hermaphroditic (2667, Hgy3), and male (859) forms was performed relative to the B10 reference genome, focusing on variation patterns associated with sex type.

Bioinformatic analysis identified a broad spectrum of genetic variants, including SNPs, insertions, deletions, and substitutions. Variant impact analysis revealed hundreds of high-impact modifications, such as frameshift and stop-gained mutations, which may directly affect protein structure and function. Venn diagram-based comparisons enabled the identification of genes harboring variants unique to specific lines, as well as those shared within female and hermaphroditic groups, revealing both distinct and overlapping patterns of genetic variation associated with reproductive type.

A key aspect of this study was the functional interpretation of these gene sets. Gene Ontology (GO) enrichment analysis uncovered a significant overrepresentation of genes involved in processes related to metabolism and growth. Notably, genetic variants were densely concentrated in pathways associated with environmental stress responses (both biotic and abiotic), DNA metabolism, and the regulation of cellular signaling. These functional insights strongly suggest that the genetic variability within these lines is closely tied to adaptive mechanisms.

The accuracy of the bioinformatic predictions was experimentally validated through PCR and Sanger sequencing. Ultimately, these findings provide new insights into the functional structure of genetic variability in cucumber and support the development of molecular markers for marker-assisted selection (MAS), contributing to improved crop adaptation and resilience.

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