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DECIPHERING CONTRACTILE FIBROBLASTS IN THE DEVELOPING MAMMARY GLAND

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The mammary gland is unique in its ability to undergo major postnatal morphogenic development, regulated by complex interactions between the epithelium and the surrounding stromal constituents, including fibroblasts[1]. Recently, we have discovered the presence of contractile (*Acta2+*) fibroblasts specifically around the growing tips of mammary epithelial ducts (terminal end buds, TEBs) in puberty and hypothesized that these peri-TEB fibroblasts form a critical microenvironment required for the growing mammary epithelium[2]. Our further scRNA sequencing and spatial mapping analyses revealed that contractile fibroblasts are only a transient population that differentiates into other subtypes of fibroblasts. However, their origin has remained unknown.

We hypothesized that the contractile fibroblasts arise from *Fgf10+* preadipocytes. Therefore, we performed lineage tracing experiments using *Fgf10-CreERT2;mT/mG* mouse model, followed by fluorescence-activated cell sorting (FACS) and immunofluorescence analyses. We found that indeed the *Fgf10+* preadipocytes differentiated into *Acta2+* peri-TEB fibroblasts, as well as peri-ductal fibroblasts and adipocytes.

CellChat analysis performed on our scRNA sequencing datasets has identified peri-TEB fibroblasts as the major producers of WNT ligands and regulators[2]. While studies have reported WNT activity in the TEB stroma during pubertal growth[3,4], the role of WNT activity in fibroblasts and its role in pubertal mammary morphogenesis has not been elucidated yet. Using WNT inhibition experiments we have found that WNT signalling is a key regulatory pathway in pubertal mammary morphogenesis influencing TEB morphology and mammary gland development, suggesting its role in regulating the functionality of contractile mammary fibroblasts.

Through this study, we elucidate the origin and regulation of *Acta2+* peri-TEB fibroblasts in pubertal mammary morphogenesis. We demonstrate that fibroblast activation, typically linked to cancer or wound healing, is crucial in organogenesis, and provoke further inquiry into fibroblast activation in normal versus pathological settings. This work lays the foundation for future studies on identification of molecular regulators for cancer and fibrosis therapy.

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WHO WILL BENEFIT FROM IMMUNOTHERAPY? THE EFFECT OF ENDOPLASMIC RETICULUM STRESS ON IMMUNE SURVEILLANCE IN CARCINOGENESIS

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Ovarian cancer (OC) is the leading cause of death among gynecologic malignancies, with a 5-year survival rate below 30% for advanced stages[1]. Although current clinical trials have failed to provide effective results, immunotherapy represents a promising approach for OC. Crosstalk between immune (IC) and cancer (CC) cells occurs within the tumor immune microenvironment (TIME), a complex and dynamic ecosystem. Various factors secreted in TIME contribute to the establishment of an immunosuppressive environment. The hostile TIME disrupts Endoplasmic Reticulum (ER) homeostasis in both CCs and ICs, leading to a state of persistent ER Stress (ERS). In CCs, chronic activation of ERS supports multiple pro-tumor attributes, whereas in ICs it impairs immune functions and promotes immunosuppressive phenotype[2]. In addition, the ability of stressed CCs to induce similar ERS in neighboring non-tumor cells has recently been described. This phenomenon is termed Transmissible ERS (TERS) and is a major contributor to cancer progression. However, the causes and effects of TERS from CCs to non-tumor cells remain unknown[3].

To investigate the role of TERS in OC and its effect on immune surveillance, we treated Peripheral Blood Mononuclear Cells (PBMCs) with conditioned medium (CM) from stressed OC cells (induced by tunicamycin). The obtained data from RT-qPCR and WB indicated that stressed CCs release soluble factors into the CM that impair the survival and efficacy of PBMCs. Alleviation of ERS by chemical chaperone TUDCA (tauroursodeoxycholic acid) reduced the effect of TERS on the effector and stress profile of PBMCs. Our findings suggest that TERS modulation may improve the function of TILs and contribute to the improvement of immunotherapy.

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A NEW PERSPECTIVE ON PLANT ADENOSINE KINASE: MONOMER/DIMER SWITCH AND ITS FUNCTIONAL IMPLICATIONS

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Adenosine (Ado) is phosphorylated in an ATP-dependent reaction catalysed by adenosine kinase (ADK). Ado is primarily synthesized through the activity of S-adenosyl homocysteine (SAH) hydrolase[1]. Since SAH hydrolase participates in the S-adenosyl methionine (SAM) cycle, the accumulation of Ado can lead to its feedback inhibition, thereby disrupting the cycle and impairing SAM-dependent transmethylation. To prevent this, ADK role in removing Ado is pivotal[2].

Here, we explored the substrate preferences, oligomeric states, and structures of ADKs from moss (*Physcomitrella patens*) and maize (*Zea mays*), complemented by metabolomic and phenotypic analyses. Our findings revealed that maize and moss ADKs can form dimers at high protein concentrations, distinguishing them from the monomeric ADKs of humans and protozoa. Structural and kinetic analyses identified a catalytically inactive dimer where mutual blocking of active sites occurs. Moss ADKs which showed a higher tendency to dimerize exhibited tenfold lower activity compared to their maize ortholog. Two monomeric structures in a ternary complex captured the transition from an open to a closed state upon substrate binding, suggesting that oligomerization can regulate moss ADK activity and potentially other plant ADKs. Furthermore, dimer formation represents a novel negative feedback mechanism that helps maintain stable levels of adenosine and AMP[3].

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DEVELOPMENT OF SOFTWARE FOR LC-MS DATA PROCESSING OF OLIGONUCLEOTIDES

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The development of advanced software for processing LC-MS data, particularly for oligonucleotides, represents a critical step forward in biomedical research and therapeutic applications. Our work focuses on creating a Python-based tool designed to streamline the analysis of oligonucleotide sequences. Upon input of an oligonucleotide sequence, the algorithm generates a library of monoisotopic masses for the sequence fragments across all charge states. These theoretical masses are then matched with experimentally measured masses within a defined ppm range using LC-MS data exported from Bruker Data Analysis software.

The algorithm has theoretical applications in inflammation and cancer diagnostics through the measurement of miRNA from the liquid body fluids. This could lead to early and precise detection of inflammation or cancer progression, offering a promising avenue for non-invasive diagnostic techniques. Moreover, the significance of this software extends beyond basic research since the oligonucleotide therapeutics have emerged as a vital class of drugs, offering targeted therapeutic strategies. However, the accurate interpretation of complex mass spectra remains a challenge in the field. This software will address these challenges by providing reliable and efficient tools for mass spectrometry data analysis, potentially improving the accuracy of oligonucleotide characterization.

Our methodology will enable quantitative and qualitative analysis of the complex biological matrix. The software's graphical user interface (GUI) will simplify interaction, making it accessible to researchers with varying levels of computational expertise.

Our software can aid ongoing efforts in oligonucleotide research and demonstrate the integration of computational tools in modern biomedical sciences, thereby fostering innovation and improving healthcare outcomes.

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INTERACTIONS OF POT1 MUTANTS WITH ALTERNATIVE TELOMERE REPEATS, CONCURRENTLY OCCURING IN CLL

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Telomeres, the ends of linear chromosomes, terminate in single-stranded DNA overhangs that can be mistaken for double-stranded breaks by DNA repair machinery[1]. The shelterin protein POT1 prevents this recognition by binding to telomeric DNA via two OB folds in its N-terminal region[2]. In chronic lymphocytic leukemia (CLL), mutations have been identified in these evolutionary conserved domains of POT1[3,4]. Simultaneously, CLL cells prolong telomeres through the alternative lengthening of telomeres (ALT) mechanism[5], that proceeds via homologous recombination and can generate nucleotide substitutions within telomeric repeat. The effect of these substitutions on POT1 ability to bind telomeric DNA remains unclear. To investigate this, we selected five mutations affecting four amino acid positions in POT1 protein that are associated with CLL and are predicted to affect its binding to wild-type telomeric DNA. The effects of these protein mutations on DNA binding were validated using ELISA-based assays. Additionally, we chose the five most abundant telomeric repeat sequences in ALT-positive cells and demonstrated their ability to form G-quadruplex (G4) structures, when occurring in four consecutive tracts. We examined how the changes in DNA sequence and G4 forming properties affect the interaction with POT1 protein and its mutant variants.

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SOLVING THE MYSTERIES OF NOVEL TWO-DOMAIN LECTINS FROM HUMAN PATHOGENS

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LecB (PA-IIL) is one of two characterized lectins (saccharide-binding proteins) from the bacterium *Pseudomonas aeruginosa*. Both proteins (LecA and LecB) play a significant role in bacterial infection and biofilm formation in patients with immune deficiencies (e.g. cystic fibrosis patients)[1]. Several LecB homologs were described in the past, for example, lectins produced by *Burkholderia cenocepacia*[2]. Nevertheless, there are still uncharacterized LecB-like proteins in the pathogenic bacteria, some of which contain an additional domain of unknown function. Their characterization could provide insights into the mechanism of infections and lead to the development of novel approaches to disease treatment.

The aim of this project is the functional and structural characterization of three potential two-domain lectins containing a LecB-like domain with emphasis on their binding properties. The genes encoding these hypothetical carbohydrate-specific proteins were identified by bioinformatic analysis, cloned into expression vectors, and expressed in *Escherichia coli*. In addition, new gene constructs were prepared to characterize each domain separately. A variety of methods were used to investigate thermostability (nanoDSF), homogeneity (DLS, AUC), and binding properties (ITC, AUC) of the purified proteins. Several crystallization screens were performed to obtain the crystals of the separate domains. The initial hits for X-ray crystallography are currently optimized to obtain well-diffracting crystals. For the whole proteins, electron microscopy methods are planned because of the expected high dynamics of the whole system,

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POLYHYDROXYALKANOATES AS FUNCTIONAL BIOMATERIALS FOR 3D-PRINTABLE HYDROGELS

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Hydrogels represent a fascinating and crucial class of materials, consisting of a hydrophilic cross-linked polymer network that retains a significant amount of water within its structure. Their unique properties have driven extensive research leading to the development of innovative hydrogel materials for biomedicine, such as scaffolds or implants. In this regard, additive manufacturing, including 3D printing, has become a powerful technology for fabricating patient-specific implants with the potential to heal critical-sized defects[1].

Polyhydroxyalkanoates (PHAs) have attracted significant interest in the biomedical industry as well[2]. As intracellular bio-polyesters produced by microorganisms, they stand out for their biodegradability, biocompatibility and wide range of mechanical properties. Since the final mechanical properties of hydrogels depend on their (bio)polymeric material, PHAs seem to be a very suitable source for their use in biomedicine. But is it even possible to prepare a hydrogel, a hydrophilic material from hydrophobic PHAs without the need for their chemical modification?

Their hydrophobic nature is incompatible with hydrogel principles. Herein, we overcame this limitation using a solvent-exchange method[3]. This method involves dissolving the polymer in a suitable water miscible solvent and exchanging the solvent with water to form hydrogels (Fig. 1). We designed hydrogels based on different types of PHAs. The effect of varying the composition, additives, solvent or solvent-exchange method on mechanical properties of hydrogels has been compared. The use of PHAs in 3D printing of hydrogels has been described as well.

Fig. 1. **Solvent exchange in PHA gel – DMSO** (left) **to water** (right)

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BIOCHEMICAL AND STRUCTURAL MODIFICATIONS INDUCED BY BRACONID WASP VENOM IN *DROSOPHILA MELANOGASTER*

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The venom of the minute wasp *Habrobracon hebetor* (HH venom) is a potent toxic cocktail that paralyzes the victim[1] and suppresses its humoral and cellular immune responses[2], typically targeting lepidopteran and coleopteran species. Consequently, the immobilized victim serves as a "living can" for developing a new generation of this ectoparasitic insect. However, the detailed mechanisms underlying HH venom's effects remain unclear, partly due to the incomplete characterization of its toxin components[3]. Notably, HH venom has been shown to possess these effects across various insect species, with evidence suggesting that adipokinetic hormone (AKH) can alleviate its detrimental impacts[4].

This study investigated the HH venom impact on certain properties of thoracic and central nervous system (CNS) tissues in *Drosophila melanogaster*, a well-established biological model, under *in vitro* conditions. The HH venom treatment significantly influenced the examined tissues´ biochemical, physiological, and structural parameters. The venom altered the activities of superoxide dismutase (SOD), catalase (CAT), and relative cell viability. Moreover, it also suppressed the expression of immune-related genes, including *Keap1*, *Relish*, *Nox*, *Eiger*, *Gadd45*, and *Domeless* in the brain, as well as in thoracic muscles, apart from the *Nox* gene. Ultrastructural analysis revealed severe deterioration in muscle cell architecture, primarily affecting mitochondrial integrity. Interestingly, co-treatment with *Drosophila melanogaster*-adipokinetic hormone (Drome-AKH) modulated many of these effects. The addition of Drome-AKH partially mitigated the venom's suppression of SOD activity, restoring it to near-normal levels. In the brain, Drome-AKH application protected from suppression of the immune-related genes, except *Gadd45*. Alas, in thoracic muscles, the protective effect was observed only for the *Eiger* gene. Furthermore, it seems that Drome-AKH improved mitochondria structure in muscle cells, comparably to the control. These findings demonstrate the general toxic effects of HH venom on *D. melanogaster* and highlight the partial protective role of Drome-AKH in mitigating these harmful impacts[5].

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A UNIQUE MECHANISM OF EXORIBONUCLEASE INHIBITION IN SARS-CoV-2: INSIGHTS FROM PHENOLIC PHYTOCHEMICAL SCREENING

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The SARS-CoV-2 exoribonuclease (ExoN) plays an essential role in viral replication by proofreading RNA synthesis and contributing to RNA recombination. This enzymatic activity is located within the N-terminal domain of non-structural protein 14 (nsp14) and relies on its interaction with the nsp10 cofactor to gain most of its activity. Given its importance in viral replication, ExoN has been highlighted as a potential drug target. Despite this, no clinically viable ExoN inhibitors have been identified[1]. Computational studies have proposed phenolic phytochemicals as candidates for ExoN inhibition[2–4], yet their efficacy remains largely unexplored.

In this study, we screened a diverse panel of phenolic phytochemicals and their precursors, such as simple phenolics, flavonoids, and naphthoquinones, for ExoN inhibition. To evaluate these compounds, we employed a dual-assay approach combining thermal stability analysis with nuclease activity assays. Despite promising *in silico* predictions, most compounds failed to inhibit ExoN. Nevertheless, we identified three inhibitors in the panel, of which compound 50 (cmp50) demonstrated an IC50 in the single-digit micromolar range. Moreover, the dual-assay approach indicated a novel mechanism for cmp50 involving disruption of the nsp10-nsp14 complex.

This observation was thus followed by a comprehensive analysis of cmp50 using enzyme kinetics, microscale thermophoresis, thermal denaturation methods, chemical crosslinking, and analytical ultracentrifugation. The summary data confirmed the presumed mode of action. Additionally, the results strongly suggest that cmp50 targets the nsp10 cofactor, promoting its transition to an inactive dimeric state. These findings provide new insights into the inhibition of ExoN, thereby establishing a foundation for developing novel drugs against SARS-CoV-2 and other coronaviruses.

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THE FUNCTIONAL ROLE OF WNT SIGNALING IN FIBROBLAST-REGULATED MAMMARY GLAND MORPHOGENESIS

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The mammary gland is a dynamic organ that experiences developmental changes throughout a female's life[1]. Mammary gland development mostly occurs postnatally and is strongly regulated by the stromal environment[2]. Terminal end buds (TEBs) are highly proliferative epithelial structures at the tips of the ducts that drive mammary gland growth during puberty[3]. Recent studies indicate that fibroblasts – stroma residents – are directly involved in mammary gland growth and branching[4,5]. Fibroblasts secrete a wide range of signaling molecules that initiate and support mammary development[6,7], including WNTs[8]. Yet, the regulation of mammary gland morphogenesis by WNT signaling remains incompletely understood.

To assess the role of WNT signaling in mammary gland morphogenesis we utilized a mouse model and performed a 5-day intraperitoneal injection of the WNT inhibitor LGK-974. On day 6 mammary glands were harvested for further analysis. The LGK-974 treatment decreased epithelial penetration and altered the quantity and morphology of TEBs (Fig. 1). Immunohistochemical analysis revealed that WNT inhibition depleted the fibroblast subpopulation surrounding the TEBs (Fig. 2).

These results suggest that inhibition of canonical WNT signaling in puberty impairs the population of peri-TEB fibroblasts, resulting in aberration of murine mammary gland development. Further analysis of the role of fibroblast WNT signaling in mammary morphogenesis is pending.

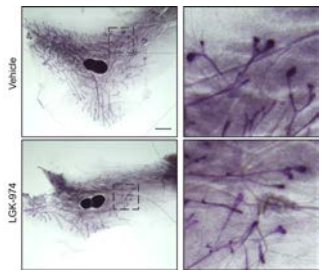

Fig. 1. **Morphological changes of murine mammary gland after treatment with WNT inhibitor LGK-974.** Zoom-in area depicting the TEBs. Scale bar, 2mm.

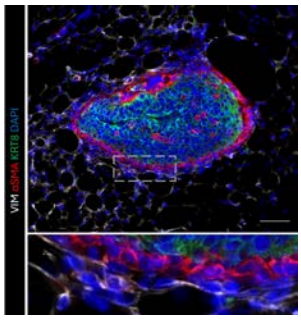

Fig. 2. **A representative image of immunohistochemical analysis of murine TEB microenvironment. White: vimentin (stromal fibroblasts); red: αSMA (basal cells and contractile fibroblasts); green: KRT8 (luminal cells); blue: DAPI-stained nuclei.** Scale bar, 50 μm.

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SUPRAMOLECULAR INTERACTION OF AZAPHTHALOCYANINES

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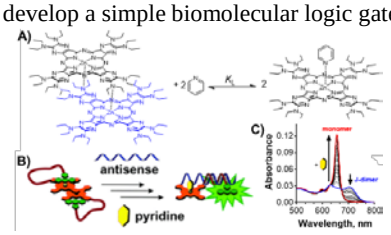
Azaphthalocyanines (AzaPcs) are planar macrocyclic compounds with interesting photophysical properties thanks to their large aromatic system. AzaPcs bearing coordinating peripheral substituent are capable of forming unique supramolecular assemblies known as J-dimers. These rare assemblies arise from the interaction between the coordinating moiety of one AzaPc molecule and the central metal cation of another (Fig. A and C). J-dimer formation affects substatnially spectral and photophysical properties which could be exploited in the development of new switching systems or logic gates.

To fully understand the self-assembly of AzaPcs, we examined the stability of J-dimers as a function of the size of their peripheral substituents. A clear correlation was observed between the dimerization constant and the bulkiness of the peripheral binding sites. Subsequently, the disassembly of J-dimers into monomers was studied under various external stimuli, including elevated temperatures, the presence of ligands with higher binding affinities to the central metal cation, or protonation of the binding sites by acids. These investigations allowed us to elucidate structure-activity relationships and explore strategies for designing systems with tailored binding strengths within J-dimers.

Finally, we succesfully attached J-dimer-forming-AzaPcs to oligodeoxynucleotide probes where they served as an effective dark quenchers of the fluorescence emitted by the reporter (fluorescein). The strong propensity of AzaPcs to form J-dimers was demonstrated even in buffer solutions. Interestingly, unprecedent heterodimers consisting of AzaPc and fluorescein were also identified (Fig. B). The spectral changes associated with the disassembly of these J-dimers upon the addition of a coordinating solvent were subsequently utilized to develop a simple biomolecular logic gate.



This work was supported by GAČR (23-06177S).

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SGLT-I MODIFY EXOSOMAL PROTEIN COMPOSITION: A POTENTIAL MECHANISM FOR CARDIOPROTECTIVE EFFECTS

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Sodium-glucose cotransporter inhibitors (SGLT-i) are widely used in diabetes treatment. Beyond their glucose-lowering effects, they provide broader benefits, including significant cardioprotective effects. SGLT-i help manage heart failure and reduce cardiovascular complications. However, the precise mechanisms underlying these additional effects remain unclear[1].

Exosomes, small membrane vesicles all cells produce, play a critical role in intercellular communication[2]. We hypothesize that SGLT-i influence exosomal composition, which could be one of the mechanisms underlying their beneficial effects.

To explore this hypothesis, we conducted *in vitro* experiments on HepG2 hepatocytes and HEK293T kidney cells treated with SGLT-2i (dapa-, empagliflozin) and a dual SGLT-1/2i (sotagliflozin). Exosomes were isolated from the culture media of treated cells and analysed through proteomic profiling using mass spectrometry and Nanoparticle Tracking Analysis (NTA).

Proteomic analysis revealed significant beneficial changes in exosomal protein composition following SGLT-i treatment. Specifically, we observed an enrichment of ribosomal proteins implicated in cell cycle regulation, stress response, and processes associated with various diseases. Additionally, increased levels of mitochondrial proteins were detected, suggesting that SGLT-i promote the release of mitochondria-containing exosomes, which have been linked with reduced inflammation and improved heart function. We further noted a reduction in transforming growth factor-β content, supporting the attenuation of fibrotic processes. NTA confirmed that treatment with SGLT-i did not affect the quantity, size, or distribution of exosomes.

Our results suggest that SGLT-i treatment positively modifies exosomal protein cargo, potentially revealing a novel mechanism underlying their cardioprotective effects.

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SELENOESTERS AS POTENTIAL ADJUVANTS IN THE TREATMENT OF AMINOGLYCOSIDE-RESISTANT STAPHYLOCOCCAL INFECTIONS

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Staphylococcus aureus infections can no longer be routinely treated with antibiotics due to high levels of antimicrobial resistance (AMR)[1]. Adjuvant therapy attempts to counter AMR by combining an antibiotic with a resistance enzyme inhibitor[2]. Because the bifunctional AAC/APH enzyme, fusion protein comprising of aminoglycoside acetyltransferase and aminoglycoside phosphotransferase, is a key player in *S. aureus* resistance to aminoglycosides[3], we assess the ability of selenoesters (74 compounds) to inhibit this enzyme and reverse the resistance to aminoglycosides. Preceding high-throughput screening of potential adjuvants uncovered the synergic effect of selenoester compounds with tobramycin against MRSA clinical isolate *in vivo*. To confirm their specificity for the AAC/APH enzyme, an *E. coli* strain expressing AAC/APH was prepared by genetic engineering methods. A structure-activity relationship (SAR) study of the selenoesters was performed on the purified enzyme to determine their main features. Enzymological methods were used to study the mechanism of interaction between the enzyme and the most effective substance – fluorinated nitryl of selenoester. This inhibitor was revealed to be a competitive inhibitor of both AAC/APH domains with K_I in low micromolar range. Protein-ligand docking of selenoesters onto the protein was performed to investigate the accessibility of the binding site, aided by funnel-metadynamics simulations. Both domains of the enzyme were also separated to reveal the crystal structures with bound inhibitor. Thus, our results suggest that selenoesters are promising compounds for the adjuvant therapy of aminoglycoside-resistant staphylococcal infections and could even bring these antibiotics back into play for clinical practice.

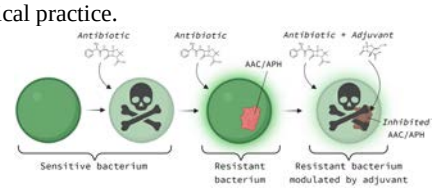

Fig. 1. **The principle of adjuvant therapy** (Created with BioRender.com)

The project was financed by the NIVB (EXCELES Program, Project ID No. LX22NPO5103) - Financed by the EU - Next Generation EU.

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APPLICATION OF LIBS FOR DETECTING MICROPLASTICS

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Plastics have become an indispensable part of modern life, serving critical roles in various sectors such as industry, transportation, and agriculture. However, the degradation of plastics into microplastics, particles ranging from 1 to 1000 µm, has raised significant environmental and health concerns. These particles undergo complex degradation and contamination processes, presenting unique challenges for their analysis and monitoring in ecosystems. The biomonitoring of microplastics involves the careful assessment and identification of small plastic particles in association with living organisms and ecosystems. This methodical process aims to understand how widely microplastics are distributed, how they might interact with organisms, and how this affects the wider environment. Establishment of robust monitoring tools are important for guiding effective mitigation strategies and ensuring a more sustainable coexistence with this material[1,2].

This study utilizes laser-induced breakdown spectroscopy (LIBS) as the primary analytical technique for analyssis of the microplastics. LIBS is a spectroscopic technique that functions by creating a plasma plume through the interaction of a laser with matter. This interaction causes atoms and ions to become excited and emit photons of distinct energy as they transition to lower energy levels. The emitted light is then gathered by collection optics before being transmitted to a spectrometer. Unique spectral lines are allowing the identification of elements present in the sample[3].

LIBS parameters were optimized to achieve high-resolution analysis of microplastic particles, allowing for the detailed characterization of spatial variations in elemental composition.

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BIOMARKERS OF RELAPSE IN MULTIPLE MYELOMA: INSIGHTS FROM MIRNA EXPRESSION AND MASS SPECTROMETRY ANALYSIS

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This study explores the potential of miRNA and mass spectrometry profiles as biomarkers of early relapse in multiple myeloma, a malignancy characterized by the uncontrolled proliferation of monoclonal plasma cells. Disease relapse is frequently driven by the selective pressure of treatment, which allows resistant subclones to thrive[1]. A major challenge in managing multiple myeloma is the absence of reliable biomarkers for early relapse detection, hindering the ability to develop timely and effective treatment strategies[2].

We examined the expression profiles of miR-16-2-3p, miR-92b-3p, and miR-598-3p in paired peripheral blood samples from 56 patients, collected at diagnosis and first relapse. Patients were categorized into fast, intermediate, and slow relapsing groups. RT-qPCR revealed statistically significant differences in miR-92b-3p and miR-598-3p expression profiles across these groups. MALDI-TOF MS (matrix assisted laser desorption/ionization-time of flight) analysis of mass spectra initially showed overlapping profiles across all patient groups. However, after excluding the intermediate relapse group, likely due to its heterogeneous relapse dynamics, PLS-DA (partial least squares-discriminant analysis) revealed two distinct clusters, effectively separating fast and slow relapsing patients at the time of first relapse.

Combining miRNA expression profiles with mass spectrometry and clinical data resulted in clear separation between slow and fast relapsing patients at both diagnosis and first relapse with PLS-DA. These findings suggest that specific miRNAs may reflect the dynamics of clonal evolution in multiple myeloma. When combined with mass spectrometry and clinical data, miRNA profiling could improve early relapse detection and enhance patient stratification for targeted treatments.

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CHARACTERIZATION OF *EX VIVO* AND *IN VITRO* MODELS TO STUDY THE CROSSTALK BETWEEN THE ENDOCANNABINOID AND IMMUNE SYSTEMS IN THE PLACENTA

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The endocannabinoid system (ECS) is increasingly recognized for its role in immune regulation and inflammatory responses, yet its involvement in placental inflammation remains largely unexplored. This study aims to characterize the expression of ECS components across different placental models and investigate how endocannabinoids influence inflammation in the placenta. Using qRT-PCR, ddPCR, immunofluorescence, and western blot, we analyzed the gene and protein expression of key ECS components in various placental models, including term placenta explants, first- and term-trimester homogenates, primary trophoblasts, and placental cell lines (BeWo, HTR-8/SVneo, and ACH-3P). Our results highlight significant variability in ECS expression across these models, underscoring the importance of careful model selection when studying placental ECS function and its regulatory mechanisms. Following this characterization, term placental explants were treated with the endocannabinoids anandamide (AEA) or 2-arachidonoylglycerol (2-AG) at concentrations of 0.1, 1, 10, and 20 µM for 48 hours. To assess the impact of ECS modulation on inflammation, lipopolysaccharide (LPS) was then used at 1 µg/mL for 4 hours to induce an inflammatory response. The expression and secretion of key pro-inflammatory cytokines, including TNF-α, IL-6 and IL-1β, were evaluated using qRT-PCR and ELISA. Our findings suggest that while AEA and 2-AG modestly reduced TNF-α and IL-1β secretion in placental explants, they had minimal effects at the gene expression level. These results indicate that ECS modulation may play a subtle yet potentially relevant role in regulating placental inflammation. Further research is required to delineate the precise mechanisms underlying ECS involvement in placental immune responses and to explore its therapeutic potential in managing maternal inflammation and related pregnancy complications, such as preterm birth.

This study was funded by the GA of CUNI (GAUK 170-050/235011) and GAČR 23-07094S.

SERVING OR STEALING: DYNAMICS OF MITOCHONDRIAL TRANSFER IN B CELL MALIGNANCIES

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Tunneling nanotubes (TNTs) are tiny, tube-like structures that allow cells to communicate directly with each other by transferring materials like mitochondria. This process has major implications in cancer biology. While originally observed in solid tumors, recent studies show that TNTs can also form in blood cancers like Acute Myeloid Leukemia[1] (AML).

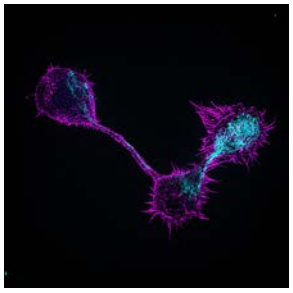

Fig 1. **Formation of tunnelling nanotube between CLL cells Magenta-actin, Cyan-anti TOMM20 (mitochondria)**

In our study, we explored this phenomenon in Chronic Lymphocytic Leukemia (CLL). We found that CLL cells, both from laboratory models and patient samples, consistently formed TNTs with T cells, which are crucial for the immune response. Through these TNTs, mitochondria were transferred from the T cells to the cancerous CLL cells. This "mitochondria theft" could be one reason why T cells become exhausted, meaning they lose their ability to fight effectively, which is a problem in CLL treatments like CAR-T cell therapy. CAR-T therapy, which uses genetically modified T cells to target and kill cancer cells, may be less effective because of this mitochondrial transfer.

We believe that by blocking the formation of TNTs, we could potentially prevent T cell exhaustion, making treatments like CAR-T therapy more effective against CLL. This finding opens new avenues for improving immune-based therapies for blood cancers.

This work was supported by the project MUNI/A/1685/2024 and by the GACR, project no. 22-35273S.

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TRI-OXO-TRIANGULENE, A PROMISING CATALYST FOR SELECTIVE REDUCTIVE AND OXIDATIVE PHOTO-CATALYSES

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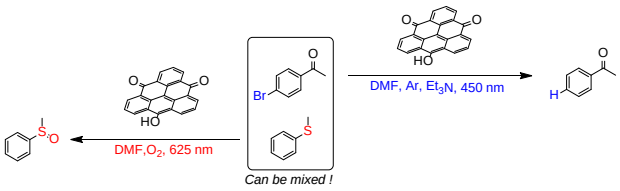
Photochemistry is a powerful technique for reactions that heat alone cannot achieve. However, substrates do not necessarily include chromophores enabling the absorption of visible light. Thus, investigations for photocatalysts are still a vibrant research field. Flavins, transition metals, complexes or polyaromatics have already demonstrated good performances. Despite their potential, issues such as complex synthesis, strict reaction conditions, toxicity, cost, and accessibility must be addressed.

Triangulenes[1] are polyaromatics known for their conjugated scaffold, which excludes two carbons, resulting in highly stable radicals or biradicals. They exhibit notable optical properties, including broad absorption in the visible light spectrum. These characteristics make triangulenes promising candidates for applications in spintronics, magnetic materials, semiconductors or photocatalysis. A derivative, tri-oxo-triangulene[2] (TOT), can be synthesised on a multigram scale[3] in just four steps, with minimal purification.

In this work, we use TOT as photocatalyst to achieve the challenging reduction of 4-bromoacetophenone but also the oxidations of thioanisole to its sulfoxide derivative. We discuss both performance and selectivity and detailing a selective sequential photo redox system including both substrates where we control the outcome by tuning the irradiation wavelength and the atmosphere. To the best of our knowledge, this is the first example of photocatalysis using triangulene derivatives. We believe this work paves the way for new materials in selective heterogeneous catalysis.



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STUDY OF DEPOSITED POLY(PHENAZINE) FILMS FOR LATENT FINGERPRINT VISUALIZATION

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Fingerprint visualization on metallic substrates is a critical challenge in forensic science, traditionally addressed using techniques such as cyanoacrylate fuming. However, this method is time-consuming and poses health risks due to the release of harmful by-products[1]. Deposition of poly(phenazine) films, including poly(neutral red) and poly(toluidine blue) prepared from monomer (Fig. 1), provides a suitable and efficient alternative[2].

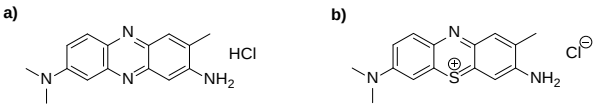

Fig. 1. **Structure of monomeric form: a) neutral red, b) toluidine blue**

These stable-colored films are electrochemically prepared and deposited on brass substrates around the sebum creating a negative image of the fingerprint due to the grease blocking electron transfer and polymer film formation[3].

In this study, poly(phenazine) films were prepared using electrochemical deposition techniques under various conditions, including the deposition method (cyclic voltammetry or chronoamperometry), applied potential, deposition time, solution composition, and monomer concentration. These films were characterized using FTIR and Raman spectroscopy to analyze their molecular properties, while SEM, stereomicroscope, and profilometry provided insights into their morphology. The effects of deposition parameters on film adhesion, stability, and color contrast were systematically evaluated.

This study shows the potential of poly(phenazine) films as efficient materials for fingerprint visualization. The findings support the adoption of this electrochemical method in forensic practice as a safer and faster alternative to conventional techniques.

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SHEDDING LIGHT ON THE SECRETS OF NANOLUC, ITS MECHANISM, AND ALLOSTERIC BEHAVIOUR

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NanoLuc luciferase, a small enzyme renowned for its exceptionally bright bioluminescence, has found widespread applications in biotechnology and biomedicine since being designed in 2012 (cit.[1]). However, the mystery behind NanoLuc's light-emitting reaction, crucial for developing next-generation bioluminescent systems, remained unsolved. Here, we made significant progress in understanding NanoLuc's mechanism by combining various laboratory and computational techniques, including crystallography, kinetic measurements, molecular docking, and molecular dynamics simulations with enhanced sampling.

One of the most intriguing features of NanoLuc is its small size, consisting of just 171 amino acid residues, in contrast to luciferases from sea pansy *Renilla reniformis* (311 residues) and firefly *Photinus pyralis* (550 residues). We confirmed that NanoLuc is monomeric in solution but can also crystallize as a homotetramer under certain conditions. We have also identified two distinct binding sites for the substrate molecule: the catalytic site, which is buried in the core of the NanoLuc monomer, and an allosteric binding pocket shaped on the oligomerization interface of NanoLuc crystals[2]. Importantly, we have demonstrated that introducing mutations in the allosteric site can enhance the bioluminescent reaction occurring in the active site.

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ARYLDIFLUOROSILICATES FOR IMPROVED NUCLEOPHILIC FLUORINATION OF SECONDARY SUBSTRATES

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Organofluorine chemistry has a key role in the development of new pharmaceuticals and agrochemicals[1]. Nucleophilic fluorination is a widely used method for introducing fluorine into organic compounds.

Tetrabutylammonium difluorotriphenylsilicate (TBAT) is a non-hygroscopic organic salt serving as a nucleophilic fluorine source[2]. Compared to other commonly used nucleophilic fluorinating reagents such as tetrabutylammonium fluoride (TBAF), TBAT shows better chemoselectivity.

With the aim of improving its reactivity and chemoselectivity, we prepared several TBAT analogues modified with aryl groups containing electron-donating or electron-withdrawing substituents (Fig. 1)[3].

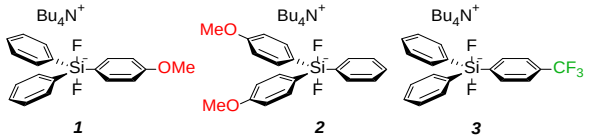

Fig. 1. **Structures of novel difluorosilicates**

Selectivity of fluorination of the newly prepared TBAT analogues was compared on a series of secondary alkyl (pseudo)halides with standard reagents such as TBAF and TBAT under unified conditions. The conversion and fluorination/elimination ratio were determined using ^{1}H NMR spectroscopy.

Our results showed that silicates ***1*** and ***2*** substituted with electron-donating groups gave comparable or better conversion and/or selectivity than TBAT with most substrates. On the other hand, silicate ***3*** showed lower reactivity compared to TBAT[3]. Introduction of more electron-donating groups on the aryl rings led to unstable reagents and decomposition into HF_2^- ions. Difluorosilicate ***2*** thus represents the borderline structure, that offers a promising alternative to conventional fluorinating reagents.

This work was supported by the GACR (reg. No. 21-29531K).

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SUBCELLULAR FRACTION-SPECIFIC INTERACTOMES OF THE TICK-BORNE ENCEPHALITIS VIRUS CAPSID PROTEIN

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The *Orthoflavivirus encephalitidis* (*Flaviviridae* family; *Orthoflavivirus* genus), previously known as tick-borne encephalitis virus (TBEV), is an arthropod-borne virus transmitted by ticks. This virus attacks the central nervous system, causing a disease called tick-borne encephalitis, whose incidence has been steadily increasing in Europe and Asia in recent years. Since there is a lack of a specific treatment for TBEV infection, attempts have been made to identify prospective candidates for drug targeting. One of these is the capsid protein (TBEV C), responsible for the formation of nucleocapsids. Its ability to bind the viral RNA and function in promoting the proper assembly of infectious particles makes it a crucial regulatory viral protein in infected cells.

Even more attention has been paid to this protein since its nuclear localization was documented[1]. Almost all TBEV C functions take place in the cytoplasm or endoplasmic reticulum, and to this day, only a few details are known about the role of TBEV C in the nucleus. For these reasons, the primary aim of this work was to identify the TBEV C interaction partners in subcellular fractions, with an emphasis on the nuclear and cytoplasmic binding partners.

This was accomplished through co-immunoprecipitation experiments followed by mass spectrometry (MS) analyses. To eliminate the identification of false-positive nucleic acid-mediated binding partners, benzonase was used to ensure the degradation of all DNA and RNA in samples.

Preliminary results indicate that TBEV C binds to histones (H2A and H2B) and, under stress conditions, interacts with glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase A, and transketolase. While optimization experiments yielded promising results, MS identification did not provide sufficiently relevant data for a comprehensive evaluation. Additionally, no significant differences were observed between samples treated with and without benzonase. Therefore, further optimization and research are necessary to evaluate these findings.

Supported by the MEYS INTER-ACTION LTAUSA18040, the GACR (projects 18-27204S, 22-25042S), and the Student Grant Agency of the Fac. Sci., Univ. of South Bohemia (2022).

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CRISPR/CAS-POWERED CHROMOSOME ENGINEERING: ADVANCING GENOME EVOLUTION IN BRASSICACEAE

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Large-scale chromosome rearrangements, such as deletions, inversions, play a central role in plant genome evolution and speciation. The advent of CRISPR/Cas technology has revolutionized the precise engineering of such rearrangements, offering creating unprecedented opportunities to investigate their genetic and phenotypic consequences. In this study, we utilized CRISPR/Cas tools to induce targeted chromosome rearrangements in the genome of *Cardamine hirsuta* (n = 8), a model species from the family Brassicaceae celebrated for its utility in developmental, ecological, and evolutionary research. Using Agrobacterium-mediated transformation with SaCas9 and tailored guide RNAs, we achieved various chromosomal modifications, including a 3 kb deletion, a 3 kb inversion, and a reciprocal translocation spanning regions of 0.1 to 2 Mb. These rearrangements were confirmed through PCR, Sanger sequencing, and chromosome painting, and were stably inherited across T2 and T3 generations. Our results demonstrate the transformative potential of CRISPR/Cas technology for reshaping chromosomal architecture in *C. hirsuta* and other non-Arabidopsis genomes. This work lay the foundation for future endeavours, including modifying chromosome numbers, reconstructing ancestral karyotypes, and repositioning rDNA loci. By advancing our understanding of the effects of chromosome rearrangements on genome stability, evolutionary dynamics, and speciation, this research broadens the horizons of plant genome engineering and evolutionary biology.

This project was funded by the GACR (grant numbers 24-11371S and 25-16796S to TM).

CALL A TRIAZOLE! VERSATILE TOOL FOR VINYL CATION OR KETENIMINE.

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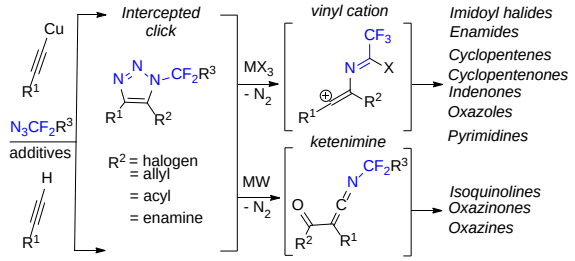
A novel approach for the synthesis of 4,5-disubstituted *N*-fluoroalkyl-1,2,3-triazoles from copper(I)acetylides or alkynes with fluorinated azides and suitable electrophilic reagents by an intercepted click reaction is described. *N*-electron-acceptor-substituted 1,2,3-triazoles showed to be excellent substrates for denitrogenative reactions under microwave reaction conditions or for Lewis acid-mediated transformations (AlX_3 or $BF_3{\cdot}OEt_2$) to provide ketenimines or vinyl cations. The choice of substitution at position 5 of the triazole – such as halogen, allyl, acyl or enamine functional group plays a crucial role in determining the subsequent reactivity of the resulting vinyl cations or ketenimines. These intermediates can lead to a variety of products including *N*-haloalkenyl imidoyl halides[1], enamides[2], multi-substituted cyclopentenes[3], indenones[4], cyclopentenones[4], oxazoles[4], isoquinolines[5], oxazines[5], oxazinones[5] or pyrimidines.



Scheme 1. **Intercepted click reaction leading to fully substituted *N*-fluoroalkyl-1,2,3-triazoles and their utilization in Lewis acid- or microwave-assisted transformation for various heterocyclic or *N*-alkenyl products via vinyl cation or ketenimine intermediates**

This work was financially supported by the Czech Science Foundation (23-04659S).

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HYALURONIC ACID AS VEHICLE FOR DELIVERY OF NANOCRYSTALS CAPABLE OF ENHANCING IMIQUIMOD SOLUBILITY AND SKIN BIOAVAILABILITY

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Dermal drug delivery remains challenging due to low drug solubility and poor skin bioavailability. One example of such type of troublesome drug is imiquimod[1], which is commonly used to treat actinic keratosis and basal cell carcinoma[2]. In recent years, we have developed several promising nanoparticles that have proven effective in dermal delivery[3]. However, their use in clinical practice is still questionable due to their low viscosity. Therefore, finding a suitable nanocarrier vehicle is essential for the next step in our research. One of the ideal candidates is hyaluronic acid (HA) due to its unique biological functions[4].

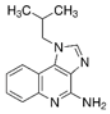

Fig. 1. **Chemical structure of imiquimod**

Here, we investigated the delivery efficiency of imiquimod nanocrystals (NC) combined with three HA gels differing in molecular weight (MW) and viscosity. The NC-HA gels were tested by *ex vivo* permeation experiment on porcine skin with evaluation of penetration kinetics in the skin tissue. The NC-HA gel with the highest MW and viscosity achieved the best skin delivery of imiquimod. In fact, in term of solubility and skin bioavailability, the obtained results were even better than those for a commercial imiquimod-based skin cream. Thus, our NC-HA system shows considerable promise for dermal drug delivery.

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ORGANOCATALYZED TANDEM REACTIONS BASED ON CYANOHYDRIN ADDITION/ACYL TRANSFER SEQUENCE

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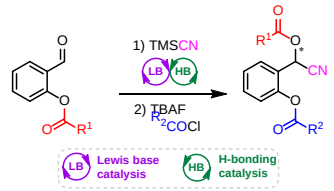
Recent advances in assymetric organocatalysis have led to previously unthinkable transformations, allowing the synthesis of challenging substrates, including *O*-substituted cyanohydrins. Those are highly valued for their versatile reactivity in synthesizing biologically active molecules[1,2]. However, despite advances in stereoselective cyanohydrin synthesis, most methods fail when targeting o-hydroxy or o-alkyl/aryloxy aromatic cyanohydrins (for a recent example, see 3)[3].

The problem with these substrates is the rapid epimerization of the resulting aromatic cyanohydrins caused by a fast reversible reaction when the aromatic aldehyde is substituted by a hydroxyl group (or a group with similar electronic properties) in the ortho position. To avoid this side reaction, we decided to trap the formed cyanohydrin adduct

immediately after its formation by intramolecular acyl transfer. Our proposed organocatalyst will serve as a nucleophile activator (enhancing TMSCN reactivity), an electrophile activator (activating aldehyde to nucleophilic attack) and an acyl functional group activator (activating intramolecular acyl transfer to the generated cyanohydrin adduct alcoholate). First results of our efforts will be presented in this contribution.



Scheme 1. **Schematic view of the targeted transformation**

Supported by the IGA of UPOL (IGA_PrF_2024_028).

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P-GLYCOPROTEIN INHIBITORS REVERSE COLCHICINE RESISTANCE IN BREAST CANCER CELLS

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Colchicine, a mitotic poison targeting rapidly dividing cells, has shown promise in cancer therapy[1,2]. However, its clinical use faces challenges, including possible cellular resistance caused by the overexpression of P-glycoprotein (P-gp)[3,4]. In this study, we evaluated the ability of colchicine combined with two P-gp inhibitors, based on aryl sulfide and dicarboxylic acid diamide structural motif, to resensitize resistant breast cancer cells. First, the combination effect was determined in MCF-7 and UFH-001 cell growing in a monolayer by WST-1 assay. The results showed that the inhibitors exhibited stronger synergistic cytotoxic effects in resistant cancer cells compared to non-resistant ones. Next, 3D models of the same cell lines in form of spheroids were used to better mimic the *in vivo* conditions. In MCF-7 and UFH-001 spheroids, combinations of colchicine with P-gp inhibitors demonstrated synergistic cytotoxic effects, which were comparable with those observed in the cell monolayers. Our results indicate that the combinations of colchicine and P-gp inhibitors has considerable potential for the treatment of colchicine-resistant breast cancer.

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COMPOSITE NANOPARTICLES FOR IMPROVED SURFACE-ENHANCED RAMAN SPECTROSCOPY

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Composite nanoparticles can integrate the properties of their components, becoming more than just the sum of its parts. The goal of this work was the synthesis of bimetallic iron oxides-silver nanoparticles and the evaluation of their potential in improved surface-enhanced Raman spectroscopic (SERS) detection of phosphorylated biomolecules. Iron oxides in acidic pH have strong affinity for phosphorylated molecules, attracting them close to silver parts of the composite nanoparticles, which selectively enhances their Raman signal.

Bimetallic nanoparticles were prepared by alcalic co-precipitation of Fe^{2+} and Fe^{3+} followed by $AgNO_3$ reduction. These colloids were prepared in different molar ratios of silver and iron oxides. The characterization of the synthetic products was conducted via electron microscopy, UV-Vis spectrometry, DLS measurements and Raman spectroscopy. Naturally, the properties of the bimetallic nanoparticles were compared to pure silver nanoparticles.

We achieved effective adsorption of phosphorylated substances on the bimetallic nanoparticles and proved their plasmonic behaviour. Furthermore, the colloid provided reasonable stability, which is highly important for its potential applications in fluidic channels.

This work was supported by the GACR (25-16617S,) by Mobility Plus Project (VAST-24-02) awarded by the CAS and by institutional support RVO 68081715 of the Inst. Anal. Chem. of the CAS.

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REGIOSELECTIVE AND STEREOSELECTIVE PREPARATION OF GANAXOLONE ANALOGUES BY TURBO-GRIGNARD CONDITIONS

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Neurosteroids play a significant role in the functioning of the brain and the entire nervous system. In recent years, synthetic analogues of neurosteroids have been approved for the treatment of conditions such as postpartum depression[1]. One of the key factors in their activity is the specific position and configuration of the hydroxyl group. Unfortunately, as endogenous mediators, neurosteroids are subject to rapid metabolic deactivation.

A common approach to address this issue is the introduction of an alkyl group near the hydroxyl group. An example of this is the steroid anesthetic ganaxolone.

When working with steroids, a major challenge is achieving stereoselectivity and regioselectivity in alkylation. To address this, the use of turbo Grignard reagents is proposed for introducing equatorial alkyl groups, along with specific additives in the synthesis of hydroxy-compounds that contain axial alkyl groups.

We will present the alkylation processes of steroids where, based on modeling, we anticipate significant neuronal activity. Compared to previously published results[2] (with yields around 30–40%), our method achieves near-quantitative, uniform conversion.

Additionally, replacing hydrogen atoms with fluorine is a crucial step in optimizing the pharmacological properties of compounds. Therefore, we are also exploring the introduction of fluoroalkyl groups.

This work was supported by the GACR, No. 23-04922S and Research Project of the CAS: RVO grant 61388963.

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DUAL PHOTOSYNTHESIS: EFFICIENCY OF PHOTOSYNTHETIC PIGMENTS IN EXTREME CONDITIONS

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Dual photosynthesis, which combines bacteriochlorophyll- and rhodopsin-based systems, represents a remarkable adaptation strategy for bacteria in energy-limited environments. These two systems complement each other: bacteriochlorophyll-based photosynthesis efficiently captures light in the near-infrared spectrum, while rhodopsin-based systems operate with a simpler, cost-effective structure that harvests green light. Such dual phototrophic mechanisms enhance survival under fluctuating light and temperature conditions, such as those in Arctic and glacial ecosystems[1]. The discovery of bacteria, such as *Sphingomonas glacialis* AAP5, encoding both systems emphasizes the ecological and evolutionary significance of this strategy, revealing pathways to optimize energy harvesting under extreme environmental stresses[2].

Sphingomonas glacialis AAP5 demonstrates a unique mechanism for adaptation to extreme conditions of prolonged absence of light, containing two fully functional photosynthetic apparatuses. One of them is based on bacteriochlorophyll with a spirilloxanthin antenna and another one is the inclusion of a proton-pumping xanthorhodopsin with a nostoxanthin antenna[3]. While xanthorhodopsin is efficiently expressed at temperatures below 16°C and in the presence of light, bacteriochlorophyll is expressed between 4°C and 22°C and only in dark conditions[4].

We investigated the dynamics of energy states in the dual photosynthetic apparatus under natural (7°C and 20°C) and extreme (45°C) temperature conditions, with and without additional LED illumination. Using time-resolved spectroscopy, we analyzed energy transfer rates by exciting specific pigments with ultrashort pump pulses (~100 fs) and detecting spectral differences between ground and excited states.

Our initial results revealed that spirilloxanthin excited at 547 nm transfers part of its absorbed energy to bacteriochlorophyll (Fig. 1a). In the second set of experiments, we studied the excited state dynamics of bacteriochlorophyll excited at 871 nm (Fig. 1b). A mild temperature effect was observed on the energy transfer rates from spirilloxanthin to bacteriochlorophyll, suggesting resilience of this process under varying conditions.

The future direction of this project involves studying the xanthorhodopsin antenna complex under the same temperature and light conditions to obtain a complete model of energy transfer dynamics and gain a deeper understanding of how these systems function synergistically in extreme environments.

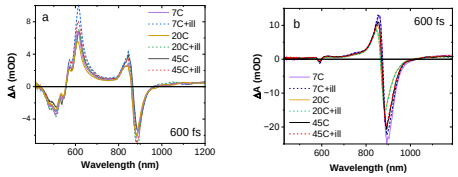

Fig. 1. **Transient absorption spectra were taken at 600 fs after 547 nm (a) and 871 nm (b) excitations**

The project is supported by the research grant GACR 24-11344S.

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DEVELOPMENT OF NOVEL PAPP-A INHIBITORS DIRECTED TO THE BINDING SITE

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Pregnancy-associated plasma protein-A (PAPP-A) plays a key role in IGF regulatory cascade. As a member of the metzincin metalloproteinase family, PAPP-A regulates IGF bioactivity by specifically cleaving IGF-binding proteins (IGFBPs), particularly IGFBP-4 and IGFBP-5. Notably, PAPP-A is the only known protease for IGFBP-4. Although predominantly expressed during pregnancy, PAPP-A is also found in various tissues, suggesting its potential to locally affect IGFs bioavailability and thereby regulate cellular processes such as growth, proliferation, differentiation, or migration. Elevated PAPP-A activity is associated with several pathologies including atherosclerosis and certain cancers, where it may contribute to disease progression[1].

Studies in *PAPP-A* knock-out mice indicate that PAPP-A deficiency contributes to prolonged lifespan and delayed aging-related pathologies. Thus PAPP-A is considered to have great therapeutic potential. Inhibitory antibodies that block IGFBP-4-binding exosite on PAPP-A have been developed and showed promising results in a reduction of atherosclerotic plaque formation in mice and inhibiting tumor growth in models of various cancers[2,3]. However, these antibodies as well as endogenous inhibitors STC1 and STC2 do not interfere with PAPP-A's active site.

We designed a new type of inhibitors based on natural peptide substrates targeting the enzyme's Zn^{2+} ion-binding active site. We prepared molecules that mimic the tetrahedral transition state intermediate during the peptide bond cleavage by replacing scissile peptide bond with a nonhydrolyzable moiety. Due to their smaller molecular weight, this type of inhibitor could offer distinct advantages and potentially help to discover PAPP-A activities independent of IGFBP cleavage.

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UNRAVELLING IMMUNE MECHANISMS DRIVING SPONTANEOUS REGRESSION IN HER2-POSITIVE BREAST CANCER: A CASE STUDY

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HER2+ breast cancer (BC) constitutes approximately 15–20 % of all BC cases and is associated with aggressive tumour behaviour and a higher recurrence and metastasis rate. Despite advances in targeted therapies, some patients experience disease relapse. The immune system plays a crucial role in the surveillance and elimination of cancer. However, tumours have evolved mechanisms to evade immune detection, creating an immunosuppressive microenvironment with various stress factors, collectively impairing immune function[1,2].

We present a rare case of spontaneous regression of histologically verified HER2+ BC, where subsequent evaluations revealed only residual fibrotic tissue lacking any tumour cells. Spontaneous remission in BC is exceedingly uncommon, with limited documented cases and no general understanding of the fundamental mechanism[3–9]. This study aims to shed light on the molecular mechanisms underpinning this spontaneous regression, focusing on immune surveillance dynamics. To investigate the underlying molecular mechanisms, immune infiltration and activation status, we conducted immunohistochemistry and immunofluorescence staining with spatial phenotyping on serially collected, paraffin-embedded tissue samples, and MALDI-TOF MS analysis on patient's serum samples collected regularly from the time of diagnosis in a two year follow up period. We correlate these results with data acquired from patients with HER2+ BC as well as healthy controls, with emphasis on identifying the key responsible molecular drivers of this rare phenomenon. Understanding the molecular basis of spontaneous tumour regression could provide valuable insights into tumour-immune interactions in HER2+ BC.

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TOWARDS NOVEL DERIVATIVES OF β-LACTAM ANTIBIOTICS – SYNTHESIS OF 1,3-DIAZETIDIN-2-ONES

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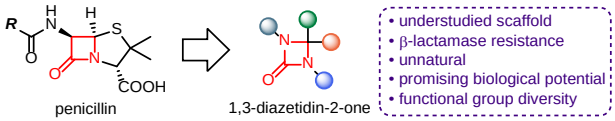
β-Lactams (azetidine-2-one) are unique small ring scaffolds well-known to organic and medicinal chemists due to their use as a key structural feature in antibiotics, such as penicillin[1]. Unfortunately, bacteria produce β-lactamase as a defense mechanism to protect against these antibiotics. The emergence of antimicrobial resistance represents one of the major global health problems[2]. Therefore, a crucial goal is to find strategies and develop new pharmaceuticals that can combat and prevent the rapid development of resistance. Our interest in this field led us to focus on the structural modification of the ring. Based on literature precedence, we attempted the synthesis of non-natural analogues of β-lactams, specifically aza-β-lactams (1,3-diazetidine-2-ones)[3].

In our contribution, we wish to disclose a new approach for the synthesis of aza-β-lactams based on a unique sequence of two intramolecular rearrangements. This method allowed us to efficiently obtain the desired products starting from *N,N*-disubstituted heterocyclic sulfonamides[4]. The scope and limitations of the method, a detailed study of the reaction mechanism and additional unexpected transformations will be disclosed.



Scheme 2: **1,3-Diazetidin-2-ones as new potent derivatives of well-known β-lactams antibiotics**

This work was supported by the European Regional Development Fund-Project "SMART Plant Biotechnology for Sustainable Agriculture" (CZ.02.01.01/00/23_020/0008497), co-funded by the EU.

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SLOW-RELEASE NITROGEN-BASED FERTILIZERS COATED WITH BIODEGRADABLE POLY(3-HYDROXYBUTYRATE)

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Fertilizers are essential for meeting agricultural food demand, but excessive nutrient leaching increases environmental pollution. This study examines slow-release fertilizers coated with biodegradable poly(3-hydroxybutyrate) (P3HB) to ensure gradual nitrogen release without soil contamination.

Pelletized urea was coated with P3HB in dioxolane, forming a durable polymer layer. Some coated pellets maintained resistance after 76 days in water. Pot experiments in Mitscherlich vessels assessed maize growth and soil nitrogen release, showing the coating did not hinder plant development[1,2].

Pellets with 50% fertilizer and 50% P3HB exhibited excellent resistance, releasing only 20% of urea after 76 days. Compared to polymer-based coatings reviewed by Lawrencia et al., our formulation demonstrated controlled fertilizer release. By adjusting filler content and coating thickness, we tailored agrochemical release, with potential applications in seed protection[3].

Results confirm coated fertilizers improve nutrient efficiency, reduce nitrogen loss, and minimize pollution while providing sustained nitrogen delivery. Large-scale pelletizing and coating processes suggest industrial feasibility. Further research is needed on soil biodegradation and long-term field effects.

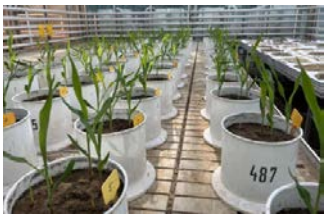

Fig. 1. **Tests of prepared P3HB coated slow-release fertilizers with maize**

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CRYPTOCHROME 2 REPRESENTS A KEY PROTEIN IN MOLECULAR CONTROL OF RETINAL DIFFERENTIATION

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Cryptochrome 2 (CRY2) is a highly conserved protein found within a wide range of species including humans. It represents one of the key proteins in regulation of circadian rhythms and its synchronization with the light-dark cyclic outer environment. Additionally, mutations in the CRY2 gene have been associated with sleep disorders and mood disturbances in humans, emphasizing its clinical relevance. In recent years, CRY2 has emerged as a multifunctional protein with roles beyond circadian clock regulation, including molecular mechanisms of development and differentiation. However, little is known about the function of CRY2 in the human retina which represents a key photosensitive organ for the synchronization of the circadian rhythms in the human body.

We use human retinal organoids as a model to describe the role of CRY2 in the human retina. Our datasets indicate that CRY2 is predominantly localized to ganglion and photoreceptor cell layers.

Using the CRISPR/Cas9 approach, we generated four CRY2 knock-out hiPSCs lines and evaluated the effect of CRY2 deficiency on retinal organoid differentiation and cell composition by immunostaining and efficiency analysis. Here we show that CRY2 deficiency leads to impaired differentiation leading to low numbers of correctly structuralized organoids or organoids with altered cell composition. Furthermore, RNA sequencing of CRY2-deficient hiPSCs revealed dysregulated gene expression, including impaired FGF signaling and downregulation of retinal-specific genes, which may contribute to inefficient retinal organoid differentiation. Notably, these findings suggest a novel link between circadian regulation and FGF signaling. These findings expand our understanding of CRY2's significance in the human retina and the differentiation of hiPSCs. The observed alterations in retinal-specific genes and FGF signaling in CRY2-deficient cells highlight the association between circadian regulation and other molecular mechanisms involved in differentiation and development.

This study was supported by the GACR (GA21-08182S) and by funds bythe Fac. Med. MU (MUNI/A/1738/2024)

SYNTHESIS OF FLUOROALKYLATED ISOQUINOLINES AND FUSED PYRIDINES FROM *N*-FLUOROALKYL-1,2,3-TRIAZOLES

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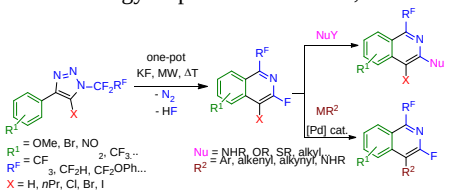
The isoquinoline core is present in various drugs and many naturally occurring alkaloids, which in many cases possess compelling biological activities. Implementing of fluorine atom or fluoroalkyl groups into a lead molecule is a widely used strategy to enhance pharmacologically relevant properties. Yet, the procedures for the preparation of 1-fluoroalkylated isoquinolines remain underdeveloped and they are often very substrate specific, low yielding, or require expensive, non-selective and atom non-economical fluoroalkylation methods and/or transition metal catalysis[1,2].

Recently, we published an efficient diverse metal-free one-pot procedure leading to 1-fluoroalkyl-3-fluoroisoquinolines[3] starting from *N*-fluoroalkyl-1,2,3-triazoles via *N*-fluoroalkylketenimines[4]. The reaction is of wide scope with possibility to fuse different aryl or heteroaryl groups to the pyridine ring. Moreover, substituents in position 3 and/or 4 of the isoquinoline ring enables further modifications with S_NAr and/or cross-coupling reactions (Scheme 1). The developed methodologies were used to synthetize the Valiglurax derivative and analogues of TRPM8 antagonists. Additionally, the radioactive ^{18}F labelling of the isoquinoline core is currently under investigation by our collaborators from the Molecular and Medical Pharmacology Department at UCLA, US.



Scheme 1. **Synthesis of 1-fluoroalkylisoquinolines from triazoles**

This work was financially supported by GACR – grant No 23-04659S and from the grant of Specific university research – grant No A2_FCHT_2024_001.

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NOVEL CUMATE-INDUCIBLE MODELS OF MYC-DRIVEN NEUROBLASTOMA ENABLE UNCOMPROMISED MITOCHONDRIAL SYNTHETIC LETHALITY SCREENS

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Mitochondria are vital for cancer cell survival and drug resistance. We recently identified mitochondrial ribosomes as a synthetic lethality target to overcome multidrug resistance in MYC-driven neuroblastoma (NB), a highly aggressive childhood tumor[1]. However, available inducible MYC NB models use inducers that interfere with mitochondrial functions, limiting their use in synthetic lethality screens. Here, we introduce novel cumate (*p*-isopropylbenzoate)-inducible MYC overexpression models to study synthetic lethality, avoiding mitochondrial side effects seen with common inducers like tetracyclines.

First, we confirmed that cumate, even at its highest recommended doses, exhibits no off-target effects on NB cell viability, mitochondrial membrane potential, mitochondrial morphology, and the expression of mitochondrial proteins. This suggests cumate as a preferred inducer for mitochondrial research. Next, MYC cDNA was subcloned into a cumate-inducible lentiviral vector, MYC non-amplified NB cell lines were transduced using lentiviral particles. Established stable cumate-inducible MYC models were characterized by immunoblotting, validating a marked upregulation of c-MYC protein following cumate induction compared with non-transduced and empty vector controls, in both transduced cell lines. Importantly, cell viability assays in these models provided unbiased mechanistic evidence that elevated c-MYC levels sensitize NB cells to cell death upon mitochondrial ribosome inhibition. Protein expression and confocal microscopy analyses revealed that this sensitivity is not driven by differences in mitochondrial stress signaling or morphology but is linked with a dramatic downregulation of c-MYC, observed specifically in cumate-induced MYC-overexpressing NB cells treated with doxycycline.

In conclusion, our cumate-inducible MYC models offer an excellent platform for investigating mitochondrial MYC-dependent synthetic lethality and may facilitate drug repurposing screens for MYC-driven tumors.

Supported by GACR (GA25-16629S) and Grant Agency of MUNI (MUNI/C/1790/2023).

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AN ADVANCED *IN VITRO* BLOOD-BRAIN BARRIER MODEL FOR PREDICTING THE TRANSFER OF POTENTIAL ANTIEPILEPTIC DRUGS

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The blood-brain barrier (BBB) regulates transport between the blood and central nervous system, protecting it from harmful substances but also restricting drug delivery[1]. This challenge arises in pharmacoresistant epilepsy, which is often linked to the overexpression of efflux pumps in BBB endothelial cells and elevated levels of drug-metabolizing enzymes[2,3]. Existing *in vitro* BBB models fail to replicate these structural changes[4]; therefore, we aim to develop a pharmacoresistant *in vitro* BBB model to screen antiepileptic compounds that either bypass or inhibit efflux pumps.

The model is created by co-culturing three human cerebral cell lines – immortalized endothelial cells, astrocytes and vascular pericytes – on transwell inserts. Barrier integrity is monitored *via* long-term transendothelial electrical resistance (TEER) measurements, with fluorescein permeability tested at the end of cultivation to confirm barrier functionality.

Our findings suggest that the most effective BBB model setup involves a contact cell arrangement, with endothelial cells seeded on the apical side and astrocytes and pericytes co-cultured on the basolateral side. Inserts are pre-coated with collagen and further coated with fibronectin (apical side) and poly-L-lysine (basolateral side). The insert size significantly affects barrier integrity, as 24-well inserts yield much lower TEER values than 12-well inserts. Pharmacoresistance in the BBB model is induced by long-term cultivation of endothelial cells in sub-toxic concentrations of antiepileptic drugs, which is expected to upregulate efflux pumps and drug-metabolizing enzymes.

This project was supported by the Technology Agency of the Czech Republic, No. TN02000109, and by the Academy of Sciences of the Czech Republic, grant RVO 61388963.

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TOWARDS THE PHYTOHABITOLS A-C TOTAL SYNTHESIS

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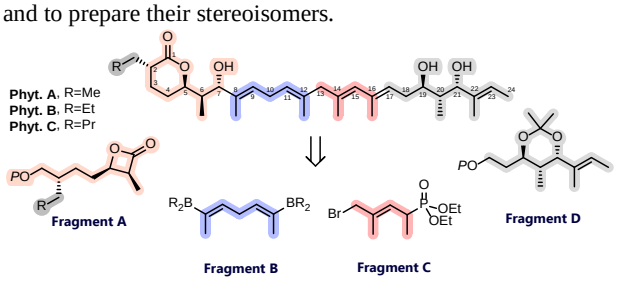
Over the past few decades, actinobacteria have proven to be a reliable source of biologically active substances[1]. To this day, many new biologically active compounds and scaffolds have been isolated. A case in point: Phytohabitols A-C, a trio of δ-lactone polyketides, isolated from actinobacteria of the genus *Phytohabitans*[2]. In initial screenings of their biological activity, these molecules inhibited the migration of cancer cells and displayed promising antitrypanosomal activity[2].

One of the long-term aims of our research group is to study the synergistic effects between polyketides and high-molecular-weight peptides. The ultimate goal is to reveal the mechanism by which polyketides, in the presence of peptides, can enhance their observed biological activity. The first step in these efforts is to achieve a short, reliable, and modular route towards the studied polyketides.

In this contribution, we wish to present our proposed synthetic approach towards Phytohabitols A-C. The proposed approach is designed to be convergent and allows us to stereospecifically install all stereocenters present in the targeted molecules. This approach should also enable us to confirm the absolute configuration of the isolated molecules and to prepare their stereoisomers.



Scheme 1: **Retrosynthesis of Phytohabitols A-C**

This work was supported by the European Regional Development Fund-Project "SMART Plant Biotechnology for Sustainable Agriculture" (No. CZ.02.01.01/00/23_020/0008497), co-funded by the European Union.

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PREVENTION OF IMMUNOTHERAPY-RELATED TOXICITIES AND PREDICTION OF RESPONSE IN PATIENTS WITH TRIPLE-NEGATIVE BREAST CANCER USING IMMUNE PROFILING AND MS FINGERPRINTING

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Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by the absence of estrogen and progesterone receptors and low expression of HER2 receptors, making it difficult to treat with conventional therapies like hormone therapy or targeted therapy[2,4]. Recent progress in immunotherapy is transforming treatment strategies, particularly using checkpoint inhibitors like anti-PD-L1[1,2]. Anti-PD-L1 immunotherapy can regulate immune cell activity to trigger a response against cancer cells. The addition of pembrolizumab, an anti-PD-L1 agent, to neoadjuvant chemotherapy, has demonstrated improved survival rates in clinical trials for advanced-stage TNBC[1]. This synergistic use of pembrolizumab in a neoadjuvant setting can result in improved survival for some patients[1]. However, challenges remain, particularly the need to early predict the incidence of immune-related adverse events (irAEs) and the ability to identify patients who will benefit most likely from the treatment[2].

In this project, we focused on analyzing the immune microenvironment of TNBC to better distinguish between responders who achieve a pathological complete response (pCR) and non-responders. Tumor tissue samples from 20 TNBC patients were analyzed using immunofluorescence staining (IF) to visualize tumor-infiltrating lymphocytes (TILs) and their markers (e.g., CD3, CD4, CD8, and PD-L1 positivity)[2]. Additionally, we employed fluorescence *in situ* hybridization (FISH) to detect stressed T-cells and other critical markers (e.g., HSPA1A, BAG3, and IL7R)[2].

Furthermore, we employed advanced mass spectrometry techniques to predict the patient's response to therapy. We analyzed serum samples from 20 patients with TNBC, along with a control cohort of 20 healthy individuals using MALDI MS to generate patient-specific spectral fingerprints and identify treatment responses and irAEs[3]. We performed multidimensional statistical analyses, such as principal component analysis (PCA) and hierarchical clustering analysis (HCA), alongside machine learning algorithms, including artificial neural networks (ANN) and random forests[3]. Leveraging these analyses, we develop a predictive model for treatment response and irAEs risk.

By integrating these results, we aim to advance the understanding of TNBC's immune microenvironment and develop personalized approaches to improve treatment outcomes while mitigating adverse effects.

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α-SYNUCLEIN CONFORMATIONS FOLLOWED BY VIBRATIONAL CHIROPTICAL SPECTROSCOPY

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α-Synuclein (αSyn) is a neuronal protein linked to Parkinson's disease, where it aggregates into amyloid fibrils. While its function remains unclear, αSyn interacts with neuronal synaptic vesicles, transitioning from an intrinsically disordered state to a partial α-helix upon membrane binding, which protects it from aggregation into β-sheet-rich fibrils[1]. The pathological role of this protein is closely linked to its conformational behavior. However, due to the intrinsic disorder of αSyn, conventional structural methods are often ineffective. To address this, we employed vibrational circular dichroism (VCD) and Raman optical activity (ROA) spectroscopies to probe its structural properties.

We recorded experimental VCD and ROA spectra of αSyn in disordered, α-helical, and fibrillated (β-sheet) states. To interpret the spectral patterns, we performed simulations using molecular dynamics and density functional theory calculations. We found that disordered αSyn adopts polyproline II helix with distinct spectral signatures (Fig. 1). Notably, we observed a strong ROA signal at low frequencies (50-200 cm^{-1}) for the first time. VCD spectra distinguished between different αSyn fibril polymorphs, while fibrils also exhibited an unexplained ROA signal[2].

Our results highlight the value of chiroptical spectroscopy and computational modeling in characterizing αSyn conformations.

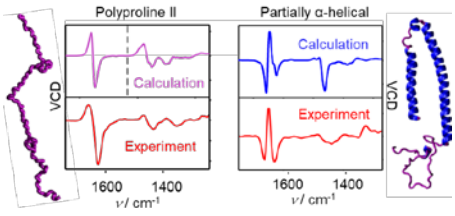

Fig. 1. **Calculated and experimental VCD spectra of αSyn in polyproline II and partially helical conformations measured in D_2O**

The work was supported by the Czech grant agency, 24-10558S.

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SYNTHESIS AND PROPERTIES OF HELICENE-INDENOFLUORENES

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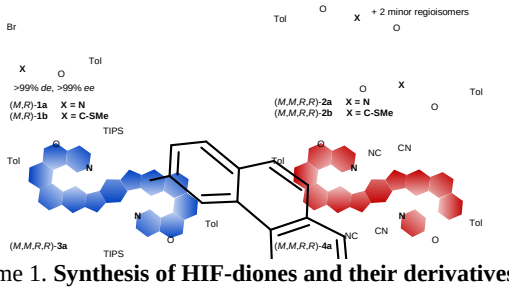
Over the past twenty years, there has been growing interest in efficient organic materials, particularly for use as active layers in devices such as OFETs and OLEDs. Helicene-indenofluorene (HIF) hybrids show significant potential as functional molecular materials. The helicene structure may play a role in chiral-induced spin selectivity and circularly polarized luminescence, while the indenofluorene core – an antiaromatic counterpart to pentacene – can enhance electronic conductivity[1].

Here, we report synthesis and properties of the nitrogen or sulfur modified HIF-diones ***2a*** and ***2b***, respectively, and the corresponding HIF hybrids ***3a*** and ***4a*** (Scheme 1). Helicenes (*M*,*R*)-***1a*** and ***1b*** were prepared with excellent enantio- and diastereoselectivity by diastereoselective [2+2+2] cyclotrimerization of enantiopure aromatic triynes developed in our group[2]. HIF-diones ***2a*** and ***2b*** were synthesized from the corresponding helicenes ***1a*** and ***1b*** and bis(boronic acid pinacol ester) by Suzuki cross-coupling and intramolecular Friedel-Crafts acylation. HIF-diones and their regioisomers showed promising (chir)optical properties, including large Stokes shifts (*ca.* 200 nm) and moderate g_{lum} factors (10^{-3}). The nucleophilic addition of the lithiated TIPS-acetylene to HIF-dione ***2a*** and dearomatization provided HIF ***3a***, while Knoevenagel reaction led to HIF ***4a***. HIF-diones ***2a*** and ***2b*** and their regioisomers, HIFs ***3a*** and ***4a*** will be explored in molecular electronics.



Scheme 1. **Synthesis of HIF-diones and their derivatives**

This work was supported by the EC (Grant Agreement No. 859752), GACR (reg. No. 20-23566S and 24-10787S), and IOCB CAS (RVO:61388963).

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AZAPHTHALOCYANINE FLUORESCENCE QUENCHING ENHANCED BY SUPRAMOLECULAR INTERACTIONS

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Phthalocyanines and their aza-analogues are synthetic dyes that can be used as photosensitizers in photodynamic therapy, fluorescence sensors or fluorescence quenchers depending on the substituents and coordinated metal. For development in the field, it is desirable to study conventional and unconventional mechanisms of quenching to design new and improved analyte-activatable fluorescence sensors and "smart" photosensitizers.

It was reported that photoinduced electron transfer (PET) between a strong electron donor, such as ferrocene, and a phthalocyanine acceptor leads to fluorescence quenching[1]. The additional nitrogen atoms in the structure of azaphthalocyanines increase the electron-deficient character of the macrocycle compared to phthalocyanines. This difference in the electronic properties makes them more susceptible to PET with electron donors.

In this study, we tested quenching of fluorescence of an azaphthalocyanine derivative with ferrocene-based quenchers in acetonitrile. The structure of both the azaphthalocyanine, and the quencher is designed to favour their interaction by additional supramolecular forces (Fig. 1). For this purpose, we chose formation of a charge-transfer complex (CT-complex) based on π-π interactions of an electron-rich and an electron-poor aromatic compounds (derivatives of 2,6-naphthalenediol and viologen, respectively).

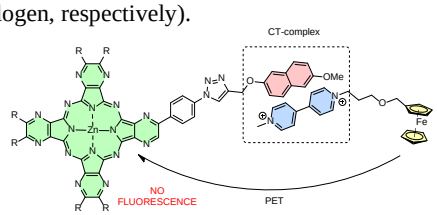

Fig. 1. **The depiction of the quenched CT-complex with the structures of the azaphthalocyanine and the quencher**

The quenching in our system was compared to the control systems lacking the key structural motives and proved to be more efficient.

This work was supported by CUNI GA (No. 170223).

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HOW BINDING SITE FLEXIBILITY PROMOTES RNA SCANNING BY TbRGG2 RRM: A MOLECULAR DYNAMICS SIMULATION STUDY

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RNA recognition motifs (RRMs) are a key class of proteins that primarily bind single-stranded RNAs. In this study, we applied standard atomistic molecular dynamics simulations to obtain insights into the intricate binding dynamics between uridine-rich RNAs and TbRGG2 RRM using the recently developed OL3-Stafix AMBER force field, which improves the description of single-stranded RNA molecules. Complementing structural experiments that unveil a primary binding mode with a single uridine bound, our simulations uncover two supplementary binding modes in which adjacent nucleotides encroach upon the binding pocket. This leads to a unique molecular mechanism through which the TbRGG2 RRM is capable of rapidly transitioning the U-rich sequence. In contrast, the presence of non-native cytidines induces stalling and destabilization of the complex. By leveraging extensive equilibrium dynamics and a large variety of binding states, TbRGG2 RRM effectively expedites diffusion along the RNA substrate while ensuring robust selectivity for U-rich sequences despite featuring a solitary binding pocket. We further substantiate our description of the complex dynamics by simulating the fully spontaneous association process of U-rich sequences to the TbRGG2 RRM. Our study highlights the critical role of dynamics and auxiliary binding states in interface dynamics employed by RNA-binding proteins, which is not readily apparent in traditional structural studies but could represent a general type of binding strategy employed by many RNA-binding proteins.

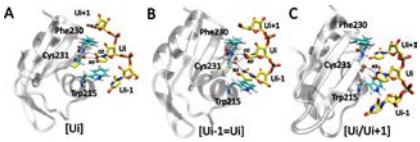

Fig. 1. **Representative structures of the three dominant states of the TbRGG2 RRM-poly(U) binding interface as observed in MD simulations.** (A) Single uridine (observed also in the experiment), (B) the vertical state, and (C) the horizontal state. The H-bonds characterizing each state are indicated with black dashed lines, and the protein is shown as a transparent gray ribbon. To annotate the three states, we use symbolic representations of binding pocket structure, where "[" and "]" refer to the clamp formed by the Trp215 and Phe230 side

chains, respectively, while "=" and "/" indicate vertical and horizontal arrangements of the uridines, respectively. The "i–1," "i+1," and "i±1" indices indicate upstream, downstream, and either of the nucleotides, respectively. This annotation is utilized throughout the study. Where relevant for the description, "i" is replaced with a specific uridine residue number.

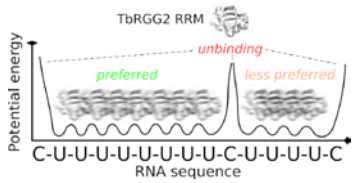

Fig. 2. **Scheme of the suggested equilibrium dynamics of the TbRGG2 RRM bound to U-rich RNA.** The flexible binding pocket allows low-barrier transitions along the continuous poly-(U) regions of the RNA on the microsecond time scale at physiological temperatures, where it will essentially form a single flat, mildly undulated basin on the energy landscape. The fuzzy protein images above the individual poly-(U) stretches indicate the protein is rapidly shifting within the region. Meanwhile, the encounter with non-uridine nucleotide destabilizes the complex and can lead to unbinding and another binding attempt. We suggest this mechanism allows the protein to quickly locate the longest stretches of uridines, which then become the preferred binding sites by entropic effects. Utilization of only a single binding pocket ensures noncognate substrates are rapidly discarded, while the entropically advantageous equilibrium dynamics accessible only for the cognate sequence guarantees high specificity. The one-dimensional free energy plot is merely illustrative, being deduced from the simulation analyses; it is not a result of any direct measurements or computations.

This work was conducted in the sustainability period of the project SYMBIT No. CZ.02.1.01/0.0/0.0/15_003/0000477 as its follow-up activity.

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NEWLY ISOLATED PRENYLATED ISOFLAVONOID FROM *FICUS CYATHISTIPULA* SHOWS ANTI-INFLAMMATORY POTENTIAL IN *IN VITRO* AND *IN VIVO* COLITIS MODEL

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Prenylated (iso)flavonoids were isolated from the roots of *Ficus cyathistipula* Warb. (Moraceae) at the Department of Natural Drugs, Faculty of Pharmacy, Masaryk University. Among these, five compounds were isolated for the first time. Their potential anti-inflammatory effects were investigated in an *in vitro* study. The initial screening was conducted using the human leukemia monocytic cell line THP1-Blue™ NF-κB, stimulated with lipopolysaccharide (LPS). Results demonstrated that seven compounds exhibited the same or greater inhibitory effect on the pro-inflammatory transcriptional factor NF-κB signaling pathway compared to the standard drug prednisolone. Two of the most active compounds were selected for further investigation. The inhibition of nuclear translocation of NF-κB, as one of the potential mechanisms of action, was also investigated. Decreasing effect on NF-κB activity was confirmed by reducing inflammatory cytokine TNFα mRNA levels in macrophages derived from THP1-Blue™ cells. The most active compound, a newly isolated prenylated isoflavonoid, was further evaluated in a 3D *in vitro* model of intestinal inflammation (Fig. 1). This model was based on a co-culture of differentiated Caco-2 cells and THP-1 derived macrophages in a Transwell® system. An inflammatory response was induced using a combination of LPS and interferone gamma (IFNγ), resulting in a decrease in transepithelial electrical resistance. After 24 hours of co-culture, this compound significantly inhibited TNFα mRNA levels in Caco-2 cells. Finally, a pilot *in vivo* study using a mouse model of dextrane sodium sulfate-induced colitis indicated that this compound attenuated clinical manifestation of the disease. The obtained results opened the door for further research of the anti-inflammatory potential of this compound.

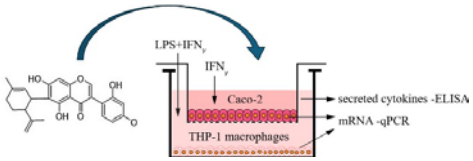

Fig 1. **Newly isolated prenylated isoflavonoid; 3D *in vitro* model of intestinal inflammation**

This work was supported by the GACR (GA23-04655S).

IMPACT OF NEWLY CHARACTERISED L-ASPARAGINASES: A FOCUS ON FOOD INDUSTRY APPLICATIONS

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L-Asparaginases (L-ASNases), a diverse group of enzymes that catalyze the hydrolytic deamination of L-asparagine (L-Asn), have found widespread applications, e.g., in food biotechnology to mitigate toxic acrylamide (AA) formation by reducing the availability of its precursor, L-Asn[1].

With the stricter legislation on AA levels currently under discussion, manufacturers will need to implement new AA-reduction strategies[2]. Rapid detection of L-Asn levels in raw materials, e.g. by L-ASNase-based biosensors, will also be essential[3]. This study, as a part of COST Action CA21149: ACRYRED, an international project addressing the complex challenge of reducing AA formation through multidisciplinary research, is based on our team's bioinformatic analysis, which reflects the abundance, diversity, and typical characteristics of L-ASNases, leading to a project postulating a new classification of L-ASNases to replace the current, inadequate one in the future. Focusing on the less-studied group previously known as *Rhizobium etli*-type L-ASNases, such as from *Paenibacillus thiaminolyticus* and *Arthrobacter polaris*, this study explores their potential for industrial applications, including modification through genetic engineering. These L-ASNases exhibit unique properties, including no glutaminase activity, retained activity after immobilization, and characteristics suitable for biosensor construction.

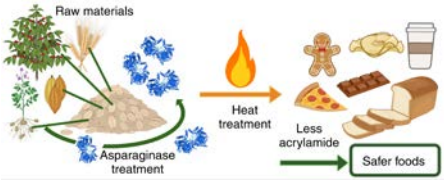

Fig. 1. **Acrylamide mitigation by L-asparaginase treatment** (Created with BioRender.com)

Supported by the grant of Specific university research (grant No A2_FPBT_2024_024, A1_FPBT_2024_001), MEYS (grant No LUC23140), and TA CR (grant No TQ03000738).

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CHIRAL 1,5-DISUBSTITUTED TRIPTYCENES AS PRECURSORS FOR NHC LIGANDS

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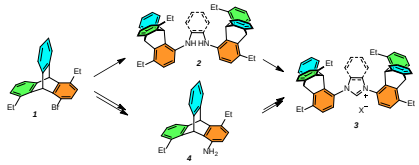
Asymmetric catalysis by transition metal complexes presents a powerful tool for achieving enantioselective transformations. Its success necessitates a continuous development of novel chiral ligands. Due to their unusual D_{3h} symmetry and rigid three-dimensional scaffold, triptycenes have been used in various areas, including molecular machines, functional polymers, host-guest chemistry, medicinal chemistry or catalysis[1,2]. Although some chiral complexes bearing NHC ligands with triptycene units have already been used in enantioselective catalysis[3], their chirality resulted from substituents at the heterocyclic core rather than the triptycene framework itself.

This work deals with the synthesis of chiral NHC precursors with unsymmetrically substituted triptycene units and their use for the preparation of chiral complexes. Based on DFT calculations, the proposed triptycene-based ligands feature a significantly larger buried volume than the carbenes in known chiral PEPPSI catalysts[4]. The key building block of the designed ligands is 4-bromo-1,5-diethyltriptycene (***1***), which can be prepared from anthracene in four reaction steps.

Dihydroimidazolium and benzimidazolium salts ***3*** were prepared from bromotriptycene ***1*** via Buchwald-Hartwig cross-coupling with ethane-1,2-diamine and benzene-1,2-diamine, followed by subsequent cyclization of diamines ***2***. Moreover, bromotriptycene ***1*** was converted into amine ***4***, which was further used for the preparation of imidazolium salt ***3***. In addition, resolution of racemic amine ***4*** was successfully performed using HPLC with chiral stationary phase. All prepared salts were studied for their potential use in the synthesis of transition metal complexes.



Scheme 3. **Synthetic pathway to NHC ligand precursors**

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PULL-DOWN ASSAY, A MOLECULAR BIOLOGY TOOL USED FOR THE IDENTIFICATION OF ENVIRONMENTAL ENDOCRINE DISRUPTIVE POLLUTANTS

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Pull-down assay is a powerful tool for the identification of environmental pollutants causing risk to ecosystems and human health[1,2]. This molecular biology-based technique uses selective protein-affinity purification of ligands from complex mixtures. For the pull-down assay, the quality of *in-house* produced protein designed together with additional tags serving for better protein solubility, folding, protein purification, and pull-down assay is crucial. For this purpose an optimized protein sequence is designed into a plasmid and

transformed into *E. coli*, where the protein is expressed in suitable conditions. Afterwards the protein is isolated and purified using optimized purification buffer for proper protein function. Protein quality is further verified by i.e. binding of standard ligand or an artificial mixture. In pull-down assay ligands specifically bind to the protein's ligand-binding domain, while non-relevant chemicals are washed away during purification steps. As the protein with bound ligands contains a purification tag (His-tag), it is bound to the capturing nickel particles and eluted only by a specific competitor. Before the identification of protein ligands using non-target analysis, protein is chemically degraded (Fig.1).

Pollutants originating from complex environmental mixtures are introduced to the environment by population growth, industrialization, pharmaceutical usage, and agricultural activities. Such pollutants interact with key proteins in the body having an impact on our health through different mechanisms like endocrine disruption (hormonal imbalance) i.e. in case of transthyretin protein (TTR). TTR transports thyroid hormones from thyroid gland to tissues e.g. brain tissue, or fetus. Disruption of this process, caused by competitive binding of pollutants to TTR, is linked to neuro/developmental disorders[3,4,5]. Although TTR inhibition is a widely measured bioassay-based endpoint, identifying specific TTR ligands among environmental pollutants remains insufficient. Applying pull-down assay helps to assess wastewater treatment efficacy and water pollution levels across various low- and high-density urban localities as well as changes in climate or lacking sewage systems and treatment plants (Africa). Beyond TTR, we have expressed and utilized other crucial proteins like androgen receptor or arylhydrocarbon receptor.

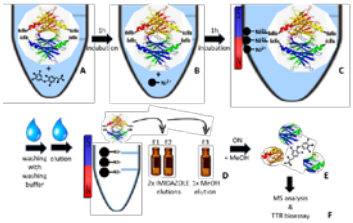

Fig. 1. **Scheme of the pull-down assay**

The project has received funding from GACR under grant agreement GX20-04676X.

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CASTING X-RAYS ON A JACALIN-RELATED LECTIN: A FUN GUY AMONG THE FUNGI

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This study focuses on the investigation of the properties and structural analysis of the lectin, a saccharide binding protein, derived from the mushroom *Calocera viscosa* (CalVL). Mushroom lectins have been extensively studied over the decades for their potential applications in biomedicine and diagnostics. Through their glycan-binding abilities, these lectins demonstrate significant biological activities, including antiproliferative, antimicrobial and mitogenic effects[1].

Lectin CalVL was determined to adopt a β-prism fold I, a structural characteristic shared by all the members of the jacalin-related lectins (JRLs) family. JRLs are typically divided into two groups based on their saccharide preference: gJRLs and mJRLs, preferring D-galactose or D-mannose[2].

CalVL was produced in the *Escherichia coli* expression system and subsequently purified through affinity chromatography using a mannose-agarose resin. Agglutination assays demonstrated that CalVL can agglutinate both yeast cells and human erythrocytes due to the interaction with their surface saccharides. Further analysis of the binding properties by a glycan array resulted in narrow range of biantennary complex *N*-glycans which are likely its optimal binding partners. CalVL was successfully crystallized in various conditions using the vapour diffusion method, particularly the sitting drop technique. X-ray diffraction data were collected at the synchrotrons PETRA III in Hamburg and BESSY II in Berlin. Preliminary structures of CalVL, both in ligand-bound and ligand-free forms, are nearing the completion of the refinement process. The phase problem was solved through molecular replacement, employing a CalVL model predicted by AlphaFold2. To the best of our knowledge, CalVL represents the first structurally characterized fungal JRL among the animal and plant members of this family.

We acknowledge CF Biomolecular Interactions and Crystallography of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042) and Europ. Regional Dev. Fund-Project CZ.02.01.01/00/23_015/0008175.

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RATIONAL APPROACH TO LARGE STOKES SHIFTS IN FLUOROPHORES: EXCITED STATE MOLECULAR GEOMETRY CHANGE IN XANTHENES

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Organic dyes are typically developed using the well-known electronic effects allowing to shift the absorption and emission maxima of the dye as designed. Their Stokes shifts, difference of absorption and emission maxima, are typically small, reflecting very similar geometry of ground and excited states. However, the systems with large Stokes shifts are important in high-resolution techniques, e.g., multicolor imaging. Up to now, the strategies on how to increase the Stokes shift are hardly explored and rarely mentioned in the literature.

In this work, we focused on 9-iminopyronins with large Stokes shifts[1] and we are separating the effect of 9-acyl-imino group (in position 9, Fig. 1) from the bridge group (position 10). The –O– bridge at position 10 has no significant effect on the photophysics of xanthene, unlike sp^3 bridge, e.g., –$Si(CH_3)_2$ (cit.[2]).

Our combined spectroscopic and theoretical approach allows understanding of the structure-property relationships in xanthene fluorophore and allow to set out the step-by-step rules for design of the xanthene-related dyes with large Stokes shifts upon: C=N exocyclic bond length change; central ring puckering; and reversible protonation[3].

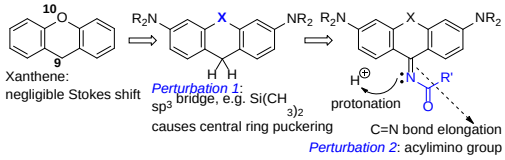

Fig. 1. **A combination of two perturbations introduced to xanthene core led to xanthene fluorophore with rationally facilitated increase of Stokes shifts**

This work was supported by the GACR (GJ20-30004Y), CETOCOEN EXCELLENCE Teaming 2 (CZ.02.1.01/0.0/0.0/17_043/0009632 and EU H2020: 857560), and RECETOX RI (LM2023069).

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TARGETING MULTIDRUG RESISTANCE IN BREAST CANCER: THE POTENTIAL OF MONOTERPENE-INDOLE ALKALOID DERIVATIVES AS P-GLYCOPROTEIN INHIBITORS

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Breast cancer is the most frequently diagnosed cancer among women and the leading cause of cancer-related deaths globally. Despite notably advances in therapy options, chemotherapy continues to be the primary method of treatment[1]. However, the effectiveness of breast cancer chemotherapy is often compromised by multidrug resistance (MDR)[2]. A major contributor to MDR is the overexpression of P-glycoprotein (P-gp). This protein actively pumps out chemotherapeutic agents, reducing their accumulation inside cells and diminishing their cytotoxic effects[3]. Considering that monoterpene-indole alkaloids (MIAs) have been recognized for their potential antitumor properties and ability to modulate MDR4, we prepared and evaluated twenty-one MIA derivatives as potential chemotherapy adjuvants. We assessed their capacity to enhance the effectiveness of doxorubicin in breast cancer cells that overexpress P-gp (MCF-7R) at concentrations of 10, 1, and 0.1 µM. The compounds were tested for their ability to lower the IC_{50} values of doxorubicin. P-gp inhibition was measured using the rhodamine 123 accumulation assay. The most effective derivative underwent further testing in homotypic and heterotypic spheroid models to more accurately replicate *in vivo* tumor conditions. Ten MIA derivatives showed significant chemosensitizing effects, with the most potent compound decreasing doxorubicin IC_{50} by 7.5-fold in MCF-7R cells. The rhodamine 123 assay validated their P-gp inhibitory activity, and their effectiveness in both spheroid models further underscored their potential to combat MDR in breast cancer. These results emphasize the therapeutic promise of MIA derivatives as adjuvants to chemotherapy and present a new strategy to enhance treatment outcomes in resistant breast cancer.

This project was supported by Fundação para a Ciência e a Tecnologia (FCT), Portugal (project: 2022.05718.PTDC).

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NEW RNAS THAT REGULATE TRANSCRIPTION IN ACTINOBACTERIA

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Actinobacteria represent one of the largest taxonomic groups within the bacterial kingdom. Despite our extensive knowledge of the secondary metabolites, especially antibiotic compounds produced by this phylum, our understanding of the transcription machinery and its regulation remains limited.
The key enzyme involved in bacterial transcription is the RNA polymerase (RNAP). RNAP is a multi-subunit enzyme composed of two large subunits, beta and beta´, two alpha subunits and omega. This complex is called RNAP core.
For the recognition of specific promoters in the genome, the RNAP core needs to be associated with a sigma factor. The complex of RNAP core with sigma factor is called RNAP holoenzyme.

Bacterial transcription can be regulated by small RNAs that directly interact with RNAP core or holoenzyme. Two such RNAs, 6S and Ms1, have been identified so far. 6S RNA is ~ 180 nt RNA that binds the RNAP holoenzyme and has been discovered in *E. coli*. Later, 6S RNAs were found in many other bacterial species, e. g. *Bacillus subtilis* (6S-1 and 6S-2 RNAs), *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Staphylococcus*, *Streptococcus*, but not in actinobacteria.

In *Mycobacterium smegmatis*, which belongs among actinobacteria, Ms1 RNA has been identified. Ms1 is a sRNA with a length of ~300 nt that bind RNAP core in *M. smegmatis*.

Recently, a novel type of similar RNA was discovered in our lab in the actinobacterial species *C. glutamicum*. We have found a new, 516 nt structured RNA and called it CoRP RNA (Corynebacterium RNA polymerase binding RNA)[1]. CoRP can bind both the RNAP core and the RNA polymerase holoenzyme with the primary sigma factor SigA. I showed that *C. glutamicum* lacking CoRP RNA has impaired growth and reduced RNA polymerase levels compared to the wild type.

I also use RNA immunoprecipitation with Illumina Next-Generation Sequencing (RIP-seq) to reveal if any other, new type of RNAP-associated RNA exist in other actinobacterial species (*B. bifidum*, *Micrococcus luteus*, *Micromonospora qiuiae*) in which no such RNA has been identified yet. In addition to regulatory RNAs binding to the transcription machinery, I also detect proteins that co-immunoprecipitate with RNAP or the primary sigma factor. In *C. glutamicum*, I found that RNAP associates with phosphofructokinase, an enzyme involved in glycolysis, suggesting a direct link between transcription and carbon metabolism.

RIP-seq can be potentially applied to all the bacterial species and will increase our knowledge about bacterial transcription and its regulation.

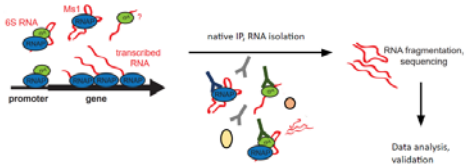

Fig. 1. **Graphical scheme representing the workflow of the RIP-seq approach**

This work was supported by grant No. 23-05622S (GACR).

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A ROLE OF PLANAR POLARITY SIGNALING IN ACTOMYOSIN CONTRACTILITY DURING VERTEBRATE NEURULATION

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Neurulation is an essential process during vertebrate development, in which a precursor of the brain and spinal cord is created. Its significance is highlighted by the fact that in the Western world, approximately 0.5–2 per 1000 pregnancies end by death because of neural tube defects[1].

The process of neurulation normally starts as a plain layer of cells, which undergoes huge rearrangement and bending to create a 3D structure of highly organized tissue, i.e., the neural tube. To create such a complex structure, cells must appropriately change their shape. First, they shrink on their apical surface, which is a process called apical constriction (ApCo)[2]. On the molecular level, this change of shape is mediated by cytoskeletal proteins such as actin and myosin that together generate a mechanical force. This mechanical force is predominantly generated by the conformational change of the myosin light chain (MLC), which is triggered by its phosphorylation[3]. Although the mechanism of phosphorylation itself in many cases is well described, it is still not well understood, which signaling pathway triggers this process during vertebrate development. In this work, we focus on the family of WNT/Planar Cell Polarity (PCP) proteins as the most likely candidates for triggering neurulation in vertebrates, pictured in Fig. 1.

PCP is a protein-mediated signaling characterized by an asymmetrical distribution of proteins in the cell. This serves in organisms as a compass because one protein complex is localized anteriorly (towards the head) meanwhile the other protein complex has posterior localization (towards the tail) in the neural plate. Since this asymmetry is established before neurulation starts, PCP proteins are good candidates for initiating the folding process[4].

Specifically, this work aims to discover whether and how PCP proteins can induce phosphorylated MLC (pMLC) in order to cause apical constriction and therefore trigger the process of neurulation. Here, we show that cytoplasmic PCP proteins Prickle and Dishevelled not only interact with MLC, but they can induce pMLC, which is necessary for a change of cell shape. This phosphorylation of MLC occurs via Casein kinase 1 ε and Rap1 GTPase activating protein 2 (Rap1GAP2)[5]. Our model is based on our experimental data in MDCK cells and in the neural plate of *Xenopus* embryos.

In sum, our work is connecting the PCP signaling pathway with a specific actomyosin-driven process in vertebrate development.

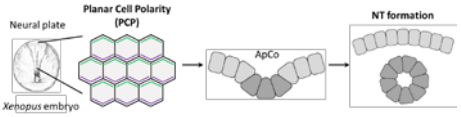

Fig. 1. **Planar Cell Polarity proteins are candidates for triggering the neural tube formation**

I would like to thank the Erasmus students A. Hernandez Ramos and C. Gonzalez for their help with preliminary data and Dr. A. Smolenski for providing the Rap1GAP2 plasmid.

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UTILIZING MULTI-OMICS APPROACHES TO INVESTIGATE THE RESISTOME OF THE CHICKEN GUT MICROBIOME

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Antimicrobial resistance (AMR) poses a global challenge, threatening human health, food security, and modern medicine[1]. Over six decades, widespread antibiotic use in human and veterinary medicine has accelerated AMR development, driven by horizontal gene transfer facilitated by mobile genetic elements (MGEs) like plasmids. MGEs enable bacteria to exchange antibiotic resistance genes (ARGs) and adapt to environmental pressures, outcompeting other microorganisms[2]. Antibiotic use selects for resistant bacteria, creating ARG reservoirs or resistomes, which are dynamic, diverse, and often poorly characterized. These resistomes can mobilize over time, leading to multidrug-resistant bacteria of clinical significance. Moreover, heavy metals and disinfectants in livestock farming environments can co-select for ARGs and resistance genes for metals and biocides, promoting persistence and dissemination[3,4].

Animal gut microbiomes, particularly in livestock like chickens and swine, are key yet underexplored ARG reservoirs. These ARGs may transfer to humans through pathways like animal waste. In poultry, sterile hatcheries create niches for bacterial colonization, including pathogens acquiring ARGs. Decades of antibiotic use in farming have intensified this, despite EU bans on growth promoters (2006) and preventive antibiotics. Global farming practices still sustain diverse resistomes in animal microbiomes[5,6].

Advancements in molecular approaches have enhanced understanding of AMR mechanisms. Metagenomics analyzes genetic material from environmental or host samples, identifying uncultivable microorganisms and novel ARGs. Whole-genome sequencing (WGS) reveals genomes, identifying ARGs, MGEs, and transfer pathways. Metaproteomics links expressed proteins to phenotypic resistance traits, while metabolomics profiles microbial metabolites, offering insights into resistance-driven biochemical changes and potential biomarkers or therapeutic targets[7].

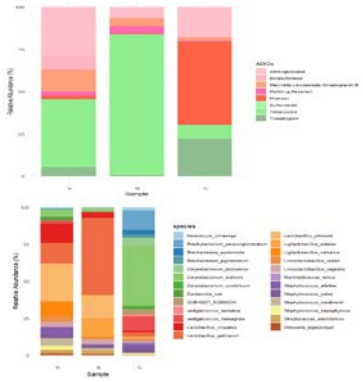

Fig. 1. **Preview of metagenomic analysis of chicken feces. All samples were derived from the same barn, showing the dynamics of ARGs and microbiota abundance at the ages of 2 (A), 3(B), and 4 (C) weeks of life**

In this project, 44 chicken broiler feces samples were collected weekly over five weeks from three barns in a Czech commercial farm (broiler lifespan: ~40 days). DNA was sequenced using shotgun metagenomics (10 GB/sample). ARG presence and abundance were assessed via KMA mapping to the PanRes database, which indexes resistance genes for antibiotics, heavy metals, and biocides. Preliminary analysis revealed a diverse resistome, predominantly linked to Tetracycline, Aminoglycoside, and Phenicol classes. Microbial community profiling, conducted using MetaPhlAn v2, identified species such as *Lactobacillus*, *Ligilactobacillus*, and *Corynebacterium* as most prevalent. Microbial and resistome profiles were dynamic, influenced by chicken health, diet, environment, and parental health, underscoring complex interactions between microbes and resistance mechanisms. The results show the dynamic nature of antimicrobial resistance in livestock gut microbiomes, highlighting the influence of various environmental and

biological factors on the dissemination and persistence of ARGs.

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DECODING THE BIOSYNTHETIC PATHWAY OF THE ANTICANCER ALKALOID PIPERLONGUMINE IN *P. FIMBRIULATUM* PLANT

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Piperaceae plants are rich sources of bioactive alkaloids with anticancer properties. *Piper fimbriulatum* is a member of *Piperaceae* family from Central America living in symbiotic life with *P. bicornis* ants. By exploring *P. fimbriulatum* alkaloid chemodiversity using mass spectrometry and computational tools we discovered that this plant produces piperlongumine, an alkaloid that exhibited anticancer activities in numerous preclinical studies. Piperlongumine inhibits the GSTP1 enzyme, causing accumulation of ROS and consequently inducing apoptotic and autophagic cell death selectively in cancer cells. Recently, this molecule also demonstrated selective anti-COVID activity in a mouse model. We discovered that besides piperlongumine, the plant produces diverse piperlongumine analogues and cyclobutane dimers. Decoding their biosynthetic pathways would enable production of piperlongumine and its derivatives using bioengineering as a more sustainable strategy compared to traditional technologies. Following our biosynthetic hypothesis based on the known biosynthetic pathway of piperine, we initiated our search from the amidation step. We presumed that in the case of piperlongumine, amidation could be carried out by an enzyme homologous to the BAHD acyltransferase piperine synthase. To find the enzyme responsible for amidation, we approached RNA isolation to obtain the transcriptomes of various tissues of *P. fimbriulatum*. After obtaining mRNA sequencing data, we assembled their transcriptomes *de novo*. We further used homology search to identify BAHD candidate enzymes. We are currently conducting their functional characterization using agrobacterium-mediated heterologous expression in tobacco plants.

We thank Klára Lorencová, Petr Vacík and Ludvík Bortl from the Prague Botanical Garden for their support with the sample collection. T.P. was supported by the GA CR grant 21-11563M and by the EU's Horizon 2020 research and innovation programme under MSC grant agreement No. 891397.

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INVESTIGATING THE ROLE OF SORLA IN ALZHEIMER'S DISEASE USING INDUCED NEURONS

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting millions of people worldwide. The hallmarks of this disease are the accumulation of amyloid beta (Aβ) and the presence of hyperphosphorylated neurofibrillary tangles of tau protein[1]. Mutations in *APP* and *PSEN1/2* genes are known to cause a familiar form of AD. Recent studies showed a connection between pathological variants of *SORL1* and AD development. Therefore, *SORL1* is now considered the "fourth" AD-causing gene[2].

Protein SORLA, encoded by the *SORL1* gene, is an intracellular sorting receptor, and one of its functions is regulating the amount of amyloid precursor protein (APP) and degradation of pathological Aβ[3]. Clinical studies of patients with AD discovered many different mutations of SORLA; however, it remains unknown how they differ and what is their effect on AD[4].

This project investigates the impact of p.G1732A mutation of the SORLA using *in vitro* neuronal models. We hypothesize that this mutation disrupts its function and can lead to AD development. To verify this hypothesis, we introduced p.G1732A mutation of SORLA into induced pluripotent stem cells (iPSCs) using CRISPR/Cas9 technology. This specific mutation was selected according to previous studies published by *Andersen et al.* describing p.G1732A as highly likely risk-increasing for AD[5]. We then differentiated obtained iPSC lines into 2D neurons and analyzed the effect of p.G1732A on SORLA maturation, shedding and localization in cell compartments. Moreover, we observed the early pathological hallmarks of AD, such as the

production of Aβ, endosomal swelling and accumulation of APP in early endosomes. These preliminary data confirm that altered SORLA function directly causes the formation of AD-specific hallmarks and thus potentially leads to the development of AD.

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IRrIS – A VERSATILE BIOIMAGING TOOL INSPIRED BY A NATURE-EVOLVED BIOLUMINESCENCE RESONANCE ENERGY TRANSFER COMPLEX

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Bioimaging is an essential tool for biotechnology and biomedicine, using biological light for *in vivo* real-time imaging of gene expression, biomolecular interactions, disease progression and a plethora of further otherwise hard-to-visualise biological phenomena. Nevertheless, fluorescence and bioluminescence entail drawbacks, such as the need for intensive sample-damaging excitation radiation, a capricious enzyme reaction yielding light with spectral characteristics restrictive for *in vivo* use or high measurement background due to autofluorescence of biological samples[1,2].

Bioluminescence resonance energy transfer (BRET), an efficient mechanism enabling the powering of a fluorescent protein light output by a luciferase reaction, is the most promising solution to the presented problems. However, native BRET is poorly understood. Thus, the state-of-the-art BRET--based systems were created through trial-and-error, resulting in low efficiency of the BRET, wasting the supplied energy[2].

We report the first-ever experimentally characterised native macromolecular BRET complex, which we rationally engineered into a standalone and fully genetically encodable BRET bioimaging probe – IRrIS. Our system uses a generic, affordable luciferin, requires no cofactors, and features bright, long-lasting, green emission with the possibility of its further redshift, ~100 % energy transfer efficiency and high functional stability, enabling its reliable use *in cellulo* or *in vivo*. Furthermore, the simple composition of the system, comprising a typical beta-barrel fold fluorescent protein and a well-behaved cnidarian luciferase put together creatively, ensures high flexibility and a good engineerability of IRrIS.

This combination of properties, achieved for the first time in a BRET-based system, predetermines IRrIS as a superior bioimaging probe and a pioneering template for the construction of a new generation of bioimaging probes or highly orthogonal building blocks for synthetic biology bearing the property of customisable BRET-based bioluminescence.

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PROTEOMIC ANALYSIS REVEALS THE ROLE OF CREATINE KINASE B IN REGULATING CELL MIGRATION IN OSTEOSARCOMA

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Creatine kinase B (CKB), a key metabolic enzyme involved in energy supply in tissues with high demand, exerts multifaceted influence on cellular processes, including cell cycle regulation or immune system function. Altered CKB expression has been observed in various cancer types. However, the precise function of CKB in regulation of growth and metastatic activity of osteosarcoma cells is still not yet fully understood[1].

The study aims to clarify the role of CKB in osteosarcoma progression. We derived SAOS-LM5 osteosarcoma cells with depleted expression of *CKB* using the CRISPR/Cas9 approach. Subsequently, we examined the effect of CKB deficiency on growth, colony forming capacity, migration, and metastasis of these cells. As a complementary approach, CKB expression/activity was transiently inhibited using siRNA or cyclocreatine (CCr). Afterwards we performed proteomic analysis of control and *CKB* depleted/inhibited cells to identify down-stream molecules of CKB.

Depletion of CKB expression using CRISPR-Cas9 resulted in slower cell migration *in vitro* and lower metastatic ability in immunodeficient mice. In contrast, it didn´t affect proliferation and chemosensitivity of SAOS-LM5 cells. Acute inhibition by CCr or siRNA resulted in decrease of both, cell proliferation and migration. Proteomics and follow-up Gene Ontology (GO) analysis identified proteins involved in regulation of cell migration, cell adhesion or cytoskeleton organization as a possible down-stream molecules of CKB. One of them, N-cadherin, a mesenchymal marker associated with poor prognosis and more frequent metastases in osteosarcoma, was confirmed as a target of CKB using both CKB depletion/inhibition.

Our findings suggest that CKB is an important factor affecting the migration and metastasis of osteosarcoma cells and can be used as a therapeutic target in the future.

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CHIRAL TRIPTYCENE DERIVATIVES AS LIGANDS FOR TRANSITION METAL COMPLEXES

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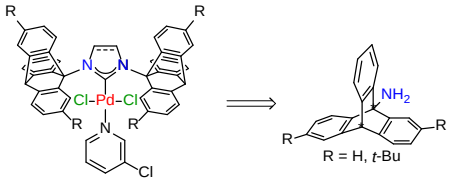
Stereoselective synthesis of enantiomerically pure compounds is crucial for production of pharmaceuticals, agrochemicals and other fine chemicals. This synthesis is usually achieved by means of transition metal catalysts bearing chiral ligands[1]. Phosphines could be considered the most used ligands and although they are very powerful and versatile in their performance, unfortunately, they are prone to undesired oxidation by air. As an alternative to phosphines, chiral *N*-heterocyclic carbene ligands (NHCs) are often used for asymmetric synthesis[2]. PEPPSI catalysts, i.e., palladium complexes bearing both an NHC ligand and a pyridine ligand, have found their application mainly in Suzuki-Miyaura cross-coupling reactions[3]. However, an asymmetric version of this reaction using modified PEPPSI catalysts to produce axially chiral biaryls remains to this day a herculean task[4].

NHC ligands based on chiral triptycene derivatives are attractive building blocks for the construction of asymmetric PEPPSI analogues, due to triptycene bulkiness and rigidity. These ligands could be easily generated from the corresponding (dihydro)imidazolium salts. In this work we have developed straightforward synthesis of a dihydroimidazolium salt containing achiral triptycene units (R = H) starting from 9-bromoanthracene and using a microwave assisted Diels-Alder reaction with benzyne precursor, 2-(trimethylsilyl)phenyl triflate, as the key step. Moreover, a chiral triptycen-9-amine derivative (R = *t*-Bu) was successfully synthesized in five steps from anthracene. Preparation of triptycene based palladium complexes is now in progress.



Scheme 1. **Triptycene based PEPPSI analogues and their building blocks**

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HYALURONIC ACID-MOLYBDENUM CLUSTER CONJUGATE: NEW STRATEGY IN DELIVERY OF MOLYBDENUM CLUSTERS INTO THE CANCER CELLS

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Hyaluronic acid (HA) is a naturally occurring polysaccharide in the human body. Composed of D-glucuronic acid and *N*-acetyl-D-glucosamine linked by β-glycosidic bonds, HA is a key component of the extracellular matrix and is renowned for its excellent biocompatibility. HA's ability to bind with the CD44 antigen, overexpressed in various cancers such as breast cancer, enables targeted drug delivery with high specificity[1]. Due to its polar nature, it is readily soluble in water and can act as a carrier for photosensitizers such as molybdenum clusters (Mo_6).

This study used hyaluronic acid to develop a novel formulation of molybdenum clusters (Mo_6). These transition metal complexes consist of six molybdenum atoms forming an octahedral core, surrounded by eight inner iodine atoms and six apical ligands, which are exchangeable. Molybdenum clusters possess unique luminescent properties, with emission in the red-near infrared region, and the ability to produce singlet oxygen upon illumination in the visible/UV region, making them promising candidates for photodynamic applications. Notably, they can be excited not only by light but also by X-rays, offering a versatile platform for X-ray-induced

photodynamic therapy, expanding their potential in advanced medical treatments[2,3].

For the preparation of the HA-Mo_6 conjugate, we utilized the sodium salt of hyaluronic acid (1,600 kDa), which was depolymerized into lower molecular weight chains using sonication. The conjugation process involved a nucleophilic substitution reaction, where the iodine atom in $(TBA)_2[Mo_6I_{14}]$ was replaced through interaction with the carboxylic groups of hyaluronic acid, resulting in the formation of a stable HA-Mo_6 complex. The uptake, subcellular localization, toxicity, phototoxicity, and radiosensitizing effect of this novel material will be described.

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TROPOMYOSIN 2 ISOFORM TPM2.3 IS A TUMOUR SUPPRESSOR AND A GOOD PROGNOSTIC FACTOR IN OSTEOSARCOMA

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Osteosarcoma, the most common primary malignant bone tumour in children and adolescents, is characterized by a high risk of disease recurrence and metastasis. While many genes contribute to the development of osteosarcoma, reliable markers for predicting its progression remain unidentified.

Tropomyosin 2 (Tpm2) is a coiled-coil protein that polymerizes along actin filaments and regulates their organization and dynamics. Tpm2 has been associated with tumorigenesis and progression of various cancer types[1,2]. However, its role in osteosarcoma has not been described, yet.

This study examines the expression of Tpm2 variants in non-metastatic and highly metastatic osteosarcoma cell lines. Tpm2.3 is one of the dominant isoforms expressed in osteosarcoma cell lines, with lower levels in highly metastatic cells. Tpm2.3 overexpression inhibited tumour growth and lung metastases in immunodeficient mice and reduced cell proliferation and migration *in vitro*. Moreover, higher expression of Tpm2.3 slightly increased sensitivity to doxorubicin, a widely utilized chemotherapeutic agent in osteosarcoma therapy.

Analysis of Tpm2 expression in patient tissue samples demonstrated large interindividual heterogeneity. High Tpm2 expression correlates with better prognosis of the disease.

These findings suggest that Tpm2.3 acts as a tumour suppressor in osteosarcoma and Tpm2 expression could be considered a prognostic factor in this type of bone tumour.

This work is a joint research project supported by the Czech Science Foundation, grant no. 23-06303K and National Science Centre, Poland, grant WEAVE-UNISONO project number 2022/04/Y/NZ5/00064

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DYSREGULATION OF microRNA IN WALDENSTRÖM MACROGLOBULINEMIA

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Waldenström macroglobulinemia (WM) and multiple myeloma (MM) are both hematological malignancies. Despite the different cellular origins—WM is a lymphoplasmacytic lymphoma, and MM is a malignancy of plasma cells—these diseases may present overlapping clinical features[1,2].

MicroRNAs (miRNAs) are short, non-coding RNAs that play a role in gene regulation and have been implicated in the pathogenesis of various cancers. Moreover, miRNAs have the potential to serve as biomarkers that not only help differentiate between WM and MM but also provide insights into their underlying molecular mechanisms[3].

Our study aimed to identify differentially expressed miRNAs between bone marrow (BM) cells from WM and MM patients. Small RNA-seq was employed to profile miRNA expression. Differentially expressed miRNAs were further validated using RT-qPCR. Additionally, BM cells from WM patients were analyzed for gene mutations, CNVs, cnLOH, deletions, translocations, and rearrangements using the custom LYmphoid neXt-Generation Sequencing (LYNX) panel.

Small RNA-seq identified eight differentially expressed miRNAs ($p < 0,01$) between WM and MM (Fig. 1). RT-qPCR validation was performed on a new dataset of patients. Certain miRNA expression levels showed significant correlations with clinical parameters, suggesting their potential roles in disease severity and progression.

Furthermore, analysis of WM samples revealed the presence of the *MYD88* mutation in a high proportion of samples, confirming its potential role in disease pathogenesis.

This study highlights the distinct miRNA expression profiles in WM compared to MM, with specific miRNAs correlating with clinical parameters indicative of disease severity and progression. Additionally, the frequent occurrence of the *MYD88* mutation in WM patients further differentiates WM from MM. The findings suggest that miRNA profiling with genetic mutation analysis, can enhance the understanding of the molecular mechanisms underlying WM and MM. This could lead to improved diagnostic accuracy and personalized treatment approaches.

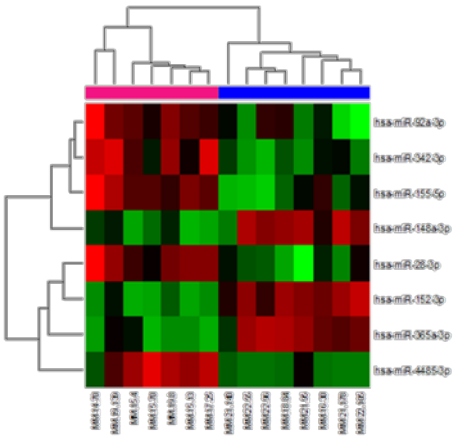

Fig. 1. **Heatmap and dendrogram of 8 differentially expressed miRNAs between WM and MM**

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FROM PROBIOTICS TO NANOCARRIERS: UNLOCKING THE MEDICAL POTENTIAL OF MEMBRANE VESICLES

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Membrane vesicles (MVs) are nanoscale lipid bilayer particles secreted by Gram-positive bacteria, offering a safe and non-replicative alternative to live probiotics. These vesicles are non-infectious, can deliver bioactive molecules in controlled doses, and maintain immunomodulatory properties, making them ideal candidates for medical applications such as nanocarriers and vaccine platforms. However, variations in MV characteristics across bacterial growth phases have hindered their standardization and clinical application.

In this study, MVs were isolated from *Lacticaseibacillus rhamnosus* CCM7091 cultures at three distinct growth phases: early exponential (6h), late exponential (12h), and late stationary (48h). MVs were characterized using advanced biophysical techniques (MADLS, Cryo-EM) and proteomics. Their immune-boosting properties were assessed *in vitro* through cytokine production (TNFα, IL-6, IL-10) in macrophages, and the role of lipoteichoic acid (LTA) in Toll-like receptor 2 (TLR2) signaling was evaluated[1].

The MVs from the late stationary phase (MV48) exhibited the most robust immune-boosting properties, significantly increasing cytokine production and nitric oxide release in macrophages. Proteomic analysis revealed distinct protein profiles in MV48, including elevated levels of LTA, which was identified as a key immunomodulatory molecule driving TLR2 activation. Additionally, MV48 uptake by intestinal epithelial cells was significantly higher, highlighting their suitability for targeted delivery.

Our study demonstrates that the bacterial growth phase critically impacts the composition and functionality of MVs. The enhanced immunomodulatory properties and safe, non-replicative nature of late-phase MVs position them as a promising therapeutic platform for applications in drug delivery, immune modulation, and vaccine development. These findings bridge microbiology, molecular medicine, and nanotechnology, advancing the potential of MVs to address global health challenges.

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AUTOMATED GC-MS FOR PARALLEL VOLATILE PROFILING & FATTY ACID QUANTIFICATION

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Accurate quantification of short and branched-chain fatty acids (S&BCFA) is desired for their pivotal role in health & disease. A fully automated gas chromatography-mass

spectrometry (GC-MS) assay integrating robotic sample preparation in-line with MS data acquisition is presented.

Serum/plasma samples are extracted via liquid-liquid extraction using a Thermo TriPlus RSH coupled with single quadrupole GC-MS. Extracts (4 uL) are injected into a programmable temperature vaporization (PTV) inlet & analytes are separated on the RTX-WAX column. Electron ionization (70eV) used & MS operated in full-scan single ion monitoring (FS-SIM) mode (scan 40–250 m/z), with ≥2 ions per S&BCFA.

Nine S&BCFA are absolutely quantified with a sample-to-sample runtime of 18 minutes (Fig. 1), i.e., 80 samples/24h.

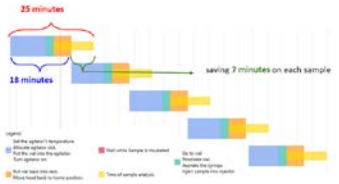

Fig. 1. **Overlapping sample preparation with MS data acquisition saves 7 minutes analysis time per sample. Blue: automated sample preparation; turquoise: aspiration of extract & injection; orange & yellow: MS data acquisition**

Key features include i) PTV injector for increased analyte loading, reducing sample volume needs, ii) direct measurement of native S&BCFAs upon RTX-WAX column, iii) high sensitivity target analysis in parallel with volatile profiling via FS-SIM mode, iv) in-line automation that reduces runtime, operational costs and manual labor which minimizes handling errors, improves reproducibility and provides potential for easier adoption & replication in clinical laboratories for routine analysis.

Authors thank the RECETOX Research Infrastructure (No LM2023069) financed by the MEYS for supportive background. The work was supported from Programme JAC - project SALVAGE (CZ.02.01.01/00/22_008/0004644) financed by the MEYS – Co-funded by the EU.

FLAVIN-CATALYZED OXIDATIVE COUPLING OF SILYL ENOL ETHERS

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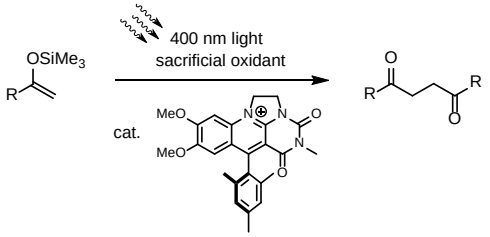
The use of light in chemical reactions allows the delivery of a large amount of energy in a targeted manner. Photoredox catalysis uses a catalyst to scavenge the light's energy to provide an oxidation or a reduction of a substrate. This enables us to perform otherwise difficult transformations under mild conditions, without harsh reagents and in a more sustainable manner[1].

The oxidative coupling of silyl enol ethers provides a path to the synthetically valuable motif of 1,4 diketones from easily accessible starting materials. Although several oxidative coupling methods have been reported[2-4], metal-free approaches have generally been underexplored and, where tested, have produced subpar results[4]. Here, we propose a novel photo-organocatalytic system that uses flavin derivatives as a prospective and highly modifiable group of catalysts[5].

This work comprises reaction conditions optimization, choosing of a suitable flavin catalyst, reaction scale-up and scope with different silyl enol ethers and silyl bis-enol ethers and a discussion of reaction mechanism. On the whole, we provide a non-metal-based alternative for the oxidative coupling of silyl enol ethers on a preparative scale while expanding the scope of flavin catalyzed reactions.



This work was supported by the GACR (GA21-14179S) and Specific university research (IGA A2_FCHT_2023_050).

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INTEGRATING ISOTHERMAL AMPLIFICATION TECHNIQUES AND LNA-BASED AI-ASSISTED ELECTROCHEMICAL BIOASSAY FOR ANALYSIS OF KRAS G12V POINT MUTATION

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Point mutations in the *KRAS* gene have been identified in a variety of cancers, including colorectal (CRC), lung and pancreatic tumour. They are often associated with poorer prognosis and resistance to treatment[1]. The G12V mutation in colorectal cancer serves as a predictive biomarker for targeted T-cell receptor (TCR) therapy, which enables the development of TCRs that can recognise and target cells with this mutation[2].

Standard methods for detecting point mutations in the *KRAS* gene include e.g., next-generation sequencing (NGS), amplification refractory mutation system (ARMS) or real-time PCR[3,4]. However, these methods are associated with several

disadvantages, such as high cost, expensive instruments, time consumption, need for complex data analysis or sensitivity to reaction inhibitors. An interesting option can be isothermal amplification techniques, which tend to be fast, simple, sensitive and inexpensive to equip[5].

In this study, we present a bioassay that enables rapid and reliable detection of the G12V mutation in the *KRAS* gene using isothermal rolling circle amplification (RCA) technique for probe-mediated selective amplification of either wild-type or G12V mutant targets. This is then combined with electrochemical readout employing high-affinity LNA capture probes to bind the amplified product. Streptavidin-horseradish peroxidase polymer, which can bind biotin incorporated into the target DNA during the RCA reaction, can then catalyse an enzymatic reaction, which is detected amperometrically. Finally, we present an artificial intelligence (AI) model using logistic regression classifier for human-free discrimination of the presence or absence of the G12V mutation. A major advantage of our study is that in addition to synthetic targets, we applied the bioassay to 7 cancer cell lines and 11 CRC patient samples. The results obtained were in complete agreement with the data from the previous NGS analysis of the patient samples.

Our proposed bioassay has good analytical sensitivity with the limit of detection of 61 pM, excellent selectivity towards the G12V mutation and the ability to detect < 1% of G12V sequence in the excess of wild-type sample, making it suitable for possible future liquid biopsy analysis. Employment of clinical samples is what sets our study apart from most electrochemical papers.

The financial support of the CHRC (No. NU21-08-00078), GACR (25-15990S), project SALVAGE (OP JAC; CZ.02.01.01/00/22_008/0004644) – co-funded by the EU and by the State Budget of the Czech Republic, BBMRI.cz (No. LM2023033) and MH CZ – DRO (MMCI, 00209805) is greatly acknowledged.

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TISSUE ENGINEERING SCAFFOLDS PREPARED BY PRESSURE INDUCED PHASE SEPARATION FROM POLYHYDROXYALKANOATES.

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Pressure induced phase separation is a promising method of tissue engineering scaffold preparation without the use of organic solvents. Sponge-like scaffolds with high porosity and pore interconnectivity can be prepared. The most common materials are synthetic biodegradable polymers such as polylactide (PLA) or polycaprolactone (PCL)[1]. Another promising biomaterial for scaffold preparation is poly(3-hydroxybutyrate) from the group of bacterial polyhydroxyalkanoates and its copolymers with other polyhydroxyalkanoate[2]. Its degradation product, β-hydroxybutyric acid is a keto body, naturally found in various tissues[3]. The main disadvantage of polyhydroxyalkanoate compared to synthetic polymers is their low thermal resistance, which might become a problem during PIPS, since it is typically done near the melting temperature. The degradation might be further enhanced in the presence of bioactive glass[4]. Bioactive glass is used in scaffolds for bone tissue engineering, since it provides sufficient stimulation for bone regeneration without the use of growth factors[5]. The aim of this work was to test preparation of scaffolds of poly(3-hydroxybutyrate) copolymers for a wide range of applications. Several compositions were prepared, both from synthetic and bacterial polymers. Their mechanical properties and degradation during processing were assessed. Suitable bioactive glass that did not induce too much degradation was found and a composite prepared. On the other hand, also highly flexible and ductile scaffolds for soft tissue regeneration were prepared.

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BIOCONJUGATES OF UPCONVERSION NANOPARTICLES FOR CANCER BIOMARKER DETECTION

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Sensitive detection of clinical biomarkers is crucial for the diagnosis of numerous illnesses. High sensitivity is of the utmost importance in the case of cancer biomarkers, as monitoring slight changes in their concentrations allows for early-stage cancer diagnosis and the assessment of treatment response.

Due to their high specificity conferred by antibodies, immunochemical assays have proven indispensable for biomarker detection. However, conventional immunoassay labels, such as enzymes or fluorophores, are typically not sensitive enough for the detection of low-abundance cancer biomarkers.

To enhance the sensitivity, various kinds of nanoparticles are often employed as labels. Photon-upconversion nanoparticles (UCNPs) stand out as one of the most promising options. These lanthanide-doped nanocrystals can convert near-infrared radiation into light of a shorter wavelength (anti-Stokes emission), significantly reducing the optical background. Moreover, their emission spectra can be easily tuned by altering the composition of dopant ions.

In immunoassays, the heterogeneous assay format is typically preferred due to its high specificity and sensitivity. These desirable properties are ensured by immobilization and washing steps, which significantly prolong the procedure. On the other hand, homogeneous immunoassays omit these time-consuming steps, however, at the cost of reduced specificity and sensitivity.

To combine the advantages of each assay format, we have developed a novel artificial intelligence-aided homogeneous immunoassay based on massively parallel spectroscopy (MPS). This single-molecule method utilizes two different UCNP-antibody labels with distinct emission spectra binding to the analyte molecule; only sandwich immunocomplexes containing the analyte molecule and both labels are detected. We have successfully employed MPS in assays for prostate-specific antigen and protein p53, two important cancer biomarkers. This immunoassay format was utilized for the first time, showing a strong potential to become a convenient and high-throughput method for biomarker detection.

NOVEL ELECTROPHOTOCATALYTIC APPROACH IN SELECTIVE FLAVIN-BASED OXIDATIONS

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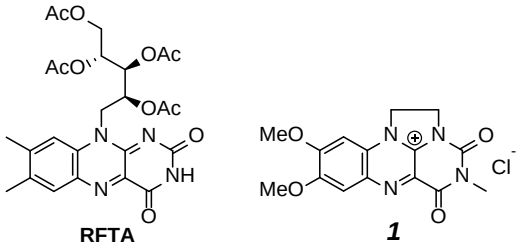
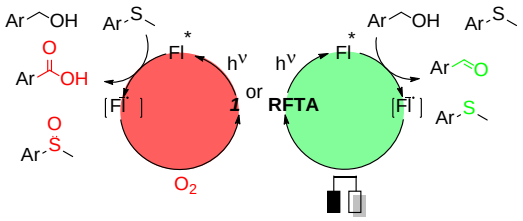
Flavins, particularly riboflavin tetraacetate (**RFTA**), have demonstrated significant efficacy in photocatalytic oxidation reactions[1]. Common examples are the oxidations of electron-rich benzyl alcohols to aldehydes or even carboxylic acids[2], or the Bayer-Villiger oxidation of cyclobutanones[3]. Some flavin derivatives, like flavinium salt ***1***, exhibit remarkable oxidative capabilities and can oxidize substrates with E_{ox} > 2.5 V, such as electron-deficient benzyl alcohols[2] in the excited state (Scheme 1). Until now, with one example[4], all these photooxidative systems with flavins use oxygen as a sacrificial oxidant for catalyst regeneration.



Scheme 1. **Structure of RFTA and isoalloxazinium salt *1***

Regeneration of photocatalysts by O_2 brings significant drawbacks in photocatalysis, such as deactivation of catalyst or lack of selectivity due to overoxidation or side-oxygenation. This issue can be solved with electrochemical regeneration under inert atmosphere. Herein, we present an electrophotocatalytic setup that operates without additives, only the substrate, catalyst, and supporting electrolyte. Our approach demonstrates selectivity in alcohol oxidation to carbonyl compounds while preventing overoxidation to carboxylic acids and unwanted oxidation of other easily oxidized heteroatoms, such as sulfur in sulfides (Scheme 2).

This work was supported by the GACR (23-06465S) and by grant of Specific university research (1100882409).



Scheme 2. **Common photocatalytic cycle (left) and electrophotocatalytic cycle (right)**

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THE USE OF BIOINFORMATICS TOOLS IN DEVELOPMENT OF A NEW CLASSIFICATION FOR L-ASPARAGINASES

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L-Asparaginases are a diverse group of enzymes that catalyse the hydrolysis of L-asparagine. In medicine, they play a pivotal role in the treatment of acute lymphoblastic leukaemia, while in the food industry, they are used to reduce neurotoxic acrylamide levels in processed products. Given their significant value, extensive research efforts are dedicated to discovering novel L-asparaginases and engineering the properties of those already in clinical or industrial use. The rapid advancement of high-throughput DNA sequencing has the potential to further accelerated this progress, generating a wealth of sequence data that remains largely underexplored.

Existing classification systems for L-asparaginases, however, are outdated, contradictory, or limited in scope, failing to reflect the full diversity of L-asparaginases or leverage modern genomic insights. To address this gap, we utilized a range of bioinformatics tools to propose a comprehensive phylogenetically driven classification that integrates existing biochemical knowledge with expanding sequence data. This system not only resolves historical ambiguities but also enables a systematic approach to discovering L-asparaginases with novel functional or structural properties.

Intriguingly, our framework has revealed multiple phylogenetically distinct families with no biochemically characterized representatives. We are investigating one of these families, which is distinguished by compact tertiary structures; a novel feature that may offer practical benefits for medical or industrial use. To help advance research in this field, we are developing an online database that incorporates this classification, providing organized L-asparaginase sequences within their phylogenetic families along with biochemical annotations for characterized enzymes.

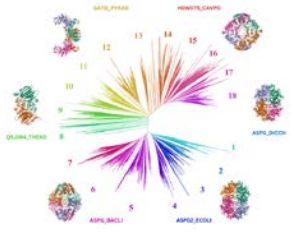

Fig. 1. **Phylogenetic tree of Class 1 L-asparaginases**

A STUDY OF THE DEEXCITATION PATHWAYS OF SEMICROCONAINE DYES

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Polymethines are a large family of fluorescent dyes, where fluorescence is increasing with molecular rigidity. However, semicroconaines do not follow this trend. Despite containing a rigid croconate subunit, semicroconaines exhibit very weak fluorescence (quantum yields < 1%). The underlying cause of this unusual behaviour was not understood. Yet, if the reason of this behaviour is identified and mitigated through external physico-chemical factors, semicroconaines could serve as highly effective turn-on fluorescent probes.

We investigated the excited-state dynamics of a library of semicroconaines with different auxochrome substituents. With a combined experimental/quantum-chemical approach, we assessed potential deexcitation pathways and discovered that isomerization of the methine bridge is the primary deexcitation pathway, leading to non-radiative S_1-S_0 relaxation through a conical intersection. We also observed a significant increase in fluorescence in solutions with higher viscosity, an external factor which restricts the intramolecular motion of the dye.

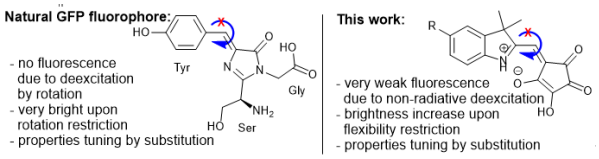

Fig. 1. **HBDI (left) and semicroconaine (right), when their rotation is confined, the fluorescence increases substantially.**

This work was supported by the GACR (GJ20-30004Y), CETOCOEN EXCELLENCE Teaming 2 (CZ.02.1.01/0.0/0.0/17_043/0009632 and EU H2020: 857560), and RECETOX RI (LM2023069).

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CERAMIDE EOP ENRICHED CEROSOMES FOR ADVANCED THERAPY OF SKIN DISEASES

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Skin diseases such as psoriasis or atopic dermatitis are related to a damaged skin barrier and reduced ceramide levels in the *stratum corneum* (SC)[1,2]. Direct ceramide administration offers a promising alternative to dermally applied corticosteroids but faces challenges due to ceramides´ poor solubility and low bioavailability into the skin. Our research team has introduced cerosomes (CRs), novel vesicular lipid carriers mimicking SC lipid composition, to effectively deliver ceramides[1]. To date, CRs have contained only the most common ceramides in the SC (NP and AP). The current goal is to enhance CRs with ceramide type EOP, the amount of which is dramatically reduced in diseased SC. Moreover, the EOP molecule is extremely rigid, crystalline and poorly soluble, so its embedding into the CRs structure is highly challenging. Here, we present new CRs enriched with EOP (EOP-CRs), prepared by thin lipid film hydration. All formulations have consisted of different ceramide type (EOP, NP and AP) concentrations in equimolar mixtures with cholesterol and fatty acids. Cholesterol and fatty acids (stearic and lignoceric acid) are essential parts of SC, and they improve the stability of CRs structure.

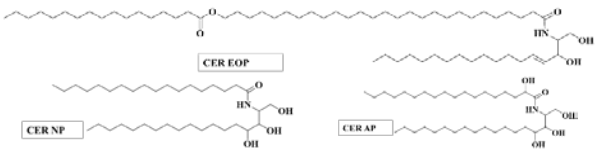

Fig. 1. **Chemical structure of ceramides**

EOP-CRs size was around 1 µm, determined by dynamic light scattering. Their morphology and crystallinity of lipids in the formulations were monitored by optical and transmission electron microscopy. Furthermore, the EOP-CRs efficacy in SC barrier function recovery was verified by an *ex vivo* skin restoration test which used chemically damaged porcine skin as a model of diseased human skin. In summary, EOP-CRs were more successful in skin restoration compared to a simple suspension of EOP.

This work was supported by project reg. number CZ.02.01.01/00/22_008/0004607: New technology for translation research in pharmaceutical sciences/NETPHARM, co-founded by EU and GAČR 25-17203S.

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DEVELOPMENT OF NEW PROTEIN THROMBOLYTIC DRUGS USING HIGH-THROUGHPUT SCREENING (HTS)

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Stroke is the second leading cause of death globally, with alteplase being the only available thrombolytic drug despite its limitations. These include high production costs, a short half-life in circulation that requires continuous infusion, suboptimal recanalization rates, and a high risk of systemic hemorrhage due to low fibrin targeting[1]. Staphylokinase, a bacterial protein with superior fibrin specificity, resistance to natural inhibitors, and cost-effective production, offers a promising alternative, though its immunogenicity and low activity have historically limited its clinical use. Recent advances in protein engineering present new opportunities to address these challenges[2].

My PhD research focuses on identifying the most clinically viable staphylokinase variant. To achieve this, I have optimized and integrated several characterization techniques into a robust high-throughput screening (HTS) platform. These include activity measurements using fluorogenic substrates and thrombolytic efficiency assessments on real fibrin clots. Comprehensive profiling also involves collecting data on stability, aggregation propensity, yield, and immunogenicity. So far, nearly 40 variants have been fully or partially characterized, with activity levels ranging from completely inactive to 150% of the wild type. Stability has also improved, with the best variant showing a remarkable 28°C increase in melting temperature compared to the wild type. The next step is to combine these beneficial mutations with previously developed non-immunogenic templates, THR174 and SY155, to create superior, well-rounded candidates[3].

At the same time, the platform is being adapted to work directly with cell cultures to allow the screening of large mutational libraries. Efforts are also underway to develop a high-throughput microfluidic system. All data from these screens will be analysed by my collaborators using molecular modelling and AI to predict improved variants. The integration of HTS and AI is increasingly popular, gaining momentum in the pharmaceutical industry, and promising to revolutionize drug development[4,5].

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INNOVATIVE BIOFERTILIZER FORMULATION UTILIZING SELF-GELATING AZOTOBACTER VINELANDII

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The demand for sustainable agriculture necessitates innovative methods to maintain yields while minimizing conventional fertiliser use, thereby enhancing environmental sustainability and protecting fertile land. One such innovation is the development of biofertilizers that utilize plant growth-promoting rhizobacteria (PGPR) to enhance soil quality through various beneficial mechanisms. We have proposed a novel biofertilizer production method employing *Azotobacter vinelandii* for in-situ self-encapsulation within a protective gel carrier through alginate crosslinking during bacterium culture. This approach streamlines preparation, potentially lowering costs and enhancing process competitiveness. Strains of *A. vinelandii* were selected for bioinoculant preparation and characterization. Among them, *Azotobacter vinelandii* CCM 289 was chosen for its superior alginate production, effective gelation, and confirmed ability to generate PGP metabolites, such as indole-3-acetic acid and siderophores. To confirm the positive effect on plant growth, three pot-cultivation experiments were executed on *Lactuca sativa L.*, incorporating four PGPR carrier formulations, with each group comprising 8 plants. These experiments demonstrated positive effects of these formulations mainly under environmental stresses, including low irrigation and nutrient deficiency. Lyophilized gel formulations significantly improved soil microbial diversity, plant growth under stress and plant dry mass. These results highlight the potential of alginate-based PGPR formulations for sustainable agriculture.

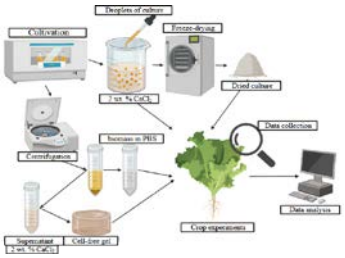

Fig. 1. **Scheme of the of the experimental part of research**

Funded by the project GA23-06757S (GAČR). Martin Súkeník: JCMM Brno Ph.D. Talent.

ROLE OF RIBOKINASE IN IMMUNOMETABOLISM OF *DROSOPHILA MELANOGASTER*

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Ribokinase (RBKS) is an enzyme responsible for phosphorylation of ribose which can be acquired either through dietary intake or via degradation of nucleotides in the salvage pathway. This makes RBKS a pivotal protagonist for ribose utilization contributing to essential cellular processes such as the pentose phosphate pathway, nucleotide synthesis and energy production[1].

Studies show RBKS deficiency does not impair viability. In *Arabidopsis thaliana*, RBKS mutants accumulated ribose but grew normally[2]. *Trypanosoma brucei* survived with low RBKS levels[3], and in mice, RBKS mutations led to ribose accumulation, increased gut motility, and reduced body weight without affecting survival[4]. Similarly, our finding show that *Drosophila* remains viable despite RBKS loss, raising the question of why RBKS is so evolutionarily conserved[5].

To explore this, we investigated whether RBKS plays a role in immunity, an aspect not yet studied. We generated mutations in both RBKS homologs present in *Drosophila melanogaster* and used parasitoid wasp *Leptopilina boulardi*, which injects its eggs into *Drosophila* larvae, triggering an immune response.

Since RBKS contributes to nucleotide biosynthesis, one key experiment assessed the efficiency of immune cell proliferation during parasitoid infection, a process with high nucleotide demand. A parallel experiment tested the overall resistance of *Drosophila* to parasitoid infection.

Preliminary results indicate that while immune cell proliferation remains unaffected by RBKS mutations, overall resistance to infection is compromised. This suggests that RBKS may play a significant role in immune function.

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COMBINING COLCHICINE WITH AUTOPHAGY MODULATORS TO OVERCOME DRUG RESISTANCE OF LUNG CANCER CELLS

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The inherent drug resistance of lung adenocarcinomas, combined with further development of resistance during long-

term treatment, poses a significant challenge in lung cancer therapy[1,2]. Colchicine (Col), a mitotic poison, shows promising potential in lung cancer therapy due to its cytostatic effects[3]. However, its clinical application is limited by its ability to induce cytoprotective autophagy, which contributes to chemoresistance[4].

To overcome this resistance and enhance the therapeutic efficacy of Col at non-toxic concentrations, we combined Col with either (i) the quinoline inhibitor (QIH) of Col-induced autophagy or (ii) the inducers of the cytotoxic autophagy based on quinoline (QID), or statin (SID) structure. The cytotoxicity of each combination was evaluated in inherently Col-resistant lung adenocarcinoma cells (A549) and non-cancerous lung fibroblasts (MRC-5) after 24 h. We identified combinations that showed a synergistic cytotoxic effect in A549 cells while acting antagonistically in MRC-5 cells.

Fluorescent labelling of the autophagosomes confirmed that Col/QID acted synergistically in A549 cells through the induction of cytotoxic autophagy. In contrast, stimulated emission depletion (STED) microscopy revealed that the synergistic cytotoxic effects of Col/SID were due to microtubule (MT) stabilization and condensation. Among the combinations tested, Col/QIH proved the most promising, resulting in synergistic cytotoxicity via MT stabilization and the inhibition of Col-induced cytoprotective autophagy. In fact, only Col/QIH exhibited these effects in both the A549 cells and in-house-developed A549-R cells, with further developed Col resistance after long-term treatment.

The above results suggest that the combination of Col with autophagy modulators is a potent and viable strategy for anticancer research, with the potential to overcome drug resistance and improve the clinical applicability of Col in treating lung adenocarcinomas.

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UNDERSTANDING YOUR INNER VIRUS: RETROVIRAL PROTEINS DRIVING HUMAN PLACENTA DEVELOPMENT

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The human placenta is a transient yet vital organ that facilitates the exchange of nutrients and metabolites between two genetically distinct individuals: the mother and the fetus. Remarkably, placenta development involves the expression of retroviral proteins, which originated from ancient infections of our ancestors by retroviruses. In evolution, these proteins were repurposed for a new beneficial role and are now indispensable for embryo development. One of them is Syncytin-1, a membrane protein that interacts with its receptor ASCT2 and mediates cell-cell fusion in the placenta[1]. These fusion events result in the formation of multinucleated syncytiotrophoblast, a tissue responsible for the fetomaternal exchange of molecules.

In contrast, Suppressyn, another retroviral-derived and placenta-specific protein, also binds ASCT2 and acts as an antagonist of Syncytin-1 activity. Expression of Suppressyn has been demonstrated to reduce cell-cell fusion *in vitro*[2], suggesting its potential role as a negative regulator of syncytiotrophoblast formation. However, the molecular mechanism of this inhibition remains incompletely understood.

To elucidate the inhibitory mechanism of Suppressyn, we established a quantitative fusion assay in the HEK293T cell model[3]. We have created stable cell lines expressing split fluorescent and split luciferase reporters, enabling us to measure cell-cell fusion levels by microscopy imaging and luminescence quantification. We also integrated a receptor-downregulation assay based on the luciferase activity to test if Suppressyn inhibits cell fusion through competitive binding or ASCT2 downregulation. Using these quantitative assays, we aim to describe the complex interplay between Syncytin-1, Suppressyn, and ASCT2 and their combined impact on cell-cell fusion in the human placenta. Understanding these mechanisms could explain the cause of some pregnancy pathologies, such as preeclampsia.

This work was supported by the project NIVB (Programme EXCELES, ID Project No. LX22NPO5103) and by Praemium Academiae Grant awarded to Jiří Hejnar by the CAS.

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META-EFFECT DRIVEN *o*-QUINONE METHIDE PPGS

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Ortho-quinone methides (*o*-QMs) are highly reactive, transient intermediates with broad applications in biological processes and organic synthesis[1]. They act as electrophilic units toward various nucleophiles or as heterodiene

cycloaddition partners in inter- and intramolecular Diels-Alder [4+2] cycloadditions with alkenes, yielding substituted chromans[2]. Nevertheless, their in-situ generation often results in complex product mixtures due to their inherent reactivity and reliance on UV activation (<300 nm), presenting significant limitations[3]. To address these challenges, we designed a modified *o*-QM precursor by introducing an electron-donating group (EDG) at the meta-position relative to the leaving group (LG). This strategic substitution leverages the meta-effect[4] to fine-tune electronic properties, shift absorption wavelengths into the visible region, and enhance selective photochemical reactivity. Moreover, this rational design approach provides a framework for optimizing *o*-QM precursors, offering valuable insights into the interplay between substituent effects and photochemical behavior. Ultimately, the outcomes of this study may broaden the versatility of *o*-QMs, paving the way for their use in a wider range of advanced applications across diverse fields.

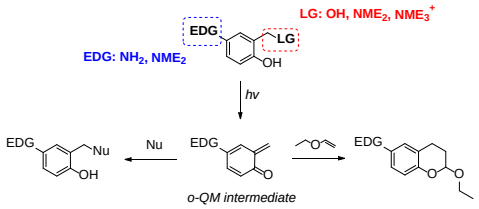

Fig. 1. **Designed meta-effect driven o-QM-based system**

This work was supported by the GACR (reg. No. 22-20319S).

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IN VIVO RAMAN SPECTROSCOPY AND MACHINE LEARNING FOR RAPID CANCER DETECTION

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Oncological diseases remain among the leading causes of death in developed countries. While biopsy followed by histopathological examination remains the gold standard for diagnosis, Raman spectroscopy has emerged as a promising and less invasive alternative for distinguishing between normal pathological tissue.

Thanks to advancements in hardware, particularly in the field of fiber-optics, a custom-designed low-hydroxyl silica fiber-optic probe coupled with sensitive CCD detector and a laser with 785 nm excitation wavelength enabled us to conduct *in vivo* measurements during routine bronchoscopies and colonoscopies. Optimal balance between patient safety and signal-to-noise ratio was achieved with an acquisition time of 2 · 3 s at 20 mW of power on the sample.

For Raman spectroscopy to be effectively adopted in clinical practice, the complex and time-intensive spectral pre-processing had to be automated. This was achieved primarily through Standard Normal Variate Transformation (SNV) and Finite Impulse Response (FIR) filtration.

In addition, classifiers trained using advanced methods of machine learning were developed to differentiate between healthy and cancerous lung[1] or colorectal[2] tissue. Principal Component Analysis (PCA) was coupled with Decision Tree (DT) and Support Vector Machine (SVM) algorithms, achieving classification accuracies (after five-fold cross-validation) of 88% and 97%, respectively.

To facilitate clinical adoption, the above-described real-time classification pipeline was integrated into a user-friendly software tool designed for non-specialists, bridging the gap between analytical research and practical medical application.

Supported by grant no. NW25-03-00441 of the Ministry of Health of the Czech Republic.

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INVESTIGATION OF BIOMEDICALLY RELEVANT HUMAN GALECTINS USING METHYL BIS(*N,N′*-DIACETYLLACTOSAMINIDE)

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This work explores an innovative strategy for the selective inhibition of human galectin-3, a well-established therapeutic target involved in conditions such as non-small cell lung carcinoma (NSCLC) and other serious diseases[1]. Building on prior research[2], we developed a novel tetrasaccharide-based inhibitor, methyl bis(*N,N′*-diacetyl-lactosaminide).

The compound was synthesized using advanced carbohydrate chemistry, involving the preparation of protected monosaccharide building blocks, their chemoselective and stereoselective conjugation[3], and stepwise deprotection. Several steps required optimization, offering insights into the reactivity of the intermediates.

The inhibitory efficacy of the synthesized molecule was assessed using an *in vitro* assay, which confirmed its high affinity and notable selectivity towards targeted human galectin-3. To understand the binding interactions, *in silico* molecular modelling techniques were employed. This approach included docking simulations (Fig. 1), molecular dynamics simulations, and energy calculations, which together provided a valuable insight into the selective inhibition of galectin-3.

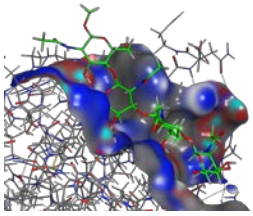

Fig. 1. **Docking of methyl bis(*N*,*N′*-diacetyllactosaminide) to galectin-3**

This work was supported by the Czech Science Foundation (reg. No. 23-05146S).

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3,4-METHANO-β-PROLINE: A BUILDING BLOCK FOR α/β FOLDAMERS

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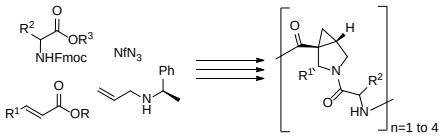
Poly-L-proline can adopt two types of structures, a right-handed helix known as polyproline I (PPI), and left-handed helix referred to as polyproline II (PPII). The PPII helix has been extensively studied, since it is essential for several biological functions, and it is an important structural motif in proteins like collagen. Moreover, synthetic polyprolines are very interesting materials that can adopt PPI as well as PPII structures, with applications in biomaterials and in collagen mimics[1].

The importance of polyproline motivated us to design a new class of foldamers (Scheme 1) alternating non-natural rigidified β-amino acids synthesized using a tandem aza-Michael addition/ [3+2] cycloaddition reaction[2], and amino acids like glycine, L-alanine and D-alanine.

The foldamers were prepared by solution phase peptide synthesis and the secondary structure was established by nuclear magnetic resonance, chiroptical methods, X-ray crystallography and computational studies.



Scheme 1. **Synthetic scheme of foldamers**

This work was supported by the IOCB of the CAS.

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PROTEIN MACHINES OVER THE RAINBOW: TOOLS FOR LOW COST, FAST AND EFFICIENT CLONING

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Conventional cloning methods rely on expensive commercial enzymes and labour-intensive selection strategies, limiting accessibility and scalability. We present an optimized workflow for rapid and cost-effective cloning using home-made polymerases and chromogenic proteins as selection markers. Using the Golden Braid cloning system, sets of vector and parts, we have produced functional enzymes that have been verified by routine laboratory workflows.

By expressing and purifying recombinant RTX[1] and Phu polymerases in-house, we can significantly reduce reaction costs while ensuring robust amplification efficiency. The polymerases are not only used for the production of inserts by PCR, but also for subsequent genotyping and further analyses, etc. This applies in particular to the robust RTX enzyme, which combines the properties of a reverse transcriptase and a thermostable DNA polymerase and can carry out PCR from crude extracts or diluted bacterial colonies without additional DNA isolation.

In addition, we are replacing conventional X-Gal-based selection with chromogenic proteins[2] from marine organisms that allow direct visual screening of successful clones without

the need for specialized substrates or equipment. This approach streamlines molecular cloning and reduces both time and resource requirements while ensuring a seamless cloning process. Our methods provide a sustainable alternative for research laboratories and industrial applications and facilitate a broader application of advanced genetic engineering techniques.

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EXPLORING COLD-ACTIVE AMYLASE FROM THE NEWLY IDENTIFIED ANTARCTIC BACTERIUM ARTHROBACTER POLARIS

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Cold regions, which constitute nearly 90% of our biosphere, are extensively inhabited by cold-adapted organisms. To thrive in such environments, these organisms had to acclimate on many different molecular levels. A key adaptation is the production of cold-active enzymes exhibiting a high catalytic efficiency at low temperatures, coupled with great flexibility and thermosensitivity. Due to their unique properties, these biocatalysts hold significant potential for applications across various biotechnological fields, including the food industry, brewing, pharmaceuticals, cosmetics, fabric production, detergents, molecular biology, and bioremediation[1,2]. Although many cold-active enzymes have been identified to date, the trend of searching for new ones displaying a unique combination of characteristics remains a compelling area of research.

Initially, an extensive solid medium screening of biotechnologically attractive enzymatic activities was conducted in selected Antarctic bacterial strains. Subsequently, we measured amylase and cellulase activities in the cell lysates at low temperatures. Based on the obtained temperature profiles, one promising bacterial strain *Arthrobacter* sp. C1-1 was selected. In this work, we successfully amplified and also sequenced the gene coding for the amylase using the isolated genomic DNA. *In silico* analyses revealed the potential presence of a signal sequence and a notable internal hydrophobic region that could complicate the production. The amylase from *Arthrobacter* sp. C1-1 was then recombinantly produced as a fusion protein with the N-terminal polyhistidine tag in *E. coli* BL21(DE3). Despite the considerably high production yield, the majority of the recombinant protein was insoluble. Consequently, various production parameters were tested to achieve a higher ratio of the soluble form, but without any key success. However, a small amount of the recombinant amylase was purified and briefly characterized. The alpha-amylase activity was confirmed using a chromogenic substrate, with its optimal temperature (30-35 °C) and pH (7,5-8). Our attention is currently focused on the production of the amylase fused with a SUMO-tag, which could enhance solubility, ensuring a sufficient amount of protein for subsequent characterization.

Since *Arthrobacter* sp. C1-1 was found to be a valuable source of cold-active enzymes, we sequenced its 16S rRNA gene, followed by the entire genomic DNA. The results of the conducted analyses exposed that the strain represents a new species of the *Arthrobacter* genus. All the necessary phylogenetic, physiological, and chemotaxonomic characteristics were determined, leading to the successful proposal of a novel species, *Arthrobacter polaris* sp. nov., with C1-1^T designated as the type strain[3].

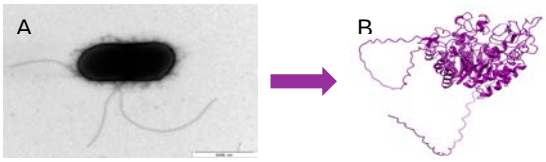

Fig. 1. **A – Transmission electron micrograph of strain C1-1^T grown in LB medium at 20 °C for 2 days. B – 3D structure of the amylase predicted by AlphaFold**

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DEVELOPMENT OF CONSTRUCTION OF POLYCYCLIC SCAFFOLDS LEADING TO GRISEMYCIN

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Natural products that exhibit interesting biological activity are a very important group of substances with significant therapeutic potential. Unfortunately, they occur in very low concentrations in natural sources and therefore cannot be sufficiently biologically evaluated. Total synthesis is therefore a very important tool for obtaining sufficient quantities of these biologically active substances.

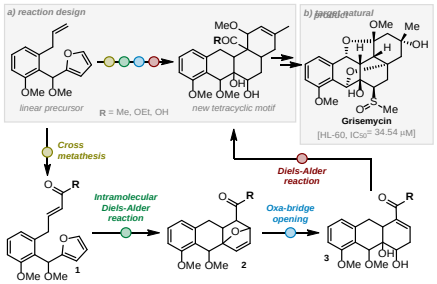

Fig. 1. **a) Synthetic platform leading to a new tetracyclic motif, b) target natural product – Grisemycin**

In this study, our focus has been on developing an innovative synthetic strategy capable of rapid assembly of polycyclic scaffolds. The synthetic pathway we have developed involves four key steps. The first step is cross metathesis, followed by the intramolecular Diels-Alder reaction and the opening of the oxa-bridge, and then further Diels-Alder reaction (Fig. 1a)[1]. Additionally, this synthetic platform represents a concise strategy for the synthesis of a tetracyclic natural product – Grisemycin, which is known for its cytotoxic effects (Fig. 1b)[2].

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DEVELOPMENT OF SYNTHETIC METHODS SUITABLE FOR THE SYNTHESIS OF FUMAGILLIN, ITS DERIVATIVES, AND RELATED NATURAL PRODUCTS

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The synergistic effect between drugs is actively searched and designed by purpose in today's pharmacology. When synergistic effects are identified, the administration of drugs not only increases efficacy, but also reduces dosage and reduces or delays the development of drug resistance[1]. In many cases, clinicians observe synergistic effects when a mixture of prescribed drugs is used with different modes of action and/or for various diseases. Such observations are in most cases based on empirical bases, and no relevant scientific follow-up is made to determine the mode of action of such drugs. However, since recently, several compounds of natural origin with expected/observed biological activity, e.g., against the bacteria or fungi, were used in the presence of certified drugs with known mode of action and the effect was studied[2].

In our group we are interested in the comprehension of synergistic effects, and we are wishing to evaluate the influence of various polyketides to the behaving of known polyaminoacid-based drugs, mostly antibiotics (commercially available and with known mode of action). In order to obtain an understanding of the mode of action, various polyketide scaffolds with known or expected biological activity should be prepared in general and modular manner. One of our targets, studied polyketide scaffolds, are compounds related to the structural motive of fumagillol (Fig. 1)[3].

In this contribution, we will present our synthetic design and first results of our general approach to a listed chemical library of targeted polyketide compounds.

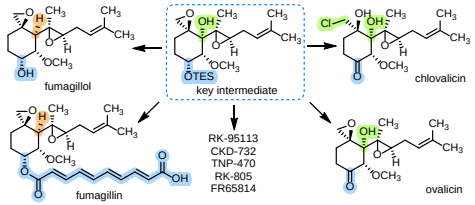

Fig. 2. **Fumagillol and related polyketides – chemical library of selected molecules targeted as a goal of our study. Targets polyketides that should be studied for their synergistic effect with polyaminoacid-based drugs**

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MODULATION OF FGF SIGNALING WITH A NOVEL DNA APTAMER

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Fundamental developmental processes in mammals, such as blastocyst formation, gastrulation, and morphogenesis of lungs, limbs and brain are regulated by fibroblast growth factor (FGF) signaling[1]. Aberrant FGF signaling causes pathologies including growth disorders, degenerative diseases and cancer[2]. Fibroblast growth factor receptors (FGFRs) are

transmembrane proteins specifically recognizing a set of FGF ligands through their extracellular domains, thus triggering tyrosine kinase activity of their intracellular domains[3]. Current FGFR-targeted therapeutics are based on small organic molecules impeding the catalytic activity of the tyrosine kinase domains of FGFRs (tyrosine kinase inhibitors, TKIs)[4]. However, TKIs exhibit low specificity, targeting all four FGFR variants as well as other tyrosine kinase receptors.

In this study, we implemented SELEX (Systematic Evolution of Ligands by Exponential Enrichment) to develop a DNA aptamer (VZ23) that specifically recognizes FGFR1b and FGFR1c, without cross-reactivity with other FGFR isoforms (FGFR2b, FGFR2c, FGFR3b, FGFR3c, FGFR4). The binding specificity towards FGFR1 variants was monitored *in vitro* and further confirmed in cellular models. VZ23 inhibits downstream FGFR1 signaling as well as regulatory processes controlled by FGFR1 (cellular senescence, proliferation, extracellular matrix homeostasis). Furthermore, we show that the inhibitory activity of VZ23 is dependent on its ability to form an unusual folding topology with an antiparallel G-quadruplex core scaffold.

Molecule optimizations enhancing the thermodynamic structure stability and reducing the nuclease sensitivity of VZ23 are the next steps towards its use as a therapeutic agent. The low production costs of aptamers, their long stability at room temperature, and the potential for covalent modification with fluorophores allow the use of VZ23 as a highly specific staining agent or as a basis for FGFR1-specific affinity matrices.

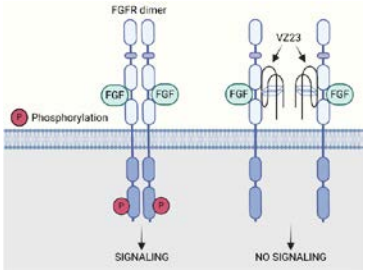

Fig. 1. **Graphical representation of VZ23-FGFR1 interaction and its inhibitory effect on the intracellular signaling**

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REMARKS AND NOTES

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Podpora vzdělávání je klíčem k budoucnosti nás všech, proto dlouhodobě podporujeme školy v rozvoji vzdělávacích aktivit a vědeckých projektů, nadšené pedagogy a nadané studenty. Rádi bychom Vás tímto informovali o grantových programech, které realizujeme a které Vám mohou pomoci ve Vašem růstu.

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O stipendium se mohou ucházet aktivní studenti, vysokoškoláci a středoškoláci, kteří se věnují chemii. Stipendium lze použít např. na nákup pomůcek ke studiu nebo mimoškolní aktivity související např. s vedením kroužku, popularizací vědy nebo v souvislosti s vědeckou aktivitou a činností s tím související.

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