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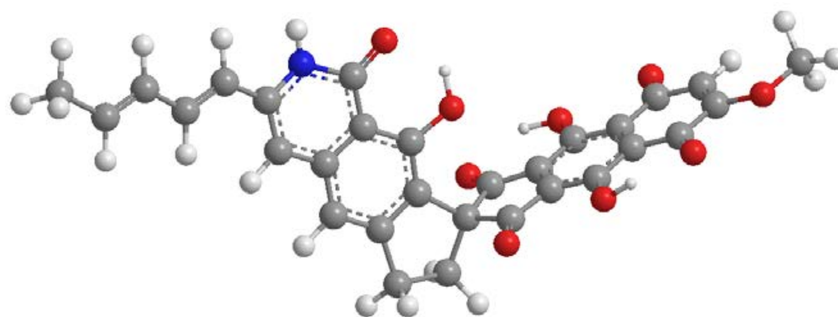

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IN SILICO STUDY (MOLECULAR DOCKING) OF INHIBITORS SARS-CoV-2 PROTEASES

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COVID-19 is a pandemic respiratory disease caused by the SARS−CoV−2 coronavirus, is a serious viral pathogen, and agents that inhibit its replication are in high demand. Among the SARS-CoV-2 components, the viral proteases such as main protease (Mpro) and papain-like protease (PLpro) represent molecules critically important in viral replication. They are promising target for antiviral therapy. For their inhibition was studied tryptanthrin derivatives and estrogen receptors modulators. Both type of compounds could be display other therapeutic effects (mitigation of the cytokine storm) independently on the protease inhibition. Also published global epidemiologic data show that while the incidence of infection is only negligibly sex-dependent with some protective role of estrogens in premenopausal women, males die more frequently than females[1].

For the docking studies, model complexes of PLpro and Mpro with the tested compounds were constructed. The 3D structures of the protease were obtained from the protein data bank database with PDB ID 3E9S and PDB ID 6LU7 for PLpro and Mpro, respectively. The tested compounds were docked to the structural model of viral proteas using the AutoDock Tools software. All the docking poses from the protein binding site were visually analysed with UCSF Chimera and BIOVIA Discovery Studio Visualiser. In this case of de novo prepared tryptanthrin derivatives, their effect on the viral replication was also studied by using in vitro Vera model.

Obtained value of binding energy (Table 1, Figure 1 and 2) suggest, that tested compounds represent promising structure motif for the inhibition of viral proteases[2,3]. In additionally, their calculated affinity of tryptanthrin derivatives for SARS-CoV-2 proteases correlated with their antiviral effectivity.

Table 1. The calculated interaction energy between SARS-CoV-2 proteases and estrogens or ERMs [kcal/mol]

Agents	Mpro	PLpro
Estradiol	−7.14	−6.86
Estrane	−7.59	−6.28
Estriol	−7.9	−6.43
Estrone	−8.96	−6.94
Bazedoxifene	−10.13	−6.07
Genistine	−7.7	−6.07
Raloxifene	−8.61	−6.14

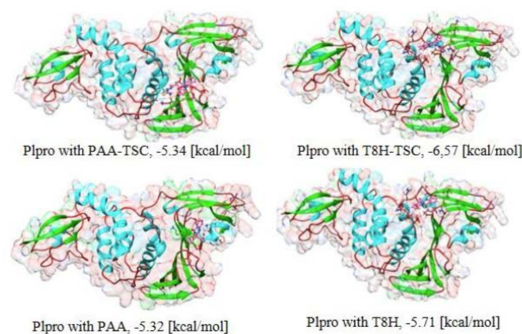

Fig. 1. **Visualisation of the docking poses and binding energy of the Plpro with tryptanthrines**

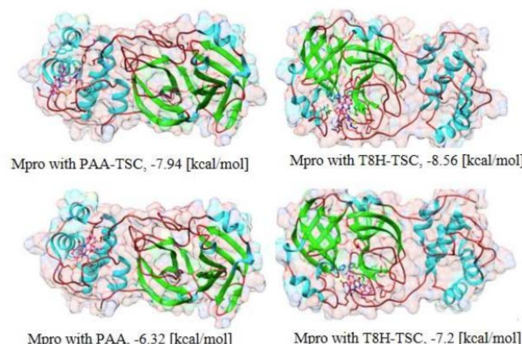

Fig. 2. **Visualisation of the docking poses and binding energy of the Mpro with tryptanthrines**

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A RADIOLIGAND RECEPTOR BINDING ASSAY FOR MEASURING OF INSULIN CONCENTRATION

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Insulin is a polypeptide hormone with a widespread impact on energy homeostasis, mainly by stimulating body cells to intake glucose from blood[1]. Therefore, accurate characterization and better understanding of insulin action on metabolism are important. We adapted a radioligand receptor binding assay for measuring insulin levels in unknown

samples. The assay enables rapid and lower cost determination of insulin concentrations compared to the existing method such as ELISA and RIA. The facts enable us to process big number of samples daily.

The principle of the IR binding assay is based on the binding competition of insulin, which is in the measured sample with unknown concentration, with a radiolabelled insulin for their receptor (insulin receptor, IR) on the surface of IM-9 cells. Both key components, radiolabelled insulin and IM-9 cells are commercially available.

The data of experimental samples obtained by IR binding assay were compared to the results determined by RIA kits and both showed a very good agreement (Fig. 1). We also prepared various insulin solutions with different concentration (from 1.37 to 1000 ng/ml, determined by UV spectrometry) and checked the accuracy and sensitivity of the method.

We utilized the new method to monitor glucose-induced insulin secretion from MIN6 pancreatic beta-cell line[2]. We observed that arginine and ornithine augment insulin secretion from MIN6 cells, whereas dopamine supress (data not shown). Serotonin has ambiguous effect on insulin secretion, dependent on the serotonin concentration.

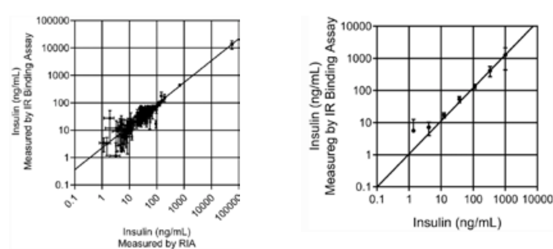

Fig. 1. **Data obtained by IR Binding assay are compared with data by RIA (left), and sensitivity of the assay was probed with different insulin concentrations (right)**

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FABRICATION OF FOLIC ACID-BASED SUPRAMOLECULAR METALLOGELS

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The challenge of delivering medications to patients efficiently, selectively, and with fewer adverse effects, motivates the need to develop novel drug delivery technologies. Among these new technologies, supramolecular gels have become a research hotspot[1]. Supramolecular gels are driven by weak and reversible non-covalent interactions. The supramolecular gels frequently display poor mechanical characteristics but can easily undergo various structural transformations upon external stimuli. For this reason, different components, such as macromolecular polymers, surfactants, or metals can be used to improve their mechanical strength[2]. The folate receptor (FR) which is anchored to the cell surface is overexpressed in a vast majority of cancer tissues, whereas its expression is restricted in healthy tissues and organs. As a result, the high affinity of folic acid (FA) for FRs offers a unique opportunity for precise targeting at cancer cells[3].

In our research, we are introducing the FA metallogels, which are stable supramolecular gels (often supragels) with various properties. Their synthesis, characterization, rheological properties, morphology, and possible structural transformations will be presented.

Our discoveries in the field expand the library of biocompatible organic building blocks with an intriguing relevance to cancer research (Figure 1).

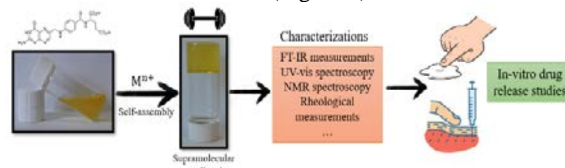

Fig. 1. **Graphical abstract**

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APPLICATION OF PHOSPHATE-BASED SELF IMMOLATIVE LINKERS IN DRUG DELIVERY

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Self-immolative linkers (spacers) are chemical constructs that undergo controlled defragmentation upon external stimuli, e.g., chemically, enzymatically, or by light. This approach can be efficiently applied for controlled drug release. Phosphate-based linkers offer a unique opportunity in prodrugging, because phosphorus enables the attachment of three different substituents contrary to only two in the case of carbamate linkers.

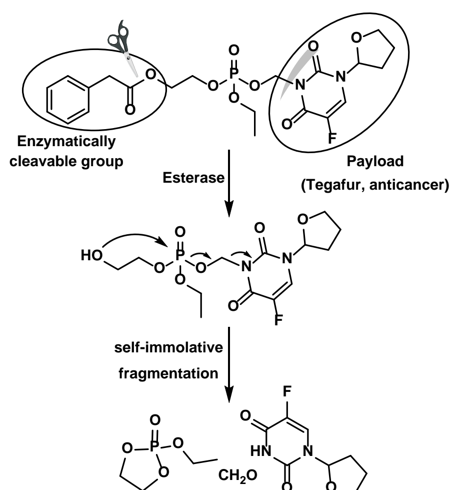

Fig. 1. **Self-immolation of tegafur-containig enzymatically cleavable linker and tegafur release**

In this work, we designed a series of novel phosphate-bridged linker, which allows delivering a broad range of drugs containing heterocyclic *exo*- or *endo*-nitrogen function, carboxylic acids, and thiols. We used ^{31}P NMR spectroscopy with *ex-situ* irradiation to monitor linker defragmentation and the payload release. We used the best-performing linkers for construction of enzymatically triggered prodrugs. As an example, Fig. 1 shows the linker for delivery of tegafur as an anticancer drug.

This work was supported by the Experientia Foundation (O.B., Start-Up grant SG-2018-1) and by the Czech Science Foundation (O.B., grant No. 20-25137Y).

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THE ROLE OF FSP1+ STROMAL CELLS IN MAMMARY GLAND DEVELOPMENT

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The development of the mammary gland is a dynamic process requiring precise cooperation of epithelial and stromal tissue compartments. Dysregulation of epithelial-mesenchymal interactions can lead to developmental defects, disruption of tissue homeostasis and, eventually, to malignancy. Stromal cells, such as fibroblasts and immune cells, play a crucial role in physiological as well as malignant tissue remodelling but their heterogeneity and roles have not been fully resolved.

In this study, we set to investigate the role of *Fsp1* (fibroblast specific protein 1)-expressing cells in the mammary gland. Previously it was suggested that FSP1 is a marker of fibroblasts[1-3]; however, our data from single-cell RNA sequencing of mammary cells suggest that fibroblasts do not express *Fsp1*.

Using a lineage-tracing approach employing *Fsp1-Cre;mT/mG* mouse model, we observed that the *Fsp1-Cre* targets mostly rounded-shape cells found dispersed in mammary stroma or in close contact with epithelium, present throughout all studied developmental time-points. Based on co-expression of F4/80, the markers of macrophages, we suggest that the FSP1+ cells belong to monocyte/macrophage population, rather than fibroblasts. Depletion of the *Fsp1*-expressing cells using *Fsp1-Cre;DTA* mouse model leads to abrogated epithelial outgrowth during puberty, and disrupted formation of alveoli resulting in severe lactation defect. This suggests the role of targeted cells in branching morphogenesis and lactation of the mammary gland.

Taken together, our work identifies *Fsp1*-expressing cells as cells of myeloid lineage with important roles in mammary gland development and function.

This work was funded by Internal Grant Agency of Masaryk University (MUNI/IGA/1311/2021), Grant Agency of Masaryk University (MUNI/G/1775/2020 and MUNI/A/1301/2022) and Brno city municipality (Brno Ph.D. Talent Scholarship).

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INFLUENCE OF THE MICROENVIRONMENT OF HEPATITIS B VIRUS-INFECTED HEPATOCYTES ON INNATE IMMUNITY’S MECHANISMS

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HBV virus is an infectious agent that replicates in the hepatocytes and causes an acute form of hepatitis, but it can

also lead to a chronical inflammation, and consequently, to a hepatocellular carcinoma. HBV virus is also described as “stealth virus” due to its ability to avoid recognition by the innate immunity system and the immune activation, as it does not cause the immune response[1].

The goal of our project is to discover the immune inhibitory mechanism, as this knowledge can be useful in clinical research.

Currently, our attention is focused on the main interferon α (IFNα) producing cells – plasmacytoid dendritic cells (pDC). After the activation of the pattern recognizing receptors (PRR), pDCs are starting the massive production of pro-inflammatory cytokines, as IFNα, IL-6, and TNFα[2]. As any other inflammatory process, pDC’s activation is strictly regulated. It was suggested that miRNA 146 is the element of negative feedback loop to control the cytokine production due to its ability to silence key proteins of the TLR signaling[3].

miRNAs are small non-coding RNAs approximately 22 nucleotide long. miRNA, together with Ago (1 - 4) proteins and Ago-bound proteins GW182, forms the RISK complex, which binds to the target mRNA and leads to its silencing[4]. It was also described that the mature miRNAs in the complex with Ago2 and GW182 can be sorted through the Multivesicular Body (MVB) into extracellular vesicles and can be transported to different cells[5,6]. Among different miRNAs, miRNA-146a plays a special role, as a major miRNA expressed in hepatocytes, having in addition immunosuppressive properties.

EVs are small single membrane vesicles of 30 - 200 nm of diameter. They contain cargo molecules that can be cellular/viral proteins, RNA, and lipids. Nowadays, EVs are considered to be part of the active cell-to-cell communication[7]. Our hypothesis is that HBV-infected hepatocytes produce the inhibitory molecules packaged in EVs, and that, in turn, are actively up-taken by pDC, leading to the inhibition of the immune response.

Our results, as the results of other laboratories[8], show that the intracellular level of miRNA 146 is higher in infected hepatocytes.

At the same time, the activation of the immune pDC, cell line, Gen2.2., after exposure to the supernatant from HBV-producing cell line HepG2.2.15, and infected hepatocytes, HepG2 NTCP cell line, is losing its effectivity. The level of IFNα production is lower after incubation with the infectious supernatant.

Our results show that HBV infected hepatocytes produce EVs that are actively up taken by the pDC cells and contains inhibitory molecules that are able to down regulate the signaling, that in turn leads to the decrease of the IFNα production.

Our future investigations will be focused more closely on the fractionation and characterization of EVs. We want to explore the content of different EV fractions by their ability to inhibit the immune response of the pDC, and by the presence of cellular/viral proteins and levels of miRNAs, or other RNA.

The work was supported by the project National Institute of Virology and Bacteriology (EXCELES, No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

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ELECTROCHEMISTRY OF TRIARYLAMINES AND THEIR OXIDATION SIDE-PRODUCTS

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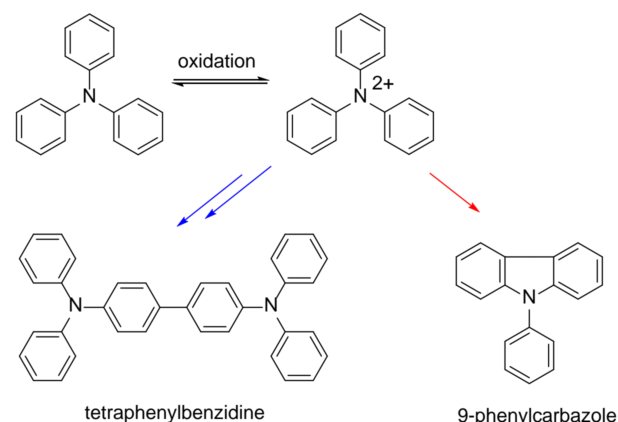
Triarylamines are electron donors, which form stable radical cations. For example, magic blue (tris-4-bromophenylamminium cation radical), can be used as a one-electron oxidant in organic synthesis[1]. Excited triarylaminium radical cations were employed as catalysts for the oxidation of arenes[2]. Triarylamines found also applications as hole-transport materials in organic electronic devices.



Scheme 1. **Formation of side-products from triarylamines**

In this work, cyclic voltammetry of a series of substituted triarylamines was measured, and the influence of substituents on the oxidation potential was studied. Electron-donating substituents shifted the oxidation potentials to lower values and vice versa. Upon oxidation, the formation of side-products

was observed for some triarylamines. Using spectroelectrochemistry as well as the comparison with published data[3], these side-products were identified as carbazoles and tetraphenylbenzidines.

This work was supported by The Ministry of Education, Youth and Sports (LTC20076).

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OPTIMISATION OF EKAREV-NLS MOUSE STRAIN FOR INVESTIGATION OF ERK SIGNALING PATTERNS DURING MAMMARY GLAND DEVELOPMENT

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Mammary gland is a dynamic tubule alveolar organ that develops through a series of changes called branching morphogenesis. Its main functional component is endothelial ductal network embedded in fibro-adipose stroma. The specific developmental patterns involved in branching morphogenesis as well as possible tumorous transformation are, among others, regulated by an extracellular signal-regulated kinase (ERK). ERK is major actor in the RTK pathways and is influenced by a number of signaling molecules. Together they form so called spatiotemporal ERK activity patterns that have been connected with specific cell behaviours[1]. Still, the understanding of this patterns in collective tissue behaviour and subsequently morphological changes is still not well explained. Here we utilise EKAREV-NLS[2] mouse strain that endogenously expresses FRET-based reporter for ERK phosphorylation activity.

The reporter construct contains cyan and yellow fluorescent proteins that negatively affect mouse growth, life span and mammary gland development. This study investigates the effects of the biosensor on the mammary gland development and offers possible solutions for rescuing the gland development. The mice were either divided into three groups and treated with hormones or crossed with different genetic background mice to boost the gland outgrowth. Following treatment mice were sacrificed while the glands were collected for histological analysis as well as for primary organoid isolation.

Finally, to validate biosensor activity under these conditions we collected the primary mammary gland organoids and cultivated them in 2D and 3D setup. Further, we imaged organoids under growth factor treatment and described patterns occurring under these conditions.

Here we show that mammary gland development can be rescued by both hormonal treatment and background change. Organoid yield is increased and biosensor activity is preserved.

This project is funded by MUNI/G/1775/2020 and MUNI/A/1301/2022.

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SELF-ENTRAPMENT OF PLANT GROWTH-PROMOTING BACTERIA IN GEL MATRIX AND THEIR USE AS NEW GENERATION BIOINOCULANTS

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Azotobacter vinelandii is a rhizobacterium (PGPR), capable of synthesizing two completely different biopolymers with great potential for applications. Polyhydroxyalkanoates (PHAs) are produced and stored in the form of intracellular granules, while alginate is produced extracellularly and used for bacterial protection. In agricultural demand, the use of PGPRs as bio-inoculants to increase yields of plants and crops can be an alternative to conventional fertilizers. Encapsulation of bacteria into hydrogel-based carriers substantially improves the application potential of the bioinoculant, as well as the viability and robustness of the bacterial culture[1].

In this work, the extracellularly produced by the bacteria, was cross-linked with a calcium chloride solution (2 % *w/w*) to form a hydrogel carrier with encapsulated bacteria. This unconventional concept, which we termed ‘self-encapsulation’, simplifies encapsulation of bacterial cells and production of hydrogel carriers, leading to the reduction of the cost, and expands its usability in numerous applications. First, we screened several strains of *A. vinelandii* for alginate production and PGPR properties (phytohormone and siderophore production and phosphate solubilisation). The strains CCM 289; DSM 87; DSM 13,529 demonstrated the best gelation potential (tested by rheological measurements) and the properties of PGPR. According to our results, the bacteria

trapped in a gel matrix seemed to be viable and cultivable, which confirms the feasibility and viability of the novel concept.

Fig. 1. **'Self-encapsulated' bacteria (left); TEM of *A. vinelandii* (right); alginate matrix (1) covering bacterial cells with intracellular granules (2)**

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RUTHENIUM(II)−TRIS-PYRAZOLYLMETHANE COMPLEXES INHIBIT CANCER CELL GROWTH BY DISRUPTING MITOCHONDRIAL CALCIUM HOMEOSTASIS

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Many anti-cancer drugs are effective as a first line treatment but fail to deliver desired outcome in recurrent disease because of cancer resistance. Therefore, new drugs with multimodal or novel mechanisms of action (MoA) are critical for treatment improvement. Metallodrugs bearing various central atoms (e.g., Pt, Ru, Os, Fe) together with wide range of bioactive organic ligands offer great variability in cancer-targeting possibilities.

In this context, several organometallic complexes based on ruthenium(II)-tris(pyrazolyl)methane scaffold were synthesized (Fig. 1). Tris(pyrazolyl)methane scaffold provides complexes with stability in aqueous media while lipophilic triphenylphosphine group favours cellular uptake. Various organic ligands further alter the physicochemical properties of complexes which are reflected in their biological activity.

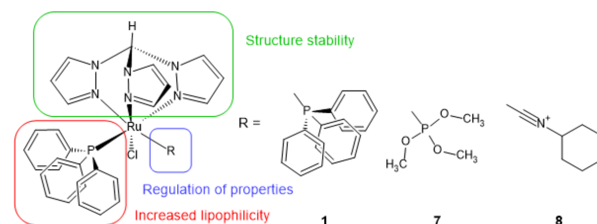

Fig. 1. **Structures of studied complexes**

Biological studies of these complexes reveal appreciable anti-cancer activity on a panel of cancer cell lines with IC_{50} values in low micromolar range (even in 3D conditions). Next, subcellular fractionation shows that complexes accumulate in membrane-rich compartments like mitochondria or endoplasmic reticulum. Together with mitochondrial membrane potential reducing properties, MoA seems to involve the disruption of mitochondria homeostasis. Furthermore, fluorescent Ca^{2+}-sensitive probes detected decreased Ca^{2+} concentration in mitochondria while elevated Ca^{2+} in cytoplasm in treated cells. These findings suggest that the studied complexes inhibit entry of Ca^{2+} to mitochondria leading to its critical accumulation in cytoplasm possibly causing death of cancer cells.

This research was supported by financial support from Czech Science Foundation (21-27514S) and University of Pisa (PRA_2020_39).

MICROFLUIDIC PLATFORM FOR SCREENING DRUG CANDIDATES AGAINST ALZHEIMER'S DISEASE

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Dementia afflicts approximately 50 million people all over the world, two-thirds of them with Alzheimer's disease (AD)[1]. A major factor behind this disease is a self-aggregating, membrane-permeating peptide amyloid beta 42 (Aβ42)[2]. Although perturbing its aggregation process is a promising concept for drug development, we lack a robust, time- and cost-effective method to monitor Aβ42's aggregation and membrane permeation activities *in vitro*, to delineate its oligomerization mechanism, and screen for candidate drugs.

For this purpose, we are developing a microfluidic platform to perform high-throughput monitoring of Aβ42 aggregation in a native-like membrane environment.

The platform consists of several microfluidic chips: (1) to create Giant Unilamellar lipid Vesicles (GUVs) by double emulsion (water – octanol – water); (2) to separate octanol from the GUVs; (3) to encapsulate GUVs together with Aβ42, its known protein partners (e.g., ApoE isoforms), and/or candidate drugs into oil droplets; and (4) to trap the droplets for fluorescence monitoring and imaging of the Aβ42 aggregation and its membrane toxicity.

Our microfluidic technology can already generate stable, high-quality, homogenous GUVs with specific lipid compositions to mimic neuron plasma membranes, and it will be used to perform a detailed, combinatorial analysis of Aβ42's aggregation kinetics and membrane permeation activities, as a function of lipids, protein partners, and potent drugs. The outlook of this project is the high-throughput *in vitro* (on-chip) validation of new Aβ42 aggregation inhibitors identified *in silico*, to discover potent drug candidates against AD.

This work was supported by the European Regional Development Fund – Project INBIO (CZ.02.1.01/0.0/16_026/0008451). JČ acknowledges the financial support for his research visit at ETH Zürich from the Czech Ministry of Education.

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DECELLULARIZATION OF PIG LUNG TO YIELD THREE-DIMENSIONAL SCAFFOLD FOR LUNG TISSUE ENGINEERING

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The human lungs are complex, hierarchically structured organs with dozens of distinct cell types designed for a particular function. The structural and physiological features of the lungs make the success of engineering an artificial lung model *in vitro* a challenge. From a slew of approaches and techniques utilized in pulmonary tissue engineering, this work focuses on the detergent-based decellularization method for obtaining a well-preserved pig lung tissue scaffold[1]. Decellularization is a method based on removing native cellular components and the entire genetic materials from a preexisting natural organ or tissue while preserving the composition, 3D microarchitecture, and biochemical and biophysical properties of the pulmonary tissue. The efficient removal of nuclear, cellular, and antigenic components is crucial for reducing scaffold immunogenicity while interacting with the host.

Further, we colonize the remaining cell-free extracellular matrix (ECM) with mesenchymal stromal cells (MSC) and human stem cell-derived expandable lung epithelial cells (ELEP)[2]. We investigate their ability to self-organize, adhere, proliferate, and differentiate into alveolar epithelial cells, epithelial cells of respiratory, and other relevant lung cell types, such as fibroblasts/myofibroblasts, which create a complex co-culture environment, using microscopic and molecular methods. Moreover, human cell incorporation ensures the best model quality, as human cells allow for more precise and reliable results when compared to cells of animal origin. These decellularized and recellularized scaffolds may serve as *in vitro* near-to-native 3D pulmonary models, hydrogels, or bio-inks for 3D printing and hold a promise for future drug testing, personalization of medicine, and a better understanding of tissue biology.

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ROLE OF BscX AND BscY IN *BORDETELLA* TYPE 3 SECRETION SYSTEM

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The type 3 secretion system (T3SS), also known as an injectosome, is a widespread macromolecular nanomachine that enables the delivery of bacterial effector proteins directly from bacterial cytosol into the cytosol of the host cells. Most T3SS subunits are genetically and structurally conserved among different Gram-negative bacteria, but there are some additional components that are distinct and species specific[1]. In the genus *Bordetella*, these include the small T3SS protein subunits BscX and BscY, homologous to the *Yersinia* YscX and YscY proteins. These proteins appear to be a part of the export gate, orchestrating the secretion of early substrates, but their structure and function in the *Bordetella* T3SS apparatus remain unknown[2].

Using nuclear magnetic resonance (NMR) spectroscopy, we determined the solution structure of the heterodimeric complex of BscX and BscY proteins, revealing that the six helical folds of BscY is wrapped by BscX made up by the N-terminal unstructured region and three C-terminal helices.

Individual Δ*bscX* or Δ*bscY* deletion mutants of *B. bronchiseptica* did not exert any cytotoxic activity towards HeLa cells, indicating that presence of the BscX-BscY heterodimer is critical for proper function of the secretion apparatus. Moreover, removal of the first 22 amino acids from BscX rendered the bacterial mutant non-cytotoxic on HeLa cells, indicating that the N-terminal unstructured region of BscX is functionally important. In summary, the BscX and BscY proteins are required for function of the T3SS apparatus and delivery of the BteA effector of *B. bronchiseptica* into host cells.

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MOLECULAR MECHANISMS LINKING METABOLIC STRESS TO GROWTH SIGNALING IN MELANOMA

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The mTORC1 signalling pathway is an essential regulator of cancer cell survival, growth, and metabolism. In addition to growth factors, energy status, and cellular stress, mTORC1 responds to the level of amino acids, sensed by specific sensors. The amino acids binding to their sensor enables the activation of mTORC1. Recently, the methionine sensor SAMTOR was identified. Unlike leucine and arginine, which directly bind their sensors Sestrin2 and CASTOR1, methionine is sensed indirectly through its metabolite S-adenosylmethionine, which binds to SAMTOR and enables mTORC1 activation[1,2].

We analyzed the impact of methionine deprivation on mTORC1 activity in *BRAF*-mutated melanoma cells. Based on the current knowledge, we expected that methionine restriction would cause mTORC1 inhibition, due to a lack of *S*-adenosylmethionine. However, we observed an unexpected increase in mTORC1 activity. Notably, the observed increase could be reverted by inhibiting methylthioadenosine phosphorylase (MTAP), an enzyme that recycles methionine metabolites back to methionine.

This surprising observation could mean that another backup mechanism is involved in the mTORC1 regulation under the methionine restriction. A similar mechanism could also respond to the restriction of other amino acids. We hypothesize that the mTORC2, AMPK, or ERK signaling pathways, all of which affect the mTORC1 activity, could be involved in this mechanism[3].

Understanding the role of individual amino acids in cancer metabolism and mTORC1 activation might lead to new approaches to melanoma therapy.

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A NEW MALDI-BASED DIAGNOSTIC METHOD FOR THE DETECTION OF *C. difficile* IN HUMANS

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Clostridium difficile is a dangerous human pathogen causing inflammation, diarrhea, or life-threatening pseudomembranous colitis[1]. Toxin B produced by the bacterium catalyzes the transfer of glucose from UDP-glucose to a threonine 37 of Ras homolog family member A protein (RhoA). The modification by glucose inactivates RhoA GTPase activity and leads to the disruption of the intestine cytoskeleton[2].

The novel approach for *C. difficile* detection is based on *in-situ* enrichment of glucosylated intact RhoA using functionalized chips prepared by ambient ion soft-landing and MALDI-ToF measurement. Neutravidin-modified chips were used prior to the enzymatic reaction for binding the biotin-tagged recombinant RhoA protein. The reaction buffer containing different concentration levels of recombinant toxin B was applied on the affinity MALDI plate and let incubate. The effectivity of the enzymatic reaction was monitored after matrix application by Autoflex speed MALDI-ToF mass spectrometer operated in linear positive mode. Data were searched for a mass shift of 162 Da corresponding to glucose modification of the RhoA. The intact RhoA was observed by a linear MALDI-ToF as a singly charged ion at m/z 25 000 and as a doubly charged ion at m/z 12 500. The glucosylated form of the RhoA was observed at m/z 25 162 and at m/z 12 581, resp. The glucose-modified RhoA was observed after *in-situ* enrichment using neutravidin chips at the lowest concentration of 2.4 ng/mL of toxin B. Changes in the workflow and several modifications of the reaction buffer allowed to keep RhoA protein intact and possible to detect by MALDI-ToF after

transferring this method to a real-life complex human stool isolate sample.

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CYCLOTRIMERIZATIONS OF ALKYNYL BORONATES IN NATURAL PRODUCT SYNTHESIS

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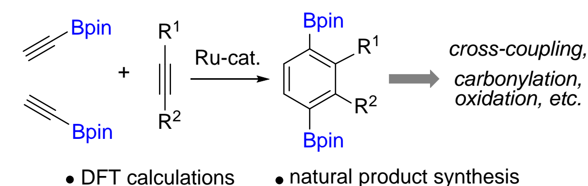
Borylated aromatic compounds represent powerful building blocks that have found numerous applications in organic synthesis (e.g. Suzuki, Chan-Lam cross-coupling reactions). Hence, development of new and more straightforward approaches for their preparation is a desirable task. In this respect, catalytic [2+2+2] cocyclotrimerizations of alkynyl boronates provide an effective atom-economical route for construction of functionalized borylated arenes[1]. However, only partially intermolecular reactions of this type have been reported so far[1-5].

We show how readily available alkynes can be selectively converted into 1,4-diborylated benzenes *via* fully intermolecular Ru-catalyzed cross-cyclotrimerization with a commercially available ethynyl boronate[6]. DFT calculations shed light on the reaction course and the origin of its remarkable regioselectivity. Lastly, we established how several 1,4-diborylated benzenes can be transformed into various products of general interest, such as natural bioactive small molecules mirandamycin and violaceoid C.



Scheme 1.

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CONTROL SELF-ASSEMBLY THROUGH SURFACES: TAILORED PROPERTIES FOR NOVEL ORGANIC (BIO-)ELECTRONICS

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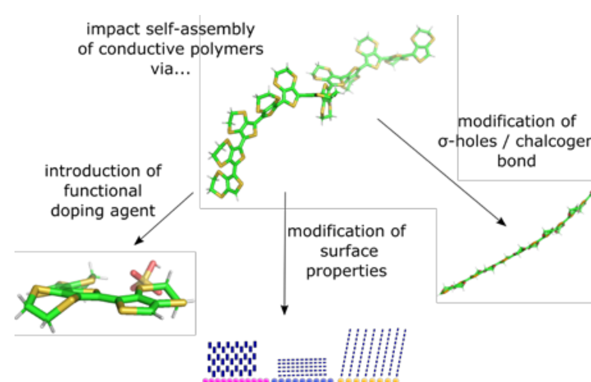
The role of organic conductors, in particular the role of conductive polymers has steadily increased over the past ten years. This is especially true for the field of biology[1]: neuro-science[2], cell signaling[3], and biomedical implants[4,5]; they all benefit from these materials outstanding properties such as bio-compatibility, stability, and combined electrical and ion-conduction.

Their performance therein, however, can still be improved. Herein we rely on surfaces to govern self-assembly in the predominant conductive polymer poly(3,4-ethylene-dioxythiophene) (PEDOT). We utilize various self-assembled monolayers (SAM's) to alter the physical properties of the deposited polymer, including its orientation, surface properties, and most importantly, its work function.[1]



Scheme 1. **Self-assembly in CP and how to govern it**

This outstanding control is achieved through a facile adaptation of oxidative chemical vapour deposition (oCVD) and can be performed with equipment readily available to biologists, chemists, and physicists alike. In this way, we make materials with tailored properties available to a wide variety of researchers.

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PLGA NANOPARTICLES FOR THE LOCAL TREATMENT OF JOINT REPLACEMENT INFECTIONS

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Musculoskeletal infections which commonly accompany orthopaedic surgery are effectively treated with a combination of systemically and locally applied antibiotics in the form of targeted delivery systems with prolonged drug release. Antibiotics administered locally provide a high drug concentration at the target site. This approach benefits from minimizing systemic drug exposure and potentially reduces resistance development[1,2].

The aim of this work was to formulate and characterize vancomycin loaded PLGA nanoparticles (NPs) for the impregnation of bone grafts used as spacers in joint replacements. For formulation of NPs commercial linear PLGA (Purasorb 5002A) and branched non-commercial, experimentally synthesized linear PLGA were used[3]. NPs were prepared by water-in-oil-in-water double emulsion solvent evaporation technique. Polyvinyl alcohol or poloxamer were used for emulsion stabilization.

NP’s size, polydispersity and zetapotential were determined using a Zetasizer Nano ZS. Encapsulation efficiency was estimated by UV-spectrophotometry directly by measuring the amount of encapsulated drug after dissolution of NPs in organic solvent and extraction of drug by water. Thermal behavior of blank PLGA nanoparticles and drug-loaded nanoparticles was studied using a DSC. The drug release into the PBS pH 7.4 at 37 °C was measured.

As a result of our work, NPs up to 300 nm in size and polydispersity below 0.2 were successfully obtained. The created NPs will be used in further tests after optimalization of other parameters, such as encapsulation efficiency and drug loading.

The study was supported by GAUK grant No. 164122/2022 and SVV 260 547.

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HIGHLY PROMISING NATURAL PHYTOHORMONES GIBBELLIN-BASED GROWTH RETARDANTS

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In modern agriculture, fertilizers, pesticides, and herbicides play an important role in influencing the development and growth of field crops. However, the use of these substances is associated with a negative impact on the environment, and other natural and non-toxic alternatives are being sought. Since gibberellins (GAs) are a key group of plant bioregulators playing a central role in controlling plant growth, they are high on the list of alternatives to their synthetic counterparts[1,2]. In principle, the control of plant growth can be influenced in two ways, by the addition of exogenous addition biologically active GAs (increase of endogenous GAs – increase of growth, germination) or by the application of growth retardants (reduction of endogenous GAs – shutting down the plant growth).

Recently, our group developed and tested *(in vitro* and field trials) a new gibberellin-based plant retardant (*anti*-gibberellin) that acts as a competitive antagonist of bioactive GAs with stronger affinity for the GA receptor (GID1) in plants than bioactive endogenous GAs[3,4]. After intensive field trials optimization (dosage, type of application, and determination of the optimal vegetative period of application; three years of field trials) on an optimal protocol for barley (20% increase (t/h) in grain yield) and wheat (7% increase (t/h) in grain yield) was developed. It should be also noted that compared to the negative control (water) no change in ear size and seed number for treated plants was observed (commercially used synthetic growth retardants such as Stabilan or Moddus showed lower numbers).

Currently, we focus on two additional issues connected with our leading structure, we need (1) to increase its bioavailability, and (2) the solubility in water. To address the

first issue, a fluorinated derivative of the lead structure was prepared with aim to improve the migration of the target molecule through the cytoplasmic membrane to the target side (GID1 receptor). The preliminary results of enzyme competitive assays show the inhibitory activity seems to be even superior to the up-to-date best compound. To achieve the second aim, to prepare a more soluble derivative of *anti*-gibberellin, we have decided to generate several ammonium salts of it and to evaluate such compounds under our field trial conditions (we expect that the salt will transform into the free acid after the salt application). Both types of modifications will hopefully address the issues of solubility and bioavailability and will allow us to decrease the amount applied in the field trials to plants. New compounds will be evaluated in field trials this year.

In this contribution, we present the latest results obtained during this project.

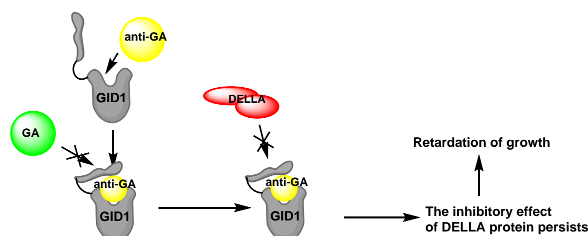

Fig. 2. **Schematic view of *anti*-GA binding and growth retardation. *Anti*-GA blocks the active side of GID1, which disables its activation. Inhibition prevents interaction with the DELLA protein (repressors of plant development) and its subsequent degradation. The inhibitory effect of the DELLA protein persists, and growth retardation occurs**

The project is financially supported by the Internal Agency of Palacký University (IGA_PrF_2022_012, IGA_PrF_2022_022) and the European Regional Development Fund-Project "Centre for Experimental Plant Biology" (no. CZ.02.1.01/0.0/0.0/16_019/0000738)

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MOLECULAR HETEROGENEITY OF ENDOPLASMIC RETICULUM STRESS PROTEINS IN PANCREATIC TUMOR CELLS

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Ductal adenocarcinoma of the pancreas (PDAC) is the most prevalent form of pancreatic cancer[1]. It is the 11th most common cancer type in the world[2]. In the Czech Republic, there is the second highest incidence of PDAC globally. We can witness that the curve of mortality almost copies the curve of incidence[3]. Treatment of PDAC is usually performed by radical resection. However, this approach shows high postoperative morbidity[4] and in addition, a high number of patients show relapse[5], usually within 2 years after resection. There is also a limited number of biomarkers connected to PDAC. Even though these markers, such as CA 19-9, can be used to predict tumour stage, resectability, overall survival and possible response of PDAC, their prediction value only matters statistically[6] and often fails as far as an individual patient is concerned.

Understanding the machinery of cellular stress response may help predict the progression of the disease, but also possible resistance to therapeutics. The major response pathway to stress of the endoplasmic reticulum (ER) is the unfolded protein response (UPR), which is mediated by several regulator proteins. The UPR is also connected to specific mitochondrial characteristics and metabolic states. If these phenomena are considered together (as the integrated stress response), they could serve as a predictor of PDAC outcome.

PDAC is a heterogenous disease, therefore special biomarker discovery methods are required. MALDI-TOF mass spectrometry providing large data sets for further mathematical analysis will be used. Such multivariate datasets contain specific patterns that can be handled and analysed by machine learning.

Here we show pilot results of the collaborative project between the University hospital in Brno and Faculty of Medicine, Masaryk University focused on biobanking and molecular and bioanalytical analysis of patient-specific pancreatic cancer cell lines. We demonstrate that PDAC cell lines differ in ER-stress and UPR machinery and the spectral patterns contain sufficient information to discriminate between distinct pathological types of PDAC.

This study was supported by Masaryk University (MUNI/A/1301/2022).

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FRET-BASED ASSAY FOR INTRACELLULAR EVALUATION OF α-SYNUCLEIN AGGREGATION INHIBITORS

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α-Synuclein (αSyn) aggregation in amyloid fibrils is a main cause of Parkinson's disease and inhibiting this aggregation is a potential treatment approach. Dozens of compounds have been developed to inhibit αSyn fibrillization, but most have not been tested in a cellular environment due to a lack of a suitable assay. The existing methods of potential inhibitor evaluation involve indirect cell viability measurements or fusion of αSyn with a fluorescent protein tag. These tags are larger than αSyn and change the aggregation kinetics, making this approach unreliable.

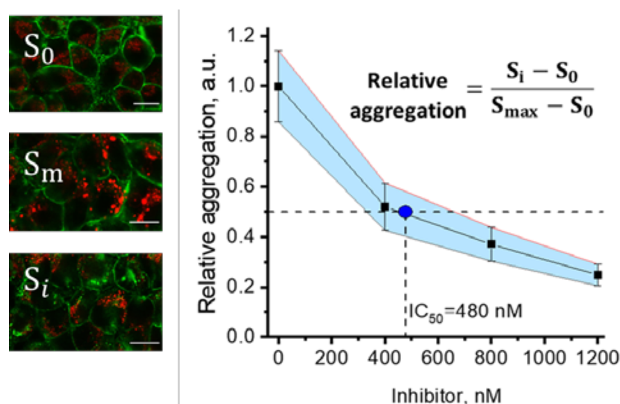

Fig 1. **Evaluation of αSyn aggregation in cells, FRET signal from aggregates is shown in red. Fluorescent signals from background (S_0) and induced-aggregation wells (S_m) are used to calculate relative aggregation in inhibitor-treated wells (S_i)**

In this work, we developed an assay for testing αSyn aggregation inhibitors in cells based on fluorescence resonance energy transfer (FRET) in fibrils between αSyn molecules labeled with small fluorescent dyes. The assay directly reports the amount of fibrillized αSyn and does not interfere with αSyn aggregation kinetics. It is also optimized for use in plate readers and testing many samples in parallel.

The developed assay can be used to exclude non-specific αSyn aggregation inhibitors from the investigation in animal models or in clinical trials. Therefore, we believe it will significantly facilitate the development of effective antiparkinsonian drugs targeting αSyn fibril growth.

2´-FLUORO-ARABINONUCLEIC ACID (FANA) AS A SENSITIVE ^{19}F NMR PROBE OF G-QUADRUPLEX FOLDING AND LIGAND BINDING

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Nucleic acids represent the fundamental biomolecular machinery that is used to transfer genetic information and regulate gene expression. Recent bioinformatics and sequencing studies revealed the simultaneous existence of G-quadruplex (G4) structures (> 70,0000 G4 sequences) within the human genome (i.e., MYC, BCL2, RET, PIM1, etc.), indicating the potential involvement in various biochemical and biological activities (replication, recombination, transcription, and splicing, etc.)[1]. G4 sequences are intrinsically polymorphic, and numerous chemical modifications have been demonstrated to elucidating and regulating G4 conformations and ligand binding.

Modified nucleotides containing 2-deoxy-2-fluoro-arabinonucleic acid (FANA) introduced by the Damha group describe an effective chemical modification to stabilize the G4 conformations[2,3]. In general, FANA-substituted DNAs prefer the anti glycosidic bond angle (C2'-endo sugar puckering) due to the replacement of the proton with more electronegative and bulky fluorine at the C2 sugar[4]. In this study, we try to use the unique capabilities of ^{19}F-NMR to explicate the conformational polymorphism of G4 and ligand binding. We choose different polymorphic G4 families from diverse genome origins, each having at least two different conformations in solution. We incorporated FANA in the sequences without disturbing the native conformational equilibrium. The 1D ^{19}F NMR clearly distinguishes the two conformational populations based on chemical shifts and intensity differences. Moreover, the ^{19}F labeling can clearly distinguish the different ligand-bound G4 sub-states, which is often tricky to interpret from ^{1}H NMR and other low-resolution spectroscopic techniques. Therefore, in the current work, we report the application of FANA labeling and ^{19}F NMR to disclose the conformational polymorphism of G-quadruplex systems. Our preliminary data suggest the potential of ^{19}F NMR to be utilized in the ligand screening assay. Currently we started using FANA modified oligos to assess the stability, folding, and ligand interactions of noncanonical nucleic acids in living cells (*i.e. Xenopus oocytes*) by ^{19}F NMR spectroscopy.

This work was supported by GAČR (grant No. 2903) and HORIZON-TMA-MSCA-PF-EF (grant No. 101068280).

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4D-MONITORING OF BIOMINERALIZATION DYNAMICS

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Teeth and bone are the main mineralized organs in vertebrates, displaying important biological functions such as body structure, protection of soft organs or mineral storage[1].

These are dynamic tissues, which get mineralized during development and, in the case of bone, remodeled during adulthood. Currently, there is not any tool to follow up the biomineralization process *in vivo*, the most spread technique is X-ray microtomography but only provides end-point data of the calcified tissue[2]. Our main focus is understanding how tooth mineralization and skeletogenesis in mammals occur by the use of mouse model.

In this work, we present a novel universal technique to trace and quantify spatiotemporal information *in vivo* of fully-calcified biological samples. This method is based on the *in vivo* administration of two fluorochromes (Calcein Green and Alizarin Red) at precise timepoints to label the newly calcified area in bone or tooth. This approach permits quantifying at micron-scale the (re)growth and repair of all hard tissues in the real biological context.

Moreover, we demonstrate that is a wide-range technique usable also in non-stereotypical models: chameleons, frogs, chickens, or zebrafish (Fig. 1).

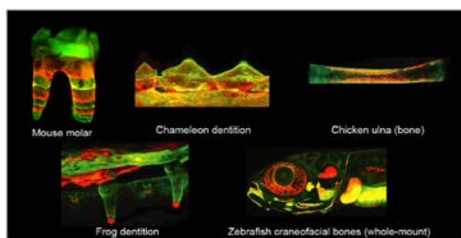

Fig. 1. **Monitorization of dental and bone tissue in representative models of vertebrates**

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LIGHT'EM UP: POLYMER-TETHERED FLUORESCENTLY QUENCHED PROBES FOR CANCER IMAGING

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Surgical resection remains a major treatment strategy for solid tumors. Intraoperative tumor identification based on brightfield imaging is the most common technique employed but alternatives are emerging. Recently, fluorescence-based techniques have proven themselves to be a promising and effective approach for intraoperative cancer detection[1]. Affinity-based unquenched probes have already reached the market while activity-based quenched probes dependent on activation by tumor-associated proteases are still under investigation.

Here, we report development of a polymer-tethered fluorescently quenched probe based on a previously reported small-molecular imaging agent. We presumed that conjugation of this small molecule to a biocompatible macromolecular carrier would offer many advantages, such as limiting unwanted lipophilic interactions or cancer tissue accumulation thanks to well-described EPR effect. The macromolecular scaffold also allows us to further tune features of the probe to obtain desired properties.

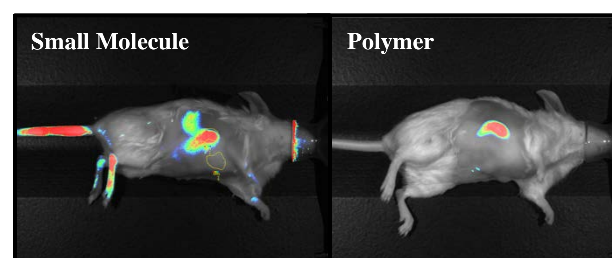

Fig. 1. **Comparison of a small-molecular probe to a polymer-tethered probe**

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HASCOFOVOMYCIN, A NEW 4-ALKYL-L-PROLINE DERIVATIVE ISOLATED FROM MARINE ACTINOBACTERIA

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Actinobacteria are important producers of structurally diverse specialized metabolites with a wide range of biological activities and potential applications in human or veterinary medicine. Especially, marine species represent an amazing and promising source of natural bioactive compounds[1,2]. 4-Alkyl-L-proline derivatives (APDs) are unusual precursors that are incorporated into the structurally and functionally diverse groups of specialized metabolites. APDs are biosynthesized in a specialized biosynthetic pathway from L-tyrosine (L-tyrosine-derived APDs) or L-leucine (L-leucine-derived APDs). L-Tyrosine-derived APDs are included in natural compounds such as lincosamides (*e.g*. lincomycin), the group of clinically used antibiotics[3], pyrrolobenzodiazepines (PBDs, *e.g.* tobramycin), antitumor agents[4], or hormaomycin, signalling molecule[5]. L-Leucine-derived APDs are incorporated into the structure of griselimycin, antitubercular compound[6], or intervencin, lincosamide discovered in our laboratory.

The rapid development of high-throughput genome sequencing methods has contributed to the development of genome mining methods and tools that have become an important part of the discovery and characterization of novel bioactive compounds[7]. Based on the analysis of the DNA sequence from a public database, we were able to identify homologous genes for the biosynthesis of L-tyrosine-derived APD from a strain of marine actinobacterium *Salinospora*. After optimization of cultivation conditions, we detected a new APD compound, hascofovomycin. The new metabolite is structurally different from the well-known lincosamides, PBDs and hormaomycin, and thus extends this group of APD compounds. The biological activity of hascofovomycin will be tested against yeast, Gram-positive and Gram-negative pathogenic bacteria.

The work was supported by the Lumina Quaeruntur Program No. LQ200202002 from CAS, by the *SEA Europe Joint Funding Scheme 4 the Joint Call for Proposals (SEAEUROPEJFS 19 IN 080), the grant of Specific university research A2_FPBT_2022_079 and by the project No. 20-09811Y from the Czech Science Foundation.*

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MOLECULAR RESPONSE OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS TO TARGETED THERAPY

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults in the Western world. The characteristic symptom of the disease is the accumulation of malignant cells in lymphoid organs and blood. The microenvironment of lymphatic tissues provides CLL cells with various pro-survival stimuli that may impair therapy efficiency. Small-molecule inhibitors targeting microenvironmental interactions represent a novel therapeutic approach. BTK inhibitor ibrutinib and PI3K inhibitor idelalisib disrupt B-cell receptor signaling (BCR), and CLL cell adhesion, CLL-T cell interactions and other microenvironmental stimuli. This leads to unprecedented clinical efficacy in CLL; however, some patients develop resistance to these inhibitors. We hypothesize that the resistance mediated by mutations in targeted kinases is preceded by a signaling adaptation that allows prolonged survival of CLL cells during therapy.

Gene expression profiling (RNAseq, Illumina) of CLL samples obtained from patients pre- and post-treatment with ibrutinib (n=22, 11 pairs) or idelalisib (n=18, 9 pairs) and subsequent *in vitro* experiments on primary CLL cells and CLL-derived cell line MEC1 allowed us to define a specific transcription factor that plays a role in adaptation of CLL cells to BCR inhibitor therapy. Inhibition of the transcription factor together with ibrutinib or idelalisib decreased CLL cells' viability and proliferation. To further uncover the role of this transcription factor in CLL, we performed Cut&Run assay, an alternative to ChIPseq, in the MEC1 cell line, to characterize genes directly regulated by this transcription factor. Apart from the regulation of the BCR signaling pathway, we noticed

a subset of genes involved in apoptosis regulation, suggesting its potential in response to other drugs such as the BH3-mimetic (BCL2 inhibitor) venetoclax that has been recently approved for CLL therapy.

Altogether, we thoroughly describe particular changes in CLL transcriptome, that provide increase in survival of leukemic cells during the BCR inhibitors therapy in both primary CLL cells and cell lines. Based on these results, we further suggest a rational combinatorial treatment of ibrutinib/idelalisib with an inhibitor targeting the transcription factor as a potential therapeutic strategy to eliminate mechanism of adaptation to BCR inhibitors.

This work was supported by the Ministry of Health of CR, grant NU20-03-00292 and by the Nat. Inst. Cancer Res. (EXCELES, project LX22NPO5102), funded by the Next Generation EU. This work was also supported by MH CZ-DRO (FNBr, 65269705) and MUNI/A/1224/2022, MUNI/C/0086/2022. The authors acknowledge the CF Genomics CEITEC MU supported by the Nat. Center for Med. Genom. NCMG) research infrastructure (LM22018132 funded by MEYS CR) and Core Fac. Bioinform. of CEITEC for their support in processing the data.

ILLUMINATING THE MECHANISM AND ALLOSTERIC BEHAVIOR OF NANOLUC LUCIFERASE

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NanoLuc is a small but bright luciferase enzyme widely used in biotechnology and biomedicine. However, the precise mechanism of its light-emitting reaction, vital for the successful development of next-generation bioluminescent systems, has not been solved. Therefore, we conducted multiple lab-based experiments to study NanoLuc. Also, we employed molecular docking and simulations using enhanced sampling to support the experimental findings and provide a bigger picture of its catalysis.

One of the declared advantages of NanoLuc is its small size of 171 amino acid residues compared to conventionally used luciferases from *Renilla reniformis* (311 residues) and firefly (550 residues). Yet, our experimental and computational findings suggest that while NanoLuc is monomeric in a substrate-free solution, it can form dimers, or even tetramers, in the presence of substrate, which should be considered when designing experiments with NanoLuc.

Our studies revealed two distinct binding sites of the substrate molecule: the catalytic site inside NanoLuc's β-barrel structure and an allosteric binding site located in a pocket formed at the dimerization interface[1]. Notably, substantial conformational changes of the enzyme are required to allow substrate binding in either the allosteric or the catalytic site, which makes simultaneous binding to both sites impossible. Interestingly, we discovered similarities between the catalysis by NanoLuc and the recently solved mechanism of *Renilla* luciferase[2], even though they are not structural homologs.

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TOWARDS A NOVEL TYPES OF ZWITTERIONIC QUINONES AND MACROCYCLES

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The well-established chemistry of classical calixarenes can be reasonably changed by the introduction of heteroatoms instead of the common CH_2 bridging units which results in significant changes in the macrocycle behaviour (cavity size, electronic properties)[1]. For instance, azacalixarenes (ACA), an evolved class of heteracalixarenes, have attracted much attention as anion receptors or as precursors of a new type of macrocycles called azacalixquinarenes (ACQ)[2].

We showed that the canonical (uncharged) quinoid rings can be converted into a stable zwitterionic form following intramolecular proton transfer (Fig. 1)[3]. Recently, we discovered that the engineering of EWG can have a strong influence on the electronic form of the quinones. Thus, because of the possible presence of both canonical and zwitterionic quinones within a single structure, ACQ possess fascinating electrochemical and optical properties, such as absorption in the red range. Therefore, the development of such systems is of growing interest for detection applications (*e.g.* anions or polar gases).

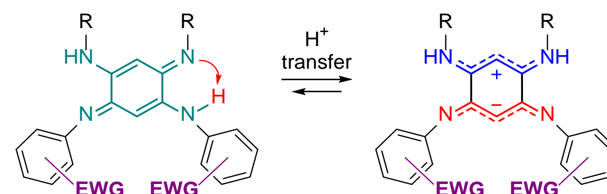

Fig. 1. **Illustration of the canonical-zwitterionic equilibrium in quinones**

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NATURAL PHENOLIC COMPOUNDS AS SOURCES OF NOVEL ANTIPLATELET DRUGS

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Antiplatelet drugs represent the keystone of prevention of ischemic cardiovascular events such as acute myocardial infarction and stroke. However, the palette of currently available drugs including acetylsalicylic acid (ASA) as the first line therapy faces many issues, such as high response variability, serious side effects and the route of administration. Polyphenol-rich diet is associated with many beneficial effects. However, they have, in general, low bioavailability. Therefore, small phenolic compounds formed by human intestinal microbiome have been suggested to be the cause of these benefits, as they achieve much higher plasma concentrations than the parent polyphenols[1]. In an initial screening study, an efficient compound derived from microbial metabolism of polyphenols has been identified. The efficacy of this compound, 4-methylcatechol, was further confirmed using various models, as well as by using a heterogenous group of healthy donors. Its mechanism of action was studied in detail and points to a disruption of cyclooxygenase and thromboxane synthase coordination[2, 3]. 4-MC was shown to be more potent than ASA and this was also confirmed in our ongoing study with patients suffering from familiar hypercholesterolemia and type I diabetes mellitus. The structure/activity relationship of phenolic compounds was also investigated with 22 structurally related derivatives of 4-MC[4]. 4-MC, a small phenolic compound derived from microbial metabolism of polyphenols is an efficient antiplatelet compound and may present, along with its derivatives, a novel avenue in antiplatelet therapy.

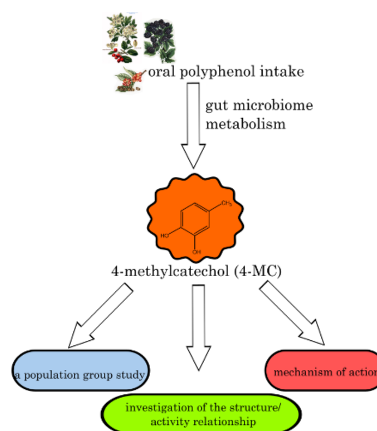

Fig. 1. **Schematic of the research workflow**

This work was supported by Charles University (grant GAUK 1322120-C3) and Czech Health Research Council (projects NU21-02-00135 a NU21J-02-00021).

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POTENTIAL NEUROPROTECTIVE PROPERTIES OF LIPIDIZED CART PEPTIDE ANALOG – *IN VITRO* AND *IN VIVO* MODELS

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Cocaine- and amphetamine-regulated transcript peptide (CARTp) is strong anorexigenic hypothalamic neuropeptide under the direct control of leptin. Due to the suggested link between obesity and Alzheimer´s disease (AD) development, anorexigenic compounds are considered as a potentially neuroprotective tool[1]. Despite therapeutic potential, anorexigenic neuropeptide delivery from the periphery to the brain is complicated because of their peptidic character and absence of physiological transporter in blood-brain barrier. Lipidization of peptides is a useful approach how to increase their stability and achieve central effect after peripheral application.

CARTp receptor has not yet been discovered. Previous work performed by Maletínská et al. described the specific

binding of CARTp to rat pheochromocytomal PC12 cells in the nM range[2].

The shortest pharmacophore of CARTp is CART(61-102) containing three disulfide bridges. Next study performed by Blechová et al. identified CART(61-102) analog with only two disulfide bridges between cys^{74}-cys^{94} and cys^{88}-cys^{101} (2-SS-CART) as ligand with preserved biological activity close to natural CART(61-102)[3]. 2-SS-CART was then modified by attaching fatty acids of different lengths (octanoic acid –octCART, myristic acid – myrCART, palmitic acid – palmCART), all of those analogs preserved high binding affinity to PC12 cells, comparable to natural CART(61-102) as shown in Table 1.

Table 1, Binding affinity of novel lipidized CARTp analogs to PC12 cells

Analog	Ki [nM]	% binding CART(61-102)
CART(61-102)	9.33 ± 4.37	100
2-SS-CART	261.30 ± 209.80	3.5
octCART	76.09 ± 17.66	12
myrCART	12.10 ± 3.40	78
palmCART	1.43 ± 0.41	652

In this study, we aimed to demonstrate the potential neuroprotective effects of our new palmitoylated CARTp analog (palmCART).

We employed an *in vitro* model of glutamate-induced excitotoxicity using PC12 cells. The cells were pretreated with palmCART [10^{-5} M], and cell viability was measured using CellTiter-Glo® assay. The signaling pathways were further examined by the method of western blot. PalmCART was also administered for 3 weeks to mouse model of obesity induced by injection of monosodium glutamate (MSG) to newborn mice, with spontaneous Tau hyperphosphorylation which is hallmark of AD, and changes in the hippocampus, first brain region affected in AD, were evaluated by the method of western blot and immunohistochemistry.

We observed that pretreatment of PC12 cells with the palmCART protected the cells from the glutamate-induced cytotoxic effect manifested by an increased viability compared to cells treated only with glutamate. Furthermore, glutamate-induced inhibition of PI3K/Akt signaling pathway was restored by palmCART pretreatment. PC12 cells exposure to palmCART for 6 h also enhanced the differentiation of the cells into neuronal-like state. 3 week-long treatment of MSG mice with palmCART resulted in body weight decrease and also attenuated Tau phosphorylation and increased neurogenesis in hippocampi of MSG, compared to MSG mice treated with saline.

Our results indicate that palmCART is a promising candidate for treatment of neurodegeneration, but the exact mechanism of action must be further studied.

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BIOPRINTING VASCULARIZED CONSTRUCTS USING RGDT-ALGINATE AND GELMA BIOINKS

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Modern methods of organoid culture allow creation of advanced tissue models, in which cells self-organize into functional tissue-like structures. Many of the current protocols however do not include vasculature, integral part of all human organs. The method of 3D-bioprinting allows also control over the spatial organization, increasing the complexity of said tissue models.

In this work we show optimization of bioink composition to prepare bioprinter- and cell friendly hydrogel environment. Two hydrogels were employed, photocrosslinkable methacrylated gelatin (gelMA) and ionically crosslinkable alginate. Through several strategies (pre-aggregation of cells, combination with non-crosslinking polymers, and especially modification of alginate with RGDT peptide[1]) we dramatically improved their printability, cell survival, proliferation, and possibility of cell-cell and cell-matrix interactions. With utilization of 3D bioprinting, we designed and fabricated both planar and three-dimensional constructs with defined geometry and cellular composition. Interconnected vascular networks were formed within such printed structures. We characterized their parameters through confocal and lightsheet microscopy, followed by 3D reconstruction and segmentation. The organization of microvascular networks was affected by the specific cell types used to form the network, but also by composition of bioink, its physical properties (*e.g.* porosity, crosslinking strategy), and by the geometry and patterning of the printed structures.

Our improvements of the bioink coupled with understanding of biological response of the printed cells allow us to fabricate highly organized 3D constructs, which are further utilized for co-culture with other cell types and organoids, creating more sophisticated tissue models *in vitro*.

This work was supported by Czech Science Foundation (GA21-06524S), Grant Agency of Masaryk University (MUNI/A/1301/2022), and the European Regional Development Fund - project INBIO (No.CZ.02.1.01/0.0/0.0/16_026/0008451).

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SYNTHESIS OF NOVEL CYTOCHALASAN ANALOGUES WITH AN ARYL GROUP AT POSITION 10

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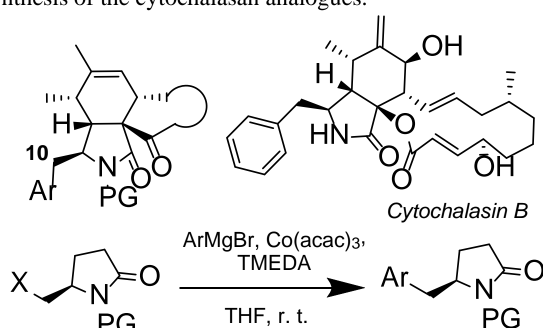
Cancer treatment currently appears to be successful, however, patients are still dying from cancer, mainly as a result of metastasis[1]. The group of substances known as migrastatics, which interfere with cancer cell invasiveness, could resolve this issue.

Cytochalasans are natural fungal products with various biological activities. Most importantly, they show migrastatic activity because they bind to the growing ends of actin microfilaments. Therefore, cytochalasans and their analogues are promising candidates for the development of migrastatics. Most cytochalasans have a tricyclic scaffold that includes a macrocycle and an isoindolone moiety[2]. By molecular docking, we found that modification at position 10 of the cytochalasan core could enhance its interaction with actin. This became the focus of our interest mainly because there has been no sufficient SAR and no universal method for the introduction of modifications to this position has been published so far.

Here we present a new synthetic route for the preparation of new cytochalasan analogues with structural modification at position 10, where different aromatic groups could be located. The new method using cobalt as a catalyst (Scheme 1) was studied on a model compound and was used in the final synthesis of the cytochalasan analogues.



Scheme 1. **Structure of the cytochalasan core itself, cytochalasin B and a new method for the introduction of aryl groups to position 10**

This work was supported by a grant of Specific university research and was financed by an institutional support of the Dagmar Procházková fund.

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STUDYING MECHANISMS OF ALZHEIMER'S DISEASE DEVELOPMENT USING CEREBRAL ORGANOIDS

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Alzheimer's disease (AD) is a neurodegenerative disorder largely associated with amyloid plaques, neurofibrillary tangles, and loss of neuronal connections in the brain[1]. Nowadays, AD is the most common cause of dementia in middle and old age, and with the gradual extension of human life, it has become a significant health problem worldwide. Despite many efforts to use various therapeutics, AD still remains an incurable disease. Therefore, scientists have embarked on the path of improving *in vitro* models that better mimic the pathophysiology of AD patients to better understand the biology of the disease. Currently, induced pluripotent stem cells (iPSCs) are widely used to study human neurodevelopment and disease. In the case of AD, iPSCs have been used to create 3D cerebral organoids (COs), which can adequately mimic AD *in vitro*[2]. Importantly, these *in vitro* models can also mimic the early development of the brain, and they can thus help to understand the initiation steps and molecular mechanisms of this devastating disease.

This project aims to uncover mechanisms that lead to the development of AD using unique *in vitro* 3D stem cell models. In our laboratory, we recently generated lines of iPSCs from patients with familial forms of AD carrying PSEN1(A246E) and PSEN2(N141I) mutations and from healthy sex- and age-matched non-demented controls (NDC)[3]. These cell lines were subsequently used to generate cerebral organoids, and we found that they adequately mimic AD pathology *in vitro*. Importantly, preliminary data showed significant changes during the development of AD-COs compare to NDC-COs. Thus, our data provide further evidence that developmental disorders and altered neurogenesis could contribute significantly to development of a familial form of Alzheimer's disease which is the key pilar of this project.

Funding for this research was provided by the Czech Science Foundation GA21-21510S; European Regional Development

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3D PRINTED CALCIUM PHOSPHATE CEMENTS ENRICHED WITH LIPOSOME-ENCAPSULATED ANTIBACTERIAL ENZYMES FOR BONE INFECTION TREATMENT

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Three-dimensional (3D) printing has been in focus in recent years. Via this method, it is possible to prepare bioactive, patient-specific porous scaffolds[1]. The process of p of the 3D printed scaffold is often accompanied by several microorganisms, which can lead to a bone infection known as *osteomyelitis*[2]. The combination of the thermosensitive copolymer, the calcium phosphate powder and suitable enzymes gives an ideal combination for 3D printing of bone implants with well-defined pore size, mechanical properties, degradation rate, as well as antibacterial activity. Therefore, as the scaffold resorbs, the antibacterial enzymes are released and the *osteomyelitis* can be suppressed.

Unfortunately, as our previous study discovered, the incorporated enzymes do not release in sufficient amounts, due to the microstructure of the 3D-printed scaffold. In this project, the antibacterial enzyme lysozyme was encapsulated into liposomes, providing additional chemical and physical protection to the enzyme during the scaffold curing.

Lysozyme was encapsulated via the ‘heating method’, which does not involve organic solvents and thereby is ideal for enzyme encapsulation. The prepared liposomes (n = 5) exhibited diameters around 117.1 ± 8.6 nm with narrow polydispersity (around 0.1) and zeta potential of −20.2 ± 5.0 mV and were stable over the course of 14 days at 25 °C. The encapsulation efficiency of lysozyme in these formulas was around 64 %.

Comparing the 3 different systems: 1. scaffold, 2. scaffold-lysozyme, and 3. scaffold-encapsulated lysozyme has shown, that coating with liposome encapsulated lysozyme improves the overall release compared to the unencapsulated enzyme. The 1st order release kinetics was determined.

At the moment, a novel antimicrobial enzyme lysostaphin known as Lysstaph-S is being encapsulated and coated onto the scaffolds. The release of Lysstaph-S is currently being evaluated.

This work was supported by the Internal Grants of BUT (SpecificResearch) Reg.No.BD622217001and by the profiBONE project (TO01000309) granted from Iceland, Liechtenstein and Norway through the EEA Grants and the Technology Agency of CR. Czech Nano Lab project LM2018110, funded by MEYS CR, is gratefully acknowledged for the financial support of the measurements at CEITEC Nano Research Infrastructure.

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OPTIMIZED HDX-MS WORKFLOW FOR PROBING ANTIBODY STRUCTURE

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Hydrogen/deuterium exchange followed by mass spectrometry (HDX-MS) is one of the most prominent and versatile techniques providing unique information about protein structure. Its advantages are no limitations in protein size and compatibility with variety of experimental conditions such as pH, temperature, protein concentration, or buffer composition. On the other hand, HDX-MS has limited spatial resolution. HDX-MS is also officially approved by several regulatory agencies as a suitable tool for validation of protein-based biopharmaceuticals. Many of them, mainly monoclonal antibodies, are considered challenging analytical targets due to their *N*-glycosylation and compact structure stabilized by disulfide bonds.

In this study, we systematically evaluated several key steps of the HDX-MS workflow, affecting its success and spatial resolution. This included disulfide bond reduction, quench buffer composition and subsequent online proteolysis conditions where different proteases, alone or in combination, were utilized. Additionally, detailed data processing workflow was developed to help in complete understanding of digest parametrization and easy cross-comparison.

The final, fully optimized conditions provided quantitative digestion, full sequence coverage, optimal peptide length and reasonable redundancy and thus improved spatial resolution of the obtained HDX-MS data.

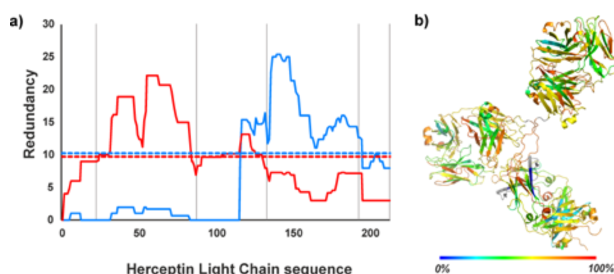

Fig. 1. **a) The distribution of redundancy across Herceptin Light Chain sequence with no denaturing agent in quenching buffer (in blue) and with addition of urea and thiourea (in red). Dashed lines represent average redundancy values. b) 3D structure of Herceptin in rainbow mode after 30min H/D exchange**

Financial support from CSF project 22-27695S and CIIB (LM2018127) is gratefully acknowledged.

LOW-COST PROGRAMMABLE PHOTOMETRIC DETECTOR FOR FIELD ASSAYS IN CHEMISTRY AND BIOCHEMISTRY

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The approach to POCT diagnostics has changed radically in recent years. As a result of the covid-19 pandemic, there has been a rush towards the transfer of analytical techniques from laboratory centres into public hands. Field assays are increasingly important, and the covid era has shown the desirability of bringing alternatives to commercial analysers to make it cheaper and more accessible to monitor various target compounds outside of the lab.

This work presented a low-cost photometer based on the Arduino UNO and a programmable RBG LED as a light source.

The instrument is suitable for measuring all various coloured solutions and for kinetic measurements of enzyme reactions. In this work, phenol red and Prussian blue were used for end-point characterisation of the analyser. The Ellman method was chosen to monitor cholinesterase activity for device characterisation in biochemical experiments[1]. Carbofuran was used as a standard for the screening of pesticides in the environment.

The sensor used in the Open-Source portable photodetection platform is a TSL230R sensor. The software was prepared to control the photometer to select the wavelength and intensity of RGB LED was prepared. Desired wavelengths were obtained by blending the red, green, and blue channel values (0 - 255).

Colour measurements of Phenol red and Prussian blue were made to compare with commercial photometers.

Cholinesterase activity measurements were made as a model use of the analyser for screening of toxicologically relevant substances in the field[2]. Two approaches to measuring enzyme reaction, end-point and continuous monitoring, were evaluated.

The prepared system was found to be comparable to the portable analyser available on the market. It was also shown that data can be collected efficiently using the Excel data streamer add-in. When interfaced with a control computer, the presented sensor system is comparable to standard analytical systems.

This work was supported by the Czech Ministry of Education, Youth and Sports [grant number SV/FVZ202103].

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NOVEL NEUROPEPTIDE IN THE INNERVATION OF SALIVARY GLANDS OF THE TICK *IXODES RICINUS*

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Ticks are hematophagous parasites and important vectors of pathogens. In tick’s life cycle, the salivary glands are especially crucial. They produce various bioactive compounds necessary for tick’s feeding, function in water homeostasis, or sex, and participate in pathogen transfer. Tick’s salivary glands together with other internal organs, including gut, are reservoirs of pathogens transmitted into the host. These organs are regulated by neurons of the central nervous system producing neuropeptides, which function as neuromodulators, neurotransmitters, or hormones[1]. Only few neuropeptides were verified so far in the innervation of salivary glands[2-4].

In this study we focused on neuropeptide PTH (parathyroid hormone-like peptide), previously characterized in the beetle *Tribolium castaneum* and later identified in the tick *Rhipicephalus microplus*[5,6]. Using bioinformatic analysis, we identified PTH and PTH receptors in genome of the hard tick *Ixodes ricinus*. We verified the PTH transcript by cloning and consecutively prepared probe for *in situ* hybridization and peptide-specific antiserum. The *in situ* hybridization and immunohistochemistry revealed expression of PTH in CNS and densely branched innervation of salivary glands, rectal sack, and muscles of the tick. Double-immunohistochemical staining of the organs revealed co-expression of PTH with other neuropeptides. Furthermore, using RT-qPCR we verified expression of PTH receptor in the salivary glands. We thus provide evidence of a novel neuropeptide in the innervation of salivary glands.

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PRODUCTION OF BIODEGRADABLE POLYMERS BY THERMOPHILIC BACTERIA

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Petrochemical plastics are widely used materials in everyone's daily life, which is why their negative impact on the environment is increasing. This is a major issue and needs to be addressed intensively. One option is to replace part of these materials with truly biodegradable plastics such as polyhydroxyalkanoates (PHA).

PHAs are biocompatible and biodegradable polymers produced by many of microorganisms. In line with the concept "Next generation industrial biotechnology" (NGIB) is also the use of thermophilic bacteria[1]. The aim of this concept is, among other things, to increase the competitiveness of industrial biotechnologies. Thermophiles are one of the promising groups of extremophiles. These microorganisms can thrive in temperatures that are inhibitory or even lethal to others. Higher cultivation temperatures bring many positive aspects, especially the reduction of sterility and cooling requirements of the process, which leads to a reduction of the PHA production costs.

For the above reasons, we paid attention to the basic screening of the biotechnological potential in selected species of thermophilic bacteria. We focused on bacteria of the genus *Caldimonas*, *Rubrobacter*, *Tepidimonas*, and *Schlegelella*. And we have achieved promising results. We tested many strains of these genera. As a result, we identified several interesting candidates, for which we performed a more detailed screening of their physiological properties and also sequenced the genome. *Schlegelella thermodepolymerans* DSM 15344^T has proven to be an excellent producer on xylose. Xylose being one of the most abundant monosaccharides in some lignocellulosic materials, may represent cheap and attractive substrate for sustainable production of PHA. The bacterial strain *Tepidimonas taiwanensis* LMG 22826^T not only appears to be a good producer of PHA, but also shows the ability to produce thermostable enzymes. On the basis of the obtained data, we further work on the construction of metabolic models and further investigate in detail their metabolic and biotechnological properties, including the possibility of their use in NGIB to produce PHA from waste substrates.

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SYNTHESIS OF UNNATURAL α-HETEROARYL α-AMINO ACIDS USING A 'MEMORY OF CHIRALITY' CONCEPT

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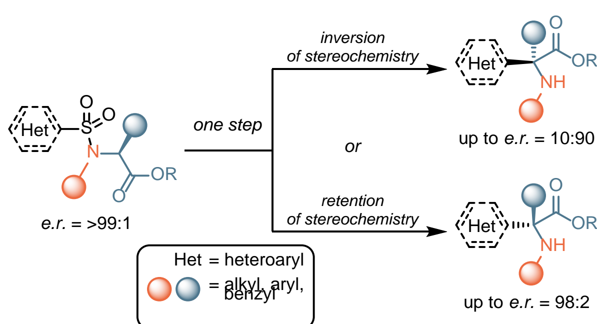
Over the past few years our group was interested in the chemistry of heteroaryl sulfonamides[1,2]. Having developed a short and efficient synthesis of those, unobvious transformations of heteroaryl sulfonamides that yielded rather surprising access to previously unknown but highly interesting molecular scaffolds could be now investigated.

In this contribution, we wish to disclose the following information. The transformation of *N*,*N*-disubstituted sulfonamides into a new, previously unknown class of amino acids – heteroaryl *α*-amino acids (HAA). The described transformation proceeds via a Smiles rearrangement-like mechanism with retention or inversion of stereochemistry of the newly formed quaternary center. Consequently, commercially available naturally occurring L-amino acids can be transformed independently into one of the two enantiomers of HAA in highly enantioselective ration. It is believed, that the stereochemical outcome of the reaction is based on the concept of 'memory of chirality'[3]. The latest achievements, including possible further application of generated compounds in the fields of organic synthesis (new methodology development) or medicinal chemistry (new class of building blocks) will be presented.



Scheme 1: **Synthesis of new heteroaryl α-amino acids (HAA)**

This work was supported by the European Regional Development Fund-Project “Centre for Experimental Plant Biology” (no. CZ.02.1.01/0.0/0.0/16_019/0000738) and by the Internal Grant Agency of Palacky University (IGA_PrF_2022_012 and IGA_PrF_2022_022).

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POLY(3-HYDROXYBUTYRATE) BASED MATERIALS FOR 3D PRINTING OF MEDICAL APPLICATIONS FOR TISSUE ENGINEERING

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One of the most advanced approaches used in contemporary regenerative medicine is tissue engineering. The key role in tissue engineering is played by a porous structure (scaffold), which serves as a support for cells and is then integrated into the human body. The scaffold must have a suitable porosity and 3D structure for the cells to grow through the entire volume of it and form new tissue[1]. In recent years, a method of 3D printing is capturing attention in the bone tissue engineering field as it allows complex tailor-made structures to be produced in a time and cost-effective manner for every specific case and patient. Some bioplastics, such as polylactic acid, poly(3-hydroxybutyrate), or polycaprolactone are appealing not only for their environmental friendliness but also for their biocompatibility[2]. Furthermore, tricalcium phosphate as well as hydroxyapatite can be used as a bioactive filler to promote *in vivo* osteogenic differentiation of mesenchymal stem cells[3].

The research focuses on 3D printing and testing of P3HB-based scaffolds. Three polymer blends were prepared based on either commercial poly(3-hydroxybutyrate) or P3HB from the chloroform-free extraction route, poly(lactic acid) and polycaprolactone, oligomeric adipate ester plasticizer, and tricalcium phosphate as a bioactive filler, and processed into the form of 3D printing filaments. The temperature tower test and warping test were conducted to determine the processing conditions for 3D printing. Mechanical tests (tensile, three-point flexural, compression) were used to study the mechanical properties of materials. Scaffolds with different surfaces were 3D printed from prepared filaments to determine the most optimal surface for cell proliferation. Optical contact angle measurement was conducted to determine the surface properties and their influence on cell adhesion, followed by the calculation of surface free energy. 3D printed surfaces were also subjected to roughness analysis by confocal microscopy to determine the effect of roughness on contact angle with water and cell growth. Finally, *in vitro* tests on scaffolds were conducted to classify the cytotoxicity of the materials and the influence of the scaffold´s surface on cell growth and proliferation.

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SYNTHESIS OF HELACENE MODELS

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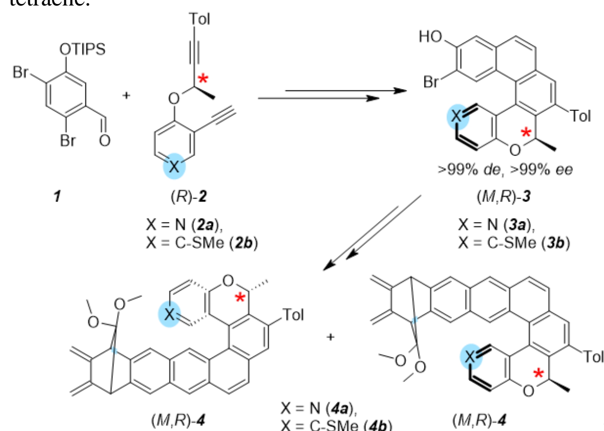
The development of functional organic materials suitable for spintronic devices is of particular interest nowadays. The materials should exhibit chiral-induced spin selectivity effect, emit circularly polarized light and be (semi)conductive[1,2]. Helacenes, hybrid molecules of helicenes and acenes, are promising candidates to show the mentioned physical properties.

Here, we report the synthesis of pyridooxa[5]helacene ***4a*** and methylthiooxa[5]helacene ***4b*** models (Scheme 1). The key steps in the preparation of (*M*,*R*)-helicenes ***3a*** and ***3b*** were Sonogashira cross-coupling reaction of dibromide ***1*** and (*R*)-diyne ***2a*** or ***2b***, propargylation of the aldehyde, and stereoselective [2+2+2] cyclotrimerization reaction previously developed in Starý group[3]. The high diastereoselectivity (>99% *de*) of

cycloisomerization was ensured by 1,3-allylic-type strain between methyl and tolyl groups. Enantiopure helacene models ***4a*** and ***4b*** were synthesized by aryne Diels-Alder reaction between helicene derivatives and corresponding tetraene.



Scheme 1. **Key intermediates in the synthesis of helacene models *4a* and *4b***

This work was financially supported by the European Commission (Grant Agreement No. 859752, HEL4CHIROLED-MSCA-ITN Project) and IOCB CAS (RVO:61388963).

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UNCOVERING THE ROLE OF PROTONATION IN BACTERIOPHYTOCHROME PHOTOREACTION

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Bacteriophytochromes have a strong potential to be used in optogenetics due their photoconversion properties[1,2]. These photoproteins could be switched between two states Pr and Pfr, absorbing red and far-red light, respectively. In bacteriophytochrome from *Deinococcus radiodurans* (DrBphP) biliverdin (BV) chromophore is covalently bound to a conserved cysteine in the PAS (Per-ARNT-Sim) domain but physically embedded within the GAF (cGMP phosphodiesteraseadenylate cyclase FhlA) domain. The PAS-GAF entity here and after is referred as a chromophore-binding domain (CBD), and a phytochrome-specific GAF related domain as PHY domain. The majority of chromophore-protein interactions are within the CBD, while an important additional chromophore-related interaction is with a “hairpin,” “tongue,” or “arm” extension of the PHY domain. The light absorption leads to ultrafast Z-to-E isomerization of the biliverdin D-ring with concomitant rotation of the C15-C16 methine bridge between the C- and D-ring that later on triggers large structural changes in photosensory module (CBD-PHY)[3,4]. The photosensory module is followed by an output effector module, which is often a histidine kinase (HK) domain in bacteria and cyanobacteria but is more variable in plants. The PHY tongue adopts a β-turn-like conformation in the Pr state but an α-helix and coil in the Pfr state, these changes relay to the effector module, thus regulating its activity.

The photoreaction is driven by the signal network between biliverdin, amino acid side chain and water molecules in a chromophore proximity[5]. Several residues play a role in tuning reaction yields and spectral features. The two histidines, H260 and H290, are conserved among phytochromes and are involved in the structural heterogeneity of the Pr-state. Other residues, D207 and Y263, form a hydrogen-bonding network around C15-C16 methine bridge between the C- and D-ring. The water network plays a crucial role in shaping the spectral responses and protonation/deprotonation events of the biliverdin.

The role of the above-mentioned residues have been studied in both chromophore-binding pocket (CBD) and photosensory modules (CBD-PHY) constructs by pH-dependent UV-VIS spectroscopy[5,6]. The site-selective mutagenesis have allowed alternating the spectroscopic properties of ground state population in several ways. For our study we have narrowed the experimental set to three variants of CBD-PHY as contrary to CBD fragment, it can enter the full photocycle: CBD-PHY, CBD-PHY-H290T, and CBD-PHY-H260A.

Studies, both experimental and theoretical, suggest that in most phytochromes, the bilin chromophore is in its fully protonated form at physiological pH. Therefore, we would like to address the role of protonation on the primary steps of phytochrome photoreaction. As it has recently been shown that a fully protonated (>99%) population for different mutants corresponds to different pH we have compare CBD-PHY-H260A (at pH 5.5) for protonated ground state populations, and CBD-PHY-H260A (at pH 9) for deprotonated populations.

Our study focuses on the primary steps of the photocycle, where we analyse the role of the key residues and protonation at the excited states reaction. Photoreaction yields are controlled by the branching among several pathways that occur on the ultrafast timescales. Time-resolved spectroscopic techniques are commonly used to elucidate the chromophore-protein interactions, photoinduced signal transduction, and other fast changes in the field of photosensors.

Here, we present the first ultrafast spectroscopic study of the initial stages of the photocycle in a photosensory module, considering the diversity of the ground state population. We

examine differences in reaction pathways and outline future research prospects.

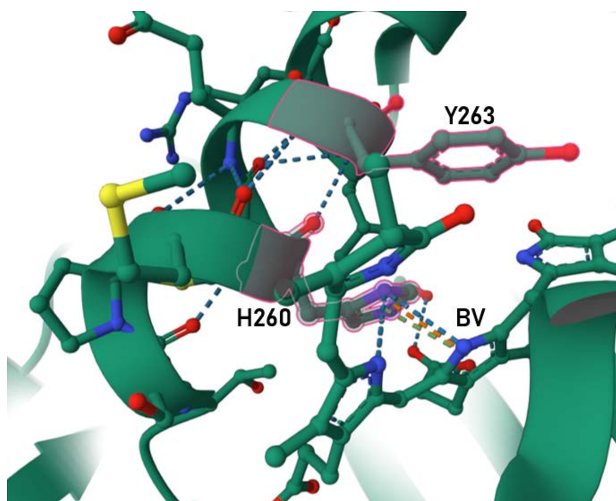

Fig. 1. **Crystal structure of chromophore-binding domain of CBD-PHY (PDB code: 4O0P) highlighting biliverdin (BV), conserved histidine (H260), and tyrosine (Y263) interaction**

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TARGETING THE INSULIN RECEPTOR WITH HORMONE AND PEPTIDE DIMERS

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Insulin and insulin-like growth factor 1 (IGF-1) are polypeptide hormones that show a high structural similarity and overlapping spectrum of biological activities. Insulin is a two-chain hormone that elicits mostly metabolic responses and IGF-1 is a single-chain hormone that exerts mostly mitogenic effects[1]. The biological actions are mediated by binding of these hormones to similar tyrosine-kinase receptors, namely insulin receptor (IR) and IGF-1 receptor (IGF-1R) and by their interaction with L1 domain, the α-CT peptide (Site 1) and Fn-III-1 domain (Site 2) of the receptors[2]. We investigated the possibility if a covalent crosslinking of two insulins or a covalent conjugation of two α-CT peptides (the C-terminus of the extracellular IR/ IGF-1R α-subunit involved in the ligand binding site) (Fig. 1) can result in potent binding to the receptors and modulation of their functional properties. Such conjugates could be IR/IGF-1R agonists or antagonists and could find applications in treatment of diabetes, growth disorders or cancer. We synthesized a series of dimers of the α-CT peptides of IR or IGF-1R and insulin dimers with different linkers via copper(I)-catalyzed azide-alkyne cycloaddition[3]. The receptor binding properties of the constructs were investigated using radioligand receptor binding assays with IR and IGF-1R and for activation of the receptors. Some of the insulin dimers showed preferential binding specificity towards the isoform A of the insulin receptor and the insulin dimers also stimulated insulin receptor more strongly than would be consistent with their binding affinities. Our results[4] suggest that designing insulin dimers may be a promising strategy for modulating the ability of the hormone to activate the receptor or to alter its specificity toward insulin receptor isoforms.

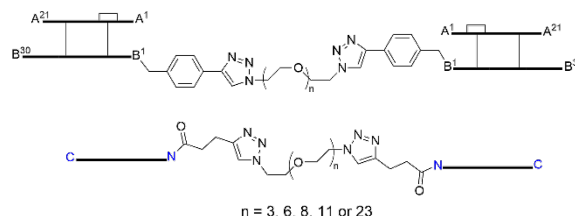

Fig 1. **Simplified representation of insulin dimers (top) and dimers of α-CT peptides and mimetic peptide (bottom). A1, B1 show the N-termini and A21 and B30 show the C-termini of the A and B chains of insulin. Blue N and C letters show the positions of the N- and C-termini of the α-CT peptides or the mimetic peptide[4]**

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CUCURBIT[7]URIL-BASED MOLECULAR DEVICES

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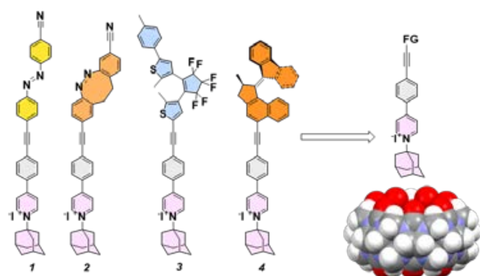
There is an increasing interest in two-dimensional arrays of molecular devices on various surfaces, which is driven by multiple possibilities of application, such as stimuli-responsive materials[1,2]. We propose that supramolecular host-guest complexation could offer a new perspective in

preparation of single-layers of molecular devices, with bulky macrocyclic host cucurbit[7]uril (**CB[7]**) separating molecular devices, while supporting surface adsorption.

Synthesized rod-shaped molecular devices ***1-4*** contain 1-adamantyl-pyridinium-based binding sites on one terminus. This moiety is known to form stable complexes with cucurbit[7]uril[3]. The second end of these rods carries either photoswitches (***1-3***) or unidirectional light-driven molecular motor (***4***). The kinetics of the switching process of devices, as well as their **CB[7]** complexes (***1-4*@CB[7]**), was followed in solution by UV-Vis spectroscopy. It was confirmed that complexation with **CB[7]** does not have significant impact on the functionality of such supramolecular assemblies. The next stage of the study involves exploration of self-assembly properties of these complexes on a gold (111) surface. These systems are analyzed by several techniques like PM-IRRAS, SERS, AFM, etc.



Scheme 1. **Synthesized molecular devices *1-4* and complexation to CB[7]**

This work was supported by the IOCB CAS (RVO: 61388963), Ministry of Education, Youth and Sports (LTAUSA19120) and the European Social Fund; OP RDE; Project: "IOCB Mobility II" (No. CZ.02.2.69/0.0/0.0/18_053/0016940).

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TELOBASE: A COMMUNITY-CURATED DATABASE OF TELOMERE MOTIFS ACROSS THE TREE OF LIFE

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Eukaryotes solve the problem of incomplete replication and protection of the ends of linear chromosomes by capping their very ends with specialized nucleoprotein structures known as telomeres. Telomere DNA typically consists of tandemly repeated minisatellite motifs that in general have $(T_xA_yG_z)_n$ structure, but recent decade of discoveries shows unexpected diversity within taxa[1-4].

Currently, there are two database sources containing information related to telomere motif diversity in organisms, Plant rDNA database[5] and older Telomerase database[6]. However, both resources primarily focus on a different type of data and cite only fragments of relevant literature published in decades of telomere research.

We have developed TeloBase database as a source of information related to telomere motif diversity in nature that gathers not only data from comprehensive literature screen but also from the analysis of available NGS data (Fig. 1). TeloBase enables easy-to-use data manipulation and visualisation that benefits from internal taxonomy created by utilizing popular on-line taxonomy databases. To tackle the issue of topicality which most databases face, TeloBase features simple data application form and curation of newly applied data that is not dependent on administrators but research community.

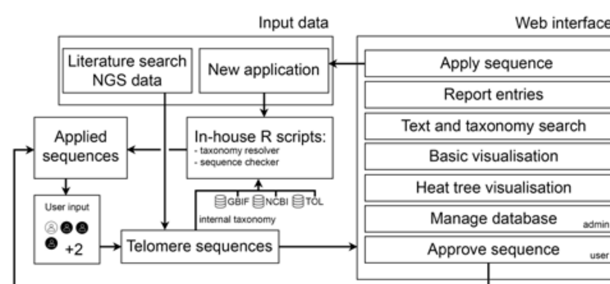

Fig. 1. **Overview of the TeloBase system**

CF Bioinformatics of CEITEC MU, Brno is acknowledged for providing computational resources. Computational resources were supplied by the project ELIXIR-CZ (LM2018131), part of the international ELIXIR infrastructure and "e-Infrastruktura CZ" (e-INFRA CZ LM2018140) supported by the Ministry of Education, Youth and Sports of the Czech Republic. This research was funded by the Czech Science Foundation, project 20-01331X, and Ministry of Education, Youth and Sports of the Czech Republic, INTER-COST project LTC20003.

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NOVEL COMPLEXES OF SILVER-BEARING *N*-HETEROCYCLIC CARBENE LIGANDS: SYNTHESIS AND APPLICATION.

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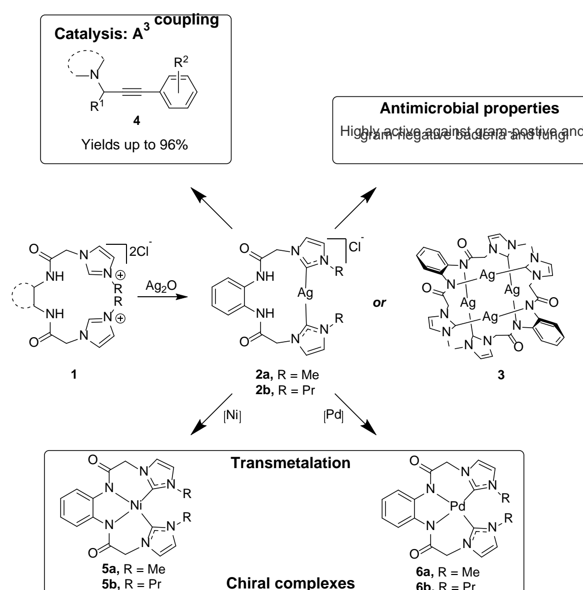
In recent years the interest in *N*-heterocyclic carbenes (NHCs) has grown significantly, especially in the organometallic field. NHCs are seen as a possible replacement or complement of phosphines. The use of metal-NHC complexes as catalysts in many chemical transformations is superior to the use of metal-phosphine[1]. Among the transition metal where NHCs have been applied, there is a growing interest in silver-NHCs (Ag-NHCs) due to simpler synthetic strategies, stability, fascinating structural diversity, and a wide range of applications[2]. In our group, we have recently developed a methodology for synthesizing a novel chelating Ag-NHC complex by containing a bisamide moiety in their backbone. The synthesis of the complex depends on the equimolar ratio of the silver source and the ligand precursor. When equal equimolar is used a ligand stabilizes the silver ion in a rare chelating mode **2**. On the other hand, if the silver source is used in excess, the reaction leads to the formation of an unprecedented tetranuclear silver complex **3**. Such a complex is stabilized by two equivalents of ligand in which silver atom is coordinated to one NHC and one amide moiety. The chelating aspect of the Ag-NHC complex **2** is a remarkable feature and is very rare to find similar chelating complexes in literature. Antimicrobial properties and the use of such complexes as catalysts in A3-coupling reactions were also studied. The complexes showed extraordinary properties in both directions. The MIC values were as low as 1 μg/ml and the A^3-coupling products were isolated with yields up to 96% using catalysts loads as low[3] as 0.1 mol%. Furthermore, Ag-NHCs complexes have been recognized as effective carbene group transfer agents. For that motive, some of the complexes were subsequently applied in the synthesis of NHC complexes of other metals, such as nickel **5** and palladium **6**, the synthesis of which failed previously[4]. Both complexes proved to have an axial chirality due to the coordination of the ligand to the metal centre in a helical way. The nickel complex **5a** proved to be configurational stable at room temperature and **5b** is stable even upon heating to high temperatures.

This work was supported by the Charles University Primus program (PRIMUS/20/SCI/017).



Scheme 1. **Synthesis and application of the developed Ag-NHCs**

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TRANSCRIPTIONAL PROFILING REVEALED IMPAIRMENT OF EARLY BRAIN DEVELOPMENT IN hiPSC-DERIVED MODELS OF ALEXANDER DISEASE

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Alexander disease (AxD) belongs to rare severe neurodegenerative disorders. It is caused by mutations in astrocytic intermediate filament protein GFAP resulting in an extensive loss of white matter[1]. Since majority of the available animal models do not accurately recapitulate the disease’s phenotype, models derived from human induced pluripotent stem cells (hiPSCs) were introduced as an effective alternative for studying AxD on the human genetic background.

We used patient-derived 2D co-cultures of neurons and astrocytes and 3D cultured 160-day-old brain organoids to investigate transcriptional changes resulting from a GFAP mutation. Leveraging single-cell RNA sequencing, we revealed previously unknown effect of the GFAP mutation on

development of the neural tissue. The analysis of cellular populations showed less mature cellular states enriched in AxD samples. Furthermore, higher proportion of cells in AxD brain organoids diverged their differentiation path into other lineages than neuroectoderm, generating cell types of e.g., mesodermal origin. Extracellular matrix and cytoskeletal components consistently appeared dysregulated in AxD samples, suggesting involvement of the mutant GFAP in mechanical properties of cells and their surroundings, which may impair their ability to develop into neural tissue[2].

Remarkably similar results were also generated by bulk RNA sequencing experiments performed on early organoids (3-9 days old), showing that the development is affected by the GFAP mutation already in early stages.

The role of the GFAP mutation in development might bring new insights into pathogenesis of the AxD and aid in design of therapeutical strategies in the future.

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INVESTIGATION OF HUMAN HSC70 OLIGOMERIZATION

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Heat shock cognate protein 70 (Hsc70) is a 71 kDa chaperone protein belonging to the ubiquitous family of heat shock proteins 70 (Hsp70). The representatives of this protein family are considered as molecular machines with ATPase activity facilitating correct folding of spatial protein structure, both in normal and stressful conditions (hypoxia, heat shock, pH fluctuations etc.). In addition, Hsc70 was identified as an uncoating enzyme for triskelion meshwork on the surface of clathrin-coated vesicles. Among other roles, Hsc70 prevents protein aggregation and assists polypeptide maturation, it facilitates the protein transport into organelles, such as endoplasmic reticulum and mitochondria. It is involved in targeting proteins for lysosomal degradation and in many others important cellular processes related to protein homeostasis [1]. Therefore, the regulation of Hsc70 and other Hsp70 proteins is believed to be highly important, especially in a context of cellular stress.

Based on the experimental observation, the mechanism of inactivation through oligomerization was hypothesized. The dimer and trimer species of Hsp70 proteins were identified both in case of prokaryotic and eukaryotic homologs[2,3]. It was also speculated that Hsp40 cofactors promote oligomerization to even higher-order oligomers[2]. This and other possible oligomerization models of wild type HSC70 and the subset of HSC70 mutants were investigated by cross-linking mass spectrometry. The distance constraints imposed by different cross-linker lengths allowed to build structural models of Hsc70 oligomeric species. To decipher between intra and intermolecular cross-links, the studied protein and its mutants were produced in ^{15}N-labeled form as well.

This project was supported by the Czech Scientific Foundation (22-27695S).

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PULL-DOWN ASSAY AS A NOVEL APPROACH FOR THE IDENTIFICATION OF COMPOUNDS INTERFERING WITH THYROID HORMONE SIGNALLING IN COMPLEX ENVIRONMENTAL MIXTURES

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Despite great efforts to improve water quality on through the Water Framework Directive (EU), where priority substances were set, there are still a lot of chemicals entering aquatic ecosystems mostly from anthropogenic activities, with the potential to cause disruption of the endocrine system (such as disruption of thyroid hormones).

Plasma protein transthyretin (TTR) plays an important role in transport of thyroid hormones (T3 and T4) in vertebrates. Some environmental pollutants can compete with natural TTR ligands, which may disrupt TTR binding and thyroid hormone transport and thus lead to neuro/developmental and metabolic disorders[1,2]. Little is known regarding the ability of chemicals occurring in aquatic environment to bind to the TTR protein[3]. Our results indicate

that complex environmental mixtures associated with discharges from wastewater treatment plants can elicit such effect, but the effect drivers are unknown.

Here, we present a novel approach to this problem, a method based on an interaction of the TTR with its ligands, called the pull-down assay (see Fig.1). In this method, recombinant TTR is prepared by inserting TTR sequence into a specifically selected plasmid and expressed in *E. coli*. The purified recombinant TTR is incubated with prioritized extracts of environmental water samples, leading to the potential binding of TTR ligands to TTR protein. The complex is bound to magnetic Ni^{2+} affinity resin via Histidine tag added to the TTR sequence. Magnetic particles with bound protein-ligand complex are attached to the surface of the reaction tube using a magnetic holder and the washing steps lead to the displacement of unbound chemicals. The complex is then eluted with imidazole solution. TTR is denatured with a solvent and ligands are released to the solution. The functionality of the high-purity recombinant protein has been confirmed in TTR binding bioassay, which is a fluorometric method, where a fluorescently labeled T4 (fT4) bound to the TTR establishes the starting level of signal, which decreases proportionally to the concentration of a competing ligand[4]. And HPLC-(ICP)-MS/MS analysis of the standard ligand (T4) and an artificial mixture of TTR ligands were used to optimize the method (PFOS, PFOA, benzophenone-2).

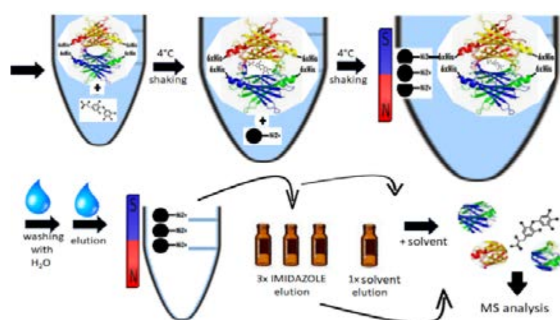

Fig. 1. **Scheme of the pull-down assay**

Pull down assay with a complex environmental sample was conducted, and the identification of ligands was performed via non-target MS analysis on LC-Orbitrap and confirmed with target MS analysis. The potential ligands could be identified using library searches and then tested for their activity in TTR binding bioassay. Finally, both known and novel ligands were identified (e.g. diclofenac and its metabolites). The presented novel pull-down assay approach proved to be a promising tool for the identification of the drivers of endocrine disrupting effects in complex environmental mixtures.

The project has received funding from Czech Science Foundation (GACR) under grant agreement GX20-04676X.

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THE GENERATION OF NEURONAL CELLULAR MODELS FOR THE ANALYSIS OF PARKINSON’S DISEASE RELATED PATHOLOGY

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Parkinson’s disease (PD) is the most common movement disorder and second most common neurodegenerative disease, which affects around 0.3% of the general population and 1 - 3% of the population over the age of 65 (ref.[1]). Progressive loss of dopaminergic neurons and accumulation of intracytoplasmic protein inclusions called Lewy bodies (LB) are the prominent hallmarks of PD. LB mainly consists of a protein called alpha synuclein (a-Syn), which is an intrinsically disordered protein predominantly expressed in the brain where it is concentrated at the nerve terminals[2,3]. Progressive accumulation of a-Syn and the formation of oligomers have been suggested to play a critical role in the pathogenesis of PD. Understanding the pathological changes, diagnosis, and treatment of PD is one of the pressing issues of present-day neuroscience and requires development of robust disease models. Cell models are efficient tools that allow the quick and multifaceted analysis of the pathological changes associated with PD.

In our present study we created novel neuronal cell lines overexpressing a-Syn (wild type or its PD-related mutated variant A53T) tagged with GFP in uniform genetic background, which will allow us to obtain more consistent data. These newly generated cell models were then separated into three types each, based on the expression levels of a-Syn using cell sorting, and analysed for cell viability and for expression levels using Western blot and qPCR. Next we comparatively analysed the biological effects and cellular changes triggered in these cell models as the result of alterations of a-Syn levels, comparing the effects of overexpression of a-Syn (WT or A53T) among themselves and with cells not overexpressing a-Syn.

Our study would contribute to the better understanding of α-Syn related pathologies in PD and to the establishment of innovative approaches for its treatment.

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EXPANDABLE LUNG-LIKE EPITHELIAL CELLS, A UNIQUE STEM CELLS-DERIVED PULMONARY STRESS AND REGENERATION MODEL

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The lung is a highly complex organ essential for numerous basic metabolic functions, such as gas exchange, protein synthesis, and conversion of vasoactive substances. Various external and internal factors, e. g. systemic metabolic disorders and inflammation, or external factors like pathogens, mechanical injury, or exposure to harmful substances can lead to a homeostasis disruption and induction of massive cellular stress. Also endoplasmic reticulum and mitochondrial disorders compromise the structure and function of lung tissue. In addition, impaired or deregulated molecular mechanisms can exacerbate or even evoke lung regeneration failure, which can be the source of many pathologies, including cancer, reduced chronic obstructive pulmonary disease or idiopathic pulmonary fibrosis.

Therefore, understanding the molecular mechanisms associated with the response to stress conditions and regeneration is essential. Here we developed a unique and robust *in vitro* model of stem cell-derived expandable lung-like epithelial cells (ELEP) to determine key factors in molecular crosstalk between cell stress pathways, where endoplasmic reticulum and mitochondria cooperate in response to alterations of lung homeostasis and differentiation.

We investigate the precise differentiation trajectories of ELEP to bronchial/alveolar lung cell types, performing a comparative analysis of the epithelial-bronchial and alveolar - phenotypes acquired during differentiation ELEP on both molecular and morphological levels. Because cell response to extrinsic or intrinsic stress determines normal or abnormal regeneration of lungs, we also reveal how cell stress response affects ELEP differentiation to bronchial-alveolar lung cell types. To understand the role of key cell stress response proteins in the regulation of ELEP differentiation, we establish ELEP clones with downregulated principal mitochondrial and ER regulators and investigate how the mutant ELEP differentiate or respond to metabolic stress. We find limitations of in vitro models in a bias introduced by (epi)genetic background of cell models, thus a further insight in identifying similarities and differences in cell stress response between ELEP differentiated from individually different hiPSC is also needed.

We believe that elucidating the connections between the cellular stress response and the terminal differentiation of lung cells, using a robust ELEP *in vitro* model, can lead to a deeper understanding of the development of pathologies and can offer new possibilities for their prevention and therapy.

This study was supported by Masaryk University (MUNI/A/1301/2022) and financial support of GA ČR (GA23-06675S). LM is supported by MHCZ–DRO (MMCI, 00209805).

DEVELOPMENT OF A SEMI-HIGHT THROUGHPUT METHOD FOR SCREENING OF NEUROSTEROIDS PLASMA STABILITY USING ANALYTICAL HPLC WITH EVAPORATIVE LIGHT SCATTERING DETECTION

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The plasma stability of individual compounds has a crucial role in drug development[1]. It is important to ensure proper distribution to target organs and pass through the blood-brain barrier. Moreover, it is essential for maintaining acceptable drug concentrations and for achieving pharmacological effects.

Neurosteroids are endogenous compounds that are according to the definition[2] compounds, the synthesis and accumulation of which occur in the brain independently of supply by the peripheral steroidogenic glands. It should be noted that all endogenous neurosteroid compounds have hydroxyl and ketone moieties on their skeleton. Consequently, these substituents could be metabolized when they are transported through the bloodstream and organs. Yet, these compounds have been identified in the plasma of humans or animals in concentrations that vary from hundreds to thousands of ng/mL. We have hypothesized that some neurosteroids may display atypical stability in plasma that may reflect their well-known neuroprotective potential[3].

The aim of this study was to develop an analytical method for screening of steroidal compounds by reversed-phase HPLC with detection by evaporative light scattering detection (ELSD) and compare this approach with generally known photo diode array (PDA) detection. The ELSD is considered an attractive alternative to conventional detection such as UV-VIS due to its versatility and universality and the fact that the majority of tested neurosteroids are non-UV-absorbing compounds. The chromatographic separation was achieved using a phenyl-hexyl column (2.1 x 100 mm; 1.7 µm). The mobile phase consisted of mixtures of acetonitrile or methanol/water with the addition of acids or buffers in gradient

elution. The comparison of both methos, method optimization, and stability results will be discussed.

This work was supported by the Czech Science Foundation GACR, No. 23-04922S and Research Project of the Academy of Sciences of the Czech Republic: RVO grant 61388963.

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MAGNETIC NANOPARTICLES WITH ANTIOXIDANT PROPERTIES FOR THERAPY AND DIAGNOSTICS

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Most prevalent problems in modern healthcare are the age-related diseases, such as neurodegenerative, oncological, and cardiovascular ones. New diagnostic and therapeutic approaches are being developed to detect these disorders at early stages and stop their progression. Among the former, superparamagnetic iron oxide nanoparticles (SPIONs) represent a highly promising drug delivery system that is not only capable of improving targeting of the active substance, but can also serve as a theranostic agent itself. Namely, the SPIONs are able to produce heat in alternating magnetic field, which allows them to kill tumors; that is the key of hyperthermia treatment. Also, it is possible to monitor the biodistribution of SPIONs in the real time using MRI.

The SPION cores were prepared by reaction of iron chlorides with ammonia solution followed by oxidation of precipitated magnetite with hydrogen peroxide in order to convert all iron ions into a non-toxic trivalent form. According to TEM, the mean size of produced γ-Fe_2O_3 cores was 16 nm with a moderately broad particle size distribution.

In order to improve colloidal stability of neat SPIONs and introduce the desired antioxidant functionality, their surface was coated with hydrophilic polymer shell, consisting of poly(ethylene glycol) possessing a bisphosphonate anchoring end group, and poly(vinyl acetate-*alt*-maleic anhydride) modified by reaction with human hormone melatonin (Fig. 1). First polymer ensures steric stabilization of the particles, while the second one provides electrostatic repulsion between the SPIONs by ionized anchoring carboxyl groups. Moreover, it has not only a superb antioxidant activity due to conjugated melatonin, but also demonstrates an anti-inflammatory effect, which is why the latter was chosen as a model drug.

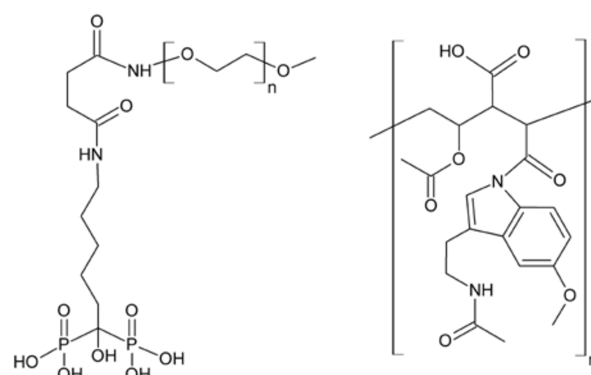

Figure 1. **Structure of polymers used for the surface modification of superparamagnetic γ-Fe_2O_3 nanoparticles**

Financial support of the International Visegrad Fund (project # 52210722) is gratefully acknowledged.

LABELING OF LIPOPEPTIDES WITH LANTHANIDES FOR THEIR SPECIFIC DETERMINATION AT VERY LOW CONCENTRATIONS IN BIOLOGICAL MATRICES BY ICP-MS

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The importance of peptide-based drugs has been steadily growing over the past years. Peptide-based drugs are similarly to their degradation products non-toxic, they have high binding affinity to the natural receptors and can be easily synthetized. On the other hand, they also have many disadvantages. Peptides in their natural form are not resistant to proteases and they are not able to cross the blood brain barrier (BBB). These deficiencies can be overcome by lipidization. Lipidization is a process consisting of an attachment of a fatty acid to the peptide. It seems to be a promising tool how to stabilize the peptide structure and overcome the natural shortcomings[1].

Anorexigenic (food intake lowering) and orexigenic (food intake increasing) peptides are being consistently studied as a potential drugs for the treatment of various diseases, e.g., obesity/cachexia, diabetes or neurodegenerative diseases. These peptides act centrally, however, in their natural form they are not able to perform the desired effect. Lipidization helps reaching the central effect after the peripheral administration[2]. Prolactin-releasing peptide (PrRP) and its lipidized analogs seems to be very attractive compounds in obesity and Alzheimer’s disease treatment[3].

Even though lipidized PrRP is a promising compound, the exact mechanism of function remains unclear. We came up with a new approach of monitoring these compounds in biological matrices, even in several orders of magnitude lower concentrations. Labelling the lipopeptides with lanthanides

allows their determination using the ICP-MS method. The goal of this study is to determine these lanthanides labelled lipopeptides in *in vitro* spiked liver and brain and then further study their concentration levels in *in vivo* models.

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ENVIRONMENTALLY RESPONSIBLE C(SP^2)–P CROSS-COUPLING ENABLED BY MULTIMETALLIC CATALYSIS IN WATER

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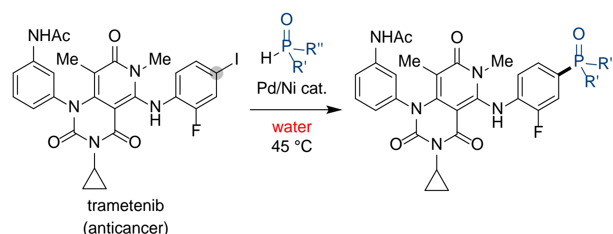
Compounds containing C(sp^2)–P bonds, such as phosphonates, phosphinates and phosphine oxides, feature numerous applications in pharmaceutical and agrochemical industries and in materials science[1]. However, their syntheses by the conventional C(sp^2)–P cross-coupling reactions rely on use of precious transition metals, often high catalyst loadings, aprotic solvents with future regulatory restrictions (*N*,*N*-dimethylformamide, *e.g.*), and rather harsh reaction conditions (heating above 100 °C)[2,3]. Moreover, most of the published C(sp^2)–P coupling methods showcase limited substrate scope, both with respect to aryl halides and H–P coupling partners. Herein, we disclose a conceptually novel, environmentally responsible C(sp^2)–P cross-coupling methodology enabled by the multimetallic catalysis approach. The developed method operates under mild conditions in water, tolerates various functional groups, and provides access to structurally diverse (hetero)aryl (thio)phosphonates, (thio)phosphinates and phosphine oxides, including those containing medicinally relevant scaffolds.



Scheme 1. **C(sp^2)–P cross-coupling in water**

This work was supported by The Czech Science Foundation (O.B., grant no. 20-25137Y).

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PROTEIN-LIPID INTERFACES IN PLANT CELL TRAFFICKING STUDIED BY MOLECULAR DYNAMICS

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Anionic phospholipids (phosphatidic acid, phosphatidylserine, phosphatidylinositol, and its phosphorylated derivates phosphoinositides) are essential regulators of many cellular processes in plants, including signalling, cell trafficking, cell growth and division. They can modulate the physical properties of membranes, establish cell polarity, act as signalling molecules and finally also mediate interactions with peripheral membrane proteins[1].

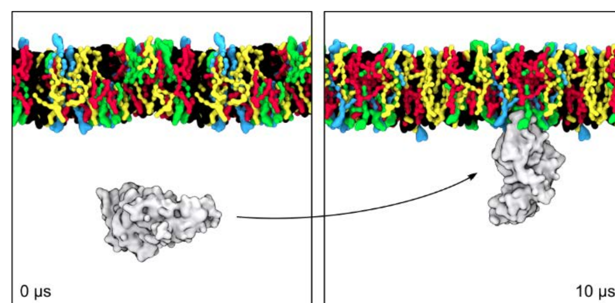

Fig. 1. **Representative snapshots of a selected coarse-grained molecular dynamics simulation demonstrating the localization of the *Nicotiana tabacum* putative clathrin assembly protein at times 0 and 10 μs. Protein is depicted in grey, phosphatidic acid in red, phosphatidylserine in yellow, phosphatidylinositol 4-phosphate in green, phosphatidylinositol 4,5-bisphosphate in blue, other lipids in black. Water and ions were left out for the sake of clarity**

This project aims to identify peripheral membrane proteins of *Nicotiana tabacum* and unravel their specificity to anionic phospholipids using growing pollen tubes as a model system. To reach the goal, we first obtained a dataset of peripheral membrane proteins of *N. tabacum* that bind to

anionic phospholipids of the plasma membrane. Since our research area of interest comprises cell trafficking events emphasizing endocytosis and exocytosis, we selected proteins possibly involved in these processes for subsequent experiments, including molecular dynamics simulations.

Molecular dynamic (MD) simulation can be seen as a powerful computational microscope enabling the study of biological systems in unprecedented detail[2]. Here, we used coarse-grained MD simulations[3] to investigate the membrane binding of several *Nicotiana tabacum* monomeric endocytic adaptor proteins that possess an ANTH domain fold (identified in our dataset). Despite the structural similarity between the proteins, MD simulations revealed differences in the ability to bind lipids, mostly in good agreement with experimental results. Moreover, this technique gave us insight into molecular details of the interactions enabling a more detailed understanding of the protein-membrane interfaces in general.

This work was supported by the grant GACR 19-21758S and 22-35680M from the Czech Grant Agency.

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ANTIBODY DC11 BINDS AD-SPECIFIC CONFORMATIONAL EPITOPE OF NEURONAL PROTEIN TAU

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The protein tau is a microtubule-associated protein predominantly expressed in neurons. Upon various posttranslational modifications, by hyperphosphorylation or truncation of its polypeptide chain, tau dissociates from microtubules to form insoluble intracellular inclusions. Presence of toxic tau aggregates together with microtubule disintegration lead to nerve cell damage. Although it has not yet been confirmed whether this process is the trigger for Alzheimer's disease (AD), the presence of insoluble tau filaments has been shown to be a hallmark of AD[1]. Therefore, designing an inhibitor of tau aggregation may be of therapeutic significance in the AD cure search process. Tau protein belongs to the group of intrinsically disordered proteins (IDPs) that exist as a conformational ensemble of interconverting structures with local structural propensities. Monoclonal antibodies stabilizing one protein’s conformation appear to be a useful tool for the investigation of IDP structure in crystallization or docking experiments[2,4]. Monoclonal antibody DC11 discriminates very strictly between physiological tau proteins and truncated tau peptides isolated from AD-brain, implying the presence of a conformational epitope of tau that carries pathological functions[2,3] in the pathogenesis of AD. Therefore, we focused on the crystallization of the Fab fragment of the DC11 antibody with $tau_{321-391}$ to determine this transient conformation of tau which represents its preaggregation form, to approximate the transition mechanism from physiological to pathological conformation. Crystals of DC11Fab gave diffraction up to 1.4 Å (Fig. 1B), while crystals of DC11Fab complexed with $tau_{321-391}$ gave diffraction up to 1.74 Å (Fig. 1A). The structure of DC11 Fab is being solved by molecular replacement and will be used for docking of tau peptides if the complex structure will not be available.

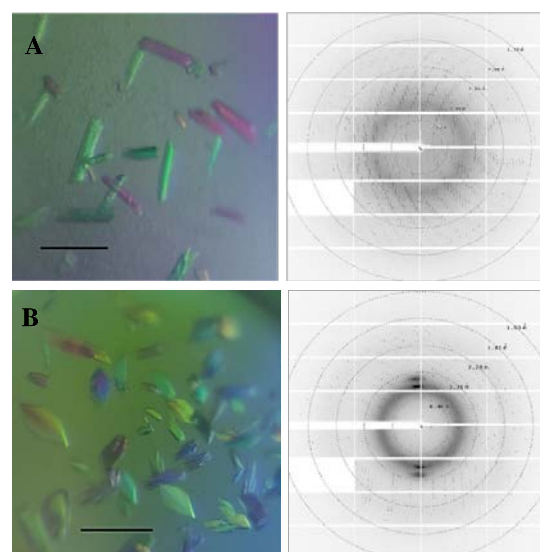

Fig. 1. **Protein crystals with corresponding diffraction pattern** **A.** Crystals of complex DC11Fab/tau321-391 grown in 0.1 M PCTP, pH 5.0, 25 % *w/v* PEG 1500. **B.** Crystals of DC11 Fab fragment grown in 0.1 M MIB buffer, pH, 25 % *w/v* PEG 1500.

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ANALYSIS OF CELLULAR RESPONSE TO SELECTIVE INHIBITORS OF HASPIN KINASE WITH FUCCI CELL CYCLE REPORTER

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The atypical kinase Haspin is required for normal mitosis progression in mammalian cells, and has a specific function in chromosome alignment, centromeric cohesion, and spindle stability. Haspin is expressed throughout the entire cell cycle, with the highest expression in non-dividing haploid spermatids. However, it is still not sufficiently explained why Haspin-null embryos develop normally and show no anatomical defects except testicular anomalies[1]. Several studies elucidating the biological role of Haspin in the cell cycle use available but insufficiently selective small-molecule inhibitors[2]. Therefore, we used novel (proprietary) Haspin inhibitors that fulfil the requirements for a selective chemical probe and allow us to address mechanistic and phenotypic questions regarding Haspin-regulated processes in cell-based assays.

We and others have demonstrated the great potential of the Fucci (= Fluorescent Ubiquitination-based Cell Cycle Indicator) reporter system in the quantitative analysis of the response of cell populations to drugs. Here we used the Fucci4 system[3], which uses 4 cell cycle sensors to label the entire cell cycle and can reveal detailed dynamics of cell behaviour in the presence of Haspin inhibitors. Moreover, we developed pipeline for automatic cell tracking and analysis of the cells in time lapse images. The results presented herein demonstrate that the new highly selective Haspin inhibitors induce a different effect on cell cycle progression, in contrast to less selective commercially available inhibitors.

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UNIQUE METHOD FOR ENANTIOSEPARATION OF CATHINONES BY CHIRAL ION EXCHANGER IN SUPERCRITICAL FLUID CHROMATOGRAPHY

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Synthetic cathinones represent a significant subset of new psychoactive substances (NPS). Due to their novelty, they are usually not controlled by international drug conventions. Therefore, consumers of such substances may be unknowingly exposed to unpredictably strong biological effects that may increase the risk of overdose events[1]. Cathinones are chiral substances. Unsurprisingly, the pharmacological activity and side effects of their individual enantiomers differ greatly. Thus, the development of rapid and effective methods for the stereoselective separation of cathinone-type NPS are of great interest. The recent progress in technology of supercritical fluid chromatography (SFC) allow for chiral analysis of drugs in hyphenation with mass spectrometry (MS). However, up to this date, very few methods for cathinones enantioseparation using SFC on various chiral stationary phases have been successfully implemented[2,3].

Recently, we have introduced unconventional gradient method for chiral separation of cathinones on chiral zwitterion ion exchangers by SFC-MS with an inversed gradient of a make-up solvent. This protocol facilitated stable electrospray ionization[3]. Moreover, we fully optimized MS detection. Since the used single-quadrupole mass spectrometer allowed limited variation of parameters, an adequate experimental setup was used to identify the best settings for three key parameters – probe temperature, cone and capillary voltage. To the best of our knowledge, this is the first example of gradient elution compensated by the inversed gradient of a make-up solvent used in SFC-MS analysis.

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BIOSTATISTIC AND MACHINE LEARNING IN MALDI MASS SPECTROMERY RESEARCH

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With increasing demands on the analyses of biological samples in complex biological matrices, high interest in developing and optimizing mass spectrometric (MS) methods also grows. MS analysis of whole cells, plasma samples, and/or tissue sample is of great importance for monitoring and elucidating biological processes in the organism and provides important information regarding organism pheno/genotype. In three topics presented herein, different techniques dealing with the complexity of such biological samples are introduced. The first project focuses on classifying of ovarian cancer cells with different percentages of cell populations with a knockout of a single gene (TUSC3). Different cell types (4 in total) from different organisms (human and mouse) were introduced to MS analysis. Intact cell MS (IC-MS) method was combined with multivariate statistical and machine learning algorithms (PLS-DA, ANN, and RF for example) using an R programming language[1]. The second project deals with the development method for the analysis of human plasma samples using MALDI-TOF MS. The aim of this project is to discriminate multiple myeloma (MM) patients and patients with similar diseases like plasma cell leukemia (PCL)[2]. The third project deals with MALDI MS imaging (MSI) of various tissues. In-house-built R script for MSI data analysis was developed and used for data processing and for segmentation of tissue section using multivariate statistical methods (mostly PCA).

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ON THE ORIGIN AND EVOLUTIONARY SIGNIFICANCE OF AUTOTRIPLOIDS IN FLOWERING RUSH (*BUTOMUS UMBELLATUS* L.)

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Whole genome duplication, the process leading to polyploidy, is a crucial feature of evolution, especially in the plant realm[1]. At least one polyploidization event occurred in the evolution of all angiosperm lineages[2]. Approximately 35 % of extant plant taxa are considered to be of recent polyploid origin[3], and many of these species retain individuals of two or more cytotypes, thus creating a real-time evolutionary "playground" called mixed-ploidy species. Even though specific mechanisms enabling cytotype co-occurrence have been determined, they cannot yet be generalized due to a low number of case studies[4].

The contact zones of cytotypes offer us a unique opportunity to study the dynamics and evolution of mixed-ploidy systems[4]. *Butomus umbellatus* (flowering rush) is such a species but also somewhat unusual. *Butomus* is one of the very few known species in which diploid and triploid (not tetraploid) cytotype is present in nature. The first known mixed-ploidy populations of *B. umbellatus* were discovered in south-eastern Slovakia – also in Latorica PLA[5], a nowadays rare area with substantially natural dynamics of marsh and other wetland biocenoses[6]. A common occurrence of both cytotypes of the model species creates a unique system for unbiased comparison of various reproductive traits and genetic diversity between diploid and triploid individuals.

The main aim of this study was to uncover the truth behind evolutionary processes that are taking place in the contact zone of diploid and triploid cytotype of *B. umbellatus* in south-eastern Slovakia, focusing mainly on the potential establishment of reproductive barrier and inter-ploidy gene flow, sexual recruitment, and the origin of triploid cytotype. Using flow cytometry and analysis of microsatellite loci, we discovered that the contact zone of cytotypes is of both primary and secondary character, and gene flow between cytotypes was also detected. These results were supported by the high production and variability of offspring detected in triploid plants in mixed populations and the excessive production of unreduced gametes in both cytotypes.

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STING AGONIST-INDUCED CD14+ MONOCYTE DEPLETION INVOLVES MULTIPLE REGULATED CELL DEATH MECHANISMS

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The immune cells respond to the double-stranded DNA (dsDNA) or cyclic dinucleotide (CDN)-mediated stimulation of the cyclic-GMP-AMP synthase – stimulator of interferon genes (cGAS-STING) pathway by secretion of type I interferons (IFNs) and proinflammatory cytokines[1,2]. The cytokines and IFNs mediate multiple innate immune processes further bridging to adaptive immune responses, among which the antiviral defence and immune surveillance are at the forefront of research interest[1,2].

Apart from the expected cytokine secretion, STING agonists also caused a complete depletion of CD14+ monocytes in peripheral blood mononuclear cells (PBMCs). Moreover, both CD14+ monocyte depletion and type I IFN secretion was blocked by the cGAS-STING pathway inhibition[3].

We have previously identified that apoptosis was involved in STING agonist-induced CD14+ monocyte depletion[3]. Hence, we further investigate the cell death mechanisms induced by the cGAS-STING pathway activation focusing on the combination of pyroptosis, apoptosis and necroptosis, so called PANoptosis[5]. First, we elaborated on the involvement of apoptosis[3] by analyzing the activation of caspases 3 and 7. Second, we demonstrated that STING agonists induce secretion of interleukin 1β (IL1β) and IL18, both cytokines that require processing by the inflammasome - a marker of pyroptosis[4]. Third, despite detected increased levels of active pyroptotic caspase 1, the involvement of necroptosis remains to be clarified. Nevertheless, based on our preliminary results, we deduce that at least two regulated cell death pathways (apoptosis and pyroptosis) are involved in the STING agonist-dependent CD14+ monocyte depletion. The cGAS-STING pathway activation-induced death of CD14+ monocytes could present an immunoregulatory mechanism, which inhibits the primary robust cytokine secretion, thus preventing potential dangers of prolonged inflammation. Moreover, as pyroptosis is of immunogenic character[4], such CD14+ monocyte cell death could further mediate activation of secondary immune processes including the adaptive immune mechanisms.

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MUTATIONS IN SORLA PROTEIN AND ITS EFFECT ON ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a number one cause of dementia and currently affecting more than 55 million people worldwide. Two main pathological hallmarks are amyloid-β plaques aggregation and Tau neurofibrillary tangles formation[1].

We distinguish between two AD variants: sporadic AD presumably triggered by the interplay of genetic and environmental factors and familial AD caused by specific mutations in *APP*, *PSEN1* or *PSEN2* genes. Recently, *SORL1* pathological variants were linked to AD development, therefore *SORL1* is considered the "fourth" AD gene[2].

SORLA protein (encoded by *SORL1* gene) is an intracellular sorting receptor, playing a huge role in sorting and trafficking of intracellular cargo between endosomes and the trans-Golgi network. SORLA directly regulates amyloid precursor protein (APP) recycling and maintains amyloid-β processing[3]. However detailed molecular mechanisms of SORLA-dependent AD development still remain unclear.

In this project, we focused on the application of stem cell-based models to examine the significance of SORLA pathogenic mutations in AD development. We introduced two different pathological mutations in the fibronectin type III (FN3) domain of SORLA into induced pluripotent stem cells (iPSCs) using CRISPR/Cas9 technology. We adapted advanced *in vitro* models of 2D inducible neurons[4] and 3D cerebral organoids. From collected samples at specific stages of differentiation, we observed decreased SORLA protein

shedding and induction of endosomal swelling, the early pathological hallmark of AD. Overall results indicate that the mutations in the FN3 domain cause malfunction of the SORLA protein resulting into phenotype typical for AD pathology.

This research was supported by the project MUNI/A/1301/2022 and NPO Project LX22NPO5107.

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CREATINE KINASE B AS A PRO-METASTATIC AND PRO-MIGRATORY FACTOR IN OSTEOSARCOMA

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Creatine kinase B (CKB) is an important enzyme involved in the cellular energy metabolism. However, this protein also has other functions, including regulation of the cell cycle or immune system response. Altered expression of CKB has been observed in various cancer types such as breast cancer, colorectal cancer, or metastatic melanoma. Nevertheless, the function of CKB in regulation of proliferation, sensitivity to chemotherapeutics or metastatic activity of osteosarcoma cells is still not fully understood[1].

The aim of this study is to clarify the role of CKB in osteosarcoma progression. We derived highly metastatic SAOS-2 LM5 osteosarcoma cells with depleted expression of *CKB*. Later, we examined the effect of CKB deficiency on growth, migration, metastasis, and energy metabolism of these osteosarcoma cells. Depletion of CKB expression decreased cell migration as well as metastatic potential in immunodeficient mice. On protein level we found altered expression of different genes related to cell migration and the epithelial-mesenchymal transition (fibronectin, Twist, p21 or p-Akt) between SAOS-LM5 *CKB* KO cells and control cells. CKB depletion did not affect proliferation rate of SAOS-2 LM5 cells, their chemosensitivity or cellular metabolism.

Similar experiments were conducted with two other osteosarcoma cell lines. In this case, however, cyclocreatine (CCr), the most effective creatine analogue, was used to inhibit CKB activity[2]. CCr-treated cells proliferated less than untreated ones. Also, the migratory ability was increased in untreated cells.

Our results showed that CKB is important in regulating the migration and metastasis of osteosarcoma cells. We also revealed that short-term inhibition of CKB by CCr affects cell proliferation.

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HYDROXYL RADICAL FOOTPRINTING OF PROTEIN/DNA COMPLEX COUPLED TO HIGH-RESOLUTION MS

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The methods of structural proteomics have undergone a remarkable growth in recent years, which had a huge impact on the field of structural and molecular biology. One of these methods, radical covalent labelling, is one of the structural techniques that has showed to be an effective analytical tool for characterization of biomolecules. In this study, we adopted the Fast Photochemical Oxidation of Proteins (FPOP) approach to study the dynamics of FOXO4 transcription factor and its DNA partner, Insulin Response Element (IRE). To study such complex, FOXO4 in the absence or presence of dsIRE was oxidized by FPOP in a quench-flow capillary reactor. Irradiated samples were further analyzed by classical bottom-up approach. To investigate IRE-FOXO4 binding and to monitor the DNA damage caused by the hydroxyl radicals, IRE alone and in the complex with the protein was exposed to FPOP oxidation. FOXO4 was digested into dipeptides using proteinase-K and IRE fragments were analyzed by LC-MS using high-resolution FT-ICR mass spectrometer operated in negative ion mode.

Analysis of oxidized peptides enabled localisation and quantification of residues directly involved in protein-DNA interaction. Analysis of separated IRE fragments revealed that hydroxyl radicals cleave the DNA nonspecifically, creating set of all possible 3’OH, 3’P, 5’OH and 5’P terminal fragment ions. Complementary fragment ions were found in the LC-MS trace and further quantified. Comparison of IRE fragment ions revealed significant protection of IRE by FOXO4 binding, predominantly in both major and minor groove of IRE. Obtaining detailed information about solvent accessibility for

IRE and FOXO4 surfaces might enable ab initio design of FOXO4/IRE structural model. This is potentially valuable because the corresponding crystal structure is currently unclear.

This work was supported by the Czech Science Foundation (grant numbers 19-16084S), Grant Agency of Charles University (359521), European Commission H2020 (EPIC-XS- grant agreement ID: 823839 and EU FT-ICR MS - grant agreement ID: 731077), and, in part, by the Czech Academy of Sciences (RVO61388971).

3D MODELS FOR THE CO-CULTIVATION OF COLORECTAL CANCER CELLS AND *BACTEROIDES FRAGILIS*

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Bacteroides fragilis (*BFr*) is one of the driver bacteria involved in colon carcinogenesis. Its enterotoxic strains produce a toxin called fragilysin and other proteases interacting with eukaryotic cells. Fragilysin promotes malignant transformation of cancer cells by cleaving E-cadherin. With E-cadherin degradation, the nuclear level of β-catenin increases. This results in cell proliferation, increased chemoresistance, and migration[1]. The impact of enterotoxic bacteria on cancer has not been sufficiently described because there are no protocols for co-cultivation of cancer-obligate-anaerobe bacteria.

We developed a novel co-cultivation system to study the interactions between *BFr* and colorectal cancer (CRC) cell lines HT-29 and HCT 116 in three dimensions. The effect of *BFr* on cancer cells was analyzed by immunohistochemistry (IHC) and western blotting. The study focuses on markers of proliferation, migration, and signaling pathways that change in cancer cells exposed to the bacteria.

We successfully co-cultivated living *BFr* with CRC spheroids for up to 6 days. We showed a decrease in E-cadherin in CRC spheroids co-cultivated with the fragilysin-positive *BFr* strain, while the fragilysin-negative strains did not have this effect on tumor cells. Moreover, upregulation of both migration markers Snail/Slug and a classical marker of epithelial-to-mesenchymal transition Vimentin was detected in spheroids exposed to *BFr.* IHC analysis also confirmed an increase in nuclear localization of β-catenin. However, these phenomena were not limited to the fragilysin-positive strain, suggesting that other potential enterotoxins also play a role. Thus, *BFr* alters signaling involved in proliferation, chemoresistance, migration, and metastasis of CRC cell lines.

Further experiments will focus on functional migration assays, bacterial localization within the spheroid, and *BFr* secretome analysis.

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BIFUNCTIONAL DIRHODIUM (II,II) COMPLEXES CONTAINING UREA MOIETY IN DIRECTED AMINATION REACTIONS

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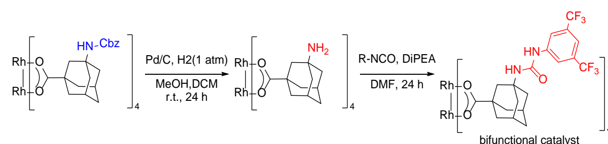
Catalytic nitrenoid insertion reactions have been a subject of study in organic chemistry as many of amine derivatives appear in natural products, artificial materials and pharmaceuticals[1].

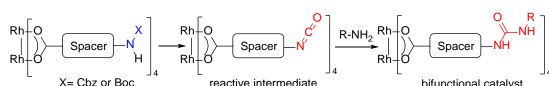
In the past two decades dirhodium (II,II) complexes are studied due to their unique catalytic properties[2]. Noticeably, the bridged, achiral dirhodium catalyst $Rh_2(esp)_2$, developed by Du Bois and co-workers has demonstrated remarkable efficiency in nitrenoid insertion reactions with catalyst loadings as low as 2 mol% (ref.[3]). Diastereoselectivity and enantioselectivity can be achieved in both intra- and intermolecular C–H amination reactions using chiral dirhodium catalysts developed by Lebel[4a], Davies[4b], Hashimoto[4c], Dauban[4d] and other groups[4f]. Bach and co-workers designed a catalyst containing hydrogen bonding moieties like quinolone, to perform regio- and enantioselective C–H aminations and aziridinations of selected substrates[5].

Recently, Hrdina's group came up with new synthesis of dirhodium complexes by reversing the traditional approach by putting the critical ligand exchange first, followed by post-functionalisation reactions. The group was able to prepare a stable dirhodium complex with a free amino group that could be subjected to post-functionalisation[6]. Unfortunately, this method was applicable only to rigid systems of dirhodium complexes with a free amino group pointing away from the axial positions of the metal centre.

In our presented work, we propose the new method of incorporating a urea moiety (hydrogen bond donor) into the dirhodium (II,II) paddlewheel complex by a post-functionalization reaction.

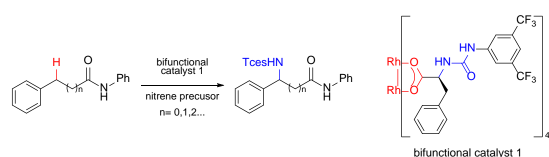


Scheme 1. **Preparation of bifunctional dirhodium (II,II) complexes using post-functionalisation of intermediate containing a reactive group**



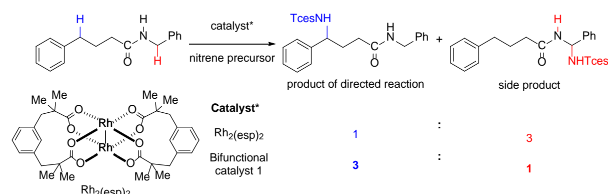
Scheme 2. **Post-functionalization of dirhodium (II,II) paddlewheel complex containing isocyanate group towards bifunctional catalyst with urea moiety**

Multiple substrates containing benzylic C−H bonds with varying distances to an amide moiety (hydrogen bonding site) were prepared and reacted with the nitrene precursor in the presence of bifunctional dirhodium catalyst to study the optimal distance between the hydrogen bonding site and the catalytic site (unique for every spacer).



Scheme 3. **Model systems to study the optimal distance between hydrogen bonding site and Lewis acidic rhodium site of the bifunctional catalyst**

Substrates containing two different benzylic C−H bonds were reacted with nitrene precursor in the presence of optimized bifunctional dirhodium (II,II) catalyst. We observe site-selectivity of nitrene insertion reaction into specific C−H bonds having optimal distance from an amide moiety, which binds to the urea moiety of the catalyst and directs the insertion reaction.



Scheme 4. **Model reactions comparing directed and undirected nitrene insertion reactions into the substrate containing two reactive C−H bonds (colour coded)**

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SAFETY AND PHOTOPROTECTIVE EFFECTS OF SILYMARIN COMPONENTS

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Accumulating evidence suggests that skin cells including keratinocytes and fibroblasts express high levels of the nuclear factor erythroid-2 related factor 2 (Nrf2), which is known to play a pivotal role in the skin homeostasis, differentiation, and metabolism during normal and pathologic conditions[1]. The Nrf2 pathway is influenced by several exogenous chemical (e.g. polyphenols) and physical (e.g. UVA radiation) stimuli (Fig. 1).

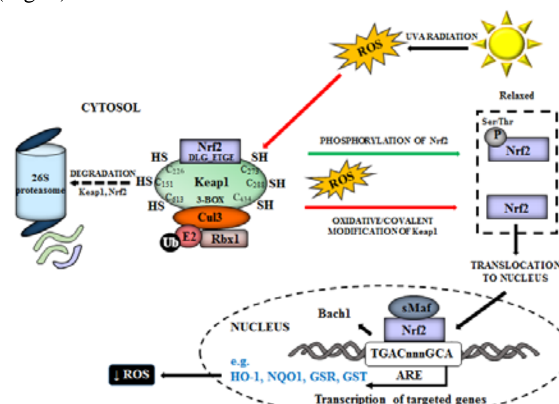

Fig. 1. **Effect of UVA radiation on Nrf2 activation**[1]

For prevention or treatment of UVA radiation-induced damage to skin abundance of natural compounds have been studied. A promising agent with beneficial effects on the skin is silymarin (SM), the extract form the seeds of milk thistle. SM is rich in polyphenols (Fig. 2) that possess antioxidant, anti-inflammatory, immunomodulatory, and detoxification

properties by targeting various cellular and molecular pathways[2].

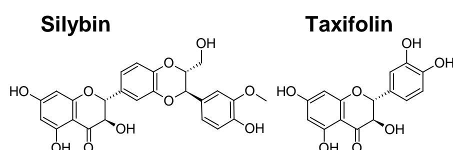

Fig. 2. **Structures of selected SM´s components**

Poor information is available on the effect of SM or its components on the Nrf2-driven signalling pathway. Thus, our study focused on SM and selected flavonolignans and their effect on the Nrf2 pathway in non-irradiated and UVA irradiated primary skin cells. The studied SM´s components included: silybin (SB), isosilybin (ISB), 2,3-dehydrosilybin (DHSB), silychristin (SC), silydianin (SD), and flavonoid taxifolin (TA). Flavonoid quercetin (QE), a structural derivative of TA, and sulforaphane (SFN), a well-known activator of the Nrf2 pathway, were used in the study.

First, stability and UVA photostability of SM and the compounds were tested. SM and pure polyphenols excluding DHSB and QE were stable in ethanol and phosphate buffers of different pH at selected temperatures and incubation times with some exceptions. The instability of QE was greater compared to DHSB. When polyphenols in buffers were exposed to UVA radiation, SM, SB, ISB, SC, SD, and TA were photostable. In contrast, DHSB and QE showed photodecomposition, which was augmented with an increasing dose of UVA radiation and in the presence of oxygen. These data correspond with observed phototoxicity of DHSB and QE on normal human epidermal keratinocytes (NHEK), dermal fibroblasts (NHDF) and keratinocyte cell line (HaCaT)[3,4].

Further, effect of UVA radiation to the Nrf2 signalling pathway in NHEK, NHDF and HaCaT was compared. Nrf2 translocation was observed in NHDF and HaCaT, not in NHEK. The UVA-modulation of Nrf2 driven genes was different in individual used skin cells. Changes in HaCaT were different from those in NHEK[1].

In all cell models, treatment with SM and selected polyphenols increased Nrf2 translocation into the nucleus. For SM and most substances, a more pronounced effect was observed at the lowest concentration used (6.25 μmol/L). The highest rate of Nrf2 translocation into the nucleus was found in QE. This flavonoid also showed the greatest ability to stimulate changes in the nuclear localization of Nrf2 protein and the expression of chosen Nrf2-driven genes NAD(P)H:quinone oxidoreductase 1 and heme oxygenase 1 in UVA-irradiated primary skin cells.

In conclusion, SM and its components could serve as photoprotective agents, however, their effect needs to be further verified *in vivo*. Similarly, the safe use of QE and DHSB needs to be verified, preferably under *in vivo* conditions, as their undesirable pro-oxidant effect may outweigh their protective effect especially in long-term use.

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FROM CO2 TO POLYHYDROXYALKANOATES VIA CYANOBACTERIA

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Carbon dioxide pollution is a topic that resonates strongly with society. More than 35 billion tons of CO_2 were emitted to the atmosphere in 2021 (ref.[1]). Capture and usage of carbon dioxide together with reducing the CO_2 emission per year could slow down warming of Earth surface.

Polyhydroxyalkanoates (PHAs) are microbial biopolymers, occur as an intracellular granules and serve as an energy storage compound. They are biodegradable, UV resistant and biocompatibile[2]. PHA itself are fragile, the properties can be improved by incorporating other monomers into the structure resulting in *in-vivo* copolymer synthesis. PHA production is financially demanding. For heterotrophic bacteria waste materials serve as a carbon source. As the cyanobacteria are capable of oxygenic photosynthesis, they do not demand carbon source and for PHA synthesis they need only CO_2 and light source.

Cyanobacteria are phototrophic gram-negative bacteria belonging to the prokaryotes. They are ecologically extremely important as they are capable of oxygenic plant-like photosynthesis. Thanks to this ability cyanobacteria played a key role in biological and in geological history of our planet. Cyanobacteria synthesize many biologically active secondary metabolites such as glycogen, pigments, lipids or PHAs[3].

In this study two cyanobacterial strains from *Synechocystis* family were tested for copolymer production. The composition of co-polymer differs and the composition of co-polymers also varies depending on the precursor concentration. *Synechocystis* sp. PCC6803 and CCALA192 cultivated with acetate and γ-butyrolactone accumulated co-polymer 3HB-co-4HB with different fractions of 4HB.

After several experiments CCALA192 seems to be very promising PHA producer. In air bubbled multicultivator we were able to gain up to 15-20 % of PHB in cell dry weight.

Role of PHAs in cyanobacterial metabolism seems to be very complex. PHAs do not serve only as an energy storage

compound, but also they help to survive the stress conditions. Deeper understanding together with well-adjusted cultivation conditions can make cyanobacteria the PHA-producers in present and future rich in CO_2.

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CONFORMATIONAL DYNAMICS OF THE TUMOR SUPPRESSOR P53 INDUCED BY THE SPECIFIC INTERACTION WITH HEME

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Heme is a vital molecule critical for various cellular processes including gas transport, catalysis, or electron transfer. Apart from these functions, heme can also act as a signal molecule. Its reversible interaction with various proteins modulates their function and consequently governs the fate of the entire cell[1].

Interestingly, the tumor suppressor p53 also belongs to the group of proteins that can specifically interact with heme[2]. p53 is a transcription factor essential for the activation of numerous genes involved in apoptosis, cell cycle arrest, metabolism, and many more. Gene encoding p53 is one of the most frequently mutated genes in human cancers with the majority of mutations occurring in its DNA-binding domain (DBD)[3]. It has been previously demonstrated that excess heme may directly interact with and destabilize p53 and significantly reduce its half-life in cells[2].

Herein, we examined the structural and functional aspects of heme-bound p53 and heme-free p53. Acquired results revealed that heme-bound p53 exhibits increased conformational dynamics and flexibility in several regions of DBD. This domain is indispensable for the proper function of p53, i.e., site-specific DNA interaction. In this study, we also employed spectroscopic techniques to characterize the properties of the heme-binding site. We propose that binding of heme to p53 renders the protein more unstable and perhaps induces structural changes such that the heme-bound p53 adopts so-called "mutant-like" conformation and loses its physiological ability to specifically bind DNA.

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FIBRIN SELECTIVE ALTEPLASE WITH IMPROVED THROMBOLYSIS AND INHIBITION RESISTANCE ENGINEERED BY RATIONAL DESIGN

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Cardiovascular diseases are the leading cause of all deaths worldwide, with stroke being the second most common cardiovascular disease[1]. Since 1996, the only FDA-approved drug for the treatment of acute ischemic stroke remains alteplase. However, alteplase still has many limitations, including incomplete recanalization, risk of intracranial haemorrhage, short half-life, and potentially neurotoxicity[2].

We implemented a comprehensive multidisciplinary workflow to develop an improved thrombolytic with increased safety and efficiency. We employed a rational protein engineering approach and computational design. The designs comprised alteplases with mutations suppressing the side effects, reconstructed ancestral alteplases, and database-mined sequences related to alteplase or a vampire bat thrombolytic desmoteplase. These candidates were biochemically characterized for the following properties: (i) enzymatic activity; (ii) stimulation by fibrinogen and fibrin; (iii) selectivity; (iv) fibrin affinity; (v) inhibition resistance; (vi) clot penetration and (vii) biological half-life.

One of the candidates, denoted as brnoteplase, showed fascinating properties: (i) enhanced stimulation by fibrin, (ii) improved selectivity towards fibrin clots, (iii) increased inhibition resistance, and (iv) improved clot penetrability by decreased fibrin affinity. Subsequent testing of blood clots lysis and recanalization *in vitro* and *in vivo* in rat models confirmed retained thrombolytic activity, while simultaneously improving useful properties of brnoteplase compared to the wild-type alteplase. Collectively, our results

provide a new modified drug candidate with a promising therapeutic potential of becoming a safer and more effective thrombolytic.

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A NEW REPORTER SYSTEM FOR HIGH-THROUGHPUT IDENTIFICATION AND VALIDATION OF eIF4F INHIBITORS

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The eIF4F translation initiation complex has a critical role in cancer. The complex enhanced activity was identified as a nexus of drug resistance and a promising therapeutic target in melanoma[1,2]. However, the spectrum of available eIF4F inhibitors is limited, and none of them is in clinical use3. One of the reasons could lie in the relative complexity of techniques used to identify such inhibitors4. Here we report a unique cell-based reporter system suitable for the high-throughput identification of novel eIF4F inhibitors in small-molecule compound libraries.

We identified several eIF4F-regulated pathways controlling melanoma cell proliferation in a proteomic screen. Then we used a promoter of one of the eIF4F-controlled genes to build a reporter system, responding to eIF4F inhibition by changes in luciferase expression in a dose-dependent manner. Subsequently, we validated the system in a panel of cell lines, determining the impact of eIF4F inhibition on luciferase activity.

Our technique is not only highly specific and suitable for high-throughput screening; it also overcomes several limitations of the current state-of-the-art eIF4F inhibitor screening assays, e.g., the proximity ligation assay. It is sensitive, less cost-intensive, significantly faster, and does not require expensive fluorescent microscopy/image analysis equipment.

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SYNTHESIS OF NEW 1,2-DIAZETIDIN-3-ONES

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1,2-Diazetidin-3-ones, small flat-like rings, are not only intriguing scaffolds for medicinal chemistry and advanced building blocks for further modifications, but mainly aza analogues to ß-lactam antibiotics. Although ß-lactam antibiotics were discovered almost 100 years ago, they continue to play a key role in clinical practice and remain one of the best studied groups of antibiotics. Their mode of action and susceptibility to resistance via ß-lactamase enzymes have been widely studied and led to the development of various derivatives and analogs[1].

1,2-Diazetidin-3-ones represent still one more underrepresented alternative to ß-lactams that places additional nitrogen into a four-membered ring[2].

1,2-Diazetidin-3-ones are currently commonly referred as pharmaceutically promising motif since it was discovered that they act as potent inhibitors of the serine hydrolase protein phosphatase methylesterase-1 (PME-1). The main inhibitor ABL127 (Fig. 1) was found to covalently inhibit PME-1 in cells through acylation of the serine nucleophile at the active site of the enzyme[3].

It is thus surprising that there are only few methods of 1,2-diazetidin-3-one synthesis that in addition suffer from severe scope limitations and/or lengthy starting material synthesis.

Recently in our research group we have addressed such issue and designed a new approach to 1,2-diazetidin-3-one based on intramolecular N-N cyclization reaction. In this approach we take an advantage of optically enriched α-heteroaryl-α-amino amides that are in one step transformed to 1,2-diazetidin-3-ones.

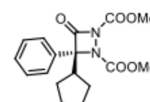

Fig. 1. Structure of the ABL127 inhibitor

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ENANTIOSELECTIVE HYDROXYMETHYLATION OF ISOINDOLINONES USING THE BENCH-STABLE FORMALDEHYDE SURROGATES

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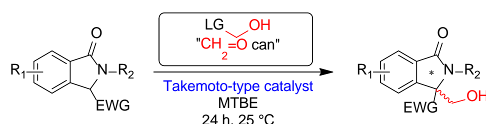
The asymmetric cross-aldol reaction with formaldehyde is one of the most efficient carbon chain extension methods. However, performing this type of reaction is far from straightforward[1,2]. Formalin solutions may cause incompatibility issues with many catalytic systems due to the water presence. On the other hand, the polymeric precursors thereof (para- or metaformaldehyde) are poorly soluble in many organic solvents causing the slow release of the reactive monomer and overall reaction slowdown. For these reasons, research on bench-stable, soluble, and reactive formaldehyde surrogates (“$H_2C{=}O$ cans”) for catalytic reactions is highly desired.

Since the formaldehyde releasers have never been systematically investigated, we synthesized and evaluated more than 20 formaldehyde surrogates in a model asymmetric methylolation of isoindolinones. Thorough screening of our catalyst library revealed that the bifunctional molecules containing basic moieties (i.e., Takemoto-type catalysts) provided the best enantioselective outcomes. Next, a series of optimizations was performed to establish the most suitable reaction conditions. A combination of the above catalysts with the triazole-based formaldehyde surrogates furnished the hydroxymethylated products within 24 h in the very good enantiomeric ratios (e.r.~95:5). Compared to the prior methodologies[3], this protocol constitutes a steep advance in the efficacy and stereoselectivity of the organocatalytic process.



The study was supported by the project CZ.02.2.69/0.0/0.0/19_073/0016943 Internal grant agency of Masaryk University (MUNI/IGA/0916/2021).

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STEALING GENES FROM VIRUSES: EXPLORING THE FUNCTION OF RETROVIRAL PROTEINS EXPRESSED IN THE HUMAN PLACENTA

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Successful development of the mammalian placenta strictly depends on the expression of genes of retroviral origin. One of them is the human *syncytin-1*, which encodes a retroviral envelope glycoprotein that can initiate the fusion of phospholipid membranes. The *syncytin-1* gene was captured during human evolution to perform a novel physiological function of fusing placental cells[1,2]. In the placental cytotrophoblast, syncytin-1 interacts with its membrane receptor – the neutral amino acid transporter ASCT2[1]. This event initiates the fusion of cytotrophoblast cells and leads to the formation of a multinucleated syncytiotrophoblast, a tissue responsible for fetomaternal nutrient exchange. The fusogenic activity of syncytin-1 is inhibited by the unrelated retroviral protein called suppressyn. It was shown that secreted suppressyn binds ASCT2 and prevents the interaction with syncytin-1 (ref.[3]). However, molecular details of the inhibition mechanism and physiological consequences for ASCT2 remain unknown.

Functional analyses of syncytin-1-induced fusion often rely on a manual counting approach. It usually utilizes fluorescence microscopy for counting all stained nuclei inside syncytialized cells to calculate the average “fusion index”. These methods are, however, very time-consuming and incompatible with the high-throughput setup. Here we present a new cell-cell fusion quantification assay, which uses a split-luciferase reporter from *Oplophorus gracilirostris*. In this approach, syncytin-1 and ASCT2 are expressed separately with different luciferase fragments in a heterologous cell-culture system[4]. If a cell-cell fusion occurs, luciferase reconstitutes and can oxidize its substrate. The resulting luminescence is ~150-fold higher than the signal produced by a firefly or *Renilla* luciferases[5]. We show that our method can be a powerful tool for obtaining structural and physiological insights into the significance of the interaction between ASCT2, syncytin-1, and suppressyn.

This work was supported by the Czech Science Foundation (grant 17/14356S), and by Praemium Academiae Grant awarded by the Czech Academy of Sciences to Jiří Hejnar.

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NOVEL METHOD FOR DETERMINATION OF METHOXPHENIDINE AND ITS MAIN METABOLITE IN BIOLOGICAL SAMPLES

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Methoxphenidine (MXP) is a new psychoactive substance that belongs to the dissociative anaesthetic group. It appeared on the black market in 2013, and MXP intoxications have been reported with at least three deaths since then. To understand the mechanism of toxicity and effects of MXP, it is important to know its pharmacological properties (e.g., toxicity, activity on receptors, and ADME) and metabolism, which may be further exploited to study its mechanism of action *in vivo*. Knowledge of the metabolism of such compounds may be useful not only in toxicological screenings but also in combination with pharmacokinetic and pharmacodynamic data to enhance understanding of how these substances can affect living organisms[1,2]. There is still very little information on MXP, and a method for the analysis of biological samples (e.g., brain, liver, lungs, and serum) remains to be developed.

Hereby, we introduce a novel UHPLC-MS method for the determination of MXP and its main metabolite norMXP in biological samples. The UHPLC-MS conditions were thoroughly optimized. Due to the complex matrix of biological samples (blood serum and body tissues), it was important to develop an extraction method with sufficient yield and low matrix effect. Liquid-liquid extraction, salting-out liquid-liquid extraction, and protein precipitation were tested separately for each type of biological sample. All new methods have been validated according to the guidelines of the European Medicines Agency (EMA). Real samples from Wistar rats (taken at certain time intervals) were analyzed. On the basis of these data, MXP and norMXP concentration curves were generated.

In conclusion, we have developed quantification and extraction methods for the determination of MXP and the major metabolite in blood serum and tissue samples from Wistar rats. The pharmacokinetic profiles for MXP and norMXP were established.

This work was part of the research project 'New psychoactive substances: forensic toxicology research center' supported by the Ministry of Interior of the Czech Republic from the OPSEC Program (grant No. VK01010212).

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IN VIVO ANALYSIS OF NANOSTRUCTURED NITINOL

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Nitinol is the metallic biomaterial formed by alloying nickel and titanium. Many different characteristics set nitinol apart from other metals and alloys, including its biocompatibility, superelasticity, strength, shape memory, fatigue resistance, and corrosion resistance. This makes nitinol widely used in medical applications, such as orthodontic appliances, vascular stents, joint replacements, and fracture fixation.

Specific health risks are associated with the presence of metal due to interactions between live tissues and implanted metal components. Metal material degradation is unavoidable and can lead to complications. When an implant is damaged (by corrosion or wear), material degradation products (micro- and nanoparticles, ions, inorganic salts, and metal oxides) are released. These products can affect the behaviour of surrounding cells and can induce loosening and osteolysis as well. To improve metal properties (e.g. implant life extension, inflammation reduction, ion/nanoparticle reduction, enhancement of biocompatibility), various surface modifications are used.

The main goal of this *in vivo* study is to better understand the processes of healing and inflammation brought on by metal ion release.

The experiment was carried out on female Wistar rats. Nitinol disc (with or without nanotube coating) 10 mm in size and 1 mm thick was placed between the *latissimus dorsi* and *scapula*. The control group also underwent an incision, but no metal implant was inserted. After the monitoring period (24 hours, 72 hours, 2 weeks, and 4 weeks), blood samples and muscle samples from direct contact with the metal were collected. ICP-MS was used to measure the kinetics of heavy

metal ion release into the tissue and circulation, and RT-qPCR was used to examine the expression levels of selected genes that play essential roles in the inflammatory response. The $2^{-\Delta\Delta CT}$ method was used to calculate changes in the expression of target genes.

This research was supported by GA20-11321S. Part of this work was carried out with the support of RECETOX Research Infrastructure (ID LM2018121, MEYS CR, 2020-2022).

SYNTHESIS OF SPIROCYCLIC COMPOUNDS BASED ON Pd-CATALYZED TANDEM CYCLIZATION/SUZUKI CROSS-COUPLING

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Spirocyclic scaffolds are widely present in many natural products and biologically active compounds, for example alkaloids like spirobacillene and pronuciferine or the well-explored antitumor antibiotic fredericamycin A[1].

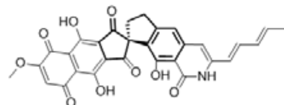
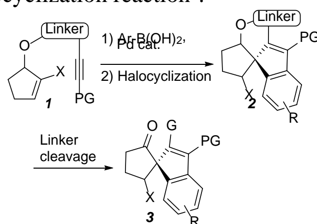


Fig. 1. **Fredericamycin A**

Since spirocycles play an important role in the discovery and development of drugs, exploring of efficient asymmetric approaches to the construction of spirocyclic compounds has attracted a lot of attention[2,3].

Herein, we suggest several routes to reach the spirocyclic core ***3*** with a quaternary carbon center (Scheme 1). Synthesis of intermediates ***2*** was performed using a Pd-catalyzed tandem cyclization/Suzuki cross-coupling of compounds ***1*** with substituted phenylboronic acids, followed by a halocarbocyclization reaction[4].



Scheme 1. **Our approach towards spirocycles**

Different types of linkers in the starting materials ***1*** and conditions of their cleavage were studied. A detailed description of these compounds and the tested linkers and specific conditions used for their formation and cleavage, as well as their properties will be presented on an oral communication.

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IMMUNE SURVEILLANCE AND CARCINOGENESIS – THE ROLE OF ENDOPLASMIC RETICULUM STRESS

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Immunotherapy has become a promising novel approach for cancer treatment; however, many patients develop resistance[1]. Various factors in the tumor microenvironment, such as low pH and hypoxia, cause cellular stress, including Endoplasmic Reticulum stress (ERS), a crucial inducer of inflammation. This leads to underexpression of MHC1 in tumor cells, increased angiogenesis, resistance to chemo- and radiotherapy, and hypoxic survival. Immune cells exposed to ERS lack antigen presentation, exhibit a suppressor phenotype, and can initiate apoptosis. Thus, in chronic inflammatory states ERS can trigger immunosuppression[2]. Modulation of ERS may be beneficial for immune surveillance and response to immunotherapy. Here, we co-cultured ovarian cancer (OC) and renal cancer (RCC) cells with peripheral blood mononuclear cells (PBMC). We modulated ERS in these co-cultures using tunicamycin (TM) and the chemical chaperone TUDCA. The alleviation of ERS by TUDCA increased the viability of cancer cells when cultured by themselves, whereas in co-culture with PBMC, their viability decreased, suggesting increased effectiveness of immune cell reaction. Moreover, TUDCA decreased the expression of the ERS markers HSPA5/BiP, DDIT3/CHOP, and EIF2AK3/PERK in these co-cultures. Next, we found that PERK and IRE1 activity were induced by TM and abolished by TUDCA. TUDCA increases the capacity of immune cells to target cancer cells by inducing PERK promoter activity in OC cells and reducing the negative effects of TM on the viability of PBMC. We relate these results to the molecular background of ERS in patient samples where we observe a positive correlation between good clinical outcomes and

higher lymphocyte infiltration at the tumor site and elevated expression of the ERS markers BiP and CHOP in tumor cells.

This study is supported by the Ministry of Health of the Czech Republic (NU21-03-00539).

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SELECTIVE α-OXYGENATION OF GLYCINE DERIVATIVES; A KEY TO SHORT PEPTIDES CONTAINING NON-NATURAL AMINO ACID

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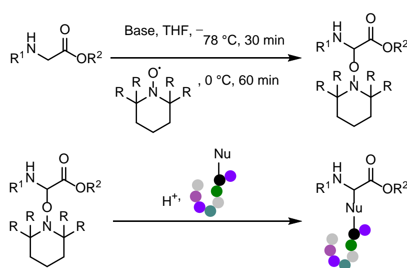
Peptides and proteins have always been important target molecules for biochemical and pharmaceutical applications. Introduction of non-natural changes to these biomolecules have gained much interest in the field since these modifications may grant them novel properties[1].

We present the methodology for the modification of glycine derivatives by a very mild oxidizing agent; the nitroxide radical, to generate glycine alkoxyamines. The methodology was extended to short peptides and interesting orthogonal reactivity of amino acids were unwrapped. The alkoxyamines can be further modified by thermal homolysis or acid-mediated heterolysis to generate a library of non-natural amino acids. Thermal homolysis was extensively studied and a correlation between the structure of glycine alkoxyamines and homolysis temperature was found. Acid-mediated heterolysis paved the way to the modification of glycine containing peptides to access non-natural peptides under physiological conditions. This novel strategy may lead to interesting potential biological applications such as peptide fusion.



Scheme 1. **Modification of glycine derivatives**

This work was supported by the Gilead Science & IOCB Research Center, IOCB Prague and the European Regional Development Fund; OP RDE; Project: ChemBioDrug (No.CZ.02.1.01/0.0/0.0/16_019/0000729).

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NOVEL DEAZAALLOXAZINES DESIGNED FOR PHOTOREDOX CATALYSIS

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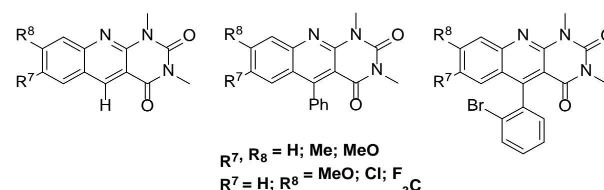
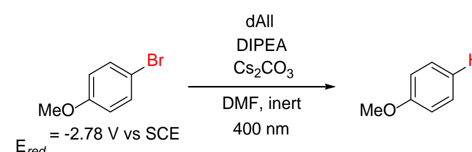
Photocatalysis with flavins (isoalloxazines, alloxazines, deazaflavins) offers a wide range of applications[1], however, still suffers from a number of disadvantages, such as insufficient photostability of flavin photocatalysts and their limited redox properties[2]. The solution comes with deazaalloxazines, the most unexplored members of the flavin family, which are characterised by significantly higher stability. However, due to absorption only in the UV region[3], simple (unsubstituted) deazaalloxazines have not been tested in photocatalysis yet. The introduction of a suitable group into the basic skeleton might be a way to make deazaalloxazines more suitable for visible light photocatalysis.

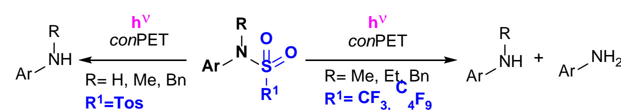
Using different approaches, we have successfully prepared visible-light-absorbing deazaalloxazines substituted on the benzene ring or in position 5 (Scheme 1).



Scheme 1. **Structure of deazaalloxazines**



Scheme 2. **Dehalogenation of challenging substrate**



Scheme 3. **Deprotection reactions of sulfonamides**

Additionaly to suitable photochemical properties, these derivatives are characterized by negative reduction potential, indicating potential applications in reductive photoredox catalysis. Thus, selected deazaalloxazines have been tested in model reductive reactions such as dehalogenations (Scheme 2) or cleavage of the N-S bond in sulfonamides, resulting in

a mild unprecedented procedure for the removal of tosyl and triflyl protecting groups (Scheme 3).

This work was supported by the Czech Science Foundation (reg. No. 21-14200K) and by grant of Specific university research (reg. No. A2_FCHT_2022_040).

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ENCAPSULATION OF MELITTIN INTO ANIONIZED POLYSACCHARIDES

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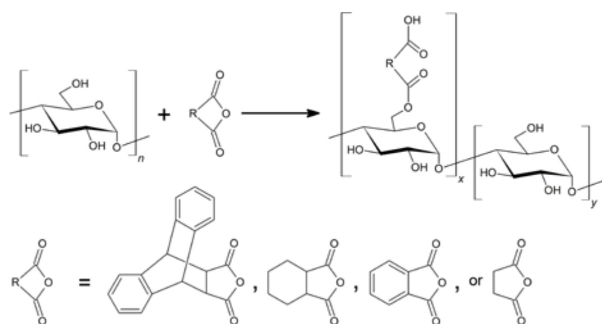
Development of functional polymers with tunable properties serving as carriers of bioactive compounds possesses high interest for a variety of biomedical purposes. Such polymer carriers should be biocompatible and biodegradable, ensuring safety of the resulting product during its application.

A series of carboxylated glycogen derivatives was synthesized for further *in vitro* assessment as encapsulators of polycationic cargos, e.g., the model peptide melittin (main toxic component of bee venom). Selected dicarboxylic acid anhydrides with different polarity were used for modification of glycogen in order to evaluate the influence of anhydride hydrophobicity on the encapsulation efficiency (Scheme 1).



Scheme 1. **Glycogen modification with selected anhydrides possessing different polarity**

The pH-sensitive properties of prepared glycogen carriers allowed to control the adsorption and release of model drug by charge regulation and multivalent interactions. DLS measurements of the size and zeta-potential of glycogen particles revealed that they adsorb melittin until precipitation occurs. *In vitro* assays confirmed that efficient inhibition of melittin’s hemolytic activity was observed with highly substituted glycogens, whereas no inhibition took place with low-functionalized samples[1].

The produced glycogen derivatives can serve as promising carriers for cationic molecular cargos, or as antidotes against animal venoms.

Financial support of Czech Science Foundation (grant #21-01090S) is gratefully acknowledged.

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SYNTHESIS OF AZIDODIFLUOROMETHYL PHENYL SULFONE AND ITS USE AS A SYNTHETIC EQUIVALENT OF AZIDODIFLUOROMETHYL ANION

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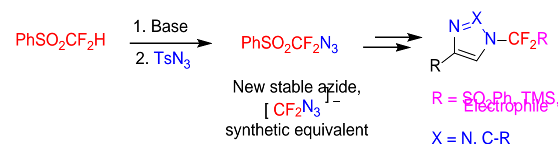
Fluorinated alkyl azides are recently rediscovered class of organic compounds and unique fluorinated building blocks[1]. After copper catalysed “click reaction” with terminal alkenes they produce *N*-perfluoroalkyl-1,2,3-triazoles[2]. Such triazoles are excellent starting material for synthesis of various nitrogen-containing heterocycles[3] or vinyl triflates/halides[4,5].

In this work, we focused on multigram scale synthesis of a new stable fluorinated azide $PhSO_2CF_2N_3$. With azide-alkyne cycloaddition we showed its utility in the preparation of *N*-difluoro(phenylsulfonyl)methyl-1,2,3-triazoles, which can be further transformed by reductive desulfonylation/silylation proces into N-CF_2TMS triazoles. The installation of the trimethylsilyl group allowed effective transfer of (triazolyl)difluoromethyl anion to various electrophiles under mild conditions. Additionally, rhodium(II)-catalyzed *trans*-annulation of N-CF_2SO_2Ph triazoles with nitriles provided *N*-difluoro(phenylsulfonyl)methyl-substituted imidazoles



Scheme 1. **Synthesis of $PhSO_2CF_2N_3$ and its further application**

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