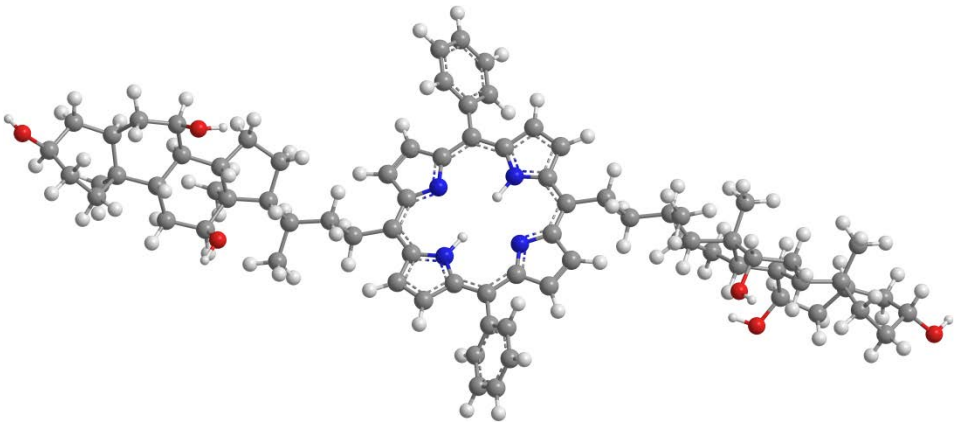

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PREDICTIVE IMPORTANCE OF TUMOR microRNAs IN HEAD AND NECK CANCER PATIENTS TREATED WITH INTENSITY-MODULATED RADIATION THERAPY

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Head and neck cancers are the sixth most common cancers worldwide mainly represented by the squamous cell carcinoma (HNSCC). Radiotherapy (RT) is very important treatment modality in HNSCC management. Closed as well as ongoing clinical trials are evaluating potential for reduced-dose. RT in less aggressive radiosensitive HNSCC defined by human papillomavirus (HPV) positivity, with promise of less acute and late toxicity. To this end, variety of different biomarkers with promising predictive value is currently investigated in HNSCC. MicroRNAs (miRNAs) are short endogenous RNAs that post-transcriptionally modulate gene expression and their deregulated expression has been observed in many cancers including HNSCC. Specific expression patterns of miRNAs have been also shown to predict prognosis and therapeutic response in HNSCC.

Aim of our study was to identify tumor tissue miRNAs which can enable prediction of locoregional control (LRC) in HNSCC patients who underwent intensity-modulated RT.
We have analyzed global miRNA expression profiles in 43 FFPE tumor biopsies collected from HNSCC patients treated with intensity-modulated radiation therapy, who were divided into two groups according to their LRC as follows: short LRC [n = 22; median 5.1 months (min 1.3, max 18.6)] *vs.* long LRC [n = 21; 60.4 (46.8, 98.8)]. This analysis has been performed using the hybridization Affymetrix GeneChip miRNA 4.0 array. Validation of miRNA candidates was performed in independent cohort of 51 HNSCC patients. MiRNA determination was carried out by RT qPCR technology using the miRNA-specific RT stem-loop primers according to the Taq-Man MicroRNA Assay protocol (Thermo Fisher Scientific).

We identified 46 miRNAs with significantly different expression between both examined groups ($p < 0.05$; average log(Fold Change) = 0.42). Based on pre-defined critera, 5 miRNAs were selected for independent validation, from which miR-15b-5p was differentially expressed between groups of patients with short and long LRC.

Our results suggest that miR-15b-5p is promising predictive biomarkers in HNSCC patients treated with intensity-modulated RT.

This work was supported by Ministry of Health of the Czech Republic, grant nr. 15-34553A, 15-33158A, 15-31627A, 15-34678A, 16-31314A and 16-31765A.

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ROLE OF RECQ4 IN GENOME MAINTENANCE

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RECQ4 has been grouped in RECQ classes of helicases, as this class is mainly involved in maintaining our blue prints so we delve into the role of RECQ4 in genome maintenance. Its address on chromosome is 8q 24.3 (cit.[1]). In spite of having conserved helicase domain it has weak helicase and ATPase activity[2,3]. RECQ4 consist of 1208 amino acids and the region between 322-400 is of immense importance because of its affinity to branched DNA structure like Holliday junctions[3], bubble[3], Y form[3], G-quadruplex and even RNA structures[4,5]. Mutation in RECQ4 causes mainly three autosomal recessive syndromes: Rothmund-Thomson[6], RAPADILINO[7] and Baller-Gerold[8] syndrome.

We tried to decipher the role RECQ4 in genome maintenance by exploiting siRNA against RECQ4 in mammalian cells, we found that depletion of RECQ4 causes appearance of micronuclei and bulky bridges, the hallmark for chromosome missegregation and this occurrence was almost 10 % more as compared to control, further we found that cells expressing RECQ4 lacking 322-400 region exhibited almost the same phenotype as exhibited by depleted RECQ4 cells. We also established that this region is involved in physical interaction with MUS81 but we couldn't find any additive affect on missegration phenotype when cells are codepleted with RECQ4 and MUS81.

RECQ4 have a role in replication also due to fact that N-terminus of RECQ4 possesses Sld2-like domain which shares sequence homology with the yeast replication initiation factors Sld2[9,10]. For this reason we checked for ultrafine bridges associated with regions difficult to replicate like centromeric ultrafine bridges and and we found that infact depleting RECQ4 produced almost double the amount of centromeric ultrafine bridges as compared to control. These results indicate important role of RECQ4 in Genome maintenance which arises mainly due to missegartion and this may be due to

centromeric assembly defects but still further investigation needed to establish this concept firmly.

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THE EFFECT OF CARBONIC ANHYDRASE IX ON THE GLOBAL MEMBRANE ENDOCYTOSIS

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Endocytosis plays an important role in many cellular processes, such as migration, invasion and signalization. Endocytosis of many signaling membrane receptors is induced by ligand activation. Oncogenicity of c-Met mutants with constitutive activity resulted not only from their activation, but also from endosome signaling[1]. Carbonic anhydrase IX (CAIX) is a tumor associated, hypoxia induced transmembrane enzyme. It conveys pro-survival and pro-migratory properties to tumor cells. CAIX contributes to the acidification of extracellular tumor environment and helps to preserve intracellular pH homeostasis[2].

Our microarray analysis of HT1080 fibrosarcoma cells with silenced *CA9* gene showed downregulation of several endocytosis related genes. Using 2D-PAGE we confirmed relation of CAIX expression with endocytosis-related proteins. Moreover, we observed strong colocalization of CAIX with HGF receptor c-Met in endosomes induced by internalizing antibody against catalytic domain of CAIX (Ab VII/20)[3]. This antibody induces clathrin-dependent endocytosis of CAIX. Perturbation of intercellular contacts proceeds with both coendocytosis of E-cadherin and c-Met as well as E-cadherin and CAIX. Stimulation of cells with HGF markedly increases clathrin mediated endocytosis of transferrin and CAIX. Upon HGF treatment the amount of CAIX in isolated endosomes was elevated in comparison to non-treated control.

Our results indicate that CAIX could be a part of "the signaling endosome" concept through its impact on the overall endocytosis, the endosomal pH modulation, or coendocytosis with receptor tyrosine kinases c-Met.

This work was supported by VEGA 2/0139/15, APVV 14-0816, APVV 15-0720.

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MOLECULARLY IMPRINTED POLYMERS SELECTIVE FOR ISOLATION OF NUCLEIC BASES

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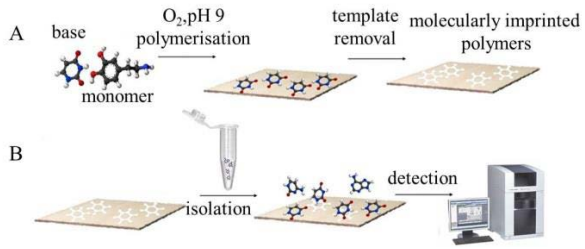
Nucleobases are components of DNA, carriers of the genetic information. Specifically, they are the parts of nucleotides, the monomers of nucleic acids without the phosphate and sugar groups. They are one of key biomolecules in all living organisms and their detection and quantification in complex matrices is of an interest of numerous scientific fields from biochemistry[1], molecular biology[2] or medicine[3,4].

In this work, the simple, sensitive and eco-friendly method for nucleic bases isolation and detection was investigated. This method that allows creating synthetic receptors for the nucleic bases is based on a technique of non-covalent molecular imprinting. Principle of whole method is shown in Fig. 1. Dopamine was used as a functional monomer. It is biocompatible, biodegradable, which undergoes a simple oxidation process and also contains several functional groups that can interact with imprinted molecules[5]. These properties make dopamine an ideal compound for these applications. The experiments focused on binding affinity and selectivity of formed polymeric layers were carried out. All experiments with dopamine imprinted polymers were evaluated by using capillary electrophoresis with absorbance detection at wavelength of 260 nm. From obtained data it was found that prepared imprinted polymers show a high binding affinity toward chosen nucleic base (uracil). Also, the selectivity in comparison with others nucleic bases was more than satisfactory.



Scheme 1. **Process of preparation (A) and use (B) of molecularly imprinted polymers**

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ALTERED 3´SPLICE-SITE SELECTION BY GERMLINE MISSENSE MUTATIONS IN A SINGLE RRM OF PUF60 ASSOCIATED WITH VERHEIJ SYNDROME

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PUF60 is a splicing factor that binds uridine (U)-rich tracts and facilitates association of the U2 small nuclear ribonucleoprotein with primary transcripts[1]. PUF60 deficiency (PD) causes a developmental delay coupled with intellectual disability and spinal, cardiac, ocular and renal defects, but PD pathogenesis is not understood[2]. Using RNA-Seq, we demonstrate that PUF60 preferentially activates alternatively spliced internal exons rich in tandem U/UG motifs upstream of their 3' splice sites (3'ss). These exons are preceded by longer AG dinucleotide exclusion zones, more distant branch sites, with a higher probability of unpaired interactions across a typical branch site location as compared to control exons. Employing a PUF60-dependent target, we also show that PD-associated amino-acid substitutions in the first RNA recognition motif (RRM) altered selection of competing 3'ss and branch sites. Finally, we propose that a differential distribution of RNA processing steps observed in cells lacking PUF60 and the PUF60-related protein RBM39[3,4] is due to the RBM39 RS domain interactions, which was required for interaction with U1-70K *in vitro* and nuclear localization. Together, these results provide new insights into the function and regulation of U-bound splicing factors network in alternative RNA processing and reveal that germline mutation heterogeneity in a single RRM can enhance phenotypic variability at the level of splice-site and branch-site selection.

This work was supported by Bloodwise (12060), VEGA (2/0057/18); patent royalties; Czech Science Foundation (P305/12/G034); internal funding (RVO68378050).

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ACTION MECHANISM OF SHORT ANTIMICROBIAL PEPTIDES AGAINST *STAPHYLOCOCCUS AUREUS* AND SYNERGISTIC EFFECT WITH ANTIBIOTICS

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The resistance of bacterial pathogens to traditional antibiotics requires search for alternative antimicrobial agents. Among them, antimicrobial peptides (AMPs), due to their unique mechanism of action, represent promising candidates to fight infections caused by multi-drug-resistant bacteria.

Here we studied the antimicrobial effect of short α-helical AMPs belonging to the group of halictine-2 (HAL-2) analogs[1] on the methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is typical example of Gram-positive bacterium that poses resistance to several antibiotics, including β-lactams. These dodecapeptides showed potent anti-staphylococcal activity which in the terms of their MIC values were in the range from 4 μM to 20 μM. This was determined by observing bacterial growth of three different strains of *S. aureus* in microtiter plate using Bioscreen C instrument.

Further we focused on the study of the synergism of HAL-2 analogs with antibiotics such as vancomycin, gentamicin, and also with a group of β-lactams. Using a checkerboard method we found that the peptides act synergistically in combination with β-lactam antibiotics (amoxicillin, ceftazidime, cefuroxime, and meropenem). With vancomycin and gentamicin they showed only additive effect.

To explain that synergistic effect, we hypothesized that these peptides must at first pass through the *S. aureus* cell wall

consisting prevalently of a peptidoglykan layer, before they reach and permeate its cell membrane. To shed the light on it, our work included propidium iodide uptake study which confirmed ability of peptides to permeate *S. aureus* cell membrane and the study of the interaction of peptides and amoxicillin with bacterial exoskeleton– sacculus. The sacculi were prepared from broken bacterial cells by purification using SDS to remove cytoplasmic components and lipids.

We hypothesize that during the combination treatment, the β-lactam antibiotic cause degradation of peptidoglycan layer which facilitates easier access of peptide to cytoplasmic membrane and its subsequent permeabilization. In addition, we observed the effect of peptides on the morphology of *S. aureus* by transmission electron microscopy.

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THE EFFECTS OF PERIFOSINE ON 3D TUMOUR MODELS OF COLORECTAL CARCINOMA

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Perifosine is a novel chemotherapeutic agent, an inhibitor of PI3K/Akt signalling pathway, which has been reported to be upregulated in several types of cancer. This pathway promotes cell growth and survival and therefore represents a specific molecular target for anticancer treatment[1]. Although the preclinical studies showed promising results of perifosine effectivity, the phase III clinical trial did not confirm its high cytotoxicity in the real-life tumour conditions.

To assess the cytotoxicity of perifosine in human solid tumours we applied a three-dimensional (3D) *in vitro* culture system called multicellular spheroids (MCS). Since the MCSs naturally possess several tumour-like characteristics, such as gradient of nutrients, oxygen, pH and proliferation and also mimic barriers for penetration of therapeutics inside the tissue, they provide a better simulation of tumour environment than two-dimensional monolayers[2].

We established a 3D model of the cell line HCT116 derived from colorectal carcinoma and studied the cytotoxicity of perifosine on these MCSs. To examine the cytotoxic effects, MTT and ATP assays were used as well as fluorescent staining by calcein-AM/propidium iodide. Furthermore, the presence of apoptotic markers was determined by protein immunoblotting.

We observed that the cytotoxicity of perifosine was increased in environment alkalised by sodium bicarbonate. The MCSs cultivated in alkalised medium also expressed increased levels of cleaved Caspase 8 and cleaved PARP, the markers of apoptosis. Overall results suggest that the antitumour effects of perifosine are environment-dependent and are particularly enhanced by increased pH of the environment.

This work was supported by the Grant Agency of Masaryk University (MUNI/G/0974/2016).

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INVESTIGATION OF COX4 ISOFORM PAIR BIOLOGICAL ROLE USING HEK293 CELL-LINE BASED KNOCK-OUT AND KNOCK-IN MODELS

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Oxidative phosphorylation (OXPHOS) is responsible for production of majority of ATP in mammalian organisms. This process is partly regulated by nuclear-encoded subunits of cytochrome *c* oxidase (COX), the terminal enzyme of respiratory chain. One of its regulatory subunits, Cox4, is an early-assembling COX component essential for the formation of catalytically functional enzyme. Moreover, regulated expression of its two isoforms (Cox4i1, Cox4i2) is hypothesized to optimize respiratory chain function according to oxygen supply. However, details of functional alterations between the two variants have not yet been described. We established HEK293 cell line-based model with complete absence of subunit Cox4 (knock-out, KO) employing CRISPR CAS9-10A technology, and characterized its impact on OXPHOS complexes. Knock-out of both isoforms Cox4i1 and Cox4i2 (COX4i1/4i2 KO clones) showed general decrease of COX subunits resulting in total absence of COX holoenzyme, making cells fully reliant on OXPHOS-independent ATP production. COX4i1/4i2 KO were subsequently utilized as a platform for knock-in of COX4i1 or COX4i2 isoform using stable overexpression. Expression of both isoforms complemented the respiratory defect of COX4i1/4i2 KO. The content of COX as well as its ability to incorporate into supercomplexes were comparable in COX4i1 and COX4i2

expressing cells. Respiratory rates of permeabilized cells in OXPHOS (coupled, state 3) and ETC (uncoupled, state 3u) states, as well as COX capacity were not distinguishable between cells expressing either isoform of COX4. However, significant changes were detected in COX oxygen kinetics. The p_{50} parameter (partial pressure of oxygen at half-maximal respiration) was approximately 2-fold increased in COX4i2 versus COX4i1 cells. These findings indicate decreased oxygen affinity of COX4i2-containing enzyme. Interestingly, we observed COX4 isoform dependent modulation of reactive oxygen species (ROS) production - COX4i2 KI clones manifested decreased mitochondrial ROS generation. Using this model, we will further focus on the ability of COX4 isoforms to serve as mitochondrial energy and redox sensors for regulation of ATP production and oxidative stress response during hypoxia.

Supported by Czech Science Foundation 16-13671S.

STRUCTURE-FUNCTION STUDIES OF THE AUX/IAA OLIGOMERIZATION DOMAIN REVEALS INTERACTION MODES FOR TRANSCRIPTION FACTORS IN EARLY AUXIN RESPONSE

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The plant hormone auxin, is a versatile small molecule that is essential during the entire plant life cycle and regulates numerous growth and development processes, mainly *via* hierarchical control of gene expression. Auxin activates primary response genes by facilitating proteolytic removal of AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA)-inducible repressors, which directly bind to transcriptional AUXIN RESPONSE FACTORS (ARF). Both proteins share highly conserved C-termini mediating homotypic and heterotypic interactions. The NMR structure of CTD of the *Pisum sativum* AUX/IAA4 revealed a globular ubiquitin-like β-grasp fold with homologies to the Phox and Bem1p (PB1) domain. The PB1 domain of wild-type PsIAA4 features two distinct surface patches of oppositely charged amino acid residues, mediating front-to-back multimerization *via* electrostatic interactions. Mutations of conserved patches on either face suppressed PsIAA4 PB1 homo-oligomerization *in vitro* and confirmed directional interaction of full-length PsIAA4 *in vivo* (yeast two-hybrid system). Mixing of oppositely patch mutated PsIAA4 PB1 monomers enabled NMR mapping of the interface of the reconstituted PsIAA4 PB1 homodimer variant, whose stoichiometry (1:1) and equilibrium binding constant (K_D ~6.4 μM) were determined by isothermal titration calorimetry[1].

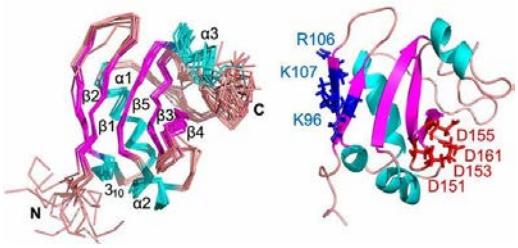

Fig. 1. **Backbone representation of the 10 lowest-energy structures. Structural elements are highlighted in color: helices (α1-α3, 310, cyan), β-strands (β1–β5, magenta), loops (salmon). (Right) Cartoon representation of the lowest-energy structure. Conserved basic and acidic residues (patches) of the canonical type I/II PB1 features are presented as blue and red sticks, respectively**

This research work was supported by DFG-GRK1026 (Deutsche Forschungsgemeinschaft Research Training School Grant).

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ANTIBACTERIAL COLLAGEN-BASED SKIN SUBSTITUTES WITH SELENIUM NANOPARTICLES DESIGNED FOR INFECTED BURN WOUNDS

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Severe burn injuries are often accompanied by a major disruption of organism homeostasis leading to dehydration and leave the organism without the barrier to fight microbial infection. Statistically, there is 42–65 % mortality in burn victims due to infection[1]. Bacteria strains from the genus *Staphylococcus* are common source of the infection. Namely *Staphylococcus aureus* (SA), an opportunistic bacterial pathogen and inhabitant of mammals' skin, belongs among the most dreaded bacteria in burn injuries causing sepsis and other life-threatening complications[2]. Overuse of antibiotics has led to the formation of the strain resistant to β-lactam derived antibiotics, so-called *Methicillin-resistant Staphylococcus aureus* (MRSA)[2].

In the presented report, we have tested two substances known for their antibacterial activity – biopolymeric chitosan and biogenic selenium nanoparticles, in combination with porous collagen matrix aiming to prepare adequate

wound dressing or skin substitute for patients with severe burn injuries. Mentioned antibacterial agents should serve as an alternative to superbug-resistant antibiotics. Prepared materials were physiochemically evaluated concerning parameters favourable in wound healing, namely porosity, swelling, degradation in presence of collagenase. These 3D-structured composites possessed high porosity reflecting in good swelling properties useful in absorbing wound extrudates and appropriate biodegradation rate. Bacterial inhibition has been tested using macrodillution broth method showing the synergistic effect of both chitosan and selenium nanoparticles towards both SA and MRSA.

This work was supported by the CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II and by the Ministry of Health under the project no. 17-29874A.

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IPSCS-DERIVED CORNEAL ENDOTHELIAL-LIKE CELLS ACT AS AN APPROPRIATE MODEL SYSTEM TO ASSESS THE IMPACT OF DISEASE ASSOCIATED *SLC4A11* VARIANTS ON PRE-mRNA SPLICING

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Congenital hereditary endothelial dystrophy (CHED, MIM #217700) is a rare autosomal recessive disorder typically presenting as corneal oedema leading to severe visual impairment from birth. The disease is caused by biallelic mutations in the *SLC4A11* gene[1].

We have used direct and whole genome sequencing to screen six probands with CHED. To analyse the effect of c.2240+5G>A on pre-mRNA splicing, and thus to prove pathogenicity of the variant, we have developed a corneal endothelial-like (CE-like) cell model differentiated from autologous induced pluripotent stem cells (iPSCs) via neural crest cells exposed to B27, PDGF-BB and DKK-2. The presence of corneal endothelial markers was evaluated using primary antibodies against ZO-1, N-cadherin and CD166. Total RNA was extracted and reverse transcriptase PCR was performed.

Collectively we identified four novel and seven previously reported disease-associated *SLC4A11* variants. CE-like cells were demonstrated to express several endothelial cell-specific markers including *SLC4A11*. The c.2240+5G>A variant was demonstrated to introduce a cryptic splice donor site leading to an insertion of six bp and the subsequent introduction of a premature stop codon (p.Thr747*).

In summary we anticipate that the use of iPSC-derived CE-like cells will be a useful tool to access the effects of variants of unknown significance on pre-mRNA splicing of corneal endothelial specific proteins and other functional outcomes such as protein function, stability and localisation.

This work was supported by GACR 17-12355S.

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INSIGHT INTO AUTO-OXIDATIVE DAMAGE IN CELLULOLYTIC ENZYMES OBTAINED BY STRUCTURAL MASS SPECTROMETRY

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Lytic Polysaccharide Monooxygenases (LPMOs) are industrially important cellulolytic enzymes used in cellulose saccharification enzyme cocktails and are promising enzymes to use in mass-production of second generation biofuels. Unlike standard cellulases and glycosidases, they degrade polysaccharides oxidatively instead of hydrolytically. Their active site harbours copper ion, which upon its reduction from Cu^{2+} to Cu^{1+} generates reactive oxygen species (ROS). Active site is located on a flat surface surrounded with aromatic amino acids facilitating substrate binding and thus guiding created ROS to precisely attack β-1-4 glyosidic bond in polysaccharides. Owing to the shape of the catalytic site, they have the ability to depolymerize flat crystalline cellulose structures not degradable by standard glycosidases, which is a bottleneck in current biofuel production. LPMOs are unfortunately notoriously unstable during the reaction due to auto-oxidative damage[1].

In this study we use structural mass spectrometry, namely Hydrogen/Deuterium Exchange Mass Spectrometry, to uncover the dynamics and structural aspects of Neurosporra crassa LPMO9c unfolding and degradation induced by its reduction and subsequent auto-oxidation. We also used mass spectrometric techniques to elucidate precise location and nature of oxidative modifications of LPMO leading to its structural changes and degradation, as well as recently reported protective effect of polysaccharide substrate[2]. Our combined observations can potentially aid in rational design of LPMO variants with higher oxidative resistance, better

substrate binding and thus increased stability and reaction yields.

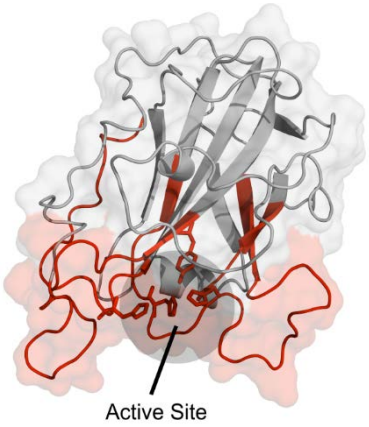

Fig. 1. **Area of oxidative damage on LPMO.** Oxidative damage occurs in regions surrounding the copper ion active site, where highly reactive ROS are generated. If no suitable substrate is present to absorb created ROS, they can react with amino acids in close vicinity, causing oxidative modifications resulting in loss of enzymatic activity and degradation of the protein

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ORGANOCATALYTIC APPROACH FOR THE SYNTHESIS OF OPTICALLY ACTIVE BENZOTHIOPHENE DERIVATIVES

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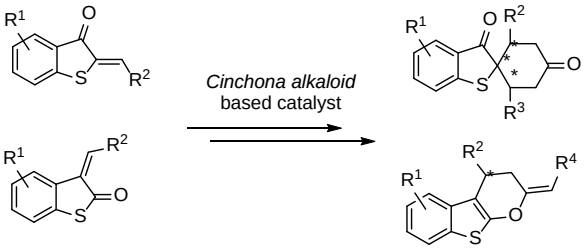
The synthesis of aromatic heterocycles represents an important and challenging goal in modern organic and medicinal chemistry. Various heterocyclic motifs are present in many natural products and molecules relevant for the life science[1]. Sulfur-containing derivatives stay among these heteroaromatic compounds as they possess remarkable biological activity. For example, thiophene and benzothiophene derivatives have found various applications in target oriented synthesis[2]. With respect to above mentioned, our group continuously pay attention to organocatalytic stereoselective reactions of benzothiophenones providing enantiomerically enriched products[3].

Herein, we would like to demonstrate cascade reactions of alkylidene benzothiophenone derivatives suitable for the preparation of functionalized cyclic and spirocyclic molecules with multiple stereocenters. *Cinchona* alkaloid based catalysts provided exclusive formation of corresponding products in high yields with excellent stereoselectivity. Furthermore, transformations to valuable species with potential biological activity will also be described.



R^1 = EWG, EDG; R_2 = EWG, EDG; R_3 = aryl, hetaryl; R_4 = EWG

Scheme 1. **Organocatalytic cascade reactions of benzothiophenone derivatives**

This work was supported by Charles University Grant Agency (1504217) and Czech Science Foundation (16-23597S).

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STUDY OF ALLOSTERIC MODULATION IN TWO-DOMAIN PROTEINS: REVERSED FUSION OF PDZ3 DOMAIN (ZO-1) WITH SH3 DOMAIN (ZO-1)

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In protein chemistry, allosteric modulators can be defined as a part of proteins (so-called domains), which modulate distal localized active (binding) site of protein, where they can increase or decrease binding affinity of active site. Well-characterized PDZ domains are ideal candidates for study of allosteric modulation mechanism affected by synergy of adjacent domains at N- or C-terminal in two-domain synthetic proteins. We used protein domains PDZ3 and SH3 from scaffold protein Tight Junction protein-1 (ZO-1), which

regulate the paracellular flux of solutes and prevent pathogen entry across cell layers.

Figure 1. **Modular organization of protein domains in ZO-1 protein, domain-couple of interest is marked in box.**

In order to biophysical characterization of fusion domains, we used circular dichroism (CD) analysis[1]. Aim of the project is understanding allostery of PDZ3 domain, which is modulated by adjacent protein domains and events connected with changes of structure, folding and substrate specificity of PDZ3 domain. We will use isothermal titration calorimetry (ITC) to determine substrate specificity of both fusion (forward PDZ3-SH3, reverse SH3-PDZ3) in interaction with proposed peptides, derived from natural ligands of PDZ3.

Scheme 1. **Two-domain proteins were used. We examined biophysical properties of naturally occurring tandem PDZ3-SH3 and also reverse combination of protein domains SH3-PDZ3, which is not found in nature.**

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EVOLUTION OF SEX-DETERMINING SYSTEMS IN GENUS *SILENE*, SECTION OTITES

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While gonochorism is very common in animals, the most of the plants are hermaphrodites. Only 6 % of angiosperms, about 15 000 species in total, are dioecious (male and female individuals present in population) but they are dispersed in 175 angiosperm families. It represents 43 % of all angiosperm families.

Plant genus *Silene* offers great opportunities to study origin of sex determination, evolution of sex determining systems and evolution of sex chromosomes. Dioecious species evolved independently in two sections of this genus (Otites and Melandrium). The sex chromosomes have evolved from different pairs of autosomes in these two sections. Our previous research has shown a switch from female heterogamety (ZW sex-determining system) to male heterogamety (XY sex-determining system) in the section Otites. Our analyses also suggest a possibility that has so far not been considered, change in heterogamety through hybridization, in which a male-determining chromosome from one species is introgressed into another one, and over-rides its previous sex determining system (ZW).

The genus *Silene* is rich in gynodioecious species and so the sex determination has likely repeatedly evolved *via* gynodioecy pathway in this genus. We have currently focused on the study in *Silene sibirica* (close gynodioecious relative of section Otites). We focus on the study of the region including locus for restoration of male fertility. We have used RNA-seq analysis for genetic mapping of chromosome carrying restorer of male fertility in *Silene sibirica*. Identification of putative male fertility restorer proceeds both *via* analysis of transcriptomic data and *via* BAC library screening. We hope that we will find connections between genetic control in gynodioecious and dioecious systems in the genus *Silene*. This information can shed light on the evolution *via* gynodioecious pathway in general.

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SYNTHESIS OF NOVEL P-CHIRAL PHOSPHINE LIGANDS FOR TRANSITION METAL CATALISYS

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There has been a growing demand for asymmetric transition metal-catalyzed reactions and phosphine ligands, such as DIOP or BINAP, were applied for the development of efficient homogeneously catalyzed reactions[1]. Their common feature is that the stereogenic element is a carbon atom but not the coordinating phosphorus atom.

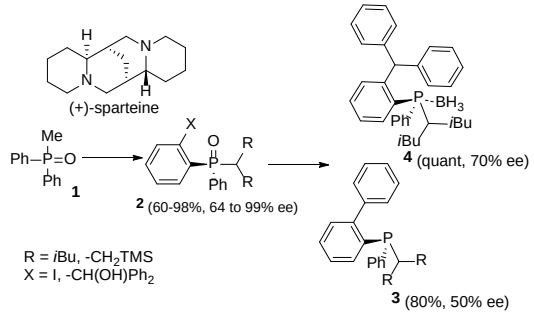
We hypothesized that P-chiral phosphines would circumvent limitations of the currently existing chiral ligands, since the coordinating atom itself is much closer to the central metal atom and chirality transfer may become more effective.

Aim of the current work is developing methodology for asymmetric synthesis of diaryl(alkyl)phosphine ligands and their application in transition metal catalysis.

P-chiral compounds ***2*** with defined configuration were successfully synthetized through directed *ortho*-lithiation with chiral amine ligands such as sparteine. Reproducible results

with good yields and moderate enantioselectivity were accomplished. Pure enantiomers can be obtained by a single crystallization of enriched compounds. X-Ray crystallography was used to determine the absolute configuration.

Compounds ***3*** and ***4*** were obtained by reduction of the corresponding benzylic alcohol or by Suzuki coupling reactions followed by reduction of the phosphine oxide. The former reactions do not affect the configuration on the phosphorous atom. Known procedures for reduction of phosphine oxides are however not applicable[2]. New reduction methods for the preparation of ***3*** and ***4*** were developed.



Scheme 1. **P-chiral phosphine ligand synthesis**

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ANTIBACTERIAL POLYMER-PHOSPHATE BONE CEMENT TARGETED ON THE OSTEOMYELITIS TREATMENT

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Calcium phosphate cements (CPCs) biomimeting the inorganic component of natural bone tissue with exceptional biocompatibility are used in reconstruction and repair bone surgery, pharmacy, dental implantology etc. Nevertheless, potential infection around bone implants called "osteomyelitis" caused mainly by *Staphylococcus aureus* (SA) and its methicillin-resistant bacterial strain (MRSA), is the main problem of modern reconstructive surgery.

In this work, polymer-calcium phosphate cement was modified by selenium nanoparticles (SeNPs). Selenium is a biogenic element and exhibits interesting antibacterial properties against both SA and MRSA[1]. SeNPs doping made the bone cement more thixotropic - injectable, flowable but cohesive. This is important for mini-invasive surgery. Furthermore, SeNPs had a positive effect on the mechanical strength of the samples of bone cement, and expedited the self-setting CPCs reaction as verified by XRD analysis. Scanning electron microscopy figured transformation of amorphous tricalcium-phosphate to flower-like crystals of hydroxyapatite. Distribution of SeNPs in the material was evaluated by secondary ion mass spectrometry imaging. Prepared antibacterial cements were very effective on a gram-positive SA and MRSA bacteria proved by disc diffusion method. The release of SeNPs from bone cement during 48 hours shows, that almost all SeNPs released within 8 hours (96.5 %), which is very promising information since general poly(methylmethacrylate) bone cements are able to release only 2 % of antibiotics.

Due to the positive effect of SeNPs on rheological, mechanical and antibacterial properties, the novel polymer-phosphate bone cement can be possibly applied in mini-invasive surgery as bone filler for the treatment of osteomyelitis.

This work was supported by the CEITEC 2020 (LQ1601) with financial support from the MEYS of the Czech Republic and by the Ministry of Health under the project no. NV18-05-00379.

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LECTIN–BASED METHOD FOR EVALUATION OF NEURAMINIDASE ACTIVITY AND ITS INHIBITORS

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Neuraminidases (NAs) play key roles in pathogenesis of various diseases and are considered viable targets mainly, but not solely in development of drugs against influenza viruses. Certain mutations allow viruses to acquire resistance towards existing anti-influenza drugs[1]. Many of commonly used methods for evaluation of NA inhibitors suffer from some drawbacks as e.g possible interference by the examined inhibitors[2]. Another problem is the fact that enzymatic hydrolysis in the course of analysis takes place in solution but majority of sialic acids (SAs) in host organisms occupy the outermost part of glycan structures lining cell-surfaces.[3]. The

aim of our research was to develop a biochemical assay, based on enzymatic hydrolysis of natural substrates by NAs and lectin-based detection of hydrolyzed substrates, suitable for screening and evaluating efficiency of NA inhibitors. Our method employs immobilized fetuin as a glycoprotein substrate for enzymatic reaction. Then, biotinylated lectins are used to bind to either galactose exposed by the cleavage of SA by NA or remaining SA. Three lectins were tested – peanut agglutinin (PNA, binding to Galβ1,3GalNAc in *O*-linked glycans), *Sambuccus nigra* agglutinin (SNA, binding preferentially to Siaα2,6Gal) and *Maackia amurensis* agglutinin II (MAA-II, binding to Siaα2,3Gal), and PNA was used for further assay development. The amount of lectin bound to fetuin is quantified *via* streptavidin-conjugated fluorescent dye. Assay is conducted in microtiter plates. Between incubations, the excess reactants and solution of enzyme and inhibitor are removed by washing with a surfactant-containing solution, thereby minimizing potential signal interference before fluorescence is measured. The developed method was applied to determine IC_{50} values of NA inhibitors (α-mangostin, quercetin, 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid) and its transformation into microarray format is in progress.

Acknowledgement: APVV-17-0239; VEGA 2/0137/18; Centre for materials, layers and systems for applications and chemical processes under extreme conditions—Stage II, ITMS: 26240120021 (R&D OP ERDF).

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PREBIOTIC SYNTHESIS OF 2´-DEOXYADENOSINE

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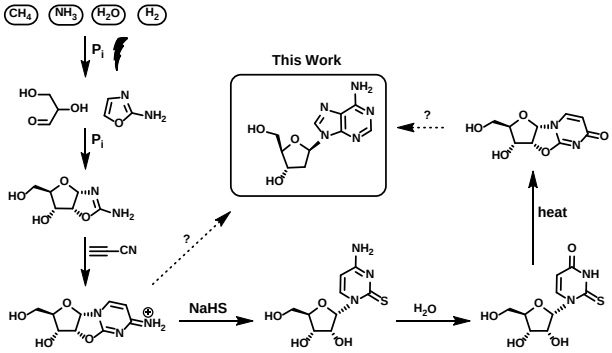
We have previously discovered a short, highly efficient route to activated ribonucleotides from plausible prebiotic feedstock molecules such as cyanamide, cyanoacetylene, glycoaldehyde, glyceraldehyde and inorganic phosphate[1]. We can accomplish the synthesis of all precursors of ribonucleotides, amino acids and lipids by the reductive homologation of hydrogen cyanide and some of its derivatives. The key steps are driven by UV using hydrogen sulphide as a reductant and can be accelerated by Cu^{I}-Cu^{II} photoredox cycling[2]. Anomerization of α-ribonucleosides to β-anomers is extremely inefficient. However, an extraordinarily efficient anomerization can be accomplished using α-2-thionucleosides[3]. Here we describe a novel prebiotic approach to 2´-deoxyadenosine.



Scheme 1. **Prebiotic synthesis of 2´-deoxyadenosine**

This work was supported by Erasmus+ funding.

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NOVEL APPROACH TO QUINAZOLINONE ALKALOIDS, TOTAL SYNTHESIS OF ARDEEMIN

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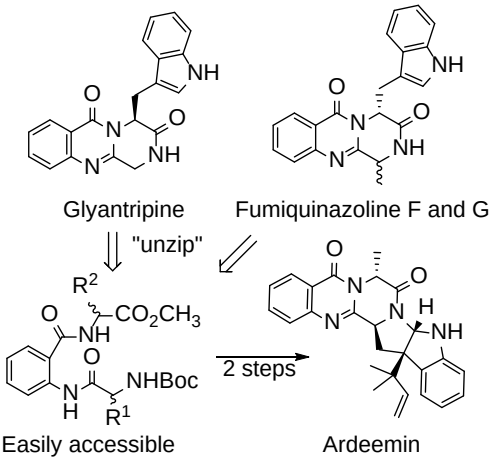
The quinazolinone family of alkaloids, having diverse biological activities, is a growing class of secondary metabolites[1]. They are biosynthesized from tryptophan and anthranilic acid by incorporation of an additional amino acid unit. The members of this class of alkaloids exhibit cytotoxic, antiviral and anti-multidrug resistance activities[2]. Therefore, practical methods that allow rapid access to larger quantities of these alkaloids and their analogs are needed.

A novel approach to quinazolinone derivatives using silica gel mediated double condensation is reported, which is successfully applied to a three-step total synthesis of glyantripine as well as fumiquinazolines F and G.



Scheme 1. **Total synthesis of natural products**

Ardeemin is a complex quinazolinone alkaloid challenging organic chemists since 1993, when it was isolated from the fungus *Aspergillus fisheri*[3].
We report the shortest total synthesis of ardeemin in only four steps starting from commercially available materials.

We thank the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences and the Gilead Sciences & IOCB Research Centre for their generous funding.

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MODIFICATION OF ALGINATE-BASED BIOINKS FOR 3D PRINTING AND STEM CELL CULTIVATION

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3D cell culture techniques provide deeper insights to cellular behavior in comparison to standard planar cultivation, since it allows more physiological cell-cell and cell-matrix interactions. 3D bioprinting is a powerful tool that gives us the possibility to create cellular/hydrogel structures with simple monolayered organization up to complex 3-dimensional tissue models.

Our research is focused on 3D modelling of lung tissue by 3D bioprinting of human stem cells (lung epithelial progenitors, endothelial cells) and alginate hydrogels. Such task is non-trivial and several aspects need to be solved. We performed optimization of key printing parameters, which are needed to achieve good print quality, reproducibility and viability of embedded cells. We generate 3D models (especially branched structures with adjustable parameters) *in silico* and print them successfully. Human stem cells printed in alginate grow for prolonged period of time, however lack of bioactive motifs decelerates their expansion rate. We are therefore working on fine-tuning of this basic material by either mixing with extracellular matrix proteins or direct modification of alginate by bioactive peptide motifs to stimulate specific cell response (adhesion, proliferation, differentiation, migration, cell aggregation) within printed objects, as well as to modify its mechanical properties.

The spatial organization provided by 3D printing and interaction of cells with hydrogel are crucial parts that move our culture system towards physiological conditions and allow for more accurate observation of biological processes involved in lung development, such as differentiation, factors directing morphogenesis and interactions between introduced cell types.

This work was supported by The Czech Science Foundation through Project No. GA18-05510S, project from Masaryk Univesity MUNI/A/1298/2017 and Brno Ph.D. Talent 2018.

EFFECT OF SOLUBLE ENDOGLIN ON CHOLESTEROL AND BILE ACIDS METABOLISM IN NASH MOUSE MODEL – A PILOT STUDY

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Plasma concentrations of soluble endoglin (sEng) are increased in cardiovascular and metabolic diseases associated with hypercholesterolemia, such as atherosclerosis, and type II diabetes mellitus, which affect liver functions and metabolism[1,2]. Therefore, the aim of the present study was to investigate the effect of high levels of sEng on cholesterol and bile acids (BA) metabolism in liver during the induction of non-alcoholic steatohepatits (NASH).

Three-months-old transgenic male mice overexpressing human sEng and wild type mice fed for 6 months either with high fat diet enriched with cholesterol and fructose (HFD) and chow diet underwent *in vivo* study with plasma and bile

collection. Plasma biochemical analysis, LC/MS of plasma BA and histology were performed. Expressions fibrotic markers was assessed by qRT-PCR.

Mice with high plasma levels of sEng fed chow diet showed increased bile flow in comparison to both HFD groups. HFD significantly increased liver weight and induced steatosis in both experimental groups. In agreement with histological changes, mRNA levels of Col1a1, a marker of steatosis but as well Nqo1, showing oxidative stress were increased. Established liver infury by HFD was proved by significantly elevated plasma cholesterol levels and activity of alkaline phosphatase (ALP) and alanine aminotransferase (ALT).

In conclusion, these pilot data demonstrated the effectiveness of HFD to evoke liver injury and development of liver fibrosis in mice. Moreover, sEng choleretic effect was suppressed by administration of high fed diet.

Supported by AZV CR number 17-31754A.

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WHOLE GENOME SEQUENCE OF THE *TREPONEMA PALLIDUM* SUBSP. *ENDEMICUM* (TEN) STRAIN IRAQ B

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Treponema pallidum subsp. *endemicum* (TEN) is the causative agent of bejel (endemic syphilis). This spirochete is highly related to other treponemes causing syphilis and yaws[1]. To date, only one strain of TEN, Bosnia A, has been completely sequenced[2]. Because of that, analysis of genetic diversity in TEN strains has not been done.

One additional strain of TEN isolated in Iraq in 1951 was available for sequencing[3]. Complete genome of the Iraq B strain was amplified and sequenced using the pooled segment genome sequencing (PSGS) method using Illumina platform.

Both Bosnia A and Iraq B strains were found to be highly related. Comparison of complete genome sequences revealed 37 single nucleotide differences indicating high genetic relatedness of both bejel treponemes.

In addition, both strains also differed in the number of repetitions in the TP0433 and TP0470 genes, and in the length of 18 homopolymeric regions.

In Bosnia A strain, deletion in the genetic region containing *tpr*F and G genes was previously found[4]. This region was identified in the subpopulation of Iraq B strain, while the larger population has the deleted version. This suggests that the deletion of this region could be underway. This region was found highly similar to corresponding region found in *Treponema pallidum* subsp. *pertenue* (TPE) strains.

Even though all TEN pathogenic strains have high genetic relatedness[5] to whole genome sequences of TPE strains, bejel strains clustered separately of both yaws and syphilis strains. This indicates a clear correlation between disease and subspecies classification.

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CHIRAL SEPARATION OF DESCHLOROKETAMINE AND BIOLOGICAL EVALUTATION OF THE ENANTIOMERS

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New psychoactive substances (NPS) have been involved in incessantly growing number of intoxication and the number of NPS on the drug market is still raising. Currently, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is monitoring more than 670 different substances.

Dissociative anaesthetics is a relatively small and hazardous group of NPS that despite the abusive potential is currently attracting the attention of many experts due to the recent scientific studies showing the potential of these substances in depression treatment. Dissociative anaesthetics are mainly represented by a group of arylcyclohexylamines. The most well-known members of the group are phencyclidine and ketamine. Deschlorketamine had been sold as a legal alternative for ketamine, which leads to its rapid expansion over the market. Differences in cytotoxicity of racemic deschloroketamine and its enantiomers were determined within this study.

Deschloroketamine was synthesized from commercially available compounds and subsequently resolved by a liquid chromatography chiral separation method. The absolute configuration of the individual enantiomers was assigned by a combination of quantum chemical calculations and an interpretation of circular dichroism spectra. Afterwards, single crystal X-ray data of both enantiomers confirmed the previously assigned chirality. The enantiomers were subjected to *in vitro* toxicity tests on a series of cell lines.

We assigned the absolute configuration of the enantiomers and determined the most preferred conformers present in the crystal by circular dichroism spectra supported by *ab initio* calculations. Cytotoxicity of (*S*)-deschloroketamine was higher in the majority of cases. For human embryonic kidney cells (HEK 293T) the (*S*)-enantiomer reached the IC50 below 1 mM concentration[1].

This study was funded by a specific university research (project MSMT No. 21-SVV/2018) and by the Ministry of Interior of the Czech Republic (project MV0/VI20172020056).

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WNT5A GOVERNS BRANCHING MORPHOGENESIS OF CHOROID PLEXUS IN THE DEVELOPING BRAIN

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Morphogenesis is a complex process arising from coordinated action of large number of signaling pathways during embryonic development. Wnt signaling pathway is one of the crucial cascades involved in proper regulation of the most fundamental developmental processes required for generation of cell and tissue diversity. Wnt5a represents one of the most well studied Wnt ligands, which was shown to play major role in execution of developmental functions associated with Wnt pathway activity, including cell polarity as well as establishment and outgrowth of multiple structures in vertebrate animals[1]. Choroid plexus (ChP), located within the lumen of brain ventricles, plays the role of the major site for the production of cerebrospinal fluid (CSF)[2]. Developing ChP exhibits high levels of Wnt5a expression and given its growth pattern and complex branching architecture, we decided to investigate potential involvement of Wnt5a-mediated signaling in the process of its development. Here we report identification of Wnt5a as a key morphogen involved in the embryogenesis of all choroid plexuses in the developing brain. We demonstrate that Wnt5a is expressed in a specific spatial pattern shared by all the choroid plexus, playing particularly prominent role in the morphogenesis of hindbrain choroid plexus. Furthermore, we show deleterious effects of Wnt5a ablation and overexpression on the hindbrain plexus formation and highlight distinct differences in non-canonical Wnt pathway activity between embryonic choroid plexuses.

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MICROFLUIDIC CHIP AS A TOOL FOR UNIFORM SPHEROID GENERATION

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Three-dimensional (3D) cell culture systems have gained increasing interest in drug discovery and tissue engineering due to their evident advantages in providing more physiologically relevant information and more predictive data for *in vivo* tests[1].

Spheroids and organoids dramatically increased interest in last years, as models with 3D cellular organization allowing to recapitulate biological properties of several human tissues or organs.. A number of methods have been developed for formation and generation of spheroids. However most of them suffer from labor-intensive protocol, difficulties in medium exchange, as well as non-uniform size of spheroids. Among the candidates, microfluidics is very promising platform for

spheroid formation and long term cultivation due to: automation, small sample volume and cost-effective fabrication. In addition, microfluidic systems are capable of continual control over medium flow in spatial and temporal domains, which allows to create precise and more *in vivo*-like microenvironments. Design of microfluidic system is created directly for purpose of the experiment.

In this work, we present microfluidic chip for formation of spheroids with divergence of size less than 20 % of diameter, For other methods, the 200 % variation is common. On the basis of a concave microwell-based PDMS multilayer chip, it enables a parallel perfusion culture of large amount of cell spheroids. Upon computer simulations (Ansys Fluent), we optimized chip designs and characterized several specific conditions in a microwells. We are able to keep fluid shear stress at very low levels for undisturbed cultivation and to ensure sufficient nutrient exchange in all microwells. Simulations are matching with experimental work on human embryonic stem cells. Nowadays we characterized proliferation rates, size uniformity morphological and specific metabolic markers by live imaging, western blot and immunohistochemistry during 7 days. Our microfluidic system will be utilized for formation and long-term cultivation of brain organoids.

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SYNERGISTIC EFFECT OF ANTIMICROBIAL PEPTIDE LL-III/43 WITH ANTIFUNGAL AZOLES ON BIOFILM FORMATION OF *CANDIDA ALBICANS*

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Candida albicans is one of the most prevalent multi-resistant pathogens nowadays. It is responsible for various types of infections such as vaginal, skin, blood stream or implant related infections[1]. This species has a great ability to form biofilm and colonize host tissues without efficient eradication by host immune system[4]. Its emerging resistance to the various types of antimicrobials like commercial antimycotics poses a severe problem of today medical practice[2]. Thus, there is a great effort to find new alternative agents, which would be able to suppress the growth of *C. albicans* and to inhibit its ability to form biofilm. Antimicrobial peptides represent such agents, since their mechanism of action is strikingly different from those of antibiotics.

This study focused on anti-biofilm activity of LL-III/43 – an analogue of α-helical antimicrobial peptide LL-III (Val-Asn-Trp-Lys-Lys-Ile-Leu-Gly-Lys-Ile-Ile-Lys-Val-Val-Lys-NH_2) which was originally isolated in our laboratory from the venom of wild bee[3]. This work studied whether LL-III/43 had synergistic effect with antifungal azoles (fluconazole, clotrimazole, vorikonazole) on prevention of biofilm formation of three strains of *C. albicans*. The inhibitory effect of the LL-III/43 alone and in combination with antifungal azoles on biofilm formation of *C. albicans* was examined using resazurin viability assay.

LL-III/43 was found to be very effective in biofilm inhibition of all used strains of *C. albicans.* The antifungal azoles had little or no effect on biofilm formation of *C. albicans* but the combination with LL-III/43 (25 μM) caused at least 80% decrease in metabolic activity of cells in *C. albicans* biofilm. Thus, LL III/43 in combination with antifungal azoles indicates a promising strategy against biofilm cells of *C. albicans.*

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NG2-GLIA PROLIFERATION AND DIFFERENTIATION AFTER DIFFERENT TYPES OF BRAIN DISORDERS

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NG2-glia, a fourth major glial cell population, are present in the adult central nervous system and display distinct morphology, antigens, and functions from other mature glial cell types. Recently, many studies have shown that these cells are multipotent *in vitro* and they also display wide differentiation potential under pathological conditions *in vivo*.

The aim of this study was to identify the rate of proliferation and differentiation after different types of brain disorders, such as focal cerebral ischemia (FCI), stab wound (SW) and demyelination (DEMY) in 3-month-old mice. We used transgenic Cspg4-cre/CAG-tdTomato mice, which after administration of tamoxifen express a red fluorescent protein

(tdTomato) in NG2-glia and cells derived therefrom. FCI was induced by middle cerebral artery occlusion, SW by sagittal cortical cut and DEMY by feeding mice with copper chelator cuprizone. To recognize the changes in expression profiles we employed single cell RT-qPCR in control mice and those 7 days after injury, followed by PCA analysis and self-organizing Kohonen maps. These methods enabled us to recognize two main populations (NG2-glia, oligodendrocytes from NG2-glia), each of them comprising 4 distinct subpopulations. Expression profiles showed that one subpopulation, NG2-glia that express a marker of reactive astrocytes – glial fibrillary acidic protein (GFAP), was present only after severe ischemic injury of the cortex (FCI), while after mild injury of the cortex (SW) and corpus callosum (DEMY) predominated subpopulations mirroring different stages of oligodendrocyte maturation. These results were also confirmed by immunohistochemistry on the coronal brain slices from control mice and those 7 days after injury, where we determined the percentage of cells that were double positive for tdTomato and one of the other marker typical for proliferation and their differentiation towards astrocytes or oligodendrocytes.

Taken together we have shown that the proliferation rate of tdTomato cells is significantly increased after all three types of brain disorders while differentiation potential fluctuates depending on the type of brain disorder.

Project was supported by GACR 17-04034S.

BONCAT AND CLICK-REACTION-ON-MEMBRANE AS A METHOD FOR IDENTIFICATION OF DIFFERENTLY EXPRESSED PROTEINS DURING TBEV INFECTION OF HUMAN NEURAL CELLS

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Changes in protein expression as a response to viral infection can be observed using many approaches, however, many of them are not able to distinguish newly synthesized proteins from the preexisting protein pool. We took advantage of BONCAT (bioorthogonal non-canonical amino acid tagging), which is based on incorporation of the unnatural amino acid L-azidohomoalanine (AHA) into newly synthesized proteins instead of L-methionine. For further analysis, AHA-containing proteins are tagged with a biotin tag by the so-called Click reaction. The biotin moiety is subsequently used for visualisation of newly synthesized proteins.

Human glioblastoma cell line was infected with tick-borne encephalitis virus, strain Neudoerfl (TBEV), and newly synthesized proteins were labelled with AHA in a 2 hour window in various intervals after infection. Proteins were separated using 2D electrophoresis followed by electroblotting. The Click reaction with biotin-alkyne was performed on a PVDF membrane and the tagged proteins were visualized using streptavidin conjugated with alkaline phosphatase. Protein spots from 2D gels were identified using MALDI-TOF/TOF.

Our optimized workflow (performing Click-on-membrane) resulted in improvement of the detection of newly synthesized proteins. Differences in protein patterns between control and infected samples were observed. Furthermore, we verified the ability of BONCAT in combination with Click-on-membrane for detection of differentially produced proteins during TBEV infection of human neural cells, some of which were identified by MALDI-TOF/TOF.

This study was supported by the MEYS of the Czech Republic INTER-ACTION projects LTARF 18021 and LTAUSA18040, the Grant Agency of the Czech Republic (18-27204S) and the Czech research infrastructure for systems biology C4SYS (project no LM2015055).

LACTATE AS A SIGNALING MOLECULE IN THE TUMOR MICROENVIRONMENT

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The main characteristics of tumor microenvironment are hypoxia, hyperlactatemia, hypercapnia and acidosis, which are caused by increased metabolism of tumor cells and abnormal tumor vasculature[1]. Recently, lactic acidosis in tumor tissue has been identified as a promising target for cancer therapy[2], because it strongly differentiates tumor tissue from the normal one. The levels of lactate in tumor tissue are from 20 to 40 times higher than the normal ones[3]. Moreover, lactate has many roles in tumors: it is an important stimulant of tumor growth, angiogenesis, and it has also an immunosuppressive role[2]. Furthermore, several reports showed that higher levels of tumor lactate correlate with a higher resistance to treatment[4], a higher incidence of metastases, poor survival of patients, and a more frequent of disease recurrence[5].

The aim of our work was to determine the effect of lactic acidosis on the sensitivity of tumor cells to radiotherapy, chemotherapy and photodynamic therapy. This feature was studied in the previously unexplored context of the lactate's ability to activate a master antioxidant and chemoprotective transcription factor Nrf2.

The cells used for the experiments were cultivated in media with different concentration of lactate anion and pH. Then, they were treated with different types of therapy, or they were used for western blot and also for isolation of mRNA followed by real time PCR. The effect of therapies on the cell viability was determined according to their metabolic activity.

Western blot and real time PCR were used for investigation of the role of Nrf2 protein in lactate-induced resistance to stress.

Our data show that the acidosis in tumor microenvironment decreases the resistance of the cells to a therapy based on the induction of apoptosis, but not to a genotoxic therapy. On the other hand, our results show a considerable variability in the sensitivity of the cells to therapy in lactate microenvironment. In some cases, lactate completely reverses the sensitizing effect of acidosis. Our preliminary data also show that the lactate treatment stimulates Nrf2 signaling pathway.

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FAITH OF AZOLES IN ENVIRONMENT

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These compounds are derivatives of 1,2,4-triazole substituted in position N4 of the triazole ring. The azole fungicides are broad-spectrum antifungal compounds used in agriculture for fruit and other crop production as well as in human and veterinary medicine for treatment of skin mycoses[2]. They affect fungi by inhibition of cell growth[1]. Azoles inhibit biosynthesis of steroids compounds such as cholesterol, ergosterol[5]. The negative effects on mammalian organisms are in genotoxicity, and carcinogenicity also they act as inhibitors of enzymes from cytochrome P450 family, 14-α-aromatase (CYP19) and 14-α-demethylase (CYP51) - the key enzymes for balance between androgens and estrogens and biosynthesis of meiosis activating sterol respectively[2]. The reactivity of azoles is influenced by presence of essential elements (e.g Cu, Zn)[3]. This study focuses on the gas-phase experiments using ESI-MS (Fig. 1) to clarify the formation and degradation of by-products, which can affect organisms in various unknown ways. As an example, we have studied the formation of zinc complexes with penconazole and the degradation of penconazole caused by presence of zinc. This key study is helpful as piece of mosaic to understanding of interactions and fate of azoles in systems that are more complex.

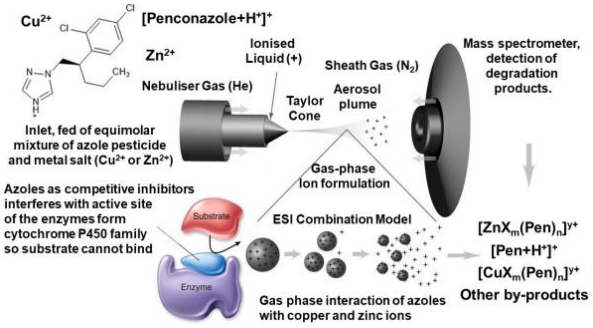

Figure 1. **Simulation of azoles reaction with Cu2+ and Zn^{2+}**

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PLAYING WITH PROPERTIES OF BIOLOGICALLY ACTIVE COMPOUNDS: HIGH-THROUGHPUT CONJUGATION OF DRUG-LIKE MOLECULES FOR CHEMICAL BIOLOGY

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Chemical biology is a progressive interdisciplinary field of modern science. It combines the application of chemical and biological techniques to prepare small molecules and determine their behaviour in various biological systems. However, it is questionable, if synthesis of small, potentially interesting compounds, is the only way how to find suitable biologically useful probes. In this regard, the alternative approach is conjugation of drug-like small molecules with a suitable label to receive conjugates with improved properties. As such examples can serve the conjugates of compounds of interests with fluorescent dye to visualize molecules in cells using a fluorescent microscopy (applied in the field of cyto-toxic terpenes)[1] or heterobifunctional PROTAC (proteolysis targeting chimeras) conjugates with thalidomide moiety able to bind two specific proteins with subsequent targeted degradation (applied in the field of protein kinases inhibitors)[2]. The high-throughput synthetic approach, recently developed in our lab, allows for fast and simple preparation of desired conjugates, without need of tedious purification. In this lecture, the synthetic concept utilizing solid-phase synthesis and *in vitro* properties of target conjugates will be reported.

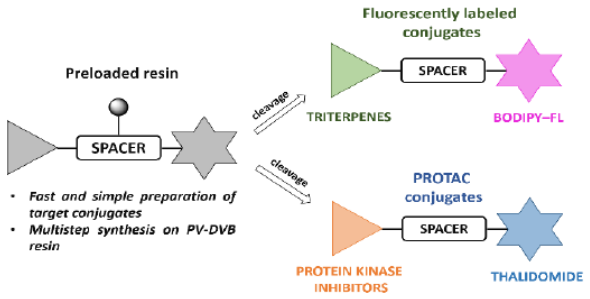

Fig. 1. **Application of preloaded resins**

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DESCRIBING A NOVEL POST-TRANSLATIONAL MODIFICATION OF PROTEIN DISHEVELLED

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Post-translational modifications (PTMs) of proteins form their variants that differ in physical and chemical properties. This is crucial for regulation of complex biological processes such as signaling pathways, where crosstalk between numerous interacting partners results in a fine-tuned cellular response. Protein Dishevelled is a key signal transducer in main branches of Wnt signaling pathway. Wnt is highly conserved pathway that is crucial in regulation of embryonic development as well as in maintenance of homeostasis in adult organisms. Its deregulation is often connected to various developmental and cancer diseases. Protein Dishevelled acts as a switch between various downstream signaling events. It consists of three structured domains and the major part of protein that is intrinsically disordered. As most of IDPs, it is target to numerous post-translational modifications that regulate its functions. We have identified proteins from Tubulin-tyrosine ligase-like (TTLL) protein family as a Dishevelled binding partners. TTLL enzymes are mostly known for regulation of broad microtubule functions by catalysis of their PTMs - polyglutamylation and polyglycylation.

Hereby we demonstrate, that polyglutamylase TTLL11 interacts with all three human homologues of protein Dishevelled and mediates their polyglutamylation in atypical manner. We have biochemically analysed this modification process. We also show, that this PTM can be decreased by deglutamylating enzymes from Cytosolic carboxypeptidase (CCP) protein family suggesting that polyglutamylation of Dishevelled is reversible. Moreover, we demonstrate that overexpressed TTLL11 has an inhibitory effect on Wnt/β-catenin dependent signaling pathway in HEK293 cell lines. To sum up, we have identified TTLL11 as both, an enzyme that catalyse atypical PTM of Dishevelled and as a novel negative regulator of Wnt/β-catenin dependent pathway. Furthermore, we biochemically describe the atypical modification process.

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MOLECULAR INTERACTIONS IN PROTEIN/RNA COMPLEXES: WHAT CAN COMPUTATIONS TELL US?

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The ribonucleic acid (RNA) molecules are involved in countless key processes in living organisms, including gene expression, cellular defense and catalysis of chemical processes. *In vivo*, RNA molecules always interact with proteins, since they are synthesized to the point of their degradation. Understanding the principles of protein/RNA interaction is therefore a matter of biologically imminent importance as it allows us to fully grasp the nature of nucleic acids and to appreciate the irreplaceable role they play in living organisms. At the same time, studying structures of protein/RNA complexes by the leading experimental methods for structure determination (e.g. X-ray crystallography, NMR spectroscopy) is inherently more complicated than determining the structure of the individual monomers. In my research, I use molecular dynamics (MD) simulations to study the protein/RNA complexes. Many biomolecular complexes are inherently dynamical, which makes MD an important tool to complement the experimental techniques of structural biology which typically provide only static ensemble-averaged pictures of the molecular complexes.

I have successfully applied MD to study dynamic recognition at protein/RNA interfaces of Fox-1, CUG-BP2, and HuR proteins. The dynamic recognition is an important element influencing the affinity of the protein/RNA complexes that is, however, not directly visible for the experimental methods. Further, I have used simulations to describe coordination of nuclease and polymerase activities in HIV-1 reverse transcriptase and to determine the structural factors of its substrate specificity. Both of these processes are critical for successful proliferation of the virus in infected cells. Lastly, I have applied MD to study hydration in the Fox-1 protein/RNA

complex and showed that water mediated interactions significantly contribute to the biomolecular structure. Yet, the fast dynamics of individual water molecules makes their study difficult by conventional structural experiments. MD simulations, on the other hand, are an ideal tool to study hydration as they allow us to observe movement of every single water molecule with essentially unlimited spatial and temporal resolution.

THE CHICKEN TVA RECEPTOR FOR AVIAN SARCOMA AND LEUKOSIS VIRUS SUBGROUP A (ASLV-A) ALSO SERVES AS RECEPTOR FOR CELLULAR UPTAKE OF COBALAMIN (VITAMIN B12)

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Deficiency of vitamin B12 (cobalamin) caused by malnutrition of mutations in the cobalamin pathway genes is associated with haematological and neurological pathologies in mammals. Cobalamin is transported from blood into cells in complex with carrier protein transcobalamin *via* a receptor encoded by the *CD320* gene. In birds, the *tva* genetic locus encodes protein belonging to the group of low density lipoprotein receptor family which is believed to be orthologous to human *CD320* gene. TVA receptor in avian cells also determines susceptibility to Avian sarcoma and leucosis viruses subgroup A (ASLV-A), however its physiological function has not been determined.

In this study we investigated whether the physiological function of the two orthologs, human CD320 and chicken TVA, is conserved despite diversification of mammals and birds more than 300 million years ago. We produced and purified recombinant chicken transcobalamin which was then incubated with radioactively or fluorescently labelled B12. The complex was then used for the import assays of B12 into chicken DF1 cells. Exogenous over-expression of TVA receptor in DF1 cells mediated by transient or stable transfection showed higher import of vitamin B12 into cells. CRISPR-mediated knock-out of endogenous TVA resulted in significantly lower cobalamin import. Further, import of vitamin B12 into cells chronically infected with ASLV-A was reduced in comparison with uninfected cells. This can be explained by lower number of available TVA receptor molecules on the cell membrane due to occupancy with viral envelope proteins. Moreover, when DF1 cells were preincubated first with transcobalamin-cobalamin complex and afterwards infected with ASLV-A, the number of infected cells was lower. In line with this, fluorescence microscopy indicated import of fluorescently labelled B12 into chicken cells. This study is the first to report of physiological function of chicken TVA receptor. Overall, our data indicate, that TVA is a part of the cobalamin metabolic pathway in chicken cells.

This work was supported by grant no. 17-23675S from the Czech Science Foundation.

PREPARATION OF DEOXOFLUORINATED GLYCOCALIXARENES *VIA* STEREOSELECTIVE CHEMICAL GLYCOSYLATION

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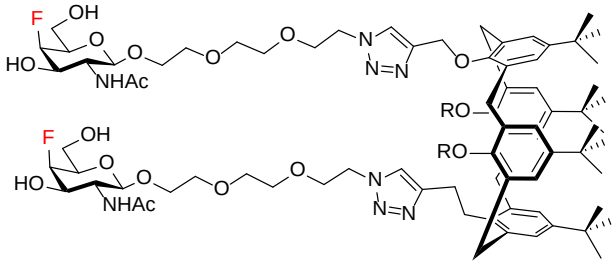
Carbohydrate-lectin interactions are essential in biological recognition events such as molecular adhesion. A typical example of this process is recognition of D-*galacto*-configured sugars *via* PA-IL lectin, which plays a crucial role in human infection by pathogenic bacteria *Pseudomonas aeruginosa*. Due to the multivalent effect is this interaction very specific and glycocalixarenes bearing D-galactose showed high affinities to PA-IL lectin[1]. Further research in this area can eventually lead to agents with anti-adhesive effect.

Deoxofluorination is a useful approach to modulate the properties of the carbohydrate molecules. Fluorine introduction has a minimal steric impact but results in a variety of electronic effects. An important step in the synthesis of glycocalixarenes bearing fluorinated *galacto*-configured carbohydrates is preparation of the corresponding deoxofluorinated donors and examination of their glycosylation stereoselectivity.

Deoxofluorinated 1,6-anhydropyranoses have been converted to the corresponding phenyl 1-thioglycoside donors. Preactivation procedure[2] utilizing diphenyl sulfoxide/triflic anhydride (Ph_2SO/Tf_2O) has been used for subsequent stereoselective chemical glycosylation of an azide-containing triethylene glycol spacer. Regioselective copper(I)-catalysed azide-alkyne cycloaddition[1] (CuAAC) has been utilized for preparation of fluorinated glycocalixarenes.



Scheme 1. **Typical example of multivalent glucocalixarene bearing 4F-GalNAc (R= 4-deoxy-4-fluoro-GalNAcTEGTriazolylmethylene)**

We thank Czech Grant Agency for support of our research (grant no. 17-18203S)

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STUDY ON STRUCTURE AND FUNCTION RELATIONSHIP OF THE HEME-CONTAINING SENSOR PROTEIN, Bach1

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Bach1 is a heme sensor protein and the first mammalian transcription factor found to bind heme. In the case of heme accumulation, Bach1 stimulates transcription of heme oxygenase, an enzyme which is responsible for free heme degradation. Under low heme concentrations, Bach1 forms a heterodimer with small Maf proteins and the complex binds to enhancers of heme oxygenase gene and represses its expression. Bach1 provides protection effect against oxidative stress damage and thus, it could act as a potential therapeutic agent of oxidative stress-related diseases. In order to understand and control such processes, it is essential to explain conformational changes induced by heme binding and signal transduction.

Hydrogen-deuterium exchange (HDX) coupled to mass spectrometry (MS) is a method which enables protein structural dynamics studying. This method is suitable for differentiating structured protein regions from those that are unstructured, and for monitoring structural changes induced by protein-protein interactions or ligand binding. HDX-MS was already successfully employed in our research of other heme sensors1. The data obtained using HDX-MS experiments for Bach1 protein are in an agreement with a general Bach1 structure: its two functional domains (BTB and ZIP domain) are rather structured, whereas the rest of the protein is without proper secondary structure. According to our analyses it is possible that a presence of DNA sequence and/or small Maf proteins is essential for noticeable conformational change of Bach1 in the course of heme interaction.

The already published results obtained by examination of Bach1 using spectroscopic approaches2 are rather controversial, so we performed more refined and detailed analyses. According to our data two molecules of heme bind to the Bach1 protein.

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EFFECT OF BINDING MOTIF ORIENTATION ON INTERACTION OF TEAD1 TRANSCRIPTION FACTOR WITH DNA

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Transcription factors mediate gene expression regulation through interactions with DNA and other regulatory proteins. TEAD transcription factors are active mainly during growth and development and induce expression of wide range of genes most of which encode proteins involved in cell proliferation, differentiation or apoptosis prevention. TEADs and many of their target proteins are also known to be upregulated in several types of cancer cells and are considered a possible target for anti-cancer therapy. Due to these properties, strict regulation of TEAD proteins activity is required to prevent tumorigenesis or developmental disorders. To date, known ways of TEAD proteins regulation include mostly interaction with other regulatory proteins - coactivators such as YAP, TAZ or Vgll. Nevertheless, the information on TEAD transcription factors activity regulation through interaction with DNA is still limited.

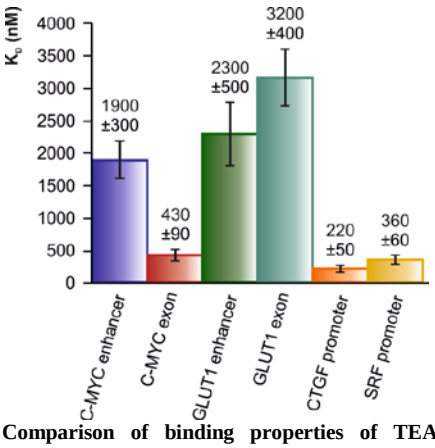

Fig. 1. **Comparison of binding properties of TEAD1-DBD complexes with M-CATs from different human genes;** Complexes with DNA duplexes with 5' to 3' oriented binding motifs (SRF promoter, CTGF promoter and C-MYC exon) had approximately ten times higher K_D than those with 3' to 5' oriented binding motif

To study the interaction of DNA binding domain of TEAD1 (TEAD1-DBD) with short DNA duplexes containing the M-CAT motif and originating from regulatory regions of different human genes, first, dissociation constant of each complex was determined using fluorescence anisotropy-based binding assay.

According to KD assay results (Fig. 1), tested M-CATs could be divided into two groups, one with approximately ten times higher affinity to TEAD1-DBD than the other, depending on the M-CAT motif orientation. For further investigation of the structural basis of this effect, structural mass spectrometry techniques, H/D exchange and chemical cross-linking, were utilized. These experiments provided information about the interaction interface, which was for all M-CATs located to H3 helix, and distance restrains needed for homology modelling and molecular docking used to explain the different binding affinities of each oligonucleotide to TEAD1-DBD. During the molecular docking, the lower affinity inverted M-CATs preferred to bind to TEAD1-DBD in 180° rotated orientation (Fig. 2) where fewer and weaker amino acid-base interaction could form leading to weaker binding affinity and thus higher dissociation constant. *In vitro* results were finally correlated with situation in living cells by the means of chromatin immunoprecipitation with qPCR quantification showing lower promoter occupancy by TEAD1 of the inverted M-CAT.

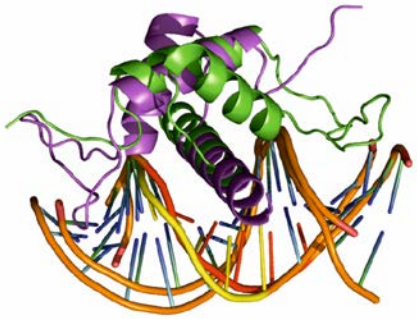

Fig. 2. **Structure superposition of TEAD1-DBD homology models docked to 3'-5' (green) or 5'-3' (purple) oriented binding motif.** Models were build based on chemical cross-linking and HD exchange results using known TEAD4-DBD/M-CAT complex structure as a template[1]

This work was supported by the Czech Science Foundation (grant 16-24309S), Charles University (project 1618218) and the Ministry of Education of the Czech Republic (project LH15010; programs "NPU II" - LQ1604 and LM2015043 CIISB for CMS BIOCEV - LTC17065)

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STRUCTURAL ANALYSIS OF CARBOHYDRATE OXIDASE COMPLEXES REVEALS DETAILS OF BINDING OF LIGAND IN ACTIVE SITE

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Carbohydrate oxidase from *Microdochium nivale* (*Mn*CO) is a flavoenzyme catalyzing oxidation of primary alcohols in various saccharides[1]. The enzyme has industrial potential owing to its ability of oxidizing oligo/polymeric saccharides.

Crystal structures of both wild type and mutant forms in complex with ligand in active site were determined. Advanced routine of paired refinement protocol[2] was performed for determination of the high resolution diffraction limits. This results in higher quality of ligand observation.

The ligand binding in the mutant form is significantly weaker than in the wild type that corresponds well with measured enzymatic activities. The crystal structures reveal details of enzyme:ligand interactions at atomic details and clarify specific properties of the enzyme with regard to catalysis conditions and substrate type.

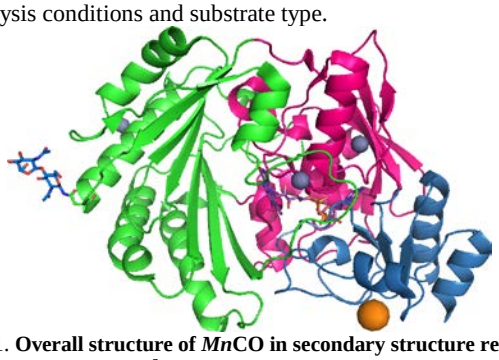

Fig. 1. **Overall structure of *Mn*CO in secondary structure representation (PDB: 3RJ8[3]). Substrate binding domain in green and two subdomains of FAD-binding domain in blue and red colors.**

This work was supported by the ERDF fund (CZ.02.1.01/0.0/0.0/16_013/0001776) and by the Grant Agency of the CTU in Prague, grant No. SGS16/246/OHK4/3T/14.

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DESIGN, SYNTHESIS AND EVALUATION OF NOVEL INHIBITORS OF KYNURENINE AMINOTRANSFERASE II

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The kynurenine metabolic pathway is believed to play an important role in the pathogenesis of psychiatric disorders and neurodegenerative diseases[1]. One of the enzymes of this pathway, kynurenin aminotransferase (KAT), appears to be a promising target for schizophrenia treatment[1], especially its isoform KAT II. Although several generations of inhibitors have been discovered, only a few chemotypes represent their structures. Therefore, using computer-aided drug design, we synthesized potential inhibitors of KAT II and evaluated their activity.

We used Molecular Operating Environment software to construct a pharmacophore model based on a structural analysis of known inhibitors. Several ligands were designed and validated using the model and molecular docking. Representative heterocyclic aminoketones achieved the best docking score.

To examine the potency of the designed compounds, we synthesized a series of aminoketone derivatives with different heterocycles and tested them for inhibition activity using *in vitro* fluorescence assay. Some of them showed good inhibition activity with IC_{50} values in the micromolar range.

Observed activities of the prepared inhibitors indicate their potential as leading structures for further optimization and possibly for the development of a clinical candidate.

This work was supported from specific university research (MSMT No 21-SVV/2018) and from ERDF/ESF PharmaBrain (No. CZ.02.1.01/0.0/0.0/16_025/0007444).

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ANTIVIRAL EFFECT OF RESVERATROL AND ITS DERIVATIVES ON TICK-BORNE ENCEPHALITIS VIRUS

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Stilbenes are polyphenolic secondary metabolites belonging to non-flavonoid phytochemicals. They can be naturally found in plants, such as wines, berries, legumes, or pines. Many beneficial effects on human health were proven for some stilbenes, including resveratrol (RSV) and its derivatives piceid (PIC) and ε-viniferin (VIN, Figure 1), specifically, antiviral, antibacterial, anti-oxidative, and anti-inflammatory effects.

Tick-borne encephalitis virus (TBEV) that belongs to the *Flavivirus* genus, causes more than 10,000 cases of tick-borne encephalitis annually in Eurasia. Despite the existing vaccine against TBEV, this number still rises, due to the low number of vaccinated people. Moreover, treatment is still unknown for the tick-borne encephalitis disease. This is the first study in which the antiviral effect of RSV, PIC, and VIN on TBEV *in vitro* and *in vivo* in laboratory mice were examined.

First of all, the *in vitro* cytotoxicity of RSV, PIC, and VIN was detected at concentrations higher than 12.5, 50, and 125 μg/mL, respectively, using the MTT assay. This method was also used for the detection of inhibition of cytopathic effect in two variants of stilbene treatment, one day before the infection (S→TBEV) or on the same day (S+TBEV). All chosen stilbenes in variants S+TBEV or S→TBEV improved cell survival. The amount of viral RNA, detected by qRT-PCR, rapidly decreased in samples treated with appropriate effective concentrations of stilbenes. Moreover, pretreatment with VIN reduced the TBEV titer which was calculated using plaque titration.

Mice were infected with TBEV and stilbenes were injected in both variants. Furthermore, the effect of interferon-β in combination with stilbenes was examined. Stilbenes prolonged the survival of mice about 2 days on average using low stilbenes dosage. Moreover, pretreatment with interferon-β together with RSV extended mice survival by about 8 days. These results suggest that RSV, PIC, and VIN can be used as the antiviral prophylactic substance against TBEV.

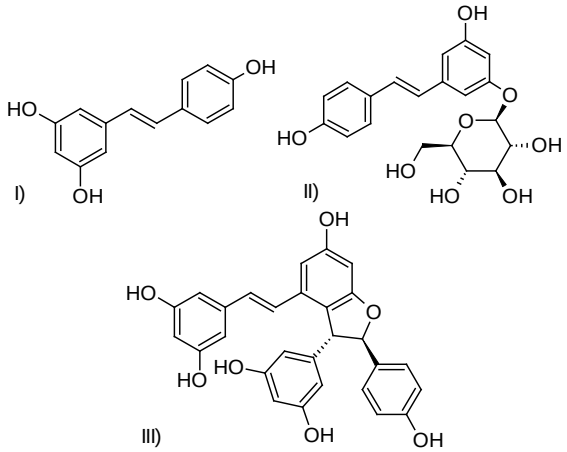

Figure 1: Structures of selected stilbenes. I) resveratrol, II) piceid, III) ε-viniferin

This study was supported by the MEYS of the Czech Republic (INTER-ACTION projects LTARF 18021 and LTAUSA18040), the Technology Agency of the Czech Republic (TG03010027),

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SYNTHESIS OF TETRABENZO[9]HELICENE AND ITS FUNCTIONAL DERIVATIVES

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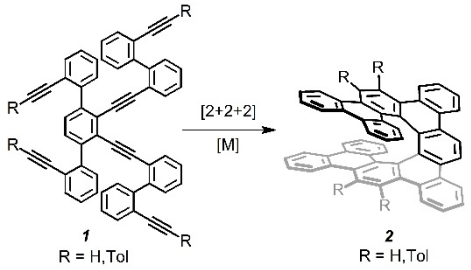
Helicenes are *ortho*-condensed helically-shaped polyaromatic hydrocarbons evincing various distinctive physicochemical properties. For instance, thanks to their π-conjugated scaffold they show intriguing optical and electronic behaviour calling for their possible application in organic electronics (organic light emitting diodes or transistors)[1]. They were also studied with respect to their single molecule conductivity to be applied in a construction of molecular-scale electronic and spintronic devices[2]. Their chiroptical properties aim for nonlinear optical applications, e.g. circularly polarised light sensors[3].

Furthermore, due to their helically chiral shape, helicenes can be used in enantioselective catalysis[1]. Therefore, a great effort has been made to find simple ways of their synthesis.

Herein, we report a straightforward synthetic approach to tetrabenzo[9]helicene ***2*** based on a series of Sonogashira and Suzuki couplings and a final key [2+2+2] nickel- or cobalt-complex mediated cyclotrimerization of hexayne ***1*** (Scheme 1). Subsequently, [9]helicene derivatives ***2*** (R = H, Tol) were resolved into enantiomers by means of chiral HPLC and their chiroptical properties were studied.



Scheme 1. **Double [2+2+2] cyclotrimerization of hexayne *1* to yield the target tetrabenzo[9]helicene *2***

This work was supported by the Czech Science Foundation (Reg. No. 19-10144S) and IOCB CAS (RVO: 61388963).

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FEATURES OF THE CYCLIZATIONS INVOLVING 3- AND 5-AMINOISOXAZOLES WITH PYRUVIC ACID DERIVATIVES

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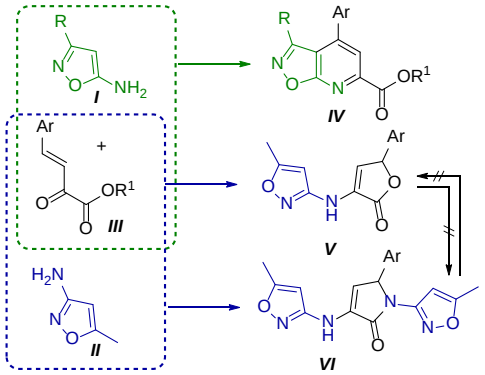
The heterocyclization reactions involving α-aminoazoles and α,β-unsaturated carbonyl compounds or their precursors are one of the best well known routes for the synthesis of new azoloazines[1,2]. The interest in the condensations of 3- and 5-aminoisoxazoles containing no substituent in the 4th position is connected with non-equivalence of the nucleophilic centres that creates ambiguity in the directions of their interactions, in particular, with ylidenes of pyruvic acid, their derivatives or synthetic precursors. It was shown that condensation reactions between 5-aminoisoxazoles ***I*** 4-aryl-2-oxobut-3-enoic acid derivatives ***III*** gave isoxazolo[5,4-*b*]pyridines ***IV***. On the other hand, heterocyclizations of 3-amino-5-methylisoxazole ***II*** led only to the unfused furan-2(5*H*)-ones ***V*** or 1,5-dihydro-2*H*-pyrrol-2-ones ***VI*** (Scheme 1); variation of the synthetic routes and reaction conditions didn't result in the formation of annulated systems. The reasons for the differences in the chemical behaviour of amines ***I***, ***II*** were studied as well.



Scheme 1. **Reactions between derivatives of 4-aryl-2-oxobut-3-enoic acid *III*, 5-aminoisoxazoles *I* or 3-amino-5-methylisoxazole *II***

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LONE PAIR-π AND ANION-π INTERACTIONS AS STABILIZATION FACTORS OF RNA TETRALOOPS

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The RNA hairpin loops are structural elements with biological role in RNA folding. UNCG and GNRA tetraloops are the most common RNA hairpin loops. Molecular dynamics (MD) simulation is a computational technique which uses empirical force field (FF) to efficiently describe time evolution of RNA during e.g. RNA folding. The accuracy of FF depends on the quality of FF parametrization and on the studied molecular system. Quantum chemical techniques (QM) are computationally more demanding compared to FF. However, they are able to study molecules with higher accuracy. Therefore, QM is able to detect inaccuracies in FF. In addition, QM accounts for electronic effects of molecules and therefore allows us to study molecular interactions in depth.

MD simulations of UNCG and GNRA folding are often problematic[1]. There are several specific interactions involved in stabilization of tetraloops which may be challenging for FF to reproduce. One of these interactions occurring in UNCG is sugar-base stacking, which involves close contact between the oxygen atom of the sugar ring and the base, and is defined as 'lone pair-π' interaction. Another interaction occurring in GNRA is phosphate-base stacking, where the close contact between the OP atom and the base has been classified as an 'anion-π' interaction.

In this study, we used highly-accurate QM methods to describe sugar-base ('lone pair-π') and phosphate-base ('anion-π') interactions occurring in UNCG and GNRA tetraloops, respectively. We mainly focused on optimal distances between the oxygen atom and the base, and the corresponding interaction energies in order to estimate the importance of these interactions in the stabilization of the tetraloops. Furthermore, we analysed the nature of sugar-base and phosphate-base stacking. Finally, we evaluated the AMBER force field – the most commonly used RNA force field – in the description of these interactions. Our results show that although stabilization coming from sugar-base and phosphate-base stacking is significant, AMBER force field should be able to describe these interactions sufficiently.

This work was supported by grant 16-13721S and by the project SYMBIT reg. number: CZ.02.1.01/0.0/0.0/15_003/0000477 financed by the ERDF.

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CARBOSILANE BUILDING BLOCKS FOR SYNTHESIS OF DENDRITIC MACROMOLECULES

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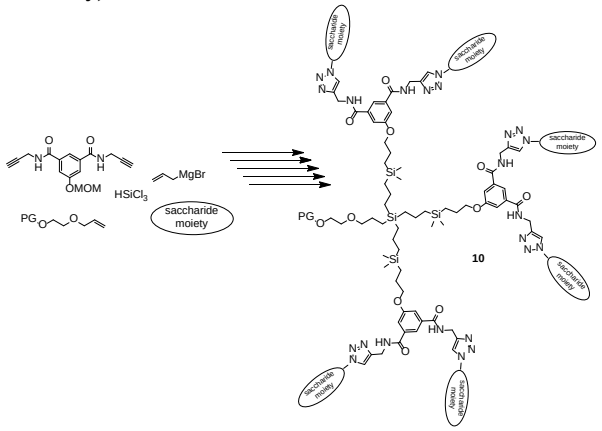
Dendrimers (DDMs) are investigated in various areas including bio- and material science[1]. Recently, a development of defined multifunctional dendritic modules may overcome issues with (regio)selective functionalization of the DDMs as the dendritic modules can be mutually combined or attached to a multivalent core. With such functionalized modules, sophisticated DDMs with novel properties can be designed[2].

Here we present a synthesis and analytical characterization of novel dendritic building blocks. The classic carbosilane structure was integrated with organic multivalent units to fine-tune the properties. Utilizing our previous experience with the synthesis of carbosilane glycodendrimers[3], we decorated the periphery of the dendritic modules with saccharide moieties.

Such readily available functionalized dendritic building blocks and intermediates can be attached to compatible surfaces, multivalent cores, or combined and easily processed to form new, highly specialized dendritic macromolecules with advanced properties.

Our dendritic modules are of great promise to be further investigated, for example, but not limited to, in theranostics (bioapplications) or in solid surface modifications (material chemistry).



This work was supported by Czech Science Foundation (GA CR) (GA15-05903S).

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A NEW FERROCENE *N*-PHOSPHINOAMIDE, ITS CHALCOGENIDES AND PALLADIUM COMPLEXES

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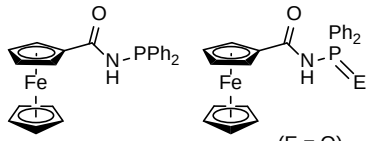
This contribution will report a successful synthesis of a new multidonor ligand, *N*-(diphenyl-phosphino)ferrocene carboxamide (compound ***1***). The preparation of this compound was optimized and the product was fully characterized. The corresponding phosphine oxide (***2***), phosphine sulfide (***3***) and phosphine selenide (***4***) were also isolated and characterized. Coordination abilities of phosphine ***1*** were tested by reactions with palladium(II) precursors.



Scheme 1. **Structures of compounds *1-4***

In particular, three different methods for converting ferrocene carboxamide[1] to phosphine ***1*** were devised. The first method was a base-catalysed substitution of amide proton by diphenylphosphine group catalysed by 4-(dimethylamino)-pyridine[2]. In the other syntheses, the amide was firstly reacted with a bis(trimethylsilyl)amide base (either lithium or sodium salt) and the *in situ* formed salt was treated with chloro-diphenylphosphine to produce compound ***1***. The best yield (53%) was obtained by the latter procedure using sodium bis(trimethylsilyl)amide. All three methods also produced the minor amounts of phosphine oxide ***2***. Phosphine sulfide ***3*** and phosphine selenide ***4*** were prepared by oxidation of ***1*** with elemental sulfur and selenium, respectively.

Phosphine ***1*** reacted with $[PdCl_2(MeCN)_2]$ and $[Pd(L^{NC})Cl_2]_2$ (L^{NC} = 2-[(dimethylamino-κ*N*)methyl]phenyl-κ*C*[1]) to give complexes featuring *P*-monodentate ligand. These compounds were reacted with silver perchlorate or potassium *tert*-butoxide, producing cationic and neutral *P*,*O*-chelating complexes, respectively.

All compounds were characterized by NMR spectroscopy, mass spectrometry, IR spectroscopy and by elemental analysis. The crystal structures of ***2-4*** and all Pd(II) complexes were determined by single-crystal X-ray crystallography.

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DETERMINATION OF AMPHETAMINE-LIKE STIMULANTS IN NEONATAL SAMPLES

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In the last decade there has been a dramatic increase in the availability and abuse of synthetic cathinones – new amphetamine-like stimulants. Interest in those compounds developed in part due to decreased availability and purity of amphetamines and they are often abused for their enhanced stimulant properties and lower price. Cathinones are particularly popular among young adults, including women of childbearing age.

Even though the abuse of amphetamine-like stimulants during pregnancy can have serious negative effects on the child development, they are currently not included in neonatal toxicological screenings. Meconium (first neonatal stool) is the specimen of choice to reveal long term exposure to illegal drugs, however, as it is a highly complex matrix, the sample preparation is a critical step preceding the instrumental analysis.

The main aim of this work was to develop a suitable extraction procedure for meconium samples utilizing the advantages of salting-out assisted liquid-liquid extraction (SALLE) and using only MS compatible ammonium salts. The optimized extraction method included the application of acetonitrile as a suitable solvent in combination with ammonium bicarbonate and ammonium formate as salting-out agents. Moreover, liquid chromatography tandem-mass spectrometry method for the determination of seven synthetic cathinones (mephedrone, methylone, butylone, flephedrone, α-pyrrolidinopentiophenone, methylenedioxypyrovalerone, and naphyrone), together with traditional stimulants (methamphetamine, amphetamine, methylendioxymetham-phetamine) has been developed.

The presented method allowed us to significantly reduce volume of toxic solvents with respect to other extraction

techniques, such as conventional liquid-liquid extraction (LLE), and it was less time-consuming than solid-phase extraction (SPE). Validation of the final analytical method was performed according to the international guidelines and requested criteria were met for all analytes except naphyrone. Finally, our analytical method was successfully applied for the analysis of real meconium samples, found positive for amphetamine, methamphetamine and methylone.

ENZYMATIC ENANTIOSELECTIVE PREPARATION OF CHIRAL SULFOXIDES

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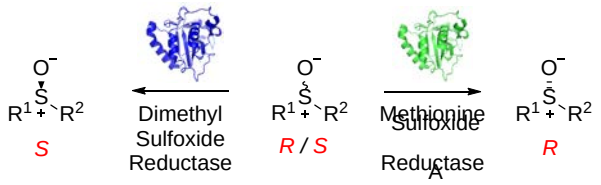
Chiral sulfoxides are important biologicaly active compounds and several of them are used as active component in pharmaceutical drugs, such as omeprazole[1,2]. Furthermore, they have also become popular as chiral ligands, catalysts, and building blocks in asymmetric synthesis[3]. Therefore, the enantioselective preparation of chiral sulfoxides is of high interest[4]. Also, despite the wealth of methods for the asymmetric synthesis of chiral sulfoxides, general protocols that provide sulfoxides with high enantiomeric purity (≥99% *ee*) remain scarce.

We have developed two new enzymatic protocols that are enantiocomplementary and provide both enantiomenrs of chiral sulfoxides with high *ee* values (Scheme 1)[5,6]. Remarkably, a wide substrate scope is observed for both methods, providing an access to enantiopure sulfoxides that are difficult to prepare by other chemical or enzymatic methods.



Scheme 1. **Enanticomplementary preparation of chiral sulfoxides**

This work was supported by Czech Science Foundation (17-25897Y).

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MECHANISMS OF LEUKEMIA CELL ADAPTATION TO TARGETED THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults in the western world. Malignant B lymphocytes are accumulating in bone marrow, blood and lymphatic tissues. The immune niche microenvironment of the CLL cells plays an important role in the disease as major pro-survival pathways are activated here, especially B-cell receptor (BCR) signalling. Recently, a novel efficient therapy has been developed for CLL targeting microenvironmental interactions. Ibrutinib is a small-molecule inhibitor of an essential BCR–associated Bruton tyrosine kinase (BTK). We have previously shown that ibrutinib interferes not only with BCR signalling but also with migration of CLL cells to the immune microenvironment[1] which leads to a gradual regression of the disease during several months. However, ibrutinib therapy does not lead to a cure, and the slow apoptosis of CLL cells exposed to ibrutinib *in vivo* allows the malignant cells to adapt and eventually become resistant to therapy.

To decipher the mechanisms that allow CLL cells to survive during ibrutinib treatment we have performed gene expression profiling (RNAseq, Illumina) of CLL samples obtained from CLL patients pre- and post-treated with ibrutinib (n=22, 11 pairs). This revealed a number of genes that are influenced by BCR-inhibition *in vivo* (N=1305, P-adjusted <0.01). The ibrutinib treatment influenced activity of pathways, such as MAPK signalling pathway ($P=5x10^{-12}$), focal adhesion ($P=5x10^{-8}$), PI3K signalling pathway ($P=1.6x10^{-3}$) or autophagy ($P=1.6x10^{-2}$), and some of this might compensate for the inhibition of BTK. Next, we selected potential candidate molecules that could be responsible for the early adaptation/relative resistance to the drug and we have mechanistically shown *in vitro* that up-regulation and activation of a PI3K-signalling regulatory molecule allows B cells to become relatively resistant to ibrutinib. Currently, this is being further validated with more primary samples from patients.

Altogether, we provide molecular evidence for a novel combinatorial therapeutic strategy with ibrutinib, which might be sufficient to achieve a deep clinical response in CLL without the use of chemo-therapy.

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DEVELOPMENT OF ATROPISOMERIC ORGANOCATALYSTS FOR ASYMMETRIC HENRY REACTION

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Our department has been interested in compounds affecting adrenergic receptors for a long period[1]. These compounds often share the structure derived from aryl-aminoethanol or aryloxy-aminopropanol skeleton. Nevertheless, the amino alcohol-based compounds are not important only in this particular group of therapeutics but occur ubiquitously among bioactive substances of natural or synthetic origin. Many of these molecules have developed an important role in clinical practice and appear also on a list of the best-selling active pharmaceutical ingredients[2].

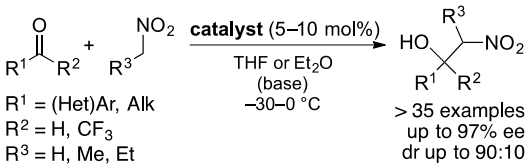
The amino alcohol unit introduces the promising pharmacophoric properties, which can be largely affected by its stereoisomeric configuration. The asymmetric organocatalyzed Henry reaction can represent a powerful method to synthesize the above compounds in a substantially effective way. In the catalytic asymmetric Henry reaction, the chiral bifunctional organocatalysts have proved their effectiveness and selectivity[3], however, many of them also suffer from serious practical disadvantages such as narrow substrate scope, relatively high catalyst loading, long reaction times, necessity of the very low reaction temperatures, and low stereoselective outcome, which provide us enough room for improvements.

Therefore we have concentrated on the identification of the novel axially chiral backbones for the organocatalysts, which represent the tunable C_2-symmetric frameworks that can help to address at least some of the aforementioned issues[4,5]. In order to find the most active and selective molecules, a large screening including 24 chiral scaffolds and 31 catalysts has been performed[6].

Under the thoroughly optimized reaction conditions, the excellent chemical yields (up to 99%), very good to excellent enantioselectivities (up to 97% ee), and remarkable diastereoselectivities (up to 10:1) were observed. It is worth mentioning that for a number of substrates, an exceptionally good catalytic performance was reached with the highest enantiomeric excesses and diastereoselectivities reported for the asymmetric organocatalyzed Henry reaction so far.

The preliminary kinetic and spectroscopic experiments were conducted in order to propose a catalytic cycle of the above transformation and gain better insight into the reaction mechanism. Moreover, interesting temperature and solvent effects were observed.

The robustness of the newly developed catalytic processes was proved in a larger scale total syntheses of enantiopure (*S*)-econazole, the late-stage intermediate of (*R*)-mirabegron, and CF_3-appended (*R*)-halostachine.



Scheme 1. **The target asymmetric Henry transformations**

Financial support was provided by the projects 50/2014/FaF (IGA UVPS Brno), 307/2015/FaF (IGA UVPS Brno), LM2011020 (MEYS CZ) and LQ1601 (MEYS CZ).

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CRISPR/CAS9 EDITED CELL LINES IN DISSECTING ROLES OF INDIVIDUAL DISHEVELLED ISOFORMS

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Wnt signalling pathway is one of the most important signalling cascades involved in embryonal development as well as adult homeostasis and regulates processes such as cell proliferation, differentiation and migration. Therefore, it is not surprising that deregulation of Wnt pathways can result in many diseases including cancers. Dishevelled (DVL) proteins

are key mediators of Wnt signalling pathways and act as a switch between individual branches of Wnt cascades. In human three isoforms of DVL (DVL1, DVL2 and DVL3) are present. All DVL proteins contain three conserved domains – DIX, PDZ and DEP, and their involvement is important for sending signal downstream to individual branches of Wnt signalling.

In our study we took advantage of CRISPR/Cas9 editing technique and created cell line that lack all three DVL isoforms (DVL1/DVL2/DVL3 TKO T-REx HEK293). This unique cell line completely lost ability to transduce Wnt signals and helped us to determine precise role of DVL proteins and processes that are dependent on presence of DVL proteins in context of Wnt signalling pathway. Moreover, with series of rescue experiment we have tested ability of individual DVL variants and their protein domains to restore response to Wnt signals[1].

As a next step, we have created cell lines that contain only one DVL isoform (DVL1-only, DVL2-only and DVL3-only T-REx HEK293). These cell lines serve as the most natural system for studying roles of individual DVL isoforms. Our cell lines enable us to test individual DVL isoforms without interference of other endogenously expressed DVLs and represent more physiological system than studies using gain-of-function experiments with overexpression of DVL. Therefore, these cell lines are useful tool for elucidating functions of individual DVL isoforms.

Our work represents complex but easy and elegant way how to dissect roles of DVL proteins in Wnt signalling pathway using CRISPR/Cas9 technique that can be applied generally in many other studies focusing on different proteins that are present in more than one isoform and their precise role is not known.

This study was supported by the Czech Science Foundation (15-21789S, GA17-16680S), by Masaryk University (MUNI/G/1100/2016) and Marie Curie ITN WntsApp (Grant no.608180). Petra Paclíková is a Brno Ph.D. Talent Scholarship Holder that is funded by the Brno City Municipality.

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DUAL TARGETING OF PROLIFERATIVE PATHWAYS IN BRAF-MUTANT MELANOMA CELLS BY PYRIDINYL IMIDAZOLES COMPOUNDS

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BRAF kinase inhibitors are clinically used to eliminate melanomas bearing the most common BRAF mutation V600E. However, in many cases, the resistance to therapy starts to appear within only a few months[1]. Recent reports indicate that simultaneous targeting different signalling pathways could be a useful strategy to combat cancer and prevent the development of drug resistance[2].

In this study, we identified the effect of pyridinyl imidazole compounds commonly used as p38 kinase inhibitors on the growth of melanoma cells with the BRAF V600E mutation. The drugs substantially decreased the levels of active ERK kinase. Mechanistically, pyridinyl imidazoles directly bound to the mutated BRAF kinase and inhibited the activation of its downstream targets MEK and ERK. Apart from the effect on ERK pathway, pyridinyl imidazoles induced vacuole formation in various cancer cell lines[3]. We found that vacuolization was caused by PIKfyve kinase inhibition, reducing cellular levels of PI(3,5)P2 and disrupting endocytosis in melanoma cells. Accumulation of endocytic vacuoles triggered delocalisation of mTOR and inactivation of mTORC1 signalling, sensitising melanoma cells to nutrient starvation and metabolic stress.

We identified molecular mechanisms, by which pyridinyl imidazole compounds affected two critical cellular pro-proliferative pathways. Moreover, our findings revealed the therapeutic potential of pyridinyl imidazoles towards BRAF-mutated melanoma cells.

This research was supported by Masaryk University (MUNI/A/1087/2018) and the Ministry of Education, Youth and Sports of the Czech Republic: the National Program for Sustainability II project Translational Medicine (LQ1605).

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GLYCAN ANALYSIS OF SERUM GLYCOPROTEINS OF PATIENTS ON PERITONEAL DIALYSIS BY LECTIN-BASED MICROARRAY

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Analysis of glycosylation status of glycoconjugates, mainly glycoproteins, may reveal the trend in changes of glycan composition which occur due to disease, aging, life style or other reason. Increasing number of studies suggest that monitoring of changes in protein glycosylation has huge diagnostic potential and information on glycosylation status can significantly increase the informative value of glycoprotein biomarkers and thus improve diagnostics, follow-up and treatment of many diseases[1]. We focused on the screening of the glycan composition of two serum glycoproteins, transferrin and fibrinogen, isolated from samples of patients on peritoneal dialysis (PD). Association of serum protein N-glycosylation with peritoneal membrane transport in PD patients was reported recently[2]. We used our lectin-based protein microarray platform enabling high-throughput glycoprofiling[3]. Whole sera (from 52 patients on PD and 38 controls) and isolated glycoproteins samples were spotted into arrays on microarray slide. The incubation was carried out with a panel of 16 biotinylated lectins with different gkycan specificity. The detection was performed after incubation with fluorescent conjugate of streptavidin using microarray scanner. The differences in glycan composition can be potential candidates as biomarkers for PD and related complications. The described method is very effective for rapid detecting glycosylation changes for a large number of samples making it useful in biomarker research and diagnostics. The intended next step is MS analysis to identify found changes in glycan structures.

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ARGON PLASMA TREATMENT INCREASES CYTOCOMPATILITY OF FLUORINATED ETHYLENE PROPYLENE

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The use of polymers in life sciences and biomedicine is often limited by their surface properties, which require tailoring to suit a particular application. Treatment with gaseous plasma comprises a fast and reproducible option of polymeric surface modification. It alters, e.g., the roughness, hydrophobicity and composition of only the surface layer and does not affect the bulk properties of a material. Fluorinated ethylene propylene (FEP) was treated with argon plasma of 3 and 8 W powers for 0-240 s and the cytocompatibility of the resulting matrices was analysed[1] using various microscopy and immunochemical methods on three model cell lines. The proliferation and morphology of cells on prepared FEP matrices was evaluated using human immortalized keratinocytes (HaCaT) and primary dermal fibroblasts (HDF) and both parameters were significantly improved in cells on modified FEP matrices compared to pristine FEP. Moreover, cell adhesion was assessed in HaCaT and HDF cells by measuring the length of focal adhesions (FAs) as well as their number per cell and percentage of cells with FAs. Cell adhesion of these cells was also significantly improved in cells grown on modified FEP matrices compared to pristine FEP. Furthermore, adipose-derived mesenchymal stem cells modified with human telomerase ASC52telo were used to evaluate the osteodifferentiation potential of the Ar-plasma modified FEP by means of Alizarin Red S staining and flow cytometry. Together, the performed experiments testify that Ar plasma modification of FEP significantly improved its cytocompatibility with respect to multiple cell models and indicate the suitability of the prepared surfaces for cell culture application.

This work was supported the Ministry of Health of the CR under project 15-33018A, specific university research MSMT No 20- SVV/2018 and projects OPPC CZ.2.16/3.1.00/24503; LO1601 and LO1304.

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ATOMISTIC DETAILS ON DYNAMICS OF HISTONE H3 RECOGNITION BY HP1 CSD

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Human heterochromatin protein 1 (HP1) is the main factor responsible for heterochromatin formation and maintenance. One of its domains, chromo-shadow domain (CSD), which is followed by a short, disordered C-terminal extension (CTE), is responsible for HP1 homodimerization. The dimer then acts as a hub, transiently binding variety of

target proteins (TPs) including methyltransferases, chromosome remodelling proteins or proteins involved in DNA replication and repair[1,2].

In this work, we focus on interactions of HP1 CSD dimer with one of its targets, histone H3 N-terminal tail. The crystal structure of the complex was resolved[3], however CTEs were resolved poorly or completely missing. Furthermore, a static, ensemble-averaged crystal structure cannot reveal the role of flexibility and structural dynamics on molecular recognition mechanisms. Thus, we study the HP1-H3 complex using *in silico* molecular dynamics (MD) simulations[4].

Our data suggests an active role of HP1 Trp174 sidechain dynamics in distinguishing residues of TPs bound in HP1 hydrophobic pockets. MD simulations with modelled CTE residues also reveal a competition between negatively charged groups of CTE residues and solvent anions binding near hydrophobic pockets. Moreover, post-translationally modified H3 phosphoTyr41 can bind to the same site. The presence of the anion site, as well as the ability of CTE to bind here, is dependent on the TP sequence, which may thus help fine-tune the recognition of different peptides. Further, our study gives insights into the ability of several contemporary pair-additive force fields to describe similar interfaces and can thus be also used as a simulation benchmark for HP1 CSD recognition.

This research was supported by the Czech Science Foundation, grant number 18-07384S.

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PROMOTER METHYLATION OF ROR1 LIGAND *WNT5A* ASSOCIATES WITH ITS EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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ROR1 (receptor tyrosine kinase-like orphan receptor-1) is up-regulated in chronic lymphocytic leukemia (CLL) and serves as a unique marker of CLL cells since it does not occur on surfaces of other peripheral blood cells (Kotaskova BJH 2016). Ligand WNT5A, which binds ROR1 and activates non-canonical WNT/Planar Cell Polarity (PCP) pathway, has very wide expression range in CLL cells (Janovska CCR 2015). Patients with positive *WNT5A* expression have significantly shorter treatment-free survival (TFS) compared to WNT5A-negative patients. The most impressive difference in TFS is within otherwise prognostically favorable IGHV-mutated subgroup (M-CLL) (P=0.0046). Within M-CLL, *WNT5A*-negative cases were significantly more frequent compared to IGHV-unmutated subgroup (U-CLL) (85% vs 50%). It has been previously published that in CLL, number of genes (*NOTCH1*, *LPL*, *DNMT3B*, etc.) has altered methylation compared to its healthy counterparts and methylation profile differs between M-CLL and U-CLL (Cahill Leukemia 2013). Altered methylation of *WNT5A* promoter was detected in some malignancies including acute lymphoblastic leukemia but has not been studied in CLL (Roman-Gomez EJC 2007).

Using bisulphite sequencing, we analyzed three CpG islands of the *WNT5A* promoter in 39 CLL patients with known *WNT5A* expression evenly divided into four groups according to IGHV status and *WNT5A* level. We revealed that the methylation level of three CpG sites within the first CpG island significantly differed between M-CLL and U-CLL (P=0.0282). At the same time, methylation differed between *WNT5A*-positive and negative patients (P=0.0036). Within M-CLL, *WNT5A* expression correlated with *WNT5A* methylation level. In *WNT5A*-negative U-CLL patients, the CpG sites were hypermethylated, however, methylation level within U-CLL *WNT5A*-positive subset did not correlate with *WNT5A* expression.

Further, we treated two B-cell lymphoblastic cell lines MINO (strongly positive *WNT5A* expression) and MAVER (*WNT5A*-negative) with demethylation agent 5-aza-2-deoxycytidine in concentrations 1, 2, 5 μM for 72 hours. The demethylation caused increase in *WNT5A* expression and *WNT5A* level rose with each day of treatment in both cell lines.

To conclude, *WNT5A* gene expression, positive in aggressive CLL cases, correlates significantly with methylation level of three CpG sites within its promoter. Global demethylation with 5-aza-2dC caused up-regulation of *WNT5A* expression in both MINO and MAVER cell lines.

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ROLE OF WNT SIGNALING IN OVARIAN CANCER

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High grade serous carcinoma of the ovary, fallopian tube and peritoneum (HGSC), the most frequent form of ovarian cancer, is the deadliest gynaecologic disease with five-year survival rate below 30 %. HGSC is characterized by early and rapid development of metastases by mainly unknown mechanisms. Deregulated Wnt signalling is a hallmark of several cancers, however, whether it underlies also progression of HGSC remains poorly studied. We used datamining in cancer databases and functional *in vitro* assays employing Kuramochi cell line (novel appropriate model for HGSC) as well as our collection of primary patient samples to investigate role of Wnt signalling in progression of HGSC. We report that Wnt/planar cell polarity pathway, but not Wnt/β-catenin pathway, proved essential for induction of stemness and migration/invasion potential of HGSC cells. Consistently, chemical inhibition as well as genetic ablation of the crucial Wnt pathway components blocked the effect of the prototypic Wnt/PCP ligand Wnt5a. Additionally, Wnt/PCP pathway components were differentially expressed between healthy and tumour tissue as well as between the primary tumour and metastases. Together, these results implicate the role of Wnt/PCP signalling in ovarian cancerogenesis including possible therapeutic potential.

This work was funded by Czech Science Foundation, Grant No. 17-11776Y.

SYNTHETIC CELL CULTURE ENVIRONMENT FOR PROPAGATION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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A diverse array of environmental factors and interaction contribute to the overall control of human pluripotent stem cell (hPSCs) activity mainly self-renew, proliferation and differentiation. This stem cell niche interactions are represented by numerous extracellular matrix components, cell junctions and soluble factors. To fulfil the potential of hPSCs as a source for regenerative medicine it is necessary to understand and characterize mechanisms of this interactions. Obtained information can help overcome actual drawbacks in hPSCs propagation in *in vitro* conditions.

In vitro cultivation of hPSCs is highly dependent on complexity of culture surfaces, which are usually treated by natural components such a supportive cell feeder layer or by surrogate extracellular matrix (e.g. Matrigel). Main disadvantage of such a culture systems is high price, low batch to batch stability and also poor biochemical characterization, which can together lead to results variability. One option how to reduce this disadvantages is using of well-defined synthetic surfaces, which provide control over surface biochemistry by implementing of short adhesive motives in precise surface concentration.

We developed the unique synthetic system combining covalently bound short amino acid sequence onto a protein-repulsive brush made of polyHEMA chains, providing specific interaction of hPSCs with immobilized adhesive motif. We analysed several ligands and concentrations. We found peptide motif RGDT successful for hPSC adhesion and long-term cultivation. Furthermore we have performed qPCR array of adhesome genes to evaluate the receptors, which take place in adhesion and propagation of hPSCs. The results were completed with WB analysis. It is very interesting, that hPSCs cultivated on synthetic surface based on 20 amino acid motif express similar proliferation rate, level of pluripotency and profile of adhesive proteins as hPSC cultivated on complex natural matrices Matrigel. Our results confirm possible application of this synthetic system for hPSC xeno-free cultivation and differentiation.

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THE ROLE OF RNF43/ZNRF3 IN NON-CANONICAL WNT SIGNALING AND ITS IMPACT ON HUMAN MELANOMA

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Proteins RNF43 and related ZNRF3 are transmembrane E3 ubiquitin ligases with an ability to inhibit canonical Wnt signalling. Their activity leads to internalization and degradation of Frizzleds, Wnt ligand receptors. Loss of function mutations of RNF43 are frequent in various cancers and they are correlated with hyper-activation of beta-catenin dependent transcription, which supports proliferation and survival. Thus, RNF43 and ZNRF3 are tumor suppressors.

In our work we have focused on RNF43 in the Wnt/Planar Cell Polarity (PCP) pathway by exploring RNF43 interactome and impact on key signalling events. Unbiased proteomic method for the detection of transient protein-protein interactions called BioID allowed us to identify that RNF43 interacts with the components of PCP receptor complex - namely Ror1, Ror2, Vangl1, Vangl2, Dvl1, Dvl2, Dvl3, Casein kinase 1ε and Casein kinase 1δ. Overexpression of RNF43 in an enzymatic dependent manner lead to desensitization of cells to Wnt5a, accompanied the by decreased level of surface and total Ror1, Vangl2 and Dvl2 and Dvl3 dephosphorylation and proteasomal degradation.

We further show that RNF43 regulates Wnt/PCP pathway in melanoma cells. Melanoma is the deadliest skin cancer that is driven by Wnt5a. Although RNF43 is not frequently mutated in this cancer, its expression dramatically decreases with cancer progression. When we overexpressed RNF43 in A375 metastatic melanoma cell line, it suppressed Wnt5a signalling and blocked migration, invasion and invadopodia formation. Therefore, we propose that RNF43 downregulation allows efficient Wnt5a signalling and is beneficial for melanoma progression.

EFFECT OF SECRETORY PATHWAY INHIBITORS ON THE ACTIVE SECRETION OF NAMPT

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NAMPT (nicotinamide phosphoribosyltransferase) is a protein which acts intracellularly as an essential enzyme involved in NAD synthesis. In addition it has also been detected in the extracellular environment where it affects the immune system and cellular metabolism, including glucose metabolism and insulin sensitivity. However, its mechanism of secretion has not been revealed yet. Our previous work has shown that NAMPT is actively secreted by cells of the immune system. Previous studies have excluded secretion by a classical secretory pathway and point to some of the non-classical secretory pathways. However, the exact mechanism of the secretion remains unknown. The aim of our work was to monitor the effect of the secretory pathway inhibitors on the secretion of NAMPT.

U937 monocytes and macrophages were used as the model cell line. The differentiation of U937 monocytes to macrophages lasted for 4 days. We used the phorbol 12-myristate 13-acetate as activator for the initiation of the differentiation. We also prepared monocyte cells that produce NAMPT in a fusion with green fluorescence protein (GFP-NAMPT), which can be observed by fluorescence microscopy. The amounts of NAMPT in the culture medium and in cell lysate were determined by the western blot method. NAMPT amount was related to the amount of GAPDH cytosolic protein to eliminate NAMPT passive release due to cell death.

The classical secretory pathway inhibitors, Brefeldin A and Monensin, caused a significant decrease in NAMPT secretion. We also tested L-asparagine, a non-classical secretory pathway inhibitor on the autophagy principle. L-asparagine also significantly reduced the secretion of NAMPT. We used fluorescence microscopy to monitore GFP-NAMPT intracellular localization and transport in macrophages U937. Non-affected cells had GFP-NAMPT located near the cytoplasmic membrane. The use of Brefeldin A or Monensin halted the transport of GFP-NAMPT to the U937 macrophage membrane. The accumulation of GFP-NAMPT near the nucleus was also observed.

Contrary to literature, our results suggest that NAMPT secretion may be linked to classical secretion pathway.

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LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION BASED ON MICROFLUIDIC CHIP

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In modern liquid chromatography, columns of smaller diameters and low flows of mobile phases are increasingly often used. The only option for hyphenation of nano- and micro-HPLC with mass spectrometry is the use of ion sources based on electrospray[1]. The disadvantage of these ion sources is the low ionization efficiency of non-polar compounds. Atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are suitable techniques for ionizing low polar and non-polar substances[2]. Commercial APCI and APPI sources operate at high flow rates compatible with conventional high-performance liquid chromatography (HPLC). However, lipid analysis is often limited by a small amount of sample, which requires low flow rate separations such as capillary or micro-HPLC. Therefore, APCI and APPI suitable for microliter-per-minute flow rates need to be developed and applied to lipids.

This present work deals with hyphenation of liquid chromatography with mass spectrometric detection based on microfluidic chip. Firstly, a miniaturized ion source for

atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) was constructed. The main component of this source was a glass microfluidic chip. Geometry and the working conditions of the chip were optimized. Since both ion sources work under the same conditions, possible advantages resulting from APCI/APPI combination were investigated. Furthermore, the performance characteristics of the sources were evaluated, and compared to the conventional high flow-rate sources. The best performing source, APCI, was then hyphenated with HPLC using low flow-rate. A method for separation of fatty acids methyl esters using Supelco 37 standard was developed. The separation conditions were as follows: C18 reversed stationary phase, and acetonitrile containing 0.1 % formic acids was used as the mobile phase. A temperature gradient was used in order to enhance the speed of the separation. The method was used for separation of fatty acids methyl esters found in transesterified samples of jojoba oil and blackcurrant oil.

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DEVELOPMENT OF PURINE NUCLEOSIDE PHOSPHORYLASE INHIBITORS AS POTENTIAL ANTICANCER THERAPEUTICS

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Purine nucleoside phosphorylase (PNP)[1] is an important enzyme involved in the purine salvage metabolism in most organisms. It catalyzes the reversible phosphorolysis of purine ribonucleosides and 2'-deoxy ribonucleosides to the free base, and ribose 1-phosphate or 2'-deoxyribose 1-phosphate, respectively. The absence of PNP in humans leads to severe T-Cell immune deficiency. Therefore, PNP inhibition might be used in the treatment of T-cell proliferative diseases, such as T-cell leukemia or T-cell lymphoma, or to suppress the T-cell response in T-cell mediated autoimmune diseases, such as rheumatoid arthritis, psoriasis, or lupus. The scope of the applications might be even extended to treatment of bacterial and protozoan infections as well[5].

Although PNP is a well-known medicinal target, which has been studied for a therapeutic utilization since the 80's, the first compound (Forodesine[2]) was delivered to the market in 2017 (in Japan only). Previously reported inhibitors[3,4] failed in preclinical and clinical trials because of poor pharmacodynamic or pharmacokinetic properties.

Novel inhibitors were designed to address these problems. We have developed novel synthetic tools, which allowed us to prepare highly active inhibitors of various PNP enzymes (human, malarial, mycobacterial).

Synthesis and structure-activity relationship study of single-digit nanomolar PNP inhibitors will be presented. These inhibitors exhibit better activities and pharmacokinetic properties than previously reported compounds. Selected compounds were evaluated *in vitro* and *in vivo* to confirm their potential as therapeutics in several medicinal areas.

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BODIPY: MORE THAN JUST A FLUORESCENT DYE

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4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene derivatives called BODIPYs are strongly absorbing small molecules that emit sharp fluorescence peaks with high quantum yields. They are widely used as fluorescent labels as they are relatively insensitive to the polarity and pH of their environment and are reasonably stable under physiological conditions[1]. We have utilized their excellent absorption properties and easy synthetic accessibility in the design of novel photoactivatable compounds (Fig. 1). Installation of heavy atoms (halogens, chalcogens) in their structure dramatically increased the triplet yields and resulted in photostable triplet sensitizers[2,3].

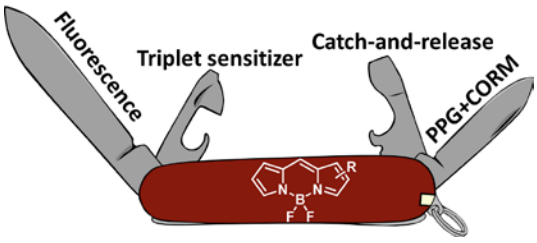

Fig. 1. **BODIPY: a multipurpose tool**

The *meso*-substituted BODIPY carboxylic acids were found to photodecarbonylate and were used as CO-releasing molecules (CORM) activated by visible light and successfully tested *in vivo*[4,5]. The structure of *meso*-methyl-substituted BODIPYs was tuned to lead to photoremovable protecting groups (PPG). Quantitative quantum yields of deprotection together with high molar absorption coefficients in the visible region (the highest known absorption cross-section) make it the most efficient photoreaction of an organic molecule including a covalent bond cleavage[6]. The optimized photoreactivity was applied in the catch-and-release system[7] for control of mRNA activity directly influencing the recovery of myocardial cells after infarction.

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NON-THERMAL PLASMA INDUCES APOPTOTIC CELL DEATH IN P53 MUTATED HEPATOCELLULAR CARCINOMA CELLS

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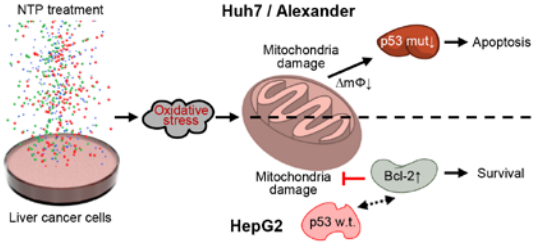
P53 protein, well-known tumour suppressor, may directly trigger apoptosis in response to stress stimuli *via* interplay with Bcl-2 family members[1]. However, p53 mutations in human tumours enhance the resistance to chemotherapeutics commonly used in clinic[2]. Thus, the inhibition of mutant p53 protein expression seems to be promising therapeutic strategy to treat human cancers[3]. Recently, non-thermal plasma (NTP), an ionized gas, containing chemically active species, has been shown as potential tool in anticancer therapy[4]. Previously, many studies have reported that NTP might be involved in regulation of different cellular processes, including cell death in tumour cells by reactive oxygen species (ROS) production[5-7]. However, the molecular mechanisms by which these physicochemical signals of plasma ROS are transmitted to cell death events remain elusive. In our study, we examined the influence of air-NTP on human hepatocellular carcinoma (Huh7 and Alexander cells) and blastoma cell lines (HepG2). Surprisingly, plasma treatment demonstrated higher anti-proliferative effect against Huh7 relative to HepG2. Our results revealed that excessive ROS generation and accumulation led to dysfunction of mitochondria and to apoptosis in Huh7. Furthermore, NTP treatment induced p53 downregulation in Huh7 (mutated p53 form) accompanied by downregulation of STAT1 and pSTAT1, supporting the activation of apoptotic cell death. On the other hand, HepG2 (wild type p53) showed higher resistance in response to apoptosis *via* NTP-mediated oxidative stress, due to overexpression of Bcl-2 protein (anti-apoptotic protein). Importantly, by blocking Bcl-2 activity using specific pharmacological inhibitor ABT-737 we sensitized HepG2 cells to NTP-induced cell death. In summary, our findings offer novel insight into plasma mediated cellular responses and proposed NTP as potential physicochemical cue for targeting of p-53 mutated cancer cells.



Scheme 1. **Tentative scheme of molecular mechanisms of Huh7 sensitivity and HepG2 resistivity to NTP induced cell death. ΔmΦ – mitochondrial membrane potential**

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LOCALIZATION OF DOUBLE AND TRIPLE BONDS IN LIPIDS USING ALDRITHIOL-2 DERIVATIZATION AND ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Lipids are essential components of living organisms and knowledge of their structure is crucial for understanding of many biochemical processes. Positions of double bonds in lipid molecules have significant implications for their chemical, biochemical and biophysical roles[1]. One of the methods for localization of double and triple bonds in lipids is their derivatization followed by mass spectrometry analysis. Most derivatization techniques have been developed for electron ionization MS but only few of them exist for electrospray ionization (ESI)[2].

We present aldrithiol-2 as a derivatization reagent that introduces permanently charged group to the lipid molecules. As a result, these derivatives are sensitively detected in ESI-MS in contrast to neutral underivatized lipids. In addition, fragmentation of aldrithiol-2 derivatives provides the diagnostic fragments which reveal the original position of double bond(s). Various lipids with double bonds were successfully derivatized, including hydrocarbons, fatty alcohols, fatty acids and their methyl esters and triacylglycerols. Positions of the double bonds were clearly established from MS^2 in all cases with the exception of triacylglycerols, where additional fragmentation step (MS^3) was required.

Recently, compounds with triple bonds were successfully derivatized and their fragmentation spectra contained diagnostic peaks as well. Thus, derivatization with aldrithiol-2 can also be also useful in structure elucidation of unusual lipids containing triple bonds.

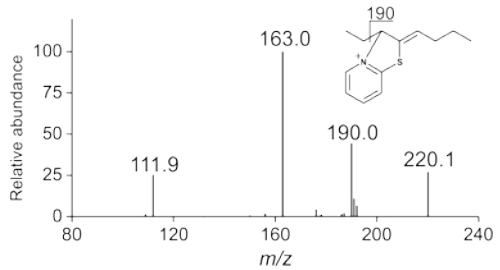

Fig. 1. **ESI-MS fragmentation spectrum of 4-octyne and aldrithiol-2 derivative with the supposed structure**

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FREE FLOATING SECTIONS - A NEW PROCEDURE OF SAMPLE PREPARATION FOR THE STUDY OF NEURODEGENERATIVE DISORDERS BY MASS SPECTROMETRY IMAGING

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MALDI mass spectrometry imaging (MSI) is a modern analytical technique capable to monitor a spatial distribution of compounds within the target tissues[1]. Collection and storage are important steps in the sample preparation. The recommended and most widely used preparation procedure for MSI is freezing samples in isopentane and storage at temperatures below -70°C (ref.[2]). However, the most common method for preserving samples in clinical practice, e.g. for immunohistochemistry, is a fixation in a solution of paraformaldehyde[3]. The use of the latter procedure would simplify tissue processing for multiple methods (including MSI) and allow for better correlation of MSI data with immunohistochemical and histological analysis.

In the present work, we optimized and evaluated paraformaldehyde fixed free floating sections for the analysis of lipids in the mouse brain and used them for the study of lipid changes in APP/PS1 mouse model of neurodegeneration.

From the free floating sections we obtained lipid images without interferences or delocalization and we demonstrated that free floating sections can be used for MSI of lipids. In the APP/PS1 mouse model, we observed colocalization of senile plaques with a changed distribution of various phospholipids by using MSI and immunohistochemistry in concert. Higher concentration of gangliosides (GM2 36:1, GM3 36:1 (Fig. 1)) and phosphatidylinositols (PI 38:4, 36:4) was observed in places, where the accumulation of the plaques occured.

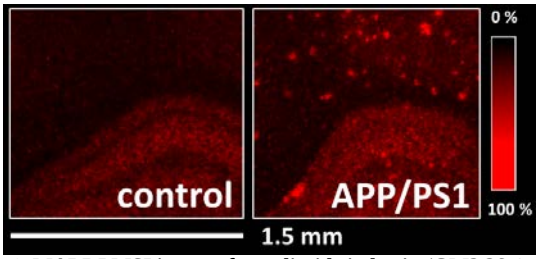

Fig. 1. **MALDI MSI image of ganglioside in brain (GM3 36:1, m/z 1179.7) at spatial resolution 15 µm**

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SEROLOGICAL ANALYSIS OF INFLAMMATORY MOLECULES IN MALIGNANT MELANOMA

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Malignant melanoma is type of a life-threatening skin cancer that develops from the melanocytes, the pigment producing cells. As other solid tumours malignant melanoma represents complicated network of tumour cells and many different cell types like cancer-associated fibroblasts, leukocytes, pericytes or endothelial cells, which together with extracellular matrix form tumour microenvironment[1].

The tumour microenvironment is being increasingly recognised as a key factor in cancer biology with impact on proliferation, invasion, angiogenesis, metastatic spread and also acquired therapy resistance[2]. Multiple bioactive molecules playing cooperative roles are promoting chronic inflammatory milieu in the tumours and this specific inflammatory setting is evident in the affected tissue.

Here we exploit the complex inflammatory response in sera of patients with malignant melanoma at distinct clinical stages of tumour using LUMINEX approach with detection of 31 proteins. We observed these targets in immunohisto-chemical profiles of primary tumours of the same patients. Further, we analysed these proteins in malignant melanoma cell lines and the principal cell population of melanoma microenvironment – cancer-associated fibroblasts.

This comparative analysis showed specific pattern of serum proteins (HGF, VEGF-A, G-CSF, IL-6, IL-8, IL-1RA and IFNalfa), which significantly differed in patients subsets from healthy controls. These results provided insight into behavior of malignant melanoma cells and its microenvironment and highlight potentially relevant targets for therapy improvement.

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GROWING THE MAMMARY TREE: A TALE OF FIBROBLAST GROWTH FACTOR 2

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The process of mammary epithelial tree formation, mammary branching morphogenesis, is the developmental counterpart of breast cancer invasive growth. The critical line between the normal and cancerous growth is drawn by the interplay of signalling networks. In this study, we investigated the role of Fibroblast growth factor (FGF) signalling in balancing between growing the mammary tree and forming a disorganized mass of cell, in other words, between the normal and cancerous state.

We employed mammary organoid culture combined with time-lapse imaging, a cutting-edge method to study three-dimensional epithelial morphogenesis *in vitro*. We used hyperstable FGF2 molecules to induce FGF2 hypersignalling and we found out that FGF hypersignalling globally disrupts mammary epithelial morphogenesis by changing the pattern formation (as defined by organoid size, branch length and frequency) and inducing hyperplastic phenotype. FGF hypersignalling also leads to increased proliferation, changed cell type distribution, ultrastructural changes and affects mammary stem cell population.

To provide a deep mechanistic insight into the FGF signalling effects, we tested the involvement of FGF receptor downstream signalling pathways (MAPK, AKT, STAT, PLC, SRC) by pharmacological inhibition. We identified MAPK signalling as the most important one for epithelial pattern formation. In hyperplastic organoids, MAPK signalling was increased and displayed altered spatial distribution of MAPK responsive cell clusters.

In sum, we propose a critical role for the FGF2-MAPK signalling axis in defining the balance between the normal branching morphogenesis and deregulated cancer-like growth.

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NOVEL LIGANDS FOR FIBROBLAST ACTIVATION PROTEIN TARGETING

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Fibroblast activation protein (FAP) is a serine protease

expressed in more than 90 % epithelial tumors. Because of its minimal or absent expression in healthy adult tissues, FAP could be a perfect platform for selective targeting of diagnostic and therapeutic tools[1].

The affinity of the best known binders is still not sufficient for FAP-targeting technologies. Thus we proposed a novel peptidomimetic FAP inhibitors with α-ketoamide warhead on Gly-Pro pattern inspired by natural FAP substrates. Since *N*-terminal part of FAP inhibitors has been widely optimized[2], we conserved the 4-quinolinoyl moiety (Fig. 1).

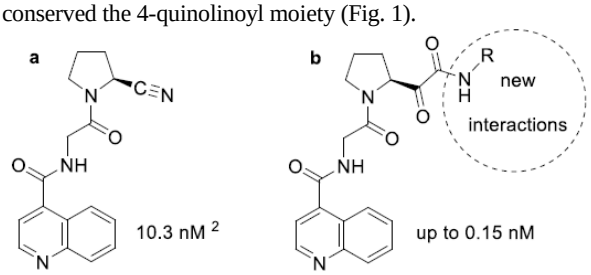

Fig. 1. **Structures of FAP inhibitors and their IC_{50} values: a) previously published FAP inhibitor bearing nitrile warhead, b) novel class of FAP inhibitors bearing α-ketoamide warhead. Black dashed line shows new space for structure optimization.**

A series of α-ketoamide compounds was synthesized *via* PADAM approach (Passerini reaction – Amine Deprotection – Acyl Migration) followed by peptide coupling chemistry and subsequent oxidation.

Resulting SAR study showed an excellent inhibition potency of several α-ketoamide compounds (IC_{50} up to 0.15 nM) that are supposed to be used for FAP targeting in diagnostic applications.

This work was supported by Ministry of Health (AZV 15-31379A) and Charles University (GA UK 1312218).

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NOVEL LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF SELECTED CORTICOSTEROIDS IN HUMAN PLASMA AND ITS DEVELOPMENT AND USE FOR ADRENAL FUNCTION TESTING

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Corticosteroids are a class of steroid hormones that are produced in the adrenal cortex. They play crucial role in regulation of various physiological processes such as maintenance of whole-body homeostasis, stress response, immune response and regulation of carbohydrate metabolism. The disorders associated with their synthesis may lead to fatal consequences. High dose synacthen test (HDST, 250 μg Synacthen test) is used in daily routine medical praxis for diagnostics of adrenal insufficiency and congenital adrenal hyperplasia.

While analytical determination of estrogens (by derivatization of phenyl group) and androgens (by derivatization of carbonyl group) is commonly used, determination of some corticosteroids (11-deoxycortisol and 11-deoxycorticosterone) is still quite challenging as the methods of their analysis are financially and time-consuming and often burdened with inaccuracies. However, accurate determination of these cortisol and corticosterone precursors may help in the diagnosis of adrenal corticosteroid production disorders.

A sensitive and specific LC-MS/MS method for determination of selected corticosteroids has been developed and validated. The method uses reactivity of primary hydroxyl functional group and allows the simultaneous determination of selected corticosteroids. Different extraction agents, derivatizing agents and reaction conditions were tried out. Also, the chromatographic and the mass spectrometer conditions were optimized.

Human plasma (1 mL) was extracted with diethylether and derivatized with fusaric acid. The reaction mixture (fusaric acid, 4-dimethylaminopyridine, 2-methyl-6-nitrobenzoic anhydride, triethylamine, tetrahydrofuran) was incubated for 1 hour at 60 °C. The sample was dissolved in methanol with addition of 5 mM ammonium formate in water, separated by liquid chromatography and subsequently analysed using mass spectrometry. Deuterated internal standards were employed for the correction of losses during sample preparation.

The method was used for determination of six corticosteroids and validated for cortisone, 11-deoxycorticosterone and corticosterone. Plasma levels of these three corticosteroids were measured in a group of 13 healthy men that underwent HDST. The method will be helpful in the diagnostics of various pathophysiological conditions, especially in diagnosis of adrenal diseases.

This work was supported by the projects MH CZ - DRO (Institute of Endocrinology - EÚ, 00023761), MH CR 17-30528 A from the Czech Health Research Council and MŠMT LO1304.

NOVEL APPROACHES FOR THE SYNTHESIS OF 2-AMINOBENZOXAZOLES AND THEIR *N*-SUBSTITUTED DERIVATIVES

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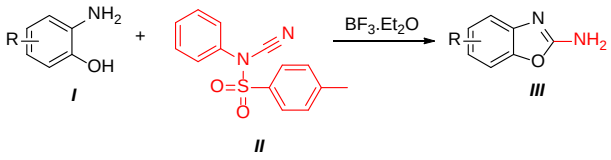
2-Aminobenzoxazoles and their *N*-substituted derivatives represent an extremely important family of compounds[1]. Many of them exhibit interesting activities that can be used in drug development, fundamental biology and material science[2,3].

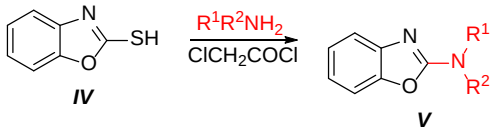
Our first synthetic strategy involves a reaction between various *o*-aminophenols ***I*** and *N*-cyano-*N*-phenyl-*p*-toluenesulfonamide (NCTS) ***II*** as a nonhazardous electrophilic cyanating agent (Scheme 1). The additional strong points are operational simplicity and wide substrate scope.



I ***II*** ***III***

Scheme 1. **Synthesis of 2-aminobenzoxazoles**

The second synthetic approach uses the Smiles rearrangement to achieve *N*-substituted derivatives (Scheme 2). Benzoxazole-2-thiol ***IV*** as an economic starting material reacts with different amines using chloroacetyl chloride as an activating agent. Wide substrate scope and short reaction times stand for the major benefits of our methodology.



IV ***V***

Scheme 2. **Synthesis of *N*-substituted 2-aminobenzoxazoles**

As a result, two novel synthetic approaches to various 2-aminobenzoxazoles and their *N*-substituted analogues, important building blocks in organic and medicinal chemistry, have been developed.

This work was supported by the Ministry of Education, Youth and Sport of the Czech Republic (projects IGA_PrF_2018_029, IGA_LF_2018_032) and the National Program of Sustainability (project LO1304).

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HYBRID ORGANIC-INORGANIC SILICA MATERIALS AND NANOFIBERS: APPLICATION IN ENANTIOSELECTIVE CATALYSIS

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Periodic mesoporous organosilicas (PMOs) are chemically and mechanically robust materials based on silsesquioxanes, which are obtained by the sol-gel process from organo-bridged alkoxysilanes (Fig. 1). Due to a tunable size of mesopores and diversity of covalently bound organic fragments in the siloxane framework, PMOs have theoretically unlimited number of applications such as catalysis, drug delivery, gas adsorption or electronics[1,2]. Here we present syntheses of various novel bis(triethoxysilanes) and subsequent preparation of corresponding hybrid organosilica materials. Alternatively, we used the bis(triethoxysilyl) precursors in the preparation of nanofibers by the electrospinning method. To our best knowledge, it is a first report of electrospun nanofibers made from organo-bridged alkoxysilyl precursors with such a high content of the organic fragments. Finally, we tested the materials and nanofibers in catalysis of e.g. nitroaldol reactions to validate their catalytic activity.

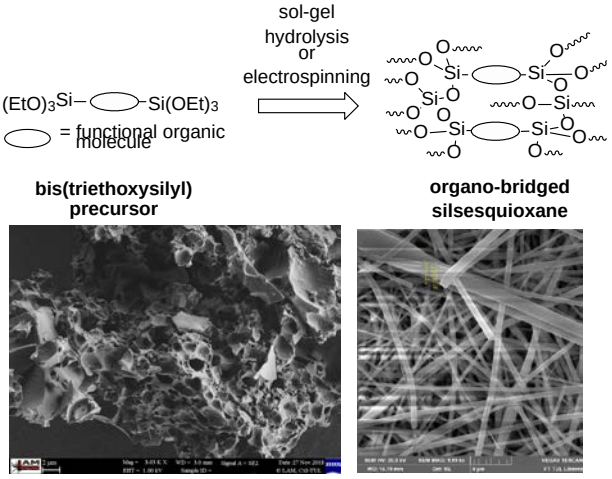

Fig. 1. **Preparation of PMOs and nanofibres from bis(triethoxysilyl) precursors; examples of SEM images of hybrid material (left) and electrospun nanofibers (right)**

Financial support from Czech Science Foundation (reg. No 18-09824S) is gratefully acknowledged.

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FACTORS INFLUENCING ALTEPLASE EFFICACY: AN *IN VITRO* STUDY

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Stroke is the second most important cause of mortality and morbidity worldwide[1]. Currently, intravenous thrombolysis using recombinant tissue plasminogen activator (rt-PA, alteplase) and/or mechanical thrombectomy present clinically approved treatment methods[2,3]. The intravenous thrombolysis holds a promising future as the mainstay in first-line treatment, largely due to the comparatively low cost and simplicity of administration. However, the successful recanalization due to pharmacological thrombolysis with alteplase remains relatively low[4]. The reasons for such low efficacy are not well understood[5,6].

In this study a simple static model of thrombolysis using red blood cell dominant clots[7] was established and set of factors potentially affecting the alteplase actions were addressed. Implications to clinical practice were drawn.

First of all alteplase induced thrombolysis was independent of clot age. The bigger clots were harder to be degraded. The *in vitro* model pointed to certain threshold of a clot size that rendered a clot to be harder to be degraded within the time of alteplase application. It appeared to be connected with the ability of alteplase to penetrate the clot. Interestingly the alteplase induced thrombolysis was not very sensitive to minor alteplase concentration manipulation justifying a reduced dose application in order to limit adverse effects. Further evidence supported no primary use of heparin for treatment of ischemic stroke treatment.

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THE USE OF PULSED MAGNETIC FIELDS TO REMOTE-CONTROL CELLULAR FUNCTIONS *VIA* MAGNETIC NANOPARTICLES

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Superparamagnetic iron oxide nanoparticles or SPIONs are widely used as contrast agents in MRI magnetic resonance imaging, hypothermia and cell kinetics studies due to unique magnetic properties[1].

Using magnetic nanoparticles became a promising tool for a variety of biomedical application, such as drug and gene delivery, cell labelling. The ability to control the movement of nanoparticles remotely and with high precision would have far-reaching implications in many areas of nanotechnology. Recent studies show that SPIONs are particularly useful for the manipulation and control of specific cellular functions under application of external magnetic fields[2,3]. However, there are still challenging questions related to optimization the methodology of cell loading, further fate of nanoparticles within the cell, its extraction or degradation and cytotoxicity[4,5].

Here we report an approach to dramatically enhance SPIONs cell labelling and to remote-control cell functions using pulsed magnetic fields (PMFs). The rate of uptake of SPIONs and transport across the cell membrane were enhanced upon application of a high intensity (7 T) short pulse

width (~15 μs) magnetic field[6,7]. We present a quantitative analysis and mechanistic explanation of how a pulsed magnetic field influences the uptake of SPIONs by cells.

From the other hand, we examined whether PMFs may exert strong enough force on nanoparticles accumulated in lysosomes that could be used for remote induction of cell death by injuring lysosomal membrane structures. Indeed, cancer cells loaded with SPIONs accumulated nanoparticles in lysosomes. Application of PMFs to SPION-loaded cells caused lysosomal membrane permeabilization, which led to extravasation of lysosomal contents into the cytoplasm, and induced apoptosis.

Our findings offer insight into the mechanics of how pulsed magnetic fields can be effectively used to optimize magnetic cell labelling and remote-control of cellular functions, which can provide a basis for better controlled biomedical applications of SPIONs.

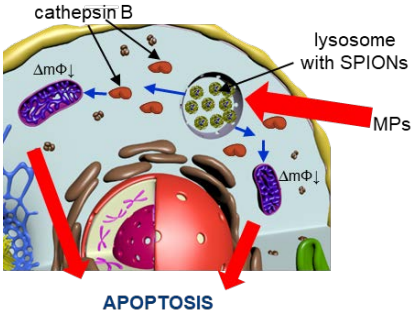

Fig. 1. **Principle of remote induction of apoptosis by PMFs. Nanoparticles accumulate inside the lysosomes. The external magnetic pulses result in lysosomal rupture and apoptosis induction**

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PUSHING THE ERK PATHWAY ACTIVITY OUT OF THE FITNESS ZONE WITH ROCAGLAMIDES

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Malignant melanoma is an aggressive form of skin cancer driven mostly by mutations in the MAPK/ERK pathway. The development of mutant BRAF kinase-targeted therapy has prolonged patient survival. However, most of the patients acquire resistance after several months of treatment. A new ERK pathway-targeted approach for melanoma therapy has been recently suggested - to push the cancer cells out of their fitness zone by over-activating the ERK signaling to levels that the cancer cells cannot sustain[1].

We recently demonstrated that melanoma cells respond to metabolic stress induced by inhibitors of cell energy metabolism by enhancing ERK pathway activity. While studying further the mechanisms responsible for this hyperactivation, we identified AMPK as an essential player in this process[2]. In the present study, we show that a small molecule rocaglamide A (rocA, identified from plant genus *Aglaia*) also potently enhanced ERK signaling at very low concentrations and inhibited melanoma cell growth and proliferation. Interestingly, when metabolic stressor or AMPK activators were combined with rocA, we observed a robust synergistic effect on the ERK signaling. The effect of rocA was observed only at the level of ERK kinase, not its upstream signaling, suggesting that rocA targeted the negative feedback regulators of ERK, the dual-specificity phosphatases (DUSPs). Further analyses revealed that the previously identified[3] inhibition of cap-mediated translation by rocA was involved in the observed effect on the ERK pathway activity.

Our results showed surprising plasticity of oncogene-driven ERK signaling in cancer cells and suggested new drug combinations that might be suitable for targeting the ERK pathway in melanoma.

This research was supported by Masaryk University (MUNI/A/1087/2018) and the Ministry of Education, Youth and Sports of the Czech Republic: the National Program for Sustainability II project Translational Medicine (LQ1605).

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A LOW COST OPTICAL SENSOR FOR MONITORING OF ELECTROCHEMICAL WATER TREATMENT

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The current trend is to simplify and increase the effectiveness of pollution monitoring in real time. Recent development in optoelectronics allows to constructs a low cost *in-situ* optical sensor for continuously monitoring[1,2].

Developed optical sensor consists from easily replaceable LED modules containing the multi-wavelength light sources (UV, RGB, IR and white LED) with replaceable three detector module positions for photodetectors. Detectors are capable to measure the light transmission in the direct pathway, the side-scattering of the light in 90° angle and to detect the fluorescence light at a proper combination of the detector and the optical filter. This configuration gives qualitative and quantitative data on the changes in the optical opacity of the water. Arduino microcontroler was used for autonomous the light source management as well as for the optical signal data measuring and recording.

Validation of the optical sensor for phytoplankton cell count determination and chlorophyll autofluorescence sensing were tested on the model suspensions of *Chlorella vulgaris* and their electrochemical elimination from water. During electrochemical elimination of *Chlorella vulgaris* a significant reduction of green colour in treated water was observed, as a result of chlorophyll degradation and cell inactivation (Fig. 1). Artificial neural network modelling suggests that the presence of various light sources with different spectral characteristics and multi detector sensing significantly increased cell count determination.

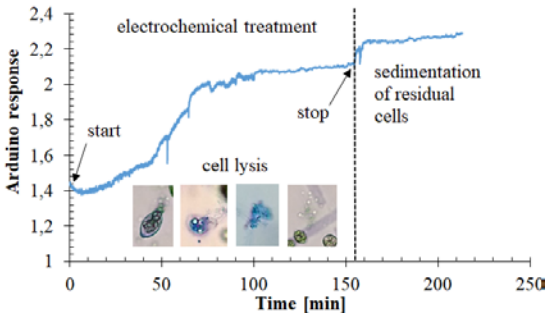

Fig. 1. **Electrochemical treatment of water contained *Chlorella vulgaris* monitored by optical sensor unit**

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THE EFFECT OF THERMORESPONSIVE SMART HYDROGEL ON THE RHEOLOGICAL PROPERTIES OF INJECTABLE CALCIUM PHOSPHATE BONE CEMENT FOR MINI-INVASIVE SURGERY

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Calcium phosphate cement (CPC) has been a subject of interest in bone tissue engineering for almost 4 decades. This hydraulic cement, composed of calcium orthophosphate, forms a mouldable paste after mixing with the liquid phase. The resulting cement exhibiting self-setting ability *in vivo* at physiological conditions has a great potential to be used *via* mini-invasive surgical procedures in the field of orthopaedics, traumatology or dentistry. However, each of these applications needs different requirements in particular for biomechanics. The advantage of CPC is the ability to tailor its properties (rheological, structural, mechanical or bioactive). In case of mini-invasive procedures, the bone cement needs to exhibit the best rheological properties such as injectability, cohesion, or setting time. One of the problems todays is poor CPC injectability and cohesion limiting its use as injectable materials in mini-invasive surgery. There are several ways to improve these features. One way is CPC modification by a thixotropic hydrogel enhancing the above mentioned properties.

The aim of this work was to compare rheological properties of commonly used water-based CPC with CPC modified by thermosensitive hydrogel capable of gelation at physiological conditions using both dynamic and steady state rheological analysis[1]. Thermosensitive biodegradable hydrogel based on poly(ethylene glycol), poly(lactic acid) and poly(glycolic acid) significantly improved thixotropic behavior of the CPC resulting in its very good homogenity and injectability *via* thin needle. Moreover, hydrogel improves also cohesion, which is responsible for keeping the cement in position after an injection into the bone defect place[2]. Based on the aforementioned results, copolymer-modified CPC seems to be useful as injectable bone cements for mini-invasive surgery.

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MASS SPECTROMETRIC DETERMINATION OF METALLOTHIONEIN BASED ON MOLECULAR IMPRINTING

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Metallothioneins (MTs) are low molecular mass (< 7 kDa), cysteine-rich proteins that play an important role in most organisms. Many studies have suggested that MT is involved in protection against metal toxicity, oxidative stress and apoptosis[1,2]. The elevated concentration of MTs has been observed in blood and/or tissues of patients suffering from diseases, such as tumour, coronary artery disease, and Alzheimer's disease[3].

Here, we used a technology of molecularly imprinted polymers as a selective purification step combined matrix assisted plasma desorption/ionization time-of-flight mass spectrometric detection and with laser ablation inductively coupled plasma mass spectrometry for analysis of MTs. MT-imprinted polymers were prepared by immobilization of the template and self-polymerization of dopamine[4] and showed good selectivity toward the template protein. This experimental setup allows to measure clinically relevant levels (μM) of MT in biological matrices (plasma).

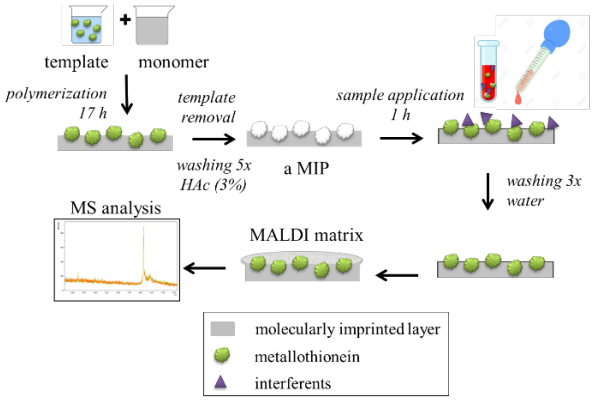

Fig. 1. **Scheme of the proposed analytical approach**

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SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF PALLADIUM(II) COMPLEXES WITH HYBRID PHOSPHANYLFERROCENE LIGANDS BEARING O-DONOR SUBSTITUENTS

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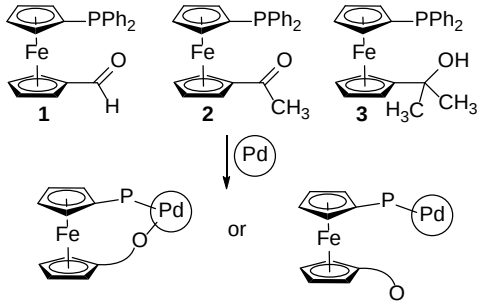
Palladium-catalysed cross-coupling reactions proceeding under the formation of new C–C bonds are powerful tools for organic synthesis. Catalytic activity of palladium species in these reaction can be controlled by the coordinated ligands. A particularly high efficiency provides 1,1'-bis(diphenylphosphino)ferrocene (dppf), a chelating ligand with two strongly coordinating groups[1]. Its molecule can be modified by replacing one phosphine moiety with another donor that could stabilize an unsaturated complex and, simultaneously, allow for substrate bonding. The resulting hybrid ligands can coordinate in hemilabile fashion[2].

This work was focused on the coordination properties of three hybrid ligands with different *O*-donor groups, compounds **1**-**3**. Although, **1** and **2** have been already prepared[3], they have not been studied as ligands. Coordination properties of **1**-**3** were probed in reactions with a $PdCl_2$ surrogate, resulting in compounds, where only *P*-donor group was coordinated. The ligands cleaved $[(L^{NC})PdCl]_2$ to give $[(L^{NC})PdCl(L\text{-}\kappa P)]$, which converted to chelate complexes upon removal of chloride ligands. Hemilabile coordination of **1**-**3** was confirmed by reactions with a Cl^- source, regenerating $[(L^{NC})PdCl(L\text{-}\kappa P)]$.



Scheme 1: **Hemilabile coordination of 1-3 in Pd(II) complexes.**

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MLST TYPING SYSTEM OF TREPONEMA PALLIDUM SSP. PALLIDUM IN THE CZECH REPUBLIC DURING YEARS 2004 TO 2017

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MLST (Multi Locus Sequence Typing) is a new typing system for *Treponema pallidum* ssp. *pallidum*, the causative agent of veneral disease syphilis, which is causing about 700 new cases in the Czech Republic and 5.6. million worldwide[1]. This system was developed recently[2] and it follows previous typing system SBMT (Sequencing based molecular typing)[3]. SBMT system used for typing TP0136, TP0548 and 23S rRNA. The MLST added locus TP0705, which increased the discriminatory power among the SS14-like clade, where majority of clinical samples belong. Also, 23S rRNA gene was removed from typing system, but it is still being examined.

In this study, we examined 674 samples collected during years 2004 to 2017 from hospitals in Brno (n = 2) and Prague (n = 2). The aim of this work was to reanalyze TP0705 locus among already typed samples with SBMT in previous studies[4,5]. For years 2015 and 2017 all three typing loci were done. In addition, 23S rDNA locus was also done. Loci were amplified with nested PCR and sequenced by Sanger sequencing.

From all examined samples, there were 281 samples positive in at least one typing locus. Among both fully and partially typed samples, the highest amplification efficiency was found for locus 23S rDNA, which is not used for typing (positive in 233 samples), followed by typing loci TP0705 (typed in 224 samples), TP0548 (typed in 208 samples) and TP0136 (typed in 204 samples).

In total, there were 136 fully-typed samples. Among them there were identified 16 different allelic profiles with 5 newly identified alleles. Totally, 145 partially typed samples contained 18 different allelic profiles with 3 newly identified alleles. Main part of positive samples was swabs from ulcers (n = 231) and only minority blood samples (n = 47) and tissue (n = 3). Among blood samples, there was found a positive association between level of RPR titer and PCR positivity, among swabs samples this association was not found. In the group of fully-typed samples, genotypes were compared genotypes with patients´ characteristics (age, sex, sexual orientation, HIV status, city, results of serological tests, primary diagnose) and status of macrolide resistance (according 23S rDNA) to found associations.

There were 3 genotypes associated with both mutations in 23S rRNA gene leading to macrolide resistance (1.3.1.; 1.26.1. with A2058G and 1.1.3. with A2058G). While an increasing number of samples with A2058G mutations was detected (86.7% in 2016/2017), the number of samples harboring A2059G mutations was found to decrease over time (3.3% in 2016/2017). On the other hand, genotype 1.1.8. was found associated with sensitivity to macrolides (no mutation found).

From patients´ characteristics, associations with gender, age or location were found.

This large collection of samples also illustrated how genotypes occurring in the population of the Czech Republic were changing during the years. It also showed differences between distant population in France, Switzerland or Cuba, but also between local populations in the Czech Republic. Population in Prague were found more diverse and the association of a genotype 1.26.1 with its occurrence in Brno was identified.

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GLUTAMINE AS A SENZITIZING FACTOR FOR CHEMOTHERAPY OF SOLID TUMORS

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Metabolic transformation of cancer cells includes an increase in the consumption of glutamine, which can even lead to a „glutamine addiction". Glutamine is the most abundant amino acid in the circulation and its role in the cell metabolism involves the synthesis and import of other amino acids, proteosynthesis, and synthesis of purine and pyrimidine bases. After sequential deamination and conversion to 2-oxoglutarate, its carbon skeleton serves as an anaplerotic substrate for the Krebs cycle. In addition to its role in energetic metabolism, glutamine can also serve as an antioxidant agent through its role as the substrate for the glutathione synthesis and for the regeneration of NADPH[1]. Depletion of glutamine can cause severe defects to the cell metabolism[2]. These phenomena are employed in the therapy of acute lymphoblastic leukemia by asparaginase, which has also a promising, yet unexplored, potential for the chemotherapy of solid tumors[3].

In this study, *in vitro* effect of asparaginase, a drug, which decreases the blood levels of asparagine and glutamine, was simulated. The effect of acute and chronic depletion of glutamine and asparagine on the cell apoptosis was studied using human cell lines derived from solid tumors, namely pancreatic adenocarcinoma (PaTu), cervix carcinoma (HeLa), and control fibroblasts MRC-5. The effect was tested in relation with oxidative stress induced by hydrogen peroxide and chemotherapeutic effect of doxorubicin, oxaliplatinum, and paclitaxel.

Our results show, that the simulation of asparaginase treatment significantly increases the sensitivity of cancer cells to oxidative stress, oxaliplatinum and paclitaxel. For this effect, a simultaneous depletion of glutamine and asparagine is required. Acute depletion of both amino acids is accompanied by a decrease of antioxidant capacity, lower activity of mTORc1 and changes in levels of intracellular amino acids and intermediate metabolites. On the other hand, all the described phenomena are absent in control fibroblasts.

In conclusion, while asparaginase is clinically used for the long-term chemotherapy of leukemia, this study suggests a potential role of its acute exposure as a chemosensitizing factor in the therapy of solid tumors.

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INVESTIGATING THE ADAR INTERACTOME

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Adenosine deaminases acting on dsRNA (ADARs) has been shown to be essential for a normal development, and to have a role in preventing innate immune response to endogenous dsRNA. ADARs deaminate adenosine to inosine by hydrolytic deamination and is known as A-to-I editing. Our group was the first to demonstrate that this editing event in endogenous dsRNA prevents the interferon (IFN) signalling cascades from RIG-I and MDA5, two cytosolic receptors for dsRNA[(1)]. In accordance, mice lacking *Adar1* have an immune phenotype with heighten levels of type-I IFN and IFN stimulated genes (ISGs). Our group rescued the embryonic lethality of *Adar1*$^{-/-}$ mice to birth by generating a double homozygous mutant between *Adar1* and *Mavs* (Mitochondrial antiviral-signalling protein), adaptor, which is downstream of RIG-I and MDA5.

In humans, mutations in *ADAR1* have been shown to cause the autoimmune disorder Aicardi Goutières syndrome-AGS[(2)], where patients display heightened levels of IFN and ISGs. Furthermore, most of the mutations were shown to reduce editing activity of the protein. An exception is *ADAR1 D1113H* mutation that is located in deaminase domain of the protein.

To investigate whether this mutation cause perturbation in protein-protein interactions, we first had to determine the protein interactome for ADARs. We prepared stable HEK293T cell lines, expressing both isoforms of ADAR1 and ADAR2. These proteins were tagged with BirA and Strep-tag at either the N or C terminal of the protein. In addition, cells were treated with HMW Poly I:C and co-immunoprecipitation of endogenous ADAR1 was also performed.

Now, we have a comprehensive data set of protein complexes in which ADAR1 and ADAR2 acts, these data are consistent for all three set. Our data is in agreement with all previously published interacting partners of ADARs. In addition, we found that tags present at different terminus can

influence interactions and protein stability.

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REMARKS AND NOTES

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