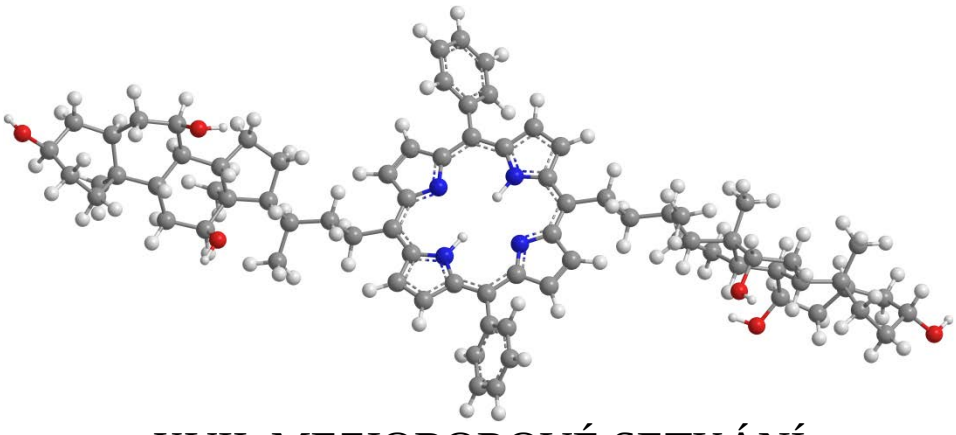

XVII. MEZIOBOROVÉ SETKÁNÍ MLADÝCH BIOLOGŮ, BIOCHEMIKŮ A CHEMIKŮ

30. 5. – 1. 6. 2017

hotel Devět Skal Milovy

sborník redigovali
Radmila Řápková, Martin Fusek, Pavel Drašar

Organizátoři konference vyslovují vřelé poděkování za podporu

IDENTIFICATION OF POTENTIAL INHIBITORS OF TAU AGGREGATION FOR ALZHEIMER'S DISEASE THERAPY

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Accumulation of amyloid beta (Aβ) and tau aggregates are two characteristic pathological hallmarks of Alzheimer's disease (AD)[1]. Tau is a microtubule-stabilizing protein, found mainly in neurons of the central nervous system. Under normal conditions, tau functions are regulated by a well-coordinated alternating cycles of phosphorylation and dephosphorylation by kinases/phosphatases. Phosphorylation of tau mainly occurs at its microtubule-binding domains, which is composed of 3-4 repeat domains (R1, R2, R3, and R4). However, in AD, dysfunctions in signaling pathways that regulate tau phosphorylation result in the hyperphosphorylation of tau and its subsequent aggregation into neurofibrillary tangles that contribute to neuronal loss in AD. Therefore, inhibition of abnormal aggregation of tau is a potent therapeutic strategy to prevent the extensive loss of neurons, and potentially to limit the progression of AD into advanced stages.

With this goal in mind, we established an in vitro tau aggregation assay for the screening and identification of potential anti-aggregation inhibitors. We used the synthetic peptides of the third repeat fragment (Tau-R3) of tau microtubule-binding domain for establishing a Thioflavin T-based assay. The Tau-R3 peptides have high self-aggregation potential, and this self-aggregation is further enhanced in the presence of polyanions, such as heparin[2]. Next, we tested the anti-aggregation activity of two known aggregation inhibitors, paclitaxel and mitoxanthrone, as positive controls to validate the assay. Thereafter, cc. 300 compounds were screened from the LOPAC®1280 – The Library of Pharmacologically Active Compounds library (Sigma Aldrich) at a single concentration of 1.25 μM. Toxicity of 12-24 h Tau-R3 aggregates and the potential therapeutic effects of selected compounds from against Tau-R3-induced toxicity were tested in SH-SY5Y cells by MTT viability assay.

Using Tau-R3 peptide-based screening assay, we identified around 15 compounds to have potent anti-Tau-R3 aggregation activity. Our preliminary studies in cell models show that Tau-R3 aggregates induce in vivo aggregation of tau in SH-SY5Y cells. Finally, some of the active compounds from the in vitro screen showed neuroprotective effects against tau-induced toxicity.

This work was supported by grants from the Czech Ministry of Education, Youth and Sports (Grant numbers: LO1304, LM2011024), and Ministry of Health of the Czech Republic (Grant number: NV15-31984A).

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THE EFFECT OF BIOLOGICALLY ACTIVE SUBSTANCES ON THE STRUCTURE AND BIOCOMPATIBILITY OF COLLAGENOUS SCAFFOLDS FOR TISSUE ENGINEERING

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The influence of biologically active additives, crosslinking agents, and enrichment with growth factors on the morphological properties of collagen-based scaffolds and their *in-vitro* bioactivity with mouse fibroblasts 3T3 have been investigated. 3D porous collagen scaffolds were modified with both antibacterial natural polysaccharides (chitosan and oxidized cellulose) and growth factors delivered in the form of blood platelet lysate. Addition of blood platelet lysate decrease the pore size of pure collagen scaffold but increased its porosity as demonstrate scanning electron microscopy pictures (Fig. 1).

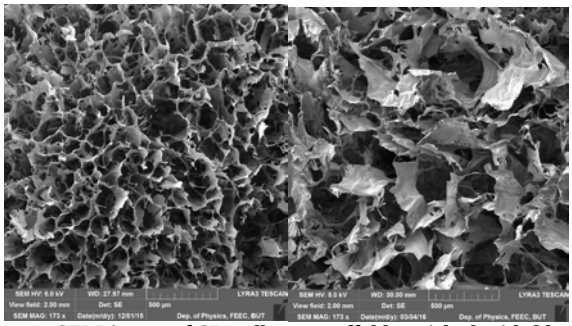

Fig. 1. **SEM image of 3D collagen scaffold enriched with blood platelet lysate (left) and without platelet lysate (right)**

Surprisingly, *in-vitro* tests of prepared scaffolds on mouse fibroblasts 3T3 showed that scaffolds enriched with platelet lysate exhibited significant synergistic effect with antibacterial additives on cells cultivation as determined by MTS assay and PicoGreen method. Therefore, these newly developed antibacterial collagen sponges involving growth factors could be used as scaffold for growing cells in systems with low mechanical loading having potential application in soft tissue engineering.

This work was supported by the Ministry of Education, Youth and Sports under the CEITEC project in 2020 LQ1601.

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WATER-SOLUBLE HETEROBIFUNCTIONAL FLUORESCENT LINKERS

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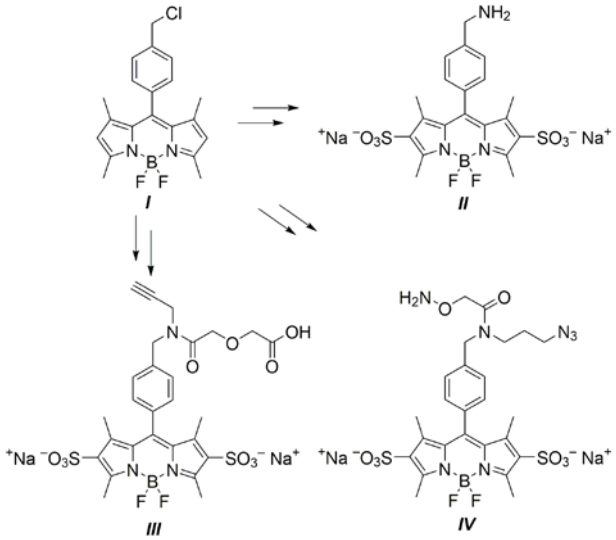
Fluorescent probes have indispensable use in molecular imaging techniques. Among their properties, which are important for bioapplications, are high aqueous solubility, excitation and emission profiles in visible or NIR region and high quantum yields[1].



Scheme 1. **Examples of heterobifunctional fluorescent linkers bearing fluorescent BODIPY cores**

Conjugation of such probes to biomolecules requires bioorthogonal reactivity. Here we show synthetic approach to heterobifunctional bioorthogonal linkers bearing BODIPY[2] motif (Scheme 1). Upon conjugation to a biomolecule, these linkers convert its original functionality to another, bioorthogonally reactive moiety (azide or alkyne useful for "click" chemistry). This new termination enables selective attachment of the construct to another biomolecule, nanoparticle or surface. The presence of BODIPY in the linker allows for direct quantification of the conjugation yield and tracking of the conjugate in biological environment.

The aqueous solubility of our BODIPY probe is mediated by presence of two sulfonic acid moieties[3,4]. We developed a reliable multigram scale sulfonation procedure of ***I***, including complex purification procedures using reverse phase flash chromatography. Compound ***I*** was further modified by alkylation of various amines using conventional or microwave synthesis. Further, reaction conditions for amidic couplings with reasonable practical yields were found. Several mono- ***II*** and heterobifunctional ***III***, ***IV*** compounds were prepared (Scheme I).

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IDENTIFICATION OF SUBSTRATE REPERTOIRE OF RHOMBOID INTRAMEMBRANE PROTEASE GlpG FROM *Escherichia coli*

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Rhomboid protease GlpG from *Escherichia coli* is the structurally and mechanistically best studied model intramembrane protease[1,2]. *glpG* gene belongs to the glpEGR operon, which is involved in glycerol metabolism. Although it is widely conserved in eubacteria including a number of pathogens (*Salmonella*, *Shigella* or *Vibrio*), its physiological function remains unknown[3].

In order to uncover the biological role of GlpG, we have used SILAC-based quantitative proteomics[4] to identify the substrate repertoire of GlpG in living *E. coli* cells. We have combined gel-free and GeLC MS analyses of periplasmic and membrane subproteomes, and improved quantitative MS data evaluation method by using several bioinformatic tools to

account for protein transmembrane topology, size and the possibility of proteolytic cleavage. A panel of resulting candidate substrates has been validated in rhomobid activity assays *in vivo*[2].

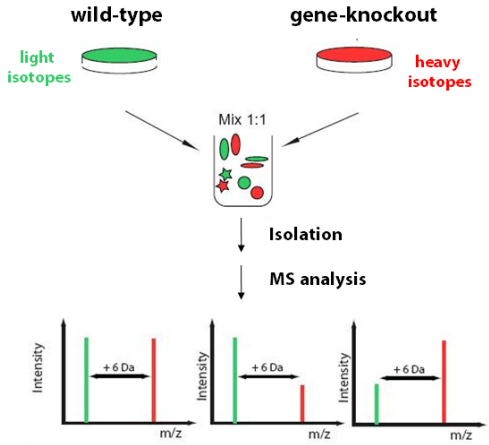

Fig. 1. **Schematic representation of SILAC-based proteomic workflow**

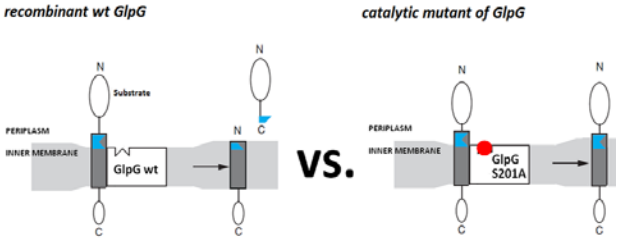

Fig. 2. **In SILAC experiment, membrane or periplasmic subproteomes of wild-type *E. coli* MC4100, grown in particular SILAC media (heavy or light), are compared with subproteomes of *ΔglpG* cells. Similarly, the subproteomes of *ΔglpG E. coli* over-expressing either recombinant wild-type GlpG or its inactive mutant are compared in concurrent experiment**

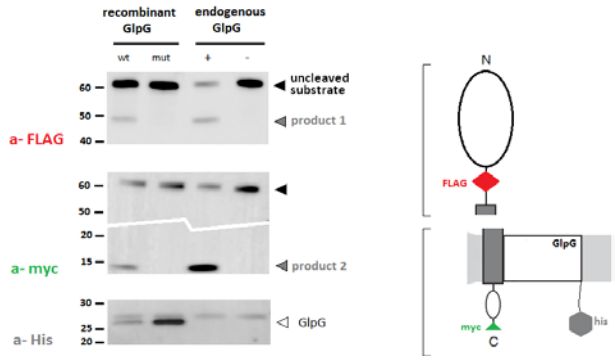

Fig. 3. **The candidate substrate is over-expressed as a chimeric N- or (and) C-terminally tagged protein in wild type and rhomboid-null *E. coli* or in the presence of C-terminally epitope-tagged rhomboid (wild type or inactive). The cleavage products are detected by immunoblotting**

The proteomes of two states (strain, condition) that need to be compared quantitatively are grown in parallel in the media containing light or heavy stable isotopes of amino acids arginine and/or lysine. In subsequent GeLC-MS/MS experiment, heavy-to-light (H/L) ratio parameter is determined for each peptide pair. If the levels of corresponding proteins are not affected in any of the compared conditions, H/L ratio values will be equal to 1.

Charles University Grant Agency (projects no. 170214 and 232313); Ministry of Education of the Czech Republic (LK11206).

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RAD51 REGULATORS: SHAPING THE FILAMENT

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DNA double strand breaks (DSBs) are among the most toxic genomic lesions. Persisting DSBs can lead to extensive genome instability, cell death or cancer. Homologous recombination (HR) represents an error-free pathway of DSB repair[1]. Among the proteins involved in key steps of HR are RAD51 recombinase and recombination mediators - including BRCA2 and RAD51 paralogues (RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 in mammalian cells). RAD51 forms helical nucleoprotein filaments capable of homology search and subsequent D-loop formation. BRCA2 nucleates RAD51 onto ssDNA, while RAD51 paralogues act downstream of BRCA2 recruitment. Similarly to BRCA2, mutations in RAD51 paralogues are associated with an increased risk of breast/ovarian cancer development and Fanconi anaemia-like disorder – a rare genetic disease[2]. However, the exact molecular mechanism of RAD51 paralogue action is poorly understood.

To address this issue, we have purified and characterized recombinant RAD51 paralogue complex. RAD51 paralogues rapidly bind RAD51-ssDNA filaments in a substoichiometric fashion - remodelling them into more stable and flexible conformation to stimulate RAD51-mediated D-loop

formation.

Our findings provide novel insights into the molecular mechanism of RAD51 paralogue action and widen our understandings how deregulation of HR could contribute to tumourigenesis and/or be utilized in anticancer therapy.

This work was supported by GACR13-26629S and GACR207/12/2323 grants.

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CONJUGATES OF BETULINIC ACID WITH PICOLYL AMINES

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Betulinic acid is an important substance for designing of new drugs. Its derivatives have anticancer, antimicrobial, anti-inflammatory and anti-HIV activity. We synthesized derivatives of betulinic acid with picolyl amines for subsequent testing of their biological activity.

We obtained two series of derivatives of betulinic acid. The synthetic protocol for the first series (Figure 1) was based on two synthetic steps: (a) Preparing the hemiester from betulinic acid and succinic anhydride; (b) Coupling of hemiester of betulinic acid with picolyl amines, activated by T3P, which was used because it allows a selective reaction with carboxyl group of hemiester in the presence of unprotected carboxyl of betulinic acid in 70-90 % yields.

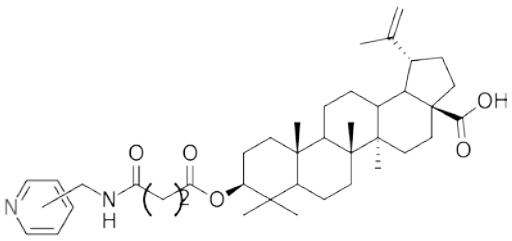

Figure 1

The second series (Figure 2) was prepared in four steps. The synthesis consists of a protection of 3-hydroxyl group by acetic anhydride, a formation of acyl chloride by oxalyl chloride, forming of picolyl amide and removing of acyl group by lithium hydroxide. The yield varied in range of 90-95 %.

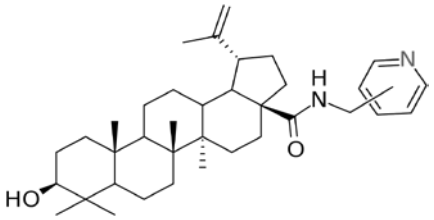

Figure 2

All target compounds were tested for cytotoxicity on the cells of human T-lymphoblastic leukemia, breast adenocarcinoma, cervical cancer, and on normal human fibroblasts for comparison. Antimicrobial tests were done in addition.

A financial support from the project COST LD15012 (MSMT), a part of the COST Network CM1407, and FV10599 (MPO).

MECHANISM OF CELL DEATH INDUCTION UPON ELECTRON TRANSPORT CHAIN INHIBITION IN PROLIFERATING AND QUIESCENT CELLS

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Mitochondrial electron transport chain (ETC) consists of four multiprotein complexes (I-IV) that drive ATP-synthase and produce the majority of ROS in a cell. Previously we showed that mitochondrially targeted vitamin E succinate selectively eliminates proliferating but not quiescent endothelial cells (ECs), and suppresses tumorigenic angiogenesis in vivo by inhibiting complex II of the ETC with subsequent ROS production.[1] This suggests that modulation of ETC activity in proliferating and quiescent cells may have different outcomes with respect to cell death induction.

To investigate this issue, we cultured ECs (Ea.hy926) and epithelial cells (MCF10A) in low (1 g/L) and high glucose (4.5 g/L) conditions, which promotes and suppresses mitochondrial respiration/ATP production, respectively. The quiescence was achieved by contact growth inhibition. Both the proliferating and quiescent cells were exposed to direct ROS inducers that do not affect the respiration (PEITC, H2O2), inhibitors of the ETC or ATP-synthase inhibitor oligomycin. Cell death and ROS production as well as various metabolic parameters, particularly mitochondrial respiration, antioxidant defence, glycolysis and ATP levels, were assessed. The role of several components of the antioxidant defence systems (SOD2- and GPx1-, TRX-reductase- and GSH) was interrogated by shRNA-mediated knockdown.

We found that proliferating cells were consistently more susceptible to cell death and ROS production in high glucose conditions than the quiescent cells. Interestingly, in low glucose conditions this pattern of cell death sensitivity was inversed with the exception of direct ROS inducers. For quiescent cells in low glucose conditions, the cell death correlated with a significant ATP depletion rather than with ROS production. While the quiescent cells have higher protein level and activity of antioxidant components (SOD2, Trx2, PrxIII, GPx) regardless of glucose content, gene silencing of SOD2 and/or GPx1 increased cell death as well as ROS production exclusively in high glucose conditions.

Our data suggest that the interference with ETC activity and subsequent lack of ATP is a major factor for cell death induction in low glucose conditions, whereas in high glucose conditions the level of ROS generation becomes dominant.

Research is supported by GAUK 98215, GAČR 16-22823S a BIOCEV European Regional Development Fund CZ.1.05/1.1.00/02.0109.

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SYNTHESIS AND SPECTROSCOPIC CONFORMATIONAL STUDIES OF Ac-Nit-NHMe DIPEPTIDE MODEL

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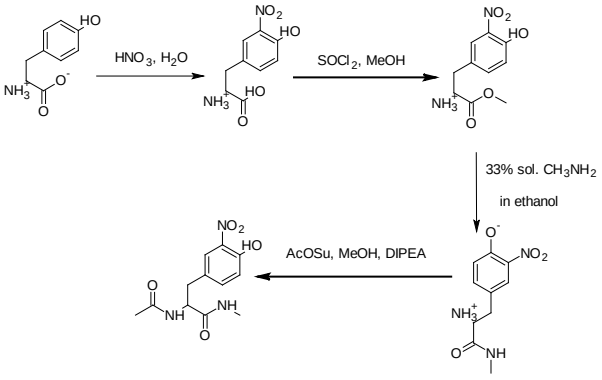
3-Nitrotyrosine, a modification of proteinogenic amino acid tyrosine, occurs naturally in organism as a result of oxidative stress. Higher abundances of nitrotyrosine in proteins have been detected in a large variety of disease conditions and are also associated with the ageing processes[1].

Nitration of proteins significantly alters their properties such as hydrophobicity and acidity[2]. A major unsolved question in the field is how tyrosine nitration affects protein structure and function.

To better understand the effect of nitration on structure of peptides, we proposed a synthesis of prototypical peptide model for peptides containing 3-nitrotyrosine residues, Ac-Nit-NHMe (Sch. 1)[3].



Scheme 1. **Synthesis of Ac-D-Nit-NHMe from H-D-Tyr-OH**

We prepared both enantiomers of Ac-Nit-NHMe and Ac-Tyr-NHMe. Their conformational spaces were compared using MD and DFT simulation in combination with various spectroscopic methods (NMR, IR, Raman, CD, VCD, and ROA spectroscopy) carried out in several solvents.

This work was supported by Czech Science Foundation (reg. no. 17-00121S).

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STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF AICHI VIRUS RNA DEPENDENT RNA POLYMERASE

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Aichi virus is a single stranded plus RNA (+ RNA) virus which causes gastroenteritis in humans. Aichi virus is also used as a model organism for studying related more virulent viruses like SARS, HCV, West Nile virus, Yellow fever virus etc. Protein $3D^{pol}$, RNA dependent RNA polymerase, is a key enzyme for the life cycle of a + RNA viruses. The $3D^{pol}$ enzymes catalyze formation of phosphodiester bond between RNA nucleotides. The picornaviral RNA replication is protein $3D^{pol}$ activity dependent. The RNA replication is curtail for both viral genome multiplication and viral protein translation. That renders $3D^{pol}$ a good target for drug design.

+ RNA viruses hijack phosphatidylinositol 4-kinases (PI4Ks) to generate membranes highly enriched in phosphatidylinositol 4-phosphate (PI4P). These viral induced membrane structures serves as structural scaffold for RNA replication and also to protect from innate intracellular immunity. We studied interaction of $3D^{pol}$ with PI4P lipid using recombinant $3D^{pol}$ from Aichi virus 1 (AiV), Poliovirus 1 (PV), Coxsackievirus b3 (CV) and Enterovirus 71 (EV). The polymerase activities of $3D^{pol}$ enzymes were compared by PETE fluorescence assay[1].

We also determined the crystal structure of Aichi $3D^{pol}$ at 3.8 Å resolution. The structure revealed an overall conserved fold. However, the Aichi $3D^{pol}$ has a different N-terminal structure compared to other related picornaviruses (PV, CV, EV), where the N-terminal residue is buried in a pocket at the base of fingers domain. Our structure reveals that Aichi $3D^{pol}$ can not stabilize itself by a slip of its first redue in the hydrophobic core of the protein.

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GENETIC CAUSES OF DEAF-BLINDNESS IN SIXTEEN CZECH FAMILIES

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Usher syndrome (USH) is the most common cause of hereditary deaf-blindness in humans with an estimated prevalence of 3-6 per 100,000 inhabitants. USH is inherited as an autosomal recessive trait and is clinically divided into three types; 1, 2, or 3, based on audiological profile and vestibular symptoms[1]. The aim of the project was to phenotype the first cohort of Czech patients with USH (16 families, 21 affected individuals) and to perform investigation into the molecular genetic cause of their disease.

Sanger sequencing of 6 frequently mutated *USH2A* exons was performed as an initial step in probands suspected to suffer from USH type II. Unsolved cases were then investigated by a range of techniques including whole-exome sequencing, targeted gene panel next generation sequencing and single-nucleotide polymorphism (SNP) array for copy number variation analysis. Detected missense mutations were evaluated for pathogenicity by six in silico tools. Mutations were verified and their segregation within the families was performed by Sanger sequencing which was also used to create a haplotype consisting of 8 SNPs in individuals with the most frequently occurring mutation.

In total 17 different mutations evaluated as pathogenic were identified in 20 individuals (95%) from 15 families, of these c.1256G>A, c.13342_13347del, deletion of exons 33 and 34 in *USH2A*, c.871G>A in *CDH23* and c.937C>T in *USH1C* were novel. A known c.11864G>A in *USH2A* was the most prevalent nutation observed, found either in a homozygous or compound heterozygous state in 9 families including an outbred pedigree showing pseudo-dominant inheritance. SNP haplotype analysis supported the hypothesis of a founder effect. Investigation into the geographic origin suggested regional clustering of the c.11864G>A allele in Southeastern part of the Czech Republic.

The proposed research will help to elucidate factors involved in the etiopathogenesis of USH, which is important for prognosis and patient counseling, management, development and introduction of novel therapies.

This work was supported by GAUK 4315/2015.

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DIFFERENT MECHANISMS OF p16/INK4A REGULATION IN HUMAN EMBRYONIC AND NERUAL STEM CELLS

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Human embryonic stem cells (hESCs) have the ability to unlimitedly self-renew which is underlied by rapid cell division and specific cell cycle regulatory mechanisms. Importantly, length of cell cycle, especially the G1 phase, and activity of specific cell cycle regulators determine the cell fate decision and differentiation (1). Molecular mechanisms behind this phenomenon are however not well described. The aim of this study was to analyse cell cycle of hESCs before and after differentiation and describe the role of cell cycle regulators during this process. To address this question, we analysed three cell types – undifferentiated hESCs, hESC-derived neural stem cells (NSC) and neural progenitor cells (NPCs). We determined the level of cell cycle regulators and curiously found that one of the regulators, p16/INK4A (p16), manifests a different expression pattern than other cell cycle inhibitors. Its level is low in hESCs and NSCs and only increases in NPCs. Protein p16 is a tumor supressor molecule which arrests the cell cycle in G1 phase. Its regulation in hESCs and NSCs has not yet been described. Therefore we focused on this molecule and found that despite absence of p16 protein, p16 mRNA is present in all three cell types. This suggests that the expression of p16 protein is regulated by currently undescribed post-transcriptional

mechanism. Our functional experiments show that expression of p16 protein is regulated differently in each cell type. For the first time we show that in hESCs, miRNAs are involved in this regulation whearas NSCs use proteasomal degradation. The regulation in NPCs remains unclear. Furthermore, we analysed hESCs edited by CRISPR/Cas system so that the gene for tumor supressor protein p53 was knocked-out. These p53KO cells demonstrated a significant upregulation of p16 (2). Also, we have focused on the relationship between p16 and Bmi1, polycomb group protein - oncogene necessary to retain self-renewal (3). Following experiments will help to understand this regulation. Alltogether, we have discovered p16 regulations in hESCs and NSCs, which will further be closely analysed as well as the role of p16 protein in p53 knock out hESCs will be further studied.

This study was supported by GJ15-18316Y and MUNI/A/1352/2015

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NEW TOOLS FOR ENRICHMENT OF *Treponema pallidum* subsp. *pallidum*, THE CAUSATIVE AGENT OF SYPHILIS, FROM CLINICAL SAMPLES CONTAINING LARGE AMOUNTS OF HUMAN DNA

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Treponema pallidum subsp. *pallidum* (TPA) is the causative agent of syphilis. Despite current major advances in culture techniques, it remains one of the last as-yet uncultured human pathogens *in vitro*. Only few published whole genomes were obtained after amplification through rabbit passages. In 2016, with introduction of new TPA enrichment methods, the number of sequenced TPA genomes increased[1,2]. Here we present a development of a new enrichment technique, which could be applied to the syphilis clinical samples containing large amounts of human DNA.

The real-time and nested PCRs for a single copy conserved gene, coding DNA polymerase, were performed to estimate number of TPA DNA copies. A DNA sample of the reference strain TPA Nichols and living cells of the strain TPA Phi-1 served as positive controls. The NGS Illumina sequencing platform was used in collaboration with University of Tubingen, Germany; University of Zurich, Switzerland; and FLIR Systems, Inc., CA, USA. BWA MEM algorithm was used for reference mapping.

We have tested positive control and clinical samples with the following enrichment methods. (i) Methods based on the separation of the TPA from the sample background on the cellular level including Micromanipulation technique, Enrichment based on selective cell type lysis and Anti-treponemal antibody enrichment. (ii) Methods based on the separation of the TPA from the sample background on the DNA level including Hybridization capture technique, Enrichment based on the selective binding of different methylation motifs and Specific DNA binding enrichment using restriction endonuclease *Dpn*I. We compared the above techniques and we present draft genomes of clinical samples isolated directly from the patients obtained by different methods with different levels of TPA DNA purity.

Despite the apparent variability in clinical symptoms, comparative genomics revealed that TPA are highly monomorphic bacteria with whole genome identity >99.8%. Since all yet available whole genome sequences of TPA came from bacteria propagated in rabbits, sequencing of syphilis genomic DNA isolated directly from the patient is required. Here we report the possible procedures for preparation of TPA DNA for NGS.

Support from GAČR (GA17-25455S to DŠ).

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SEARCHING FOR A PATH TOWARDS A HELICENE -BASED MACROCYCLE

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Vast π-conjugated macrocycles have been targets of material chemistry for a long time for their appealing

physicochemical properties, with potential applications in molecular electronics, optics and as parts of nano devices. Helicenes, *ortho*-fused helix-shaped aromatic molecules, might be attractive building blocks of such macrocycles addressing chirality issues.

Here, we report on the attempts at the synthesis of a large shape-persistent macrocycle ***1*** comprising dibenzo[5]helicene[1] units that should combine the interesting qualities of both helicenes and shape-persistent macrocycles. Palladium-catalyzed cross-coupling reactions as well as olefin metathesis were used to provide the desired macrocycle in a reasonable amount and purity. A number of dibenzo[5]helicene derivatives equipped with various solubilizing groups were synthesized. Trityl groups turned out to be optimal and allowed a large scale synthesis as well as feasible separation of the target macrocycle.

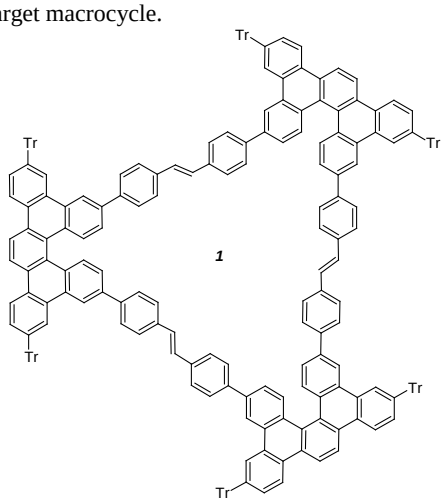

Figure 1. **Target macrocycle**

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THE NOT-SO-MISSING GENES IN BIRDS

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Thanks to the advances in high throughput sequencing methods and data analysis, genome of the huge number of species has been recently read and assembled. Despite of this, subset of essential and well described mammalian genes, such as erythropoetin, leptin or TNF alpha, have not been identified in birds and their existence has been controversial for a long time. After thorough analysis of combined sequencing data with high coverage, we were able to identify some of these genes and thus prove their existence. Interestingly, all these "missing" genes are characterized by exceptionally high GC content and long G/C stretches. Such characteristics cause difficulties in PCR amplification preventing efficient sequencing and can, therefore, lead to the absence of these sequences in databases and genome assemblies. This observation is probably general and seems to apply for a significant portion of avian genes thought to be missing. In all genes we analyzed, the GC richness was observed exclusively in the avian orthologs and not in the orthologs of other species. This arises the question what is the cause of such an extreme evolutionary driven accumulation of GC nucleotides in this subset of avian genes. We hypothesize that these regions are important for formation of G-quadruplex structures involved in the pairing of homologous chromosomes during meiosis. However, this possibility has to be further investigated. Our work also demonstrates that sequences biased in their nucleotide content are often underrepresented in sequencing data. Thus, genome assemblies should be treated with caution.

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WHAT IS THE MOLECULAR MECHANISM UNDERLYING TRANSLATION REINITIATION OF MAMMALIAN TRANSCRIPTIONAL ACTIVATOR ATF4?

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ATF4 (activating transcription factor 4) is a key player in the process called integrated stress response (ISR) which enables cells to cope with incoming stress stimuli. It has been shown that translational upregulation of *ATF4* mRNA in stress is governed by a gene-specific regulatory mechanism called translation reinitiation exploiting the short uORFs (upstream open reading frames) present in some 5´UTRs and having an ability to retain the post-termination 40S ribosomal subunit on the mRNA[1]. Notably, defective translational control of *ATF4* as well as other uORF-containing mRNAs has already been linked to several human diseases including cancer. However, the requirements for reinitiation in mammals are still not known even though uORFs are present in up to 50% mammalian transcriptomes and thus might represent important *cis*-regulatory features in translational control of eukaryotic gene expression. So far, eukaryotic reinitiation has been deeply studied only with the help of yeast

GCN4 mRNA, the yeast homologue of mammalian *ATF4* mRNA[2]. In this study we performed an extensive mutagenesis and analysis of the GFP-fused human *ATF4* constructs containing its natural 5' UTR in order to elucidate the precise roles of all its three uORFs in *ATF4* regulation. We also monitored the behavior of endogenous *ATF4* mRNA and ATF4 protein stability after stress stimuli. Finally, we wish to combine our findings with the data from ribosome profiling to obtain a complete and clear picture of the gene-specific *ATF4* translation control in time and space. We believe that this work will shed revised light on the real regulatory mechanism of *ATF4* expression upon stress and as such will have a significant impact on the field of translational control.

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NOVEL APPROACH TO QUINAZOLINE ALKALOIDS – TOTAL SYNTHESIS OF ARDEEMIN

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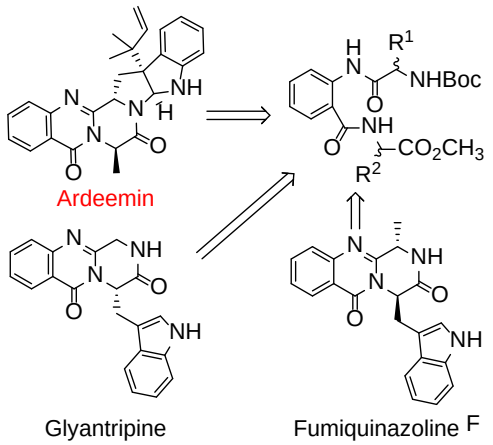
The quinazoline family of alkaloids, having diverse biological activities, is a growing class of secondary metabolites[1]. They are biosynthesized from tryptophan and anthranilic acid by incorporation of an additional amino acid unit. The members of this class of alkaloids exhibit cytotoxic, antiviral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



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

A novel approach to quinazoline derivatives using silica gel mediated double condensation is reported, which is successfully applied to total syntheses of glyantripine, fumiquinazoline F, ardeemin and its analogs.

Ardeemin is a complex quinazoline alkaloid challenging organic chemists since 1993, when was isolated from the fungus *Aspergillus fisheri*[3].
We report the shortest synthesis of ardeemin in just 4 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 generous funding.

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CHARACTERISATION OF NOVEL ANTIBACTERIAL BIOMATERIALS FOR ORTHOPEDIC IMPLANTS - TITANIUM COATED WITH TITANIA LAYERS CONTAINING SILVER

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Infection at the site of implantation is a common complication during orthopaedic surgery, which can even lead to implant failure. One of the possible ways how to prepare an antibacterial surface is to coat an implant by a layer containing silver using sol-gel method[1].

Dip-coating technique was used to create silver-doped titania coatings on titanium substrate. Sol contained two forms of silver (either nitrate or phosphate). Materials were characterised in terms of adhesion (tape test), morphology (SEM), kinetics of Ag release into a cultivation medium, total content of Ag in the layer, antibacterial effect against *Escherichia coli* and *Staphylococcus epidermidis* and also cytotoxicity towards mouse fibroblast L929 and human osteosarcoma U-2 OS cell lines (indirect test, ISO 10993-5).

The layer thickness ranged in order of hundreds of nanometres and its adhesion was very strong. SEM analysis confirmed the presence of silver particles. The biggest amount of Ag was released within first four hours, however, even after one month, the release was still detectable. Strong antibacterial effect towards *E. coli* was observed after 24h exposition for both forms of Ag in the coatings. Indirect test with extracts of

materials proved sufficient cytocompatibility of the tested materials. Thus, titania coatings seem to be promising candidates for orthopaedic biomaterials which are worth further studying.

This work was supported by TAČR (TE01020390).

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THE GUT MICROBIOME INFLUENCES THE EXPRESSION OF LIVER CYTOCHROME P450

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The gut microbiome, an aggregate genome of trillions of microorganisms residing in the human gastrointestinal tract is known to have the substantial ability to metabolize drugs and influences their stability and oral bioavailability[1]. However, many questions concerning the impact of the gut microbiota on drug metabolism, remain unanswered, namely what are the molecular mechanisms and which bacterial species are involved?

In our study, the germ-free (GF) mice, lacking the intestinal flora, and mice intentionally colonized by non-pathogenic bacteria *Lactobacillus plantarum*^{NIZO2877} or probiotic bacteria *Escherichia coli* Nissle 1917 were used to investigate the effect of microbial colonization on mRNA and protein expression and enzymatic activity of liver cytochromes P450 (CYPs), the main drug metbolizing enzymes. The specific pathogen-free (SPF) mice were used as a control group.

Our results show that the mRNA expression of *Cyp1a2* and *Cyp2e1* was significantly increased, while the expression of *Cyp3a11* mRNA was decreased under GF conditions compared to the SPF mice. The both chosen bacteria given to the GF mice decreased the level of *Cyp1a2* mRNA and normalized it to the control level. The same trend was observed in the enzymatic activity of CYP1A2. On the other hand, the monocolonization of the GF mice with both bacteria did not significantly affect the expression of *Cyp3a11* gene, which remained decreased (in comparison with SPF mice). Also the activity of CYP3A remained decreased on the level of GF mice after colonization by both chosen bacteria.

These results indicate that one of the possible mechanisms of action of the gut microbiome on drug metabolism may be its impact on the expression of hepatic biotransformation enzymes.

This work was supported by grants of the Czech Science Foundation 17- 09869S and by the internal student grant of the Palacky University LF_2016_012.

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DETERMINATION OF POROSITY FROM TOMOGRAPHIC DATA OF HYDROXYAPATITE SCAFFOLD-LIKE STRUCTURES

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In many fields, researchers take advantage of knowledge of material's porosity. For obtaining quantitative information of single, enclosed cavities, a lot of methods have been developed[1]. The situation gets complicated when pores are interconnected within the structure. This is the case of foams and scaffolds, which are used in modern biology research for tissue engineering[2].

X-ray computed microtomography (μCT) is a non-destructive method for 3D imaging of materials[3]. A result from tomographic measurement is a set of slices through the sample, where grey values correspond to linear attenuation coefficient. These slices can be stacked and then imaged and processed as 3D data.

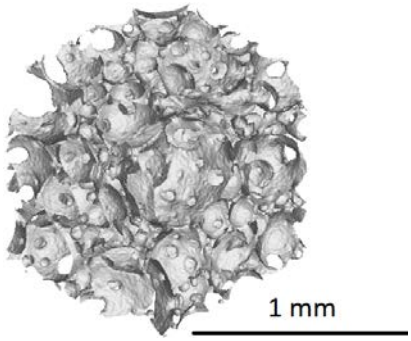

Fig. 1. **3D render of hydroxyapatite foam measured by computed microtomography**

For getting quantitative information about porosity in foams or scaffolds scanned with μCT, many software offer various approaches. We compare different software for pore analysis in terms of provided result, accessibility, quantitative analysis, field of application and user-friendliness. As a testing

sample, a tomographic data of hydroxyapatite foam for bone replacement (Fig. 1) were used.

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DEVELOPMENT OF THE SCREENING ASSAY FOR IDENTIFICATION OF COMPOUNDS TARGETING INFLUENZA A

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Influenza virus A circulates in birds and mammals and causes severe infectious disease with potential fatal outcomes. Virus circulates worldwide and triggers annual epidemics that affect from 3 to 5 million people each year[1]. There are two classes of anti-influenza drugs currently available: neuraminidase and M2 channel inhibitors. However, increasing resistance against these two types of inhibitors along with potential emergence of new viral strains and unpredictability of pandemic outbreaks emphasize an unmet need for new types of inhibitors[2].

RNA-dependent influenza polymerase serves as a novel promising target in the development of anti-influenza medications. The enzyme consists of three subunits: PB1, PB2 and PA, each of them possessing a distinct function. PA and PB2 subunits participate in a process called 'cap snatching', which is crucial for viral replication. To initiate a viral mRNA synthesis, PB2 subunit binds 7-methylguanosine cap of host pre-mRNAs which is further cleaved by PA subunit[3,4].
Therefore, we aim to develop high-throughput assays for screening of compounds targeting influenza RNA polymerase, particularly, its cap binding and endonuclease domains.

In our laboratory, we have expressed and purified recombinant cap binding domain of PB2 subunit and N-terminal endonuclease domain of PA subunit, both from H1N1 pandemic isolate, with appropriate fusion tags. Also we designed probes based on already published nanomolar inhibitors (mostly cytotoxic *in vivo*), which will be further used in screening assays. Binding properties of several probes with different types of linkers were tested by surface plasmone resonance. Our further plans are to set up the first series of screening experiments.

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SILENCING OF ONCOGENIC MIR-21 BY AN ANTIMIR COATED FLUORESCENT NANODIAMONDS

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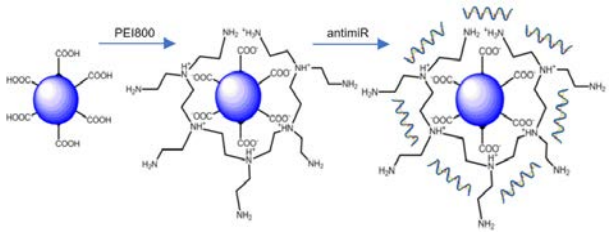
miRNAs (miRs) are short non-coding sequences of RNA which regulate gene expression through RNA interference (RNAi). RNAi is a biological process by which miRs interact with mature mRNA in the cell cytoplasm. The translation of mRNA to a protein structure is silenced through this interaction[1] and miRs are therefore able to regulate a lot of cell functions. In case that there is an overexpression of specific miRs inside cell then the tumour growth may begin. These specific miRs are known as oncomirs and they are classified as oncogens[2]. Down-regulation of an overexpressed miR may be performed by compatible sequence of RNA, specifically created to inhibit miR. This effector molecule is called antimiR. Currently known limiting factors for successful delivery of antimiRs in the cell cytoplasm is an uptake of antimiR functionalized carrier and a release of these effector molecules.

Here we report the inhibition of an oncogenic miR-21 within CT26 colon cancer cell line. As a carrier of effector therapeutic molecules (antimiRs) we used an advanced nanosystem consisting of a fluorescent nanodiamond (FND) coated with low-molecular polyethyleneimine (PEI800).



Scheme 1. **Fluorescent nanodiamond particle with noncovalent polyethyleneimine and antimiR coating used for delivery of antimiR in cancer cells. Source[3]**

We were able to observe transfection and effective release of antimiR molecules in the cell cytoplasm. The presence of antimiR in cytoplasm caused an inhibition of oncogenic miR-21 and thus decreased the silencing of the target tumour suppressor genes.

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SILK SERICIN-MODIFIED NANOFIBERS FOR THE DEVELOPMENT OF *IN VITRO* MODELS OF LUNG

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In vitro models of lung have shown to be crucial for exploration of various factors' and environmental agents' impact on the lung[1]. These models are based on scaffolds subsequently seeded with cells. The scaffolds must meet the condition of biocompatibility and should promote cell proliferation and adhesion. Silk sericin, a bioactive molecule isolated from silkworm cocoons, has been previously shown to have a positive impact on cell proliferation and to pronounce antimicrobial properties[2]. Therefore it may hold a promise as an additive to polymers to be used as cell supports.

Here we have prepared nanofibrous polymeric scaffolds from syntetic polymer, poly-ε-caprolactone, modified with silk sericin to induce bioactive properties of such cell supports. These scaffolds were designed to mimic by their morphological structure the extracelular component of alveolocapillary interface, the most distal part of lung, where the gas exchange takes place. Nanofibers were prepared by electrospinning method using Nanospider technology. The scaffolds were evaluated for their physicochemical properties as well as for their influence on cell behavior in *in vitro* cultures. Adult stem cells isolated from lipoaspirate were used for initial experiments. Lung progenitors *in vitro* derived from human embryonic stem cells were used as cells representing respiratory epithelium. To unravel the detailed morphology of the interaction between materials and cells we have used scanning and transmission electron microscopy. We quantified cell viability and proliferation with MTT and crystal violet staining.

Collectively, we have prepared biocompatible bioactive cellular supports for future development of *in vitro* models of alveolocapillary interface. Importantly, we provide quantitative measures documenting positive influence of silk sericin addition to nanofibrous polymeric structures.

This research was financially supported by funds from Ministry of Health of the Czech Republic (16-31501A), project LQ1605 from the National Program of Sustainability II (MEYS CR) and project Tissue Engineering 7 (MUNI/A/1369/2016) from Masaryk University.

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RING-CLOSING ENYNE METATHESIS OF PROCHIRAL ENDIYNES

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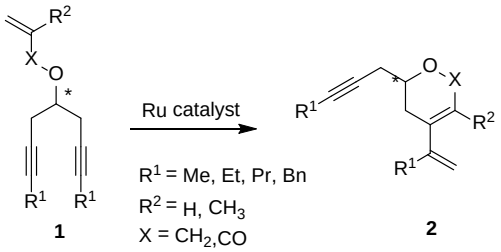
Ring-closing enyne metathesis (RCEYM) is a reaction which creates a cycle and a system of conjugated double bonds (Scheme 1). Symmetrical endiynes can serve as prochiral substrates for studying enantioselective RCEYM.

Starting from hepta-1,6-diyn-4-ol, we synthesized a small library of endiynes **1**. These endiynes were then utilized in RCEYM reactions with commercial Grubbs and Hoveyda-Grubbs catalysts, forming dihydropyrane derivatives **2** (Scheme 1). The role of ethene atmosphere ("Mori conditions")[1] in such reactions was also explored.



Scheme 1. **RCEYM of prochiral endiynes 1**

Due to their intriguing structure, the products of endiyne RCEYM metathesis (**2**) enable a number of orthogonal modifications, such as Diels-Alder reactions or Huisgen cycloadditions ("Click reactions"). We have recently started our study of such modifications (Fig. 1). Pyrane derivates **2** are promising intermediates in the synthesis of natural products and their analogues.

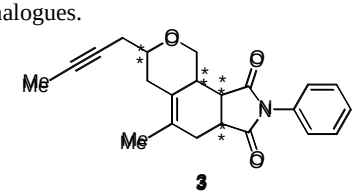


Fig. 1. **Pyrane derivative 2 modified by Diels-Alder reaction**

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DECIPHERING THE ROLES OF FIBROBLASTS DURING PUBERTAL MAMMARY GLAND DEVELOPMENT

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For decades it has been largely recognized that mammary gland stroma plays a major role in regulation of development and homeostasis of mammary epithelium. However, despite major efforts in the area, the particular roles of the individual stromal components have not been fully elucidated.

We have focused on the role of fibroblasts during pubertal mammary gland development. Our studies using mouse models revealed that modulation of receptor tyrosine kinase (RTK) signaling in mammary fibroblasts has an important role in regulation of epithelial branching morphogenesis. Upregulation of RTK signaling in mammary fibroblasts (in *Spry1* knockout mice) led to increased epithelial invasion and branching, while downregulation of RTK signaling in mammary fibroblast (in *Fsp-Cre;Spry2*-GOF mice) slowed down epithelial invasion and reduced branching.

To investigate the cellular and signaling mechanisms by which these phenotypes are induced, we used 3D cell cultures, including co-culture branching assay and organotypic invasion assay, time-lapse imaging, Western blots, and qPCR. We found that mammary fibroblasts induce epithelial branching and promote epithelial invasion into extracellular matrix (ECM) by paracrine signaling, ECM remodeling, and, intriguingly, using mechanical force.

Of several RTKs expressed in fibroblasts, we are currently investigating the role of FGFR signaling because it has not been studied in mammary gland stroma before. We found that FGF signaling promotes fibroblast proliferation, migration and ECM remodeling. Moreover, using a 3D spheroid invasion assay we found that FGF2 induces an invasive phenotype in fibroblasts through its action on actomyosin cytoskeleton. 3D organotypic co-cultures of mammary epithelial organoids with fibroblasts revealed that this invasive phenotype was also induced by mammary epithelium and represented an activated state of fibroblasts that regulated mammary branching morphogenesis through both paracrine signaling and ECM remodeling.

Taken together, our studies bring new insights into the mechanisms by which fibroblasts regulate mammary gland development and homeostasis, and deregulation of which could lead to tumor formation.

This work has been supported by the grant GJ16-20031Y (GACR).

SEQUENCE REQUIREMENTS AND GTP-DEPENDENT FORMATION OF MULTIMERIC G-QUADRUPLEXES

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Although much research has been done on nucleic acid sequences that form non-canonical structures known as G-quadruplexes (G4), the sequence requirements of higher-order G4 structures have received less attention. DNA and RNA G4 structures have been proposed to be important regulatory elements and more research may provide new insights into their potential biological roles.

For a sequence to be able to form a G4 it normally needs to contain four stretches of at least three guanine bases separated by loops of one to seven nucleotides[1]. It was previously shown that some sequences can form G4 structures despite deviating from this general sequence rule. In addition,

we recently reported that mutations in tetrads can induce formation of multimeric G4 structures[2].

To investigate the assembly of higher-order structures in more detail, we analyzed all possible variants of the central tetrad in a monomeric, parallel strand G4 on native gels. Under our conditions most of the sequences existed in a monomeric form, but approximately 10% formed higher-order structures. Mass spectrometry indicated that the two main types of structures observed were dimers and tetramers, while circular dichroism verified that the structures were G4.

Our study shows that for a variant of this G-quadruplex to form a dimer it typically needs to contain a central NNGG tetrad, whereas a central GGNN tetrad is required for tetramer assembly. This finding is of interest in light of our previous observation that G4 variants containing a central NNGG tetrad promote peroxide reactions while GGNN G-quadruplexes bind GTP[2]. Taken together, our findings support the idea that mutations in tetrads can influence the biochemical specificity of G4 structures.

Our results also suggest the intriguing possibility that GTP regulates formation of multimeric G4 structures. For several sequences containing a central GGAG tetrad, GTP inhibits tetramer formation, probably by replacing adenosine in the mutated tetrad. We propose that this enables formation of a canonical GGGG tetrad and causes the structure to behave like the monomeric reference sequence. We hypothesize that GTP-dependent formation of multimeric G-quadruplexes may regulate transcription *in vivo*, but further experiments will be needed to test this idea.

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SIRNA INHIBITION OF TISSUE FACTOR EXPRESSION REDUCES LIVER ISCHEMIA AFTER PANCREATIC ISLETS TRANSPLANTATION IN RAT

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After infusion into the portal vein, transplanted pancreatic islets (PI) exposed to recipient´s blood trigger the instant blood-mediated inflammatory reaction (IBMIR), characterized by platelet consumption, activation of the coagulation cascade, and complement system. This results in a loss of up to 60% of transplanted islets, formation of a thrombus, and local ischemic lesions in liver during a short time after transplantation (Tx). IBMIR is caused by molecules of tissue factor (TF) abundantly expressed in PI cells and culminates 90 – 120 min after Tx. Temporary inhibition of TF expression using RNA interference could reduce the IBMIR, thus improving the engraftment of PI and so the results of PI Tx as a therapeutic method. In our study, isolated pancreatic islets were transfected with anti-TF siRNA using microporation and transplanted into the rat liver to determine the effect *in vivo*. Hence, an experimental model for quantification of non-perfused liver tissue after PI Tx using magnetic resonance imaging (MRI) was created.

Brown Norway rats (~250 g, male) were used as donors and recipients. PI were isolated using collagenase digestion followed by separation in discontinuous density gradient. After overnight cultivation, PI were microporated using Neon® Transfection System (250 V/ 30 ms /2 pulses) with anti-TF siRNA. Next day, 1000 of native or transfected islets were transplanted into the liver of healthy or STZ-diabetic rat with ligated hepatic arteries (*a. hepatica propria* and *a. hepatoesophagica*). 2 h after Tx, the extent of liver ischemia was measured using contrast-enhanced MRI. The volume of non-perfused liver tissue was quantified using the digital image analysis with ImageJ software. Long-term function of islets was confirmed by monitoring of glycemia.

Ligation of hepatic arteries enables to measure the extent of rat liver ischemia after PI Tx using MRI and to quantify the amount of non-perfused liver tissue through the volumetry method, thus providing a possibility of evaluating the IBMIR level *in vivo*. Transfection of anti-TF siRNA into PI cells reduces the TF-mRNA level to 25 % after 24 h. 2 h after transplantation, the extent of liver ischemia was significantly reduced (from 25 to <10 % of liver tissue) with anti-TF siRNA transfected islets compared to non-treated islets indicating lower level of IBMIR. Transfected as well as native islets restored normoglycemia in diabetic rats in a long term.

This work was supported by grants GAUK #108214, GAUK #34216, MHCZ IKEM IN 00023001, and Czech Ministry of Health, grant no. 16-28249A.

ROLE OF CERAMIDE STEREOCHEMISTRY IN MICROSTRUCTURE, PHASE BEHAVIOR AND PERMEABILITY OF MODEL SKIN BARRIER MEMBRANES

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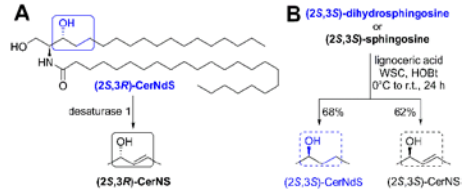
Ceramides (Cer) are key blocks in the intercellular space of the *stratum corneum*, the outermost layer of the mammalian skin. Cer prevent the penetration of unwanted substances and the loss of body water. To elucidate our simple question, *i.e.*, how important is the correct stereochemistry of skin Cer, we aimed to determine the effect of the change of C3 Cer stereochemistry on the skin barrier properties[1,2].

The synthesis of non-physiological (2*S*,3*S*)-(dihydro)Cer is described in Scheme 1. Barrier properties of both synthesized Cer and their physiological (2*S*,3*R*)-diastereomers were studied in model lipid membranes (Cer/free fatty acids C_{16}-C_{24}/cholesterol/cholesteryl sulfate). To elucidate the behavior of Cer diastereomers, microstructure of the model membranes was investigated by X-ray powder diffraction and ATR-FTIR (+deuterated lipids). Additionally, permeability of model membranes was assessed in Franz diffusion cells by using permeability markers including the opposition to electric current and water loss through the membrane. The change of configuration at C3 led to higher permeability of the model membranes; the highest values of water loss were found in (2*S*,3*S*)-CerNdS-based membranes. Additionally, using ATR-FTIR we found out that non-physiological (dihydro)Cer did not mix well with deuterated free fatty acids. This study confirmed that the physiological skin (dihydro)Cer stereochemistry is essential for the skin barrier function.



Scheme 1. **(Dihydro)Cer diastereomers. Panel A shows physiological Cer biochemistry and panel B shows the synthesis of non-physiological (dihydro)Cer**

This work was supported by The Czech Science Foundation (13-23891S) and Charles University (SVV 260 291).

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TUFTSIN-BASED OLIGOPEPTIDE CARRIERS FOR ANTIMYCOBACTERIAL ACTIVE COMPOUNDS

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Drug delivery systems (DDS) may help to overcome undesired properties of bioactive molecules, e.g., low solubility or selectivity. We have studied tuftsin-based oligopeptides as potential carriers for small antimicrobial compounds. Tuftsin derivatives are nontoxic and non-immunogenic. They enhance immune response and target specifically macrophages, thus increasing cellular uptake, activity and reducing toxicity[1].

Oligotuftsin-based carriers $([TKPKG]_n$, n=1-4) were synthesized by solid-phase synthesis (Fmoc/tBu strategy, rink amide MBHA resin, diisopropylcarbodiimide/HOBt, NMP). N-Terminus and/or side chain lysine ε-amino group(s) were substituted to obtain carriers with various properties. Carboxy-lic acids (acetic, succinic, palmitic etc.) modify lipophilicity, short peptide spacers (G_5, GFLG cleavable by cathepsin B) were used to control the cellular site where the drug is released, whereas fluorescein enables to determine cellular uptake by flow cytometry and fluorescent microscopy. One or more aminooxyacetic acid molecule(s) were coupled with peptides to provide a reactive group for the attachment of active molecules. After these modifications, peptide carriers were cleaved from the resin using 95% TFA and then purified[1].

Salicylanilides share interesting antibacterial properties, but their potential use is prevented due to their limited solubili-ty and a higher toxicity[2]. That is why salicylanilides bearing formyl or acetyl group were selected as model compounds for the evaluation of peptide carriers' efficacy. The conjugation to form acid stable oxime bond was performed in the mixture of acetate buffer/2-ethoxyethanol (1:1; rt, 24-72 h). Novel conjugates were purified and characterised (MS, RP-HPLC, elemental analysis, hydrolytic stability)[1].

These conjugates exhibited a significant *in vitro* extracellular antimycobacterial activity including for drug-resistant strains (*Mycobacterium tuberculosis*, *M. abscessus*). Moreover, they are sharply more effective against intracellular mycobac-teria than parent salicylanilides. Cellular uptake has increased substantially, too. Cytostatic and cytotoxic effects on mamma-lian cells are mostly alleviated. In conclusion, our tuftsine peptides are perspective carriers for antimycobacterial agents.

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ROLE OF CYS RESIDUES IN THE ASK1 REGULATION BY THIOREDOXIN

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Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. The function of ASK1 is

associated with the activation of apoptosis and thus it plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration and cardiovascular diseases. The kinase activity of ASK1 is regulated by many factors, including binding of thioredoxin (Trx) and the 14-3-3 protein that both function as inhibitors of ASK1. However, the mechanisms by which these binding interactions inhibit ASK1 are still unclear.

The aim of this study was to investigate the role of individual cysteine residues from TRX1 as well as from ASK1-TBD in the interaction between these two proteins. Sedimentation velocity analysis together with the site-directed mutagenesis revealed that from five cysteine residues in human TRX1 molecule, the residue Cys32 is crucial for TRX1 binding to ASK1-TBD in reducing conditions. Formation of disulfide bond between Cys32 and Cys35 has a major effect on complex dissociation under oxidative stress. ASK1-TBD contains seven Cys residues from which Cys200, Cys206, Cys250 and one from the pair Cys225 or Cys226 seem to be well accessible at the surface of the ASK1-TBD molecule. Oxidation of ASK1-TBD leads to the formation of two intramolecular disulfide bonds, Cys200-Cys206 and Cys225-Cys226, which induce subtle but significant structural changes of ASK1-TBD. On the other hand, serine-scanning mutagenesis of ASK1-TBD showed that Cys250 is the only cysteine residue which is critical for the stability of ASK1-TBD:TRX1 complex[1,2].

This work was supported by The Czech Science Foundation (Project 14-10061S) and The Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology).

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SECRETED SERINE PROTEASE SMSP2 OF THE BLOOD FLUKE *Schistosoma mansoni*: BIOCHEMICAL CHARACTERIZATION, LOCALIZATION AND HOST PROTEIN PROCESSING

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Schistosomiasis caused by parasitic blood flukes of the genus Schistosoma is the second most important parasitic infection after malaria with more than 240 million people infected. Treatment relies on a single drug – praziquantel. Hence, there is a pressing need to develop additional therapeutics. Our work is focused on *S. mansoni* serine protease 2 (SmSP2). It was localized in the tegument and esophageal glands, ovaries, testes and vitelaria of adult schistosomes by immunofluorescence microscopy and *in situ* RNA hybridization. Enzyme activity measurements and immunoblotting identified SmSP2 in the excretory/secretory products. Recombinant SmSP2 was produced in the *Pichia pastoris* expression system and its cleavage specificity was investigated using combinatorial substrate libraries and 3D model analysis. SmSP2 was found to activate plasmin, the key component of the fibrinolytic system, and releases vasoregulatory kinins from kininogen. Our results suggest that SmSP2 plays a role in host-parasite interactions and represents a potential target for inhibitory drugs.

This work was supported by the grant LD15101 and the project InterBioMed LO1302 and the institutional project RVO 61388963.

TRANSDIFFERENTIATION OF NON-ENDOCRINE PANCREATIC CELLS

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Transdifferentiation of non-endocrine pancreatic cells (NEPCs) into insulin-producing cells (IPCs) represents a promising approach for the treatment of diabetes mellitus. Transdifferentiation towards IPCs requires effective delivery of specific bioactive molecules into appropriate cells and their nuclei. However, not all methods of the bioactive molecules administration are safe and efficient enough for clinical applications.

Here we report a comparison of two DNA non-integrating methods applicable for various cell manipulations. We have evaluated the efficiency of the intracellular delivery and activity of Cre recombinase (Cre) either in a form of synthetic mRNA (syn-mRNA-Cre) or fusion recombinant protein containing protein transduction domain (PTD-Cre). The efficiency of Cre was determined using genetically modified HEK293, BRIN-BD11 and PANC-1 cell lines.

All three cell lines were derived by integration of RFP loxP STOP loxP GFP sequence by piggyBac plasmid. Synthetic mRNA coding for Cre were prepared by MEGAscriptT7 Kit. PTD-Cre was expressed in competent E. coli. Cell lines (n=3)

were transfected with syn-mRNA-Cre by Lipofectamine MessengerMAX mRNA Transfection Reagent. PTD-Cre was added directly into the culture medium. The efficiency of Cre was determined by the detection of GFP expression by flow cytometry. GFP expression was induced by Cre upon DNA recombination and excision of stop codon preceding the GFP gene.

At the maximum concentrations, PTD-Cre (15 μM) achieved appreciable recombination only in BRIN-BD11 cells (48%). In contrast, syn-mRNA-Cre (2.1 nM) induced recombination in between 29% and 71% of nuclei in all the three cell types. Repeated administration twice in two days significantly increased efficacy only in samples where the recombination was already successful with single administration and the efficiency of syn-mRNA-Cre method at the maximum concentration (2.1 nM) ranged between 66% and 90% depending on the cell type. We conclude that the syn-mRNA-based method is more robust, less cell-type dependent, and required less preparation than the PTD-fusion alternative for delivering DNA-targeting proteins to cell nuclei.

Our data indicate that transfection with bioactive molecules such as mRNA represents a promising and safe approach for cell manipulation. Moreover using mRNA is effective enough for IPCs generation.

This work was supported by Ministry of Health of the Czech Republic, grant nr. 15-25924A and by MH CZ - DRO ("Institute for Clinical and Experimental Medicine - IKEM, IN 00023001")

RUTHENIUM COMPLEXES CONTAINING CHIRAL POLYFLUOROOXAALKANOATES

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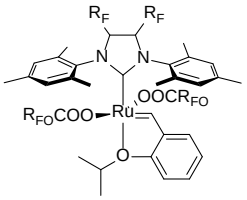
Alkene metathesis, a modern way how to prepare compounds with a strictly defined double bond, becomes increasingly popular both in organic chemistry and in pharmaceutical industry. As the main challenge, required expensive and toxic ruthenium catalysts have to be separated and preferably recycled. By an introduction of poly- or perfluoroalkanoate chains to the Hoveyda-Grubbs 2nd generation catalyst (***HG-II***)[1], medium fluorous separation method can be used with advantage.

In my work, I dealt with a synthesis of ***HG-II*** modified with racemic poly- or perfluorooxaalkanoates as anionic ligands. I prepared four complexes ***1a-d*** with medium fluorous properties and reasonable activity in model ring closing metatheses[2]. Moreover, the precatalysts ***1b*** and ***1d*** could be efficiently recycled.



1a: R_F = H, R_{FO} = CHF-O-C_3F_7
1b: R_F = $CH_2CH_2C_6F_{13}$, R_{FO} = CHF-O-C_3F_7
1c: R_F = H, R_{FO} = CF(CF$_3$)-O-C_3F_7
1d: R_F = $CH_2CH_2C_6F_{13}$, R_{FO} = CF(CF$_3$)-O-C_3F_7

My recent work concentrated on the separation of starting racemic fluoro acids into pure enantiomers, which served as a novel economical approach to chiral alkene metathesis precatalysts. Among several approaches employed, namely resolution of diastereomeric ammonium salts and separation of diastereomeric esters or amides by column chromatography, the former method gave best results providing enantiomerically enriched fluoro acids. Its transformation to silver salt, followed by reaction with commercial ***HG-II*** precatalysts results in enantiomerically enriched complexes as ***1a***.

This work was supported by the Grant Agency of the Czech Republic (reg. No. 207/10/1533) and specific university research (MSMT No 20/2015, MSMT No 20/2016).

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DIELS-ALDER APPROACH TO SUBSTITUTED DECALINES

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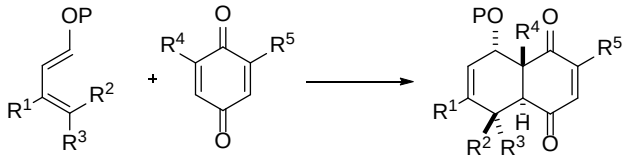
A plethora of natural products, namely terpenes and steroids, contain the decaline scaffold.[1] Herein, we report a Diels-Alder approach[2] to construct substituted decalines **3** bearing substituents R^1-R^5 on both rings A and B (Scheme 1).

Preparations of dienes **1** and non-trivially substituted quinones **2** are described, along with the conversion of the initially formed cis-decalines to the thermodynamically more stable trans-isomers and manipulation of some functional groups.



P = protecting group

Scheme 1. **Synthesis of substituted decalines**

Newly prepared decalines are envisioned to be used as intermediates in the synthesis of selected natural products and their analogs.

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SYNTHESIS OF NOVEL SALICYL-DIPEPTIDES WITH ANTIPROLIFERATIVE PROPERTIES

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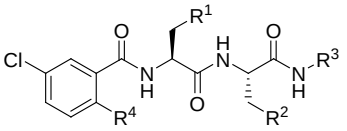
The uncontrolled growth of cells is disease which resulting in different life-threatening diseases generally called cancer. Chemotherapy is a one type of cancer treatment but it has a serious problem to developing resistance to approved drugs and therefore there is an urgent need for new drugs combinations, that will be more specific and with less side effects[1]. Recent literature described aliphatic salicylamides as epidermal growth factor receptor tyrosine kinase inhibitors[2]. In our previous research, we described salicylamide derivatives, with significant antiproliferative effects against cancer cell lines as well as induced apoptosis in melanoma cell line in single-digit micromolar concentrations[3]. Further our group investigated antiproliferative activity of new series of salicylamide terminated with aliphatic chain[4]. On the basis of these interesting results, we synthesized new analogous of salicylamides including 5-chlorosalicylic acid and neutral optically pure amino acids (L-Leu and L-Phe) eventually dipeptide (Fig. 1.). We have tested antiproliferative activity of all prepared salicylamide derivatives against five different cancer cell lines i.e. K562, CEM, MCF7, G361, THP-1.



R^1, R^2 = *i*-Pr, Ph; R^3 = subsituted aniline;
R^4 = O-Bn, OH

Fig. 1. **General structure of novel salicyl-dipeptide amide**

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OXIDATIVE INTERMEDIATE SWITCHING FOR THE TOTAL SYNTHESIS OF LIGNANS

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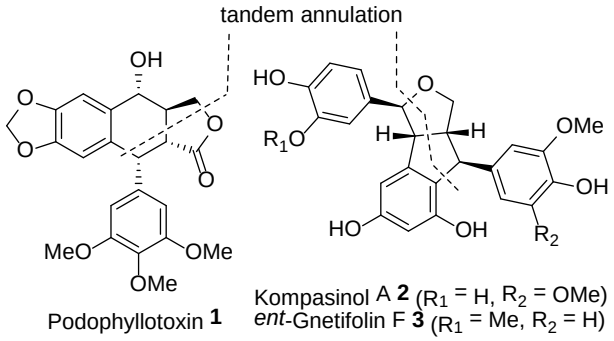
Podophyllotoxin (***1***) is a prominent member of the lignan family of natural products. The anti-tumor activity of the parent compound has been known for a long time and its glycosides Etoposide and Etopophos were succesfully commercialized[1]. Its derivatives have also been found to inhibit HIV replication[2]. Kompasinol A (***2***) and Gnetifolin F (***3***) are related members of a unique stilbenolignan subfamily that show diverse bioactivities like α-glucosidase inhibition and antiinflammatory TNF-α inhibition[3].

Despite having different biosynthetic origin, the structures of both aryltetralone lignans like ***1*** and indane stilbenolignans like ***2***, ***3*** reveal some structural similarities that may be exploited to develop a unified synthetic strategy. Because the targets are in high demand due to their low natural abundance and interesting bioactivities, a practical and atom-efficient synthesis is highly sought-after. Redox switching

between intermediates of different oxidation states enables sequencing of bond forming reactions by exploiting the orthogonal reactivity of carbanions, C-centered radicals and carbocations towards electron-rich versus electron-deficient π-systems, leading to the formation of multiple bonds and stereocenters in one synthetic operation - thus rapidly increasing molecular complexity and cutting costs. Details on methodology development and initial results toward total synthesis will be discussed in this contribution.



Podophyllotoxin **1**

Kompasinol A **2** (R_1 = H, R_2 = OMe)
ent-Gnetifolin F **3** (R_1 = Me, R_2 = H)

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IDENTIFICATION OF dsRNA MYCOVIRUSES IN PLANT-PATHOGENIC FUNGUS *FUSARIUM OXYSPORUM*

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Fusarium oxysporum is a plant pathogenic fungus causing Fusarium wilt. Persistence in soil, inefficient chemical control and lack of active treatment make this fungus a major cause for concern in agriculture worldwide[1]. The disease is particularly destructive towards tomatoes in greenhouses, tobacco field cultivation, and perhaps most importantly, banana plantations in the tropics, where a new strain endangers the dominant Cavendish banana[2]. In this study, we focused on viruses infecting *Fusarium oxysporum*, exploring the possibility of using these so called mycoviruses to debilitate the fungus, thus protecting plants from disease.

As a marker of fungal virus presence, we used dsRNA, which does not occur in cellular organisms, but is a constituent of most fungal viruses[3]. Two independent methods of dsRNA isolation have been used to indicate virus presence in isolates[4,5]. *F. oxysporum* strains were obrained from the Czech Collection of Microorganisms in Brno. Fungal strains positive and negative for dsRNA were then compared in terms of growth, microscopic and macroscopic morphology. Pathogenity on plants was tested by inoculating *Arabidopsis thaliana* plants with mycelium plugs. Viral dsRNA was reverse transcribed, amplified and cloned into *E. coli* DH5α for later sequencing.

From four fungal strains tested, one was conclusively dsRNA-positive, and thus marked as mycovirus-positive. No significant differences were observed in terms of growth or microscopic appearance. However, when inoculated in soil, only the fungus lacking dsRNA caused disease and subsequent wilt in plants. *Arabidopsis thaliana* inoculated with fungus containing dsRNA showed no signs of wilt or disease.

Our work investigated using mycoviruses to decrease virulence of plant pathogenic fungi. We see great potential in employing a yet uncharacterized fungal virus in controlling the spread of Fusarium wilt.

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THE NATURE OF STABLE EXPRESSION OF THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

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Transcription of the provirus – the host genome-integrated form of retroviral genome – is, together with integration, the central step of retroviral replication cycle. Regulation of transcription plays key role in the expression of proviral genes and is often the target of epigenetic silencing. Cells that carry silenced proviruses thus show the phenotype of latently infected cells. Latent proviruses are of particular interest in the research of human immunodeficiency virus (HIV). Patients infected with HIV and treated with

antiretroviral therapy (ART) can ostensibly clear the virus from blood to undetectable levels. However, once the ART is interrupted, the virus can rebound and spread again in the patient. Existence of latent infection is thus one of the major obstacles toward the cure of HIV infection today.

Despite of the comprehensive research the features of HIV-1 expression including its stability, sensitivity to and timing of silencing or the effect of HIV-1 expression on cellular physiology are still questionable.

In our projects, we aim to describe the effects of integration-site on the stability of the expression of retroviruses. For HIV-1 we use T-lymhocyte Jurkat cell line transduced with single-round minimal or full lenght retroviral vectors encoding for fluorescent markers of expression. Possibility of selection for marker expression, single-provirus cellular clones establishement and tracking of expressing cells by the flow cytometry give us the opportunity to follow the long-term activity and silencing of HIV-1 on the level of single proviruses.

Experiments with minimal HIV-1-derived vector show that the majority of the proviruses selected for expressional activity 3 days post infection (dpi) keep stable expression as long as 60 dpi. Using whole genome HIV-1-derived vector, however, showed rapid decrease of count of virus-expressing cells in the population in the first week after infection. Moreover, virus expressing cells had only limited ability to establish cellular clones. Surprisingly, replacement of the *gag* gene in viral genome with fluorescent marker restored the ability of expression-positive cells to sustain in the population. Stability of proviral expressional activity was also observed in established cellular clones.

Our results show that HIV-1 expression, once active after integration, is stable and not affected by silencing. We also show that *gag*-expressing virus is unable to keep stable expression in the population of dividing cells indicating the new mechanism of toxic effect of HIV-1 expression.

This work was supported by grant GA14-34873S of GAČR and grant 816216 of GAUK.

FANCONI-ANAEMIA ASSOCIATED RAD51 MUTATIONS IMPAIR REPLICATION FORK PROTECTION DUE TO DESTABILIZATION OF RAD51 FILAMENT

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Fanconi anaemia (FA) is a genetic disorder characterized by chromosomal instability and predisposition to cancer. FA pathway coordinates complex mechanism for the proper repair of DNA interstrand crosslinks (ICLs)[1]. Recently, mutations in RAD51 recombinase were reported to cause FA-like phenotype due to a nucleolytic over-processing of ICL-repair intermediates[2,3]. It remains unknown whether FA-associated RAD51 mutations display defect in the prevention of nascent DNA during normal replication and what is the exact molecular mechanism by which they alter RAD51 nucleoprotein filament properties.

Here, we report that FA-associated RAD51 mutations impair protection of nascent DNA strands from Mre11 degradation during unperturbed replication and in response to replication fork stalling in *Xenopus laevis* egg extract. Reconstitution of DNA protection *in vitro* using synthetic DNA substrates confirmed, that the defect arises directly due to properties of the mutated RAD51 filaments. Using stopped-flow technique, we show that FA-associated mutations cause destabilization of RAD51 filament. Moreover, visualization by 3D electron microscopy show pronounced structural changes within the mutant RAD51 filaments that are not stabilized by the prevention of ATP hydrolysis due to the aberrant ATP binding and/or hydrolysis.

Altogether, our results interconnects FA pathway with replication fork protection and provide a novel mechanistic insight into our understanding of the RAD51 role in recombination-independent mechanism of genome maintenance.

This work was supported by Czech Science Foundation (GA17-17720S, GA13-26629S and GAP207/12/2323), the European Regional Development Fund - Project FNUSA-ICRC (no. CZ.1.05/1.1.00/02.0123)

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CATIONIC CARBOSILANE DENDRIMERS FOR BIOAPPLICATIONS

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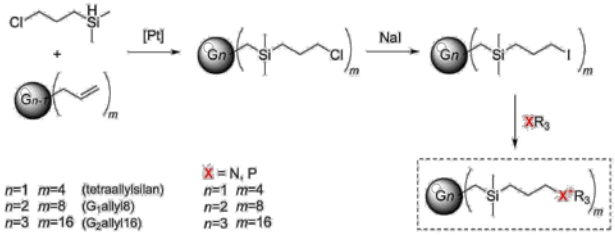
Carbosilane dendrimers represent three-dimensional branched molecules with defined structure and accurate molar weight. With their unique properties, carbosilane dendrimers are under intensive investigation for many biomedicinal applications, mainly for drug targeting and gene therapies. Carbosilane dendrimers with cationic groups at their periphery are able to interact with biomolecules and cellular structures. Scientific research in the area of gene therapies depends on the development of specific, non-toxic and secure vectors for therapeutic delivery of nucleic acids. For that purpose, carbosilane dendrimers mainly with ammonium groups were investigated[1]. Carbosilane dendrimers bearing other cationic groups attracted little attention so far.

The study is focused on the synthesis and chemical properties of cationic carbosilane dendrimers (1.-3. generation) with nitrogen- and phosphorus- onium groups at periphery[2, 3]. In addition, biochemical properties including cytotoxicity and the ability to interact with biomolecules (DNA, RNA) and cell structures have been evaluated in cooperation with biology department of the University of J. E. Purkynje in Ústí N/L[3].



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

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FLAVONOIDS AS INHIBITORS OF OATP-MEDIATED ROSUVASTATIN INTESTINAL TRANSPORT

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Statins represent the first-choice therapy for hypercholesterolemia. They are orally administered and therefore their bioavailability could be influenced by intestinal transporters. Absorption of statins from human intestine involves transport by the organic anion-transporting polypeptides (OATPs) localized at the apical membrane of enterocytes, namely hOATP2B1 and hOATP1A2. Some dietary flavonoids have been shown to inhibit hOATP transport function. Therefore, a potential influence of food flavonoids on absorption of statins mediated by hOATPs should be considered as a factor able to affect their pharmacokinetics.

The aims of the study were to confirm the active transport of rosuvastatin into enterocytes *ex vivo* and determine the potency of selected flavonoids (galangin, chrysin and pinocembrin) inhibit the hOATP2B1- or hOATP1A2-mediated transport of rosuvastatin *in vitro*.

Precision-cut intestinal slices prepared from the rat ileum were used for evaluation of the active transport of rosuvastatin. For this purpose the uptake of rosuvastatin into slices was compared at 37 °C and 4 °C. The MDCKII cells transiently transfected with hOATP2B1 and the HEK293 cells transiently transfected with hOATP1A2 were used for studying the inhibitory potency of selected flavonoids on rosuvastatin uptake.

We verified the active character of rosuvastatin transport into enterocytes since significantly lower accumulation of rosuvastatin into rat intestinal slices had been found at the temperature inhibiting energy-dependent transport processes. Both hOATP2B1 and hOATP1A2 transporters were able to transport rosuvastatin into the cells. All tested flavonoids showed the concentration-dependent inhibitory effect on hOAP2B1- and hOAP1A2-mediated rosuvastatin transport, with higher potency towards hOAP2B1. The most potent inhibitor of rosuvastatin transport was galangin (with IC_{50} 0.74 µM for OAP2B1 and 21.5 µM for OAP1A2), followed by chrysin (IC_{50} values: 2.3 µM for OAP2B1 and 63.5 µM for OAP1A2) and pinocembrin (IC_{50} values 8.9 µM for OAP2B1). The found results suggest a potential of the tested flavonoids for food-drug interactions in the human intestine at the level of absorption.

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DISCOVERY OF NEW PROTEIN KINASE INHIBITORS WITH THE FURO [3,2-*b*]PYRIDINE CORE

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Development of new protein kinase inhibitors has been a very active field in the academic as well as in the industrial sector. Up to date, 30 compounds that are currently clinically used have been identified[1]. The central hypothesis of our project was that the furo[3,2-*b*]pyridine motif could serve as a proper bioisostere of the pyrazolo[1,5-*a*]pyrimidine pharmacophore, which was successfully used in numerous series of potent and selective inhibitors of various protein kinases[2]. Interestingly, only a few series of furo[3,2-*b*]pyridine-based protein kinase inhibitors were documented in the (patent) literature[3,4]. In addition, furo[3,2-*b*]pyridines with NHR substituents at the 7 position, which are generally important for the interaction with the hinge regions of kinases, were not known at all. In order to prepare the initial set of furo[3,2-*b*]pyridines with particular substitutions patterns at positions 3, 5, 6 and 7, we optimized two known methods to assemble the furo[3,2-*b*]pyridine core and developed one new annulation methodology. While some direct analogs of known pyrazolo[1,5-*a*]pyrimidine inhibitors proved to be less potent, the series with proper substituents at positions 3 and 5 of the furo[3,2-*b*]pyridine scaffold contained some highly potent (IC_{50} < 20 nM) and selective inhibitors of CLK and HIPK kinases, which emerged only recently as possible therapeutic targets.

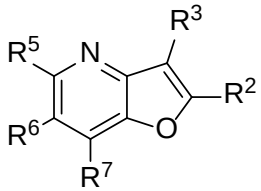

Fig. 1. **Furo[3,2-*b*]pyridine scafold**

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Enterococcus faecalis PROTEOLYTIC ACTIVITY: THE MECHANISM OF RESISTANCE TO ANTIMICROBIAL PEPTIDES

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Antimicrobial peptides (AMPs) are promising substances with the potential to replace common antibiotics, especially in the treatment of non-healing infections. Such infections (e.g. those caused by enterococci) are characterised by the presence of biofilm and increased resistance to antibiotics.

In this study, we focused on the mechanism of *Enterococcus faecalis* resistance to a short α-helical peptide, GILSSLWKKLKKIIAK-NH_2 (HYL-20), a synthetic analogue of a natural AMP previously isolated from wild bee venom[1]. This mechanism consists of the secretion of two - gelatinase (GelE) and serine protease (SprE) – able to degrade the peptide. Using RP-HPLC, we analysed proteolytic degradation of HYL-20 by three different strains of *E. faecalis*. We isolated the degradation fragments, identified them by ESI-Q-TOF MS and determined all cleavage sites. Furthermore, we investigated HYL-20 degradation after its incubation with *E. faecalis* in the presence of 1,10-phenanthroline, a specific GelE inhibitor. Our results suggest that unexpected and yet unidentified protease catalyse deamidation of C-terminal Lys and makes HYL-20 susceptible to subsequent intramolecular cleavage of Lys-Ile and Lys-Leu peptide bond by GelE. However, the involvement of SprE to the process was not proved. To confirm our findings, we designed and prepared a peptide with C-terminal Lys in D-configuration (HYL-20*k*) that resisted to degradation by *E. faecalis* and showed improved antimicrobial activity. The effect of the modification on secondary structure of the peptide was studied by NMR spectroscopy. In addition, we demonstrated that both these peptides, unlike vancomycin, eradicate also *E. faecalis* settled in biofilms. Finally, transmission electron microscopy showed the peptides cause bacterial membrane breakage leading to the loss of cell content.

The present work illustrates a multi-perspective approach to new antibacterial drug design based on studying pathogen-drug interaction.

This work was supported by the TA CR (TA04010638), Czech Health Research Council (16-27726A), and IOCB, AV CR

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THE REGULATION OF INTRACELLULAR SIGNALLING IN EMBRYONIC STEM CELLS BY HYPOXIA

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Hypoxia, the lack of oxygen, is involved in the regulation of stem cell fate. Hypoxia inducible factor 1 (HIF-1) is supposed to be a master regulator of hypoxic response[1].

Here, we focus on the effect of hypoxia on intracellular signalling pathways responsible for mouse embryonic stem (ES) cell maintenance[2,3]. We employed wild type and HIF-1α-deficient ES cells to investigate hypoxic response in the phosphorylation of proteins of various intracellular signalling pathways, including the ERK, Akt and STAT3 pathways. Cultivation in 1% O_2 for 24h resulted in the strong dephosphorylation of ERK and its upstream kinases and to a lesser extent of Akt in an HIF-1-independent manner, while STAT3 phosphorylation remained unaffected. The downregulation of ERK could not be mimicked either by the overexpression of HIF-1α or HIF-2α or by pharmacologically induced hypoxia using hypoxia mimetics (DMOG, $CoCl_2$, DFO, JNJ-42041935).

Dual-specificity phosphatases (DUSP) 1, 5 and 6 are hypoxia-sensitive MAPK-specific phosphatases involved in ERK downregulation, and protein phosphatase 2A (PP2A) regulates both ERK and Akt[4,5]. However, combining multiple approaches, we revealed only the limited significance of DUSPs and PP2A in the hypoxia-mediated attenuation of ERK signalling. Interestingly, we observed a decreased reactive oxygen species (ROS) level in hypoxia and a similar phosphorylation pattern for ERK when the cells were supplemented with glutathione. Therefore, we suggest a potential role for the ROS-dependent attenuation of ERK signalling in hypoxia, without the involvement of HIF-1.

We are grateful to prof. L. Poellinger and prof. A. Miyajima for providing the plasmids. The work was funded by the Faculty of Medicine of Masaryk University (Grant MUNI/A/1558/2014), the Ministry of Education, Youth and Sport of the Czech Republic (the project no. LQ1605 NPU II; project MSM0021622430, and by collaboration between Masaryk University and the Karolinska Institutet under the Project KI-MU CZ.1.07/2.3.00/20.0180.

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DEVELOPMENT AND CHARACTERIZATION OF THE UREA DERIVATIVES WITH UNIQUE EFFICIENCY IN DELAYING PLANT SENESCENCE

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According to the present state of the art, cytokinins are key plant antisenescence hormones[1]. Despite this highly applicable function their use in plant biotechnology is affected due to some undesirable side effects, such as the inhibition of root growth and development[2]. We present design and synthesis of a series of thidiazuron derivatives, which are more potent in delaying plant senescence than classical cytokinins and, interestingly, do not show the above-mentioned negative effect. The later fact is most probably connected with their very low cytokinin activity as revealed by screening in molecular and classical cytokinin bioassays, respectively. Most of the compounds did not activate Arabidopsis cytokinin receptors AHK3 and CRE1/AHK4 *in vitro* and only poorly activated cytokinin primary response gene ARR5 in *A. thaliana in vivo*. Notwithstanding the further research is needed to understand the mode of the action of these compounds. To the best of our knowledge the most effective compound (ASES) is more potent in delaying plant senescence than any other compounds described in the literature. We are convinced that thanks to unique antisenescence activity, the compounds could have prominent utilization in agriculture as plant stress protectants.

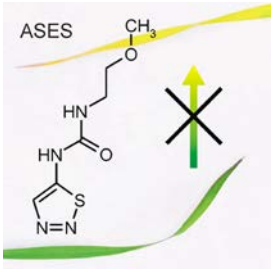

Fig. 1. **Structure of the ASES**

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SYNTHESIS AND CHARACTERIZATION OF NOVEL PORPHYRIN-OLEALONIC ACID CONJUGATES LINKED BY A 1,2,3-TRIAZOLE RING

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Porphyrins natural products, studied over the last few decades due to their importance. Photodynamic therapy (PDT) is one their application; they can act as photosensitizers activable with visible light to locally produce a highly reactive singlet oxygen radical, killing tumour cells. The photophysical properties of porphyrins allow visualization of their localization as well as of their conjugates *in vitro* and *in vivo*. The application of PDT has been limited, for several reasons: i) skin photosensitivity; ii) its limitation in treating tumours that are on or just under the skin[1]; iii) their inability to penetrate the cell membrane[2]. Consequently, light exposure mostly damages the tumour cell-wall, but rarely the nucleus of target cells[3].

We report a synthesis of novel dendritic molecules in high yields with improved water solubility bearing a porphyrin core, triterpenic acid units, and linkers by a "click chemistry" approach. Our compounds have two properties: (i) acting as a photosensitizer agent for PDT and (ii) displaying cytotoxicity in another part of molecule in the inner part of tissue. Triterpenoids have been chosen for their rigid and chiral framework, potential for functionalization, broad biological activity profile, ability to penetrate the cell membrane and bind to specific hormonal receptors[4]. For the key reaction, we chose copper-catalyzed azide-alkyne cycloaddition (CuAAC) due to its selectivity and the ability of triazole to mimic amide bond.

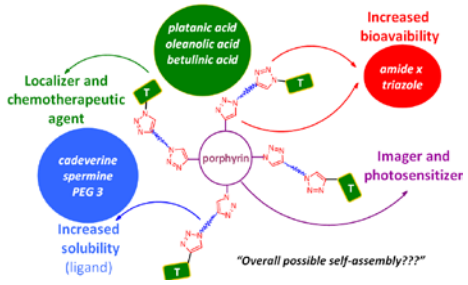

Fig. 1. **Rationale for synthesis of porphyrin dendritic molecules**

Funding: COST LD15012 (MSMT) and FV10599 (MPO).

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CREATION OF THE ARTIFICIAL CAVITY FOR SUBSEQUENT TRANSPLANTATION OF PANCREATIC ISLETS USING PLCL SCAFFOLD

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The outcomes of pancreatic islet (PI) transplantation into the portal vein of liver is still not optimal and therefore there is a need to search a better alternative site for PI administration to optimize the PI engraftment and function. One of the promising approaches is a creation of adequately pre-vascularized artificial cavity using a microporous scaffold implanted into subcutaneous tissue or into the greater omentum. The goal of our study was to test the biocompatibility of PLCL (poly-L-lactide-co-ε-caprolactone) scaffold as well as the influence of scaffold coating with heparin- and VEGF on vascular ingrowth.

Capsular-shaped anisotropic channeled porous scaffold was prepared by the thermally induced phase separation technique (Dip TIPS). Its wall was 600 µm thick and inner lumen was 4 mm in diameter. Surface of the scaffold was coated with heparin and VEGF (10 µg). Heparin-coated scaffold (n=3+3) and heparin-VEGF-coated scaffold were implanted under the skin and within the greater omentum of Lewis rats (250-300g) for 2 weeks. Perfusion of scaffolds was detected using DCE MRI after 1 and 2 weeks. Scaffolds were explanted at the end of the study and vascular and fibrous

tissue ingrowth as well as possible inflammatory reaction of the host was evaluated by histology.

Post-operative recovery and behavior of the recipients was adequate. The histological examination did not show any significant infiltration by inflammatory cells, any pathological fibrotic response around the capsules. The infiltration of scaffold by the host's connective tissue was guided through the channeled pores. While in subcutaneously implanted scaffolds the connective tissue did not reach the inner surface, in scaffolds implanted into the greater omentum the fibrous tissue could be detected within the internal cavity. Heparin and VEGF stimulated tissue ingrowth and VEGF stimulated neoangiogenesis.

PLCL scaffolds are biocompatible. Scaffolds implanted in the greater omentum created a promising artificial cavity with rich capillar network at the inner surface for subsequent islet or cell transplantation.

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NEW POSSIBILITIES IN TREATMENT OF FAILING HEART: FOCUS ON INFLAMMATORY SIGNALING PATHWAYS

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Cardiovascular diseases (CVDs), associated with chronic inflammation, are the leading cause of mortality and morbidity worldwide. Inflammation ultimately leads to poorly functioning of heart, resulting in progression of CVDs. It is becoming increasingly evidence that nitration products of unsaturated fatty acids (NO_2-FAs) represent an important class of endogenous biological mediators, which are generated as an adaptive response of organism to oxidative and nitrative stress. The purpose of our study was to define the role of nitro-oleic acid (NO_2-OA) in prevention and treatment of atrial fibrosis and pulmonary arterial hypertension in mice.

The effect of NO_2-OA was tested in different cell types (including macrophages, neutrophils, fibroblasts, endothelial, and smooth muscle cells) both *in vivo* and *in vitro*. Interestingly, our results showed that physiologically-relevant concentrations of NO_2-OA significantly improve the heart functions and overall outcome of animals with atrial fibrosis and pulmonary arterial hypertension. These effects were associated with reduced polarization of macrophages toward pro-inflammatory and immuno-regulatory subsets, decreased activation of different signaling pathways as well as production of pro-inflammatory and pro-fibrotic mediators. Moreover, we have demonstrated that NO_2-OA effectively influenced the process of macrophage differentiation induced by growth factors and thus could regulate their activation phenotype during inflammatory processes.

Further, we have characterized NO_2-OA effects on endothelium response to macrophage and neutrophil derived cytokines with consequent fibrosis development arising from vessel inflammation in different tissues. NO_2-OA prevented pathological activation of endothelial cells characterized by reduced production of pro-inflammatory cytokines and chemokines as well as expression of adhesive molecules. Accordingly, NO_2-OA can prevent transformation of endothelial cells to the pro-fibrotic phenotype through blocking of endothelial-mesenchymal transition triggered by TGF-β. Beside that, NO_2-OA significantly diminished the proliferation of vascular smooth muscle cells.

In aggregate, our study provided the unique results showing the protective effects of NO_2-OA in progression of cardiovascular inflammation, supporting that NO_2-FAs represent new drug candidates suitable for deployment against chronic and inflammatory diseases having a complex pathogenesis. Following main signaling pathways, we also helped to clarify molecular mechanism of nitro-lipids action.

This work was supported by the Czech Science Foundation (17-08066Y), by the Ministry of Education, Youth and Sports (LD15069), and is based upon work from the COST Action BM1404 Mye-EUNITER (www.mye-euniter.eu), supported by COST (European Cooperation in Science and Technology).

THE ROLE OF MICRORNA MOLECULES IN CELL PLASTICITY REGULATION

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Reprogramming somatic cells to induced pluripotent stem (iPS) cells represents a promising tool for generating autologous cell lines suitable for biomedicine research and therapy. However, low efficiency of reprogramming process, retention of epigenetic memory, partial reprogramming, increased genomic instability or possible tumorigenicity and immunogenicity represent the major hurdles for potential therapeutic applications of iPS cells[1]. Recently, a novel approach for cell reprogramming has been established, where dedifferentiated/plastic state is induced in somatic cells. In such plastic state, cells are open for external signals for differentiation towards cell line of interest. Using this approach, clinically relevant cell types can be obtained without passing through pluripotent state which eliminates the limitations associated with the use of iPS cells[2].

In this work, we study the role of microRNA (miRNA) molecules in plastic cell state induction, as miRNAs represent critical regulators of gene expression and are involved in number of cellular processes including cell fate specification. To determine which miRNAs are involved in cell plasticity regulation, we performed next generation sequencing of human dermal fibroblasts (hDFs) and hDFs in plastic state. Selected candidates were either up-regulated or inhibited in hDFs and tested for their ability to contribute to induction of plastic state. Our novel approach of miRNA modulation in differentiated cells will help to elucidate the mechanisms underlying cell fate switch and could be also beneficial for development of safe new protocols for generating cells of high clinical importance.

This work has been supported by the projects MUNI/A/1369/2016 (Masaryk University), GJ16-24004Y (Czech Science Foundation), and Brno Ph.D. Talent.

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THE ROLE OF MICRORNA-145 IN DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS-DERIVED NEURAL STEM CELLS

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Human embryonic stem cells (hESCs) are pluripotent stem cells with the ability to differentiate into any cell types of human body. MicroRNAs have been linked with the key stem cell properties - self-renewal and pluripotency. The factors included in modulation self-renewal and pluripotency are Sox2 and Oct4. The studies show that microRNA-145 downregulates these factors and that the level of microRNA-145 is low in hESCs and it only increases after differentiation[1]. Thus, our aim was to study the role of microRNA-145 in differentiation of hESCs to neural stem cells (NSCs) and neural progenitor cells (NPCs). We confirmed that the level of microRNA-145 is low in hESC. Surprisingly, we found that its expression is also low in NSCs and increases only after differentiation to NPCs. Therefore, we decided to overexpress the level of microRNA-145 in hESCs and NSCs by transfection of microRNA-145 mimic. The results from the western blot showed that the level of Sox2 and Oct4 after transfection was low in undifferentiated hESC. However, the level of Sox2 remained high in self-renewing NSCs. Curiously, the results from the real-time PCR screen confirmed the high level of microRNA-145 and mRNA for Sox2 in NCSs. Studies are therefore ongoing to reveal the role of microRNA-145 in self-renewing NSCs. This might help to uncover the molecular pathway lying behind the maintenance of self-renewing neural stem cells.

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NOVEL 'ROOM-TEMPERATURE SMECTICS': SYNTHESIS AND OPTICAL PURITY–MESOMORPHIC BEHAVIOUR RELATIONSHIP

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Liquid crystals (LCs) are frequently used as stationary phases in different types of chromatography[1], but there is only a small number of studies that concern chiral LCs as analytes[2,3] and there is no such report on using SFC for LCs analysis/separation.

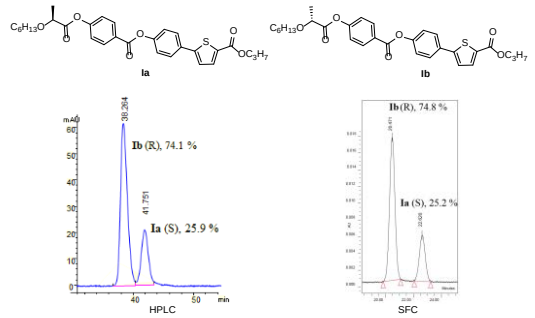

Fig. 1. **Materials structures and chromatograms of LC mixtures in HPLC and SFC mode**

In this study, we have optimized the synthesis of L-lactic acid-based chiral intermediates, which we have used in the preparation of novel room temperature ferroelectric LCs. We have chosen a material exhibiting the best physical properties ***Ia*** and synthesised its enantiomeric analogue ***Ib*** (Fig. 1). In order to investigate the influence of optical purity of the target material on mesomorphic behaviour, we have prepared mixtures with the different ratio of the enantiomers. We investigated physical properties of these mixtures using differential scanning calorimetry and optical polarization microscopy. Both optical and chemical purity of the chiral intermediates and target materials as well as the enantiomeric excess in the prepared mixtures have been determined by HPLC and SFC on a chiral stationary phase. We show that

even a trace amount of an opposite enantiomer in the optically pure LC matrix induce significant changes in the molecular self-assembly.

This work was supported by SUR (MSMT No 20-SVV/2017).

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DIRECT NANOPATTERNING OF BIOMOLECULES BY ELECTRON BEAM LITHOGRAPHY FOR STUDYING OF CELLULAR INTERACTION

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Each cell reside in physiologically defined environment, which is composed from three mains factors: extracellular matrix, cell adhesion molecules, and soluble factors. Cells respond to those components at macro, micro and nanoscale level not only in biological fashion, but also in respect to chemistry and nanotopography of these cues. Understanding of such cellular interactions is difficult and is current issue in many biology application. Previous approaches used for study of this phenomena were based on random distribution of proteins contained in cellular environment, dealing with this task at macro- and micro-scale level. Nowadays it is known that cells recognize, adhere to or communicate through receptors spatially distributed in cell membrane at nanoscale dimension. Thus, it is obvious that preparing of surface with nanoscale distribution is more biologically relevant.

The main goal of this work is develop the method for preparing patterns with a nano-scale resolution and, furthermore, modify these structures with biomolecules. For this purpose we used electron beam lithography technique (EBL) that allow creation of patterns in very precise and controlled nanoscale way. EphA2, member of ephrin receptor tyrosine kinase family, which is involved in many cellular processes, was chosen as a model molecule.

By EBL we were able to prepare surface with nanoscale properties with sub-50 nm length scale. The surface characteristics were determined by AFM and SEM microscopy. Furthermore, the surface prepared by EBL was suitable for modification with model protein EphA2. This modification was achieved by preparation of recombinant protein EphA2 fused with HaloTag, which bind whole protein complex to metal surface via thiolate linker. Binding of protein to metal surface was confirmed by AFM and immunofluorescence detection.

Finally, highly specific interaction of human embryonic stem cells with immobilized EphA2 protein on metal surface was described. This molecule had positive impact on cell adhesion and proliferation. Our results indicate great potential of systems based on directed molecule distribution in many biological application.

This work has been supported by the projects MUNI/A/1369/2016 (Masaryk University), and LQ1605 (MEYS CR, NPUII).

ALV-K: NEW VIRUS, NEW RECEPTOR?

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Coevolution of a pathogen with its host is driven by of myriads of mutations. On the host side, mutations can be characterized as restriction factors preventing effective replication of the pathogen, e.g. retrovirus. These factors operate at the levels of virion entry into the cell, integration, expression, and maturation. On the virus side, mutations allow its possessor to overcome these obstacles. One of the ways is to change the receptor recognition regions, leading to extended receptor usage. This also might be the very first step in species-to-species transmission.

In the past, avian sarcoma and leucosis viruses (ASLV) were classified based on the diversity of their envelope glycoproteins, by antigen and host specificity, into A to J subgroups. Recently, new virus emerged in Asia and it was shown to cluster with A, B and C subgroups but still genetically distant[1].

We further investigated this virus and tested its ability to infect various avian cell lines. The host range was very similar to A subgroup, but we also observed some differences, e.g. ability of new virus to infect cell lines derived from bobwhite and inability to infect cell lines derived from guineafowl, while subgroup A showed inverse infection pattern. We have also proven inability of the new virus to infect cells preinfected by subgroup A but not by other groups.

To support our observation, we have successfully infected mammalian cell line expressing Tva receptor, which are nonpermissive for any other ASLV. Futhermore, we were unable to infect chicken cell line with *tva* gene targeted by

CRISPR/CAS9 system, rendering them nonpermissive for A subgroup.

Taken together, we suggest this new virus to be designated subgroup K for its sequence distance from other groups and for its different interaction with Tva receptor.

In the past, related subgroups B/D and E faced a similar situation with receptor Tvb being used by all three subgroups, although in a different way for B/D and E. Here we present a new study focused on receptor-envelope glycoprotein interactions of evolutionarily distant subgroups A and K.

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3D CELL CULTURE OF MOUSE LUNG STEM/PROGENITOR CELLS

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The ability of adult lung tissue to maintain itself, remodel, regenerate and repair after injury is dependent on the activity of resident adult lung stem/progenitor cells (LSPCs)[1]. In adult lung epithelium, different populations of lung stem and progenitor cells (LSPCs) have been identified[2]. They reside in different anatomical regions throughout the respiratory tree and can give rise to multiple epithelial lineages of proximal and/or distal airways of the lung[2,3]. However, our understanding of cellular hierarchy in adult lung and microenvironmental signals that regulate LSPC self-renewal and differentiation remains incomplete. To properly address these questions in vitro, development of physiologically relevant 3D models has been necessary.

We developed a protocol for isolation of LSPC that takes advantage of the unique abilities of stem cells to survive in non-adherent conditions and to self-renew. In this assay, LSPCs form spheroids (lungospheres) of several distinct phenotypes, which most likely correspond to distinct parental LSPC types. Lungospheres can be serially passaged and their proliferation, self-renewal and differentiation is regulated by FGF signalling. Furthermore, lungospheres can be embedded in 3D ECM, such as Matrigel, and cultured in submerged or ALI culture. When embedded into 3D Matrigel, lungospheres proliferate and form large cystic or branched structures in response to FGF signalling.

Our lungosphere and 3D cell culture assays provide useful tools to assess stem/progenitor properties of distinct lung epithelial cell populations and to study lung epithelial-stromal interactions in vitro.

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FUNCTION OF THE STRESS SIGMA FACTOR SIGB SUBUNIT OF RNA POLYMERASE IN *Corynebacterium glutamicum*

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Corynebacterium glutamicum genome encodes 7 sigma subunits of RNA polymerase (RNAP)[1]. The primary sigma factor SigA is involved in transcription of housekeeping genes, whereas the SigB controls the expression of genes of global stress regulation in Corynebacterium glutamicum[2].

To determine reliably which sigma factors as subunits of RNA polymerase recognize promoters of various genes, we developed in vivo and in vitro methods for such assignment. We selected three supposed SigB-dependent promoters, Pfba, Ppqo, PsigE, which were tested by the developed methods in *C. glutamicum* wild type strain and sigB deletion strain. Tested promoters have similar -10 region sequences, though encoded genes belong to different biochemical pathways. The *fba* gene encodes fructose-l,6-bisphosphate aldolase, which is involved in glycolysis and the *pqo* gene encodes pyruvate:quinone oxidoreductase, which catalyze hydrolysis of pyruvate[3], The *sigE* gene encodes the SigE.

We found out that even though the promoters Pfba and Ppqo are partially sigA-dependent, they are mainly dependent on sigB. Promoter PsigE was found to be a sigB-dependent promoter with much smaller control by the SigA subunit of RNAP. Surprisingly, all three promoters were recognized by both SigA and SigB using in vitro transcription system, whereas the promoters were nearly exclusively SigB-dependent when tested by in vivo two-plasmid system.

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AMBIENT MASS SPECTROMETRY: FROM THE PLANAR TO THE NON-PLANAR SURFACE ANALYSIS

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Desorption atmospheric pressure photoionization (DAPPI) and desorption electrospray ionization (DESI) belongs to the group of ionization techniques in ambient mass spectrometry. DAPPI is a technique employing a hot jet of the solvent vapours and nebulizing gas to desorb an analyte, and a vacuum ultraviolet lamp to ionize it. DESI is an extraction-based technique using an electrospray ionization.

The original version of the ion source, which was manufactured in our laboratory, allowed a software control of motorized sample holder. This enabled the automatic motion of the analyzed object along the line (analysis along one axis), or within the confines of selected area (analysis in two axes).

The journey from the planar to the non-planar surface analysis has commenced with thin-layer chromatography (TLC) plates, practically perfect planar surfaces[1]. First, the capability of DAPPI-MS to ionize and detect various lipid classes from TLC and high-performance thin-layer chromatography (HPTLC) plates was tested. Limits of detection for lipid standards separated using normal-phase plates were established. Then, the method was utilized to examine the composition of vernix caseosa, a white creamy proteolipid biofilm that progressively coats the fetus during the last trimester of the pregnancy, and plant oils. Lipid class separation was carried out on the normal-phase plates, whereas individual triacylglycerol and wax ester species were separated on the reversed-phase plates.

Recently, an upgraded version of our former ion source now equipped with automatic travel in the *z*-axis and laser triangulation for the non-planar surface analysis in ambient mass spectrometry was constructed. Ion source has three motors to assure automatic movement in the *x*-*y*-*z*-axes. Incorporated laser triangulation system serves for measuring the sample height. This device allows the automatic analysis of various, native, irregularly-shaped samples in one run.

Different plastic objects were printed in a 3D printer and coated with 2,5-dimethoxybenzoic acid. Their analyses revealed advantages and limitations of the new ion source. Finally, food, pharmaceutical, and bio samples were studied.

The work was supported by Charles University in Prague (project GAUK232515).

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DISTINCT SENESCENCE PHENOTYPES OF TUMOR CELLS INDUCED BY DOCETAXEL IFNγ/TNFα TREATMENTS

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Cellular senescence is considered to be a principal barrier against tumorigenesis. Senescence in tumor cells can be caused by distinct inducers – chemotherapy, irradiation, and under particular circumstances, by some cytokines. Senescent cells express a number of secreted proteins, cytokines and growth factors that may stimulate or inhibit cell proliferation. To investigate deeply the process of senescence, we have compared different ways of senescence induction – docetaxel (DTX) and IFNγ in combination with TNFα. We have recently demonstrated that DTX but not IFNγ induced senescence in the TC-1 cell line. Further, both DTX and Th1 cytokines IFNγ and TNFα were able to induce cellular senescence in the B16F10 melanoma cells. Induction of this cytokine-induced senescence was associated with ROS production and NOX4 expression in B16F10 cells.

In this study, we compared in detail the impact of DTX and IFNγ in combination with TNFα in terms of senescence induction, using two C57BL/6 mice-derived tumor cell lines TC-1 and B16-F10. B16-F10 senescence cells phenotype after DTX treatment differed from that of IFNγ/TNFα. DTX-treated cells are morphologically larger, more round and flat than the untreated cells, with high granularity in the cytoplasm. Interestingly, after IFNγ/TNFα treatment B16-F10 cells become larger, flattened, elongated (spindle-shaped) cells with processes. Granularity in the cytoplasm was also present. According to β-galactosidase test, proliferation tests and *in vivo* experiments, the percentage of proliferating cells in IFNγ/TNFα-treated was higher than after the DTX-treatment. Increased expression of p21, in both cell lines after DTX and IFNγ/TNFα, was found. Finally, we evaluated the capacity to induce bystander senescence of both cell lines after the DTX- or IFNγ/TNFα treatments.

This work was supported by the grant No. 15-24769S from the Czech Science Foundation (GACR).

PREPARATION, CHARACTERIZATION AND EVALUATION OF ZINC OXIDE NANOPARTICLES LOADED BOVINE SERUM ALBUMIN NANOPARTICLES AS ANTIMICROBIAL AGENT

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The dimensions of structures in the guise of nanoscale materials has created a nano-platform for applied biomedical research in developing novel and improved nano-hybrid materials for diagnostics and therapeutics applications1. Currently, the discovery of effective topical agents poses a major challenge in the field of wound healing. The present study focuses to prepare a novel targeting nano drug delivery system for enhancing the therapeutic and antibacterial properties of Zinc Oxide Nanoparticles (ZnO NPs) loaded Bovine Serum Albumin Nanoparticles (BSA NPs) and in vitro analysis. Herein, the ZnO NPs loaded BSA NPs were prepared by modified desolvation method. The ZnO NPs loaded BSA NPs were further interrogated both qualitatively and quantitatively *via* physiochemical characterization methods, which provide a new route as a bioactive material. The particle size and surface morphologies of NPs were determined by DLS and FE-SEM. The encapsulation efficiency of ZnO NPs: BSA NPs of 20:100 ratio has given the best encapsulation efficiency 98.87±0.18 %. The antimicrobial activity of ZnO NPs has excellent antibacterial property against Bacillus subtilis and Staphylococcus aureus showing maximum zone of inhibition about 33 mm and 17 mm. This data suggests that ZnO NPs loaded BSA NPs is promising agent and have potential use as a topical agent and may have future application in the area of development in wound care.

The authors would like to thank D. Y. Patil University, Kolhapur for the funding to support our research.

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NANOFIBERS MODIFIED WITH HEXAAMINO-*CYCLO*-TRIPHOSPHAZENE FOR TISSUE ENGINEERING APPLICATIONS

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Phosphazenes are relatively new class of chemical compounds that have found their way into various applications. As biomaterials, polyphosphazenes are widely investigated, mainly for bone, nerve, and vascular engineering[1].

Objective of our study was to supplement, for the first time, commonly used polymers, poly-ε-caprolactone (PCL) and poly-L-lactic acid (PLLA), with cyclic trimeric phosphazene, the hexaamino-*cyclo*-triphosphazene, in order to form blended electrospun scaffolds with potentially enhanced qualities.

Morphology and surface properties of electrospun scaffolds were evaluated by electron microscopy, contact angle measurement, and energy-dispersive X-ray spectroscopy.

For assessment of material performance towards cells, human lung papillary adenocarcinoma cells (line NCI-H441) and normal human adipose-derived stem cells (ASCs) isolated from lipoaspirate were used and complex assessment of cell viability, morphology, ultrastructure, adhesion, metabolism, and proliferation was carried out on days 3 and 6 of cultivation. The results revealed that phosphazene indeed does not cause cytotoxicity and instead enhances cell metabolism, growth, and spreading on modified materials, which is particularly well pronounced for poly-ε-caprolactone.

This study brings new insight into phoshazene-containing biomaterials and suggests hexaamino-*cyclo*-triphosphazene as an attractive additive for modifying materials that are intended for applications in tissue engineering.

This research was financially supported by funds from Ministry of Health of the Czech Republic (16-31501A), project LQ1605 from the National Program of Sustainability II (MEYS CR), project Tissue Engineering 7 (MUNI/A/1369/2016) from Masaryk University, and project FR-TI1/413 from Ministry of Industry & Trade of the Czech Republic.

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DDI2 DEFICIENCY IN MOUSE IMPAIRES EARLY DEVELOPMENT OF ENDOTHELIAL TISSUE

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DNA damage-inducible protein 2 (Ddi2) is one of the Ddi1-like proteins that belong to the family of proteasome adaptor proteins. Adaptor proteins are important regulatory elements taking part in protein degradation, but also in stress response or DNA repair mechanisms within the ubiquitin-proteasome system. Their function is provided via structurally conserved domains – a ubiquitin-like domain (UBL) targeting the proteasome and a ubiquitin-associated domain (UBA) bound to the polyubiquitin chain on the substrate. However, Ddi1-like proteins additionally harbour a retroviral protease-like domain (RVP), which contains a catalytic triad DT/SG typical for aspartic proteases. The proteolytic activity of the human Ddi2 under proteasomal inhibition has been recently described. Nevertheless, the native function of Ddi2 remains unclear. Since murine and human Ddi2 proteins share 96% sequence identity, we chose the Black6 mouse as a suitable model to study the biological role of the human Ddi2 homolog. We have generated a novel mouse strain with a specific deletion of exon six in *DDI2* gene which causes truncation of the full-length protein and diminishes the active site of the protease domain. We further show that homozygous embryos bearing this truncation die between Theiler stages 16-18 with severe phenotype in endothelial tissue, both embryonic and extraembryonic. Overall, we present a broad biochemical and phenotyping study of the C57Bl/6N-Ddi2$^{em1/Rase}$ strain, which could shed some light on the biological role of Ddi2.

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NOVEL ORGANOMETALLIC COMPOUNDS AND THEIR EFFECT ON OVARIAN CANCER CELLS

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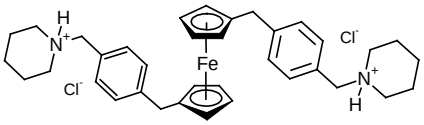
Cisplatin is the first ever and substantially successful compound containing the central metal atom in its structure (1). Despite the undeniable success against several types of tumors, cisplatin as well as its derivatives displays a number of grave side effects. A significant problem is also affecting of rapidly dividing non-cancerous cells and developing drug resistance during treatment (2). Therefore, the current research deals with synthesis and testing of new organometallic compounds containing other metals such as Fe, Ti, Ru, Zr, etc., which may in near future replace chemotherapy based on cisplatin. In this context, we cooperate with several laboratories focused on synthesis of organometallic compounds whose cytotoxicity is further tested on selected ovarian cancer cell lines in our case A2780, A2780cis and SK-OV-3 (3, unpublished data).
The most promising substances were selected to study their cumulative ability in the cells and to determine cellular processes responsible for their cellular uptake more in detail. Recently we developed new method based on pulse voltammetry to study amount of particular drugs accumulated in the cells. This data is now compared with ICP-MS method used to determine ferrocenes in the cells. Both methods confirmed elevated levels of iron in tested cancer cells (4, unpublished data).



Scheme 1. **One of the most active ferrocene nr. 10 (3)**

The important part of our work also consists in determination the mode of action of the most active substances towards cancer cells. Our preliminary experiments indicate increased development of oxygen radicals that may represent a major cytotoxic mechanism.

This project was supported by LO1413, MH CZ - DRO (MMCI, 00209805), GACR P206/12/G151 and GACR 17-05838S.

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X-RAY STRUCTURE OF HUMAN STING WITH NOVEL CYCLIC DINUCLEOTIDES AS A TOOL FOR DISCOVERING NEW TREATMENTS OF CHRONIC HEPATITIS B

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The first line of defence against microbial pathogen infection and tissue damage is the innate immune system. The system is activated by germ line-encoded pattern recognition receptors which detect pathogen specific ligands as well as pathogen associated molecular patterns. Ligand recognition leads to the production of interferons, proinflammatory chemokines, cytokines and anti-microbial peptides[1]. One of these receptors is STING (stimulator of interferon genes), a protein localized on the endoplasmic reticulum membrane. STING, as a signalling adaptor of innate immune system, is activated directly by binding cyclic dinucleotides of bacterial or host origin or through distinct DNA sensors, which are critical in the innate immune response toward microbial DNA and RNA. Activated STING regulates the induction of type I interferons (INF-α and INF-β)[2].

The therapy of chronic hepatitis B is nowadays limited to seven approved treatments: two formulations of interferon α (conventional INF-α and pegylated INF-α) and five nucleos(t)ide analogues (lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir disoproxil). The preferred first-line treatments are pegylated INF-α, entecavir and tenofovir disoproxil. Unfortunately, after stopping long-term therapy with nucleos(t)ide analogues viral relapse occurs in almost all patients. On the other hand, one year treatment with pegylated INF-α leads to viral suppression in approximately 25% patients, which suggests, that the stimulation of patient´s immune system may be the right way in developing HBV cure[3].

The aim of our work is to develop a potent STING activator. My part of this work is crystallization of human STING in complex with newly synthesized cyclic dinucleotides analogues. Structural analysis will allow us to further improve our cyclic dinucleotides analogues until compounds potent enough to be used in human medicine will be reached. To this date I have successfully crystallized human STING with 2'3'-c-GAM(PS). The crystals belonged to $P4_212$ space group and diffracted to 2.5 Å resolution. The obtained structural information is being used in molecular modelling and docking studies.

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AGR2 IS INVOLVED IN THE REGULATION OF THE EPITHELIAL PHENOTYPE BY REPRESSING EMT

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To initiate the metastatic cascade cancer cells frequently undergo the multiple and dynamic transition between different cellular phenotypes. One of these essential processes is the epithelial-mesenchymal transition. During EMT, the epithelial cells are reprogrammed and adapted into mesenchymal-like phenotype exerting increased migratory and invasive potential[1,2]. Enhanced expression of AGR2 was described in wide range of malignancies and AGR2 protein was shown to regulate several cancer-associated processes necessary for tumor development and progression including cellular proliferation, survival and drug resistance[3].

In our work we aimed to unravel the regulation of AGR2 in TGF-β induced EMT and to understand more in detail the role of AGR2 in the maintenance of epithelial cellular integrity.

We found that induction of EMT by TGF-β was associated with decreased AGR2 expression on both mRNA and protein level. Our data indicate that downregulation of AGR2 expression results from the tight cooperation between SMAD and MAPK signalling cascades, both TGF-β inducible signalling pathway.

The knockout of AGR2 expression initiated the acquisition of EMT-like characteristics by the cells *in vitro*. We observed decrease of epithelial marker E-cadherin and *vice versa* elevation of mesenchymal markers N-cadherin and vimentin. These changes were associated with the loss of cell-cell and cell-matrix junctions and enhanced invasive potential. Moreover, induction of AGR2 in the cells with mesenchymal phenotype caused the reversion of cellular phenotype from mesenchymal to epithelial-like by the loss of mesenchymal markers and re-acquisition of epithelial determinants.

Additionally, we observed that upregulation of EMT-related transcription factors ZEB1 and SNAI2 corresponds with decreased AGR2 level. Importantly, inhibition of AGR2 expression induced both ZEB1 and SNAI2 and facilitated their nuclear translocation, thus explaining how AGR2 depletion may contribute to the mesenchymal phenotype.

Taken together our results highlight a crucial role of AGR2 in maintaining of the epithelial phenotype by preventing the activation of key factors involved in the process of EMT.

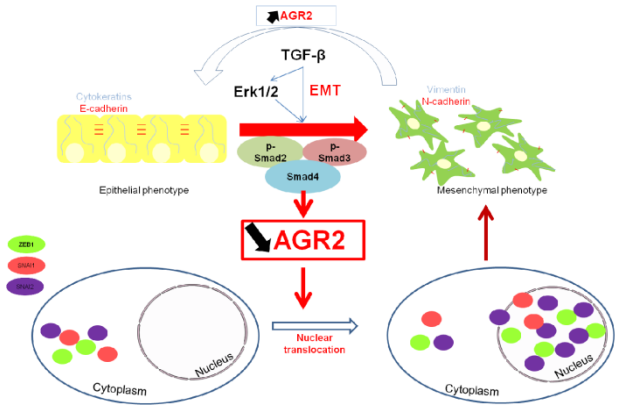

Fig. 1. **The role of AGR2 in the epithelial- mesenchymal transition**

The work was supported by the project MEYS – NPS I – LO1413 and GACR 13-00956S.

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COMPARISON OF MALDI-MSI AND LC-MS FOR PHARMACOKINETIC STUDY OF METFORMIN

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Liquid chromatography-mass spectrometry (LC-MS) is a commonly used technique used for pharmacokinetic studies of drugs. However, LC-MS suffers from lack of spatial information. On the contrary, MALDI mass spectrometry imaging (MALDI-MSI) is a modern analytical technique capable to evaluate a spatial distribution of compounds within target tissues. In our work, we used MALDI-MSI for pharmacokinetic study of metformin and obtained results were compared with data parallelly measured by LC-MS.

First, MALDI-MSI sample preparation procedure was optimized allowing determination of the distribution of orally dosed metformin in mice kidney and liver tissue sections. The optimization included a) selection of a suitable MALDI matrix, b) optimization of various parameters of MSI analysis. Dihydroxybenzoic acid was identified as the most suitable matrix. Sublimation method was successfully applied for matrix deposition (4 min, 140 °C). LC-MS method utilizing tissue homogenates was also developed. LC analysis was performed on Atlantis Silica HILIC collum. The mobile phase consisted of 0.1 % HCOOH in H_2O/ACN (20/80) with addition of $HCOONH_4$. MS analysis was carried out in a positive ESI mode employing MRM (multiple reaction monitoring).

MSI analysis revealed that the highest relative concentration of metformin within the kidney samples was found in the inner zone. The excretion of metformin from kidney started already 15 minutes after oral administration of the drug. The concentration of metformin in liver tissue sections after 15 min from the administration was relatively low. Higher amount of the drug was found after 30 min and a slight decrease was observed after 60 minutes. Drug distribution correlates very well with results found by LC-MS of tissue homogenates. We demonstrated that MSI and LC-MS are powerful complemental techniques. LC-MS provides accurate average drug concentration in the tissue fraction. However, a minimal size of the collected tissue part is limited. In contrast, laser in MALDI-MSI can be focused on a very small area of the sample therefore it gives high lateral resolution. MALDI-MSI provides information on the relative distribution of the drug in the tissue section very precisely.

DISSECTING THE ROLES OF FIBROBLAST GROWTH FACTOR (FGF) SIGNALLING IN MAMMARY BRANCHING MORPHOGENESIS USING HYPERSTABLE FGF2 VARIANTS

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Fibroblast growth factor (FGF) signalling plays a key role in regulation of mammary gland development, especially in epithelial branching morphogenesis. When deregulated, FGF signalling can lead to developmental disorders and breast cancer, and therefore it has attracted a lot of research interest. However, although multiple in vivo and in vitro approaches have been used so far to investigate the role of FGF signalling in normal mammary development and cancerogenesis, studies addressing the ligand level and availability have been scarce.

To study the effects of intensity and duration of FGF2 signalling on mammary epithelial morphogenesis, we employed hyperstable variants of FGF2 (with half-life over 24 h, in comparison to wild-type FGF2 with half-life of 6 h). In 3D cultures we tested a range of FGF2 concentrations and various modes of exposure to FGF2s.

Treatment of 3D organoid cultures with hyperstable FGF2s (1 nM; the concentration standardly used for FGF2-wt) induced a novel epithelial morphogenetic phenotype

characterized by hyperplasia and formation of massive, thick branches. This hyperplastic organoid phenotype could not be induced by wild-type FGF2 by increased frequency of medium change (frequency increased from every 24 h to every 6 h) or by increased concentration unless at 20-times higher concentration (i.e. 20 nM). Investigation of the other side of the signalling spectrum, i.e. of the minimal limiting concentrations that are able to induce epithelial morphogenesis, revealed that hyperstable FGF2s are able to induce branching morphogenesis at twenty-five times lower concentration than the wild-type FGF2. Importantly, at low concentrations, hyperstable FGF2s induce only normal epithelial branching phenotype and no hyperplasia is observed. These observations suggest that the level of FGF signalling regulates epithelial patterning for branching.

We are currently analysing the candidate cellular mechanisms that could be responsible for these morphological changes, including proliferation, apoptosis, polarity and differentiation.

Our findings will contribute to better understanding of the roles of FGF signalling in normal mammary gland development and breast cancer initiation and progression.

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TOWARDS HIGH BIOCOMPATIBILITY OF ORGANIC CONDUCTIVE MATERIALS FOR HEART ON CHIP APPLICATIONS

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Recent trend involves the use of organic conductive materials in biosensors since they have a potential to form an excellent biointerface to determine electrochemical signals arising from living cells and tissues. Though organic materials are generally regarded to be biocompatible, detailed insights into their biocompatibility are sparse as well as the kwoledge if they can support cardiomyocytes. In this study multiple aspects of biocompatibility of triisopropylsilyethynyl pentacene (TIPS), diketopyrrolopyrrole (DPP), poly 3-hexyl-thiophene-2,5-diyl (P3HT) and poly 3,4-ethylene-dioxythiophene (PEDOT:PSS) which show high potential for a biosensor construction were investigated. Stability of these materials in physiological environment and wetting properties which are preconditions for biocompatibility were determined. TIPS DPP and P3HT were stable but PEDOT:PSS produced some leachates. Native TIPS DPP and P3HT showed lowered wettability. The biocompatibility was studied by means of 3T3 fibroblasts. Native TIPS DPP P3HT and PEDOT:PSS showed limited biocompatibility compared to standard cell culture plastics. Further trials to manipulate biocompatibility were carried out. Among other procedures tested the biocompatibility could be improved remarkably by means of collagen IV coating.

Finally a culture of spontaneously beating cardiomyocytes differentiated out of murine embryonic stem cells was established at TIPS DPP P3HT and PEDOT:PSS. The collagen IV coating procedure allowed construction of highly biocompatible PEDOT:PSS based sensor. This was sensor successfully seeded with spontaneously beating cardiomyocytes. The electrical characteristic of the sensor enabled detection of an electrical signal of spontaneously beating cardiomyocytes, however, the noise to signal ratio was significant.

This work was supported by grants GACR 13-29358S and GACR 17-24707S

SYNTHESIS, CATALYTICAL ACTIVITY AND RECYCLING OF POLYFLUORINATED CHIRAL PALLADIUM COMPLEXES

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PEPPSI catalysts (i.e. Pyridine-Enhanced Precatalyst Preparation, Stabilization and Initiaiton) rank among the most important homogeneous palladium catalysts, which are being used in contemporary organic synthesis. In comparison with conventional phosphane palladium complexes, these are distinguished by higher stability, robustness and longer reaction halftime.

This project is focused on the syntheses of new PEPPSI analogues modified with several polyfluorinated chains for them to be medium fluorous. Furthermore, the target structures also bear NHC ligands with stereogenic centers. Thus, their enantiomerically pure forms are expected to catalyze enantioselective processes.

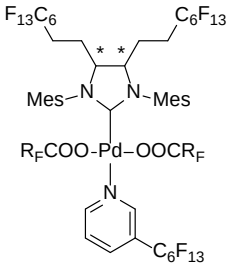
A typical example of the target structure is complex **1** (Fig. 1), which bears 5 polyfluorinated chains (heavy fluorous as witnessed by P_i(FBS) = 4) and two stereocenters in its NHC ligand.

Catalytic activity of the complexes was tested using model Suzuki coupling of 4-iodotoluene and phenylboronic acid. All the fluorous modifications turned out beneficious in terms of the catalytic activity.

In order to test the stereoselectivity of the catalyst, a new model Suzuki coupling reaction was optimized, namely the reaction of 1-naphthylboronic acid and 1-iodo-2-meth-oxynaphthalene, providing axially chiral binaphthyl products.

Last but not least, the recycling and recovery of the complexes was tested and optimized. Here we made use of a recently developed separation technique using medium

fluorous polyfluorinated ethers, which are cheap and environmentally benign.



1 $R_F = CF_3O(CF_2CF_2O)_2CF_2$

Fig. 1. **Polyfluorinated complex 1**

Financial support from specific university research (MSMT No 20-SVV/2016)

STRUCTURAL STUDIES OF NEUTRAL TREHALASE IN COMPLEX WITH 14-3-3 REGULATORY PROTEIN

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Neutral trehalase 1 (Nth1) is highly conserved enzyme that has been found in wide variety of organisms. This enzyme belongs to the class of hydrolases, and it hydrolytically cleaves the trehalose into two glucose molecules. Trehalose is a naturally occurring disaccharide serving in yeast cells as a source of carbon and energy and protects the cell membrane during heat shock or oxidative stress. Trehalose is crucial for flying insect, because trehalose is the main compound found in insect haemolymph, thus trehalase inhibitors may be used as potential selective inseticides. The Nth1 activity in *Saccharomyces cerevisiae* is regulated through a mechanism involving the phosphorylation of two serines by cAMP-dependent kinase A (PKA), Ca^{2+} and 14-3-3 protein (Bmh1) binding[1,2,3].

The aim of this study was the structure determination of Nth1 using protein crystallography. Construct containing the catalytic domain of Nth1 (residues 153-751) was recombinantly expressed, purified and crystallised using hanging-drop vapor-diffusion method. Nth1 153-751 was crystallised in the apo form and in the presence of 200 mM trehalose. Diffraction data of apo and trehalose-bound form were gathered to the 2.7 Å and 2.9 Å resolutions, respectively, at the BESSY II (MX-BEAMLINE_14.1) in Berlin. This is the first crystal structure of trehalase from eukaryotic organism, catalytic domain of Nth1 consists of $(\alpha/\alpha)_6$ barrel, as other glycosidases[4]. According to the structural models, the catalytic residues were verified to be: D478 and E674. Activity measurement of D478A and E674A confirmed that these mutant forms are catalytically dead. To verify trehalose binding to apo-trehalase, microscale thermophoresis method was performed, K_D was determined in mM range.

This work was supported by Research Project RVO: 67985823 of the Institute of Physiology of The Czech Academy of Sciences.

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3D IMAGING OF BIOPOLYMERIC SCAFFOLDS SEEDED WITH CELLS USING X-RAY COMPUTED NANO-TOMOGRAPHY

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Recently, biocompatible and resorbable biopolymer-based porous scaffolds with potential application as cell and/or drug carriers were developed in our department[1]. In addition to the chemical composition of the biopolymer scaffolds, their morphology is a very important characteristic playing a significant role in tissue regeneration. As we have previously proved[2], biopolymeric scaffolds can be visualized by non-destructive 3D X-ray micro-computed tomography (micro-CT). However, by this method, we were not able to visualize both biopolymeric scaffold and seeded cells. Therefore, in this study, we propose X-ray nano-computed tomography (nano-CT) as a novel 3D imaging technique to visualize whole volume of porous collagen scaffold seeded with mesenchymal stem cells without its destruction with a resolution of about 270 nm (Fig. 1). This new technology will help us to better understand principles of seeding the scaffold by cells, the behavior of cells in the scaffold (proliferation and differentiation in different time intervals), or changes in the morphology of the scaffold after some time exposed to

the cells, which is essential in the modern tissue engineering field.

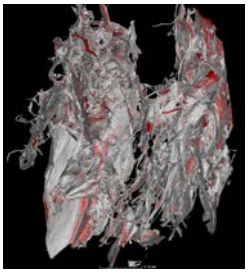

Fig. 1. **3D visualization of scaffold seeded with cells (red color).**

This research was carried out under the project CEITEC 2020 (LQ1601).

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THE USE OF MICRO AND NANO COMPUTED TOMOGRAPHY FOR IMAGING OF SOFT TISSUES IN EMBRYOS

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Imaging of soft tissues is crucial for understanding chemical and biological processes inside the living organisms. This is especially important to study initial formation during embryonic development[1]. Such imaging requires the best possible resolution and high demands on preparation of the samples. However, 2D image does not contain all sufficient information about observed structures. Recently, imaging techniques were improved considerably to allow extend traditional imaging techniques to 3D imaging[2].

One of the convenient tool for 3D imaging of embryonic samples is X-ray Computed Tomography (CT). It is a non-destructive method with spatial resolution up to 1μm. However, CT imaging has been limited by low contrast of soft biological tissue. X-ray source shows very similar absorption for unmineralized structures[2]. In this case phase-contrast tomography[3] or staining agents are applied[2].

In this work the high contrast 3D data of soft tissues in embryonic samples were obtained (Figure 1) by staining with iodine and phosphotungstic acid and subsequent tomographic analysis. This brings new possibilities for exploring and understanding the processes inside the evolving body.

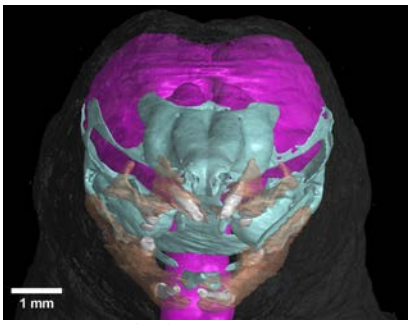

Fig. 1. **3D visualization of soft tissues in mouse embryo head.**

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NOVEL CYCLOHEXANE-1,2-DIAMINE-BASED CATALYST FOR ASYMMETRIC HENRY REACTION

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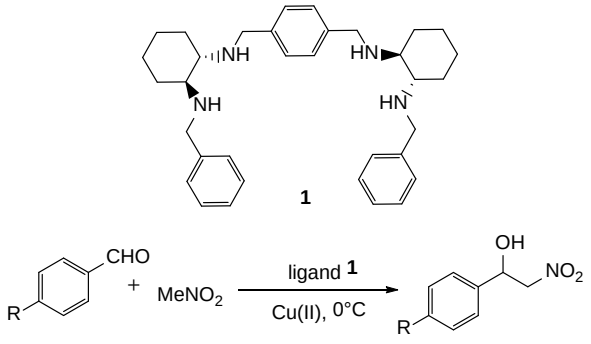
Here we report new enantiomerically pure (1*S*,2*S*)-cyclohexane-1,2-diamine derivative (***1***) as a ligand in Cu(II) complexes as catalysts of asymmetric Henry reaction (scheme 1)[1]. We have examined the role of various solvents and different metal salts as the source of cation as well as we have studied the effect of addition of bases to the reaction mixture. Afterwards, we have investigated the scope of this catalytic system in the Henry reaction of various aldehydes and different Cu(II):ligand ratio.

The nitroaldol adducts were isolated in good yields with moderate to excellent enantioselectivities. The best results have been obtained on reactions of benzaldehyde with nitromethane catalyzed by 20 mol.% of equimolar $Cu(OAc)_2$ complex with ligand ***1*** in ethanol or tetrahydrofuran (55% yield, 90% e.e. and 60% yield, 92% e.e., respectively).



Scheme 1. **Tested Henry reaction and a structure of the ligand *1***

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Rhomboids are conserved intramembrane serine proteases found in all kingdoms of life. They are important in many biological processes, such as growth factor secretion, invasion of the malaria parasite, regulation of mitochondrial dynamics or membrane protein quality control[1]. These findings suggest that rhomboids have a wide therapeutic potential, but the lack of potent, selective and pharmacologically compliant inhibitors impedes further understanding and validation of their function[2]. Based on the enzymological and structural analysis of rhomboid protease mechanism, specificity and substrate-enzyme interactions[3,4] we established a general platform for the design of novel rhomboid inhibitors. Here we present peptidyl ketoamides with substituents at their ketoamide nitrogen as new potent and selective covalent reversible inhibitors of rhomboids. They are active in low nanomolar range, exceeding the so far known rhomboid inhibitors by up to three orders of magnitude. Testing of their inhibiting potency against about a hundred human hydrolases shows that they are selective for rhomboid proteases, and kinetic and structural analysis revealed their competitive, slow-binding mechanism. Since the ketoamide warhead is a clinically used pharmacophore[5], we expect these compounds to be widely applicable in cell biology and drug discovery of rhomboid proteases.

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ABSTRACT WAS WITHDRAWN ON REQUEST OF THE AUTHORS

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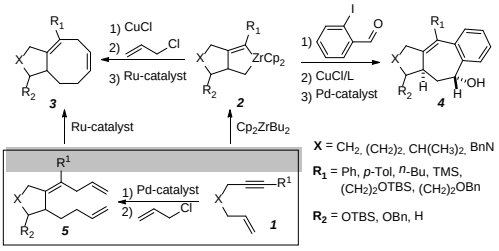
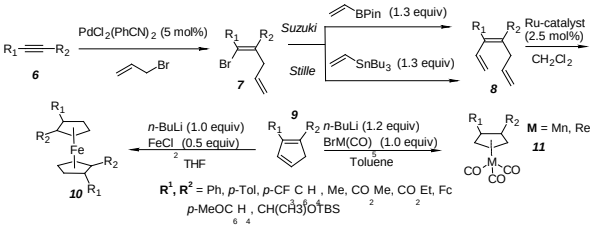
Abstract was withdrawn on request of the authors.



Scheme 1. **Preparation of fused carbocycles**

The formed compounds might serve as potential advanced intermediates for preparation of natural products. On the other hand, it was envisioned that Pd-catalyzed bis-allylation of enynes could provide intermediates ***5*** suitable for the subsequent ring closing metathesis, thus forming 5(6)-8 membered carbocycles. The mentioned approach is currently under investigation.

Furthermore, method[3] for the preparation of substituted cyclopentadienes, a versatile organic substrates in organic synthesis, based on functionalization of internal alkynes is described. As shown in scheme 2, bromoallylation of various alkynes ***6*** was carried out in the presence of Pd-catalyst providing corresponding 1-bromo-1,4-dienes ***7***. Subsequently Suzuki or Stille cross-coupling of vinyl metals with bromodienes ***5*** gave rise to 1,3,6-trienes ***8*** in 50-92% yields. Subsequent Ru-catalyzed ring-closing metathesis of ***8*** provided 1,2-disubstituted cyclopentadienes ***9***. Then metallation of the formed cyclopentadienes with *n*-BuLi and followed by reactions with anhydrous $FeCl_2$ or bromopentacarbonylmetals provided the ferrocene ***10***, manganese and rhenium complexes ***11***.



Scheme 2. **Preparation of cyclopentadienyl complexes**

This work was supported by the Czech Science Foundation (Project 13-15915S) and Charles University Grant Agency (Project 1132/2015).

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TRANSITION-METAL-CATALYZED FUNCTIONALIZATION OF ENYNES AND ALKYNES

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Formation of carbocyclic compounds featuring fused 5-7, 5-8 and other expanded ring systems by using transition-metal mediated and/or catalysed transformation of enynes ***1*** is very attractive. Herein, we would like to present conversions of bicyclic zirconacyclopentenes ***2***, easily prepared from the corresponding enynes, into the 5(6)-8[1] ***3*** and 5-7-6(Ar)[2] ***4*** membered ring systems by selective reactions of the sp^2- and sp^3C-Zr bonds with suitable electrophiles followed by the subsequent transformations (Scheme 1).

KINETIC MECHANISM OF AN ANCESTOR OF HALOALKANE DEHALOGENASE AND LUCIFERASE

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Classical enzymology describes enzymes as highly specific catalysts that accelerate one particular chemical reaction in a living organism. However, recent years have witnessed results reporting the ability of enzymes to catalyze more than one reaction – the enzyme promiscuity[1]. This feature can be observed for the ancestors of modern enzymes which can exhibit multiple activities of their descendants.

One such primordial enzyme has been reconstructed as the most likelihood ancestor of the haloalkane dehalogenases and the *Renilla* luciferase. While haloalkane dehalogenases hydrolytically cleave haloalkanes to their corresponding alcohols[2], luciferases oxidize luciferins by molecular oxygen with the simultaneous release of visible light[3]. The resurrected ancestor exhibits very interestingly dual hydrolase and oxidoreductase activity, each based on the different reaction mechanism. The enzyme is being kinetically examined in order to understand the evolution on a molecular level as well as to describe the luciferase reaction mechanism which is still not solved and could help to optimize commercial bioluminescence assays.

The latest results focused on dehalogenase activity of the ancestral enzyme helped to identify the rate-limiting step which was hydrolysis of the alkyl-enzyme intermediate. An analysis of products' release showed very fast binding of halide anion by the ancestral enzyme, but more surprisingly also by luciferase RLuc. It was shown that the residues responsible for halide binding remain preserved in the luciferase RLuc, even though it lacks the dehalogenase activity. The importance of the residues for oxidoreductase activity is unknown and is waiting for further examination.

The collected kinetic data provide a detailed molecular understanding of the catalytic mechanism of the exceptional ancestor's dual activity.

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BIFUNCTIONAL L-PROLINOL BASED ORGANOCATALYSTS: STEREOSELECTIVE MICHAEL ADDITION IN HOMOGENOUS AND HETEROGENOUS ENVIRONMENT

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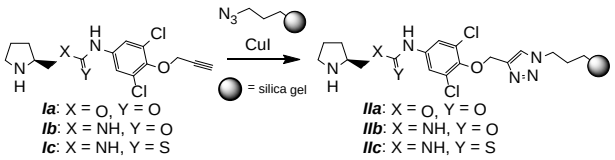
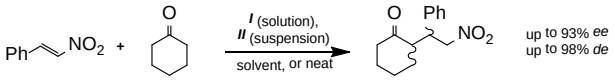
Stereoselective Michael additions[1] represent the staple reactions in the field of organic synthesis. The stereoselectivity of the reactions is usually mediated by bifunctional catalysts, which activate both the electrophile and nucleophile at the same time[2,3]. One of the many challenges in the field is to develop a suitable heterogenous catalytic system, which provides an easy way of regeneration of the catalyst by standard filtration.

Herein we present a series of bifunctional L-prolinol based organocatalysts (***Ia-c***), which have also been immobilized to azidopropyl-modified silica solid phase yielding heterogenous catalysts (***IIa-c***) by *click*-reaction (Scheme 1). The synthesis as well as the catalytic properties of both homogenous (***Ia-c***) and heterogenous (***IIa-c***) catalysts in a selected model Michael addition of cyclohexanone and *β*-nitrostyrene (Scheme 2) are discussed.

The reaction was carried out in various solvents. The experiments showed a very high impact of the solvent polarity on both yield and stereoselectivity. The less polar solvents used (hexane, toluene) delivered the highest conversion and stereoselectivity, whereas the polar solvents (dichloromethane, methanol) were significantly less effective. Nevertheless the best results were achieved in neat cyclohexanone (up to 93% *ee*; up to 98% *de*)[4].



Scheme 1. **Structure of the target catalysts**



Scheme 2. **Model Michael addition**

This work was supported by the Czech Science Foundation (reg. No. 16-17689Y).

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PROTEOMIC ANALYSIS OF RAT BRAIN CORTEX AFTER MORPHINE TREATMENT AND 20 DAYS OF DRUG WITHDRAWAL

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Morphine is a prototypical opioid which has been used as analgesic drug for centuries. Adenylyl cyclase (AC) superactivation is generally thought to represent a possible biochemical substratum for the development of opiate tolerance and dependence. We indicated a specific compensatory up-regulation of ACI (8-fold) and ACII (2.5-fold) in plasma membrane fraction (PM) isolated from forebrain cortex of rats exposed to morphine for 10 days (10-50 mg/kg) and this increase faded away 20 days since the last dose of drug[1].

The aim of our present work was to test reversibility of chronic morphine effect after 20 days of drug withdrawal in order to obtain comparable data with our previous results. From methodological point of view we used 2D gel-based analysis accompanied by MALDI-TOF MS/MS and label-free quantification algorithm called MaxLFQ.

Depending on the method used for protein detection and quantification, 28 (MALDI-TOF MS/MS) or 113 (MaxLFQ) altered proteins were identified. Importantly, in rats sacrificed 20 days since the last dose of drug, the number of changed proteins was decreased to 14 (MALDI-TOF MS/MS) and 19 (MaxLFQ)[2]. Our data indicate the high ability of living organism to return to the physiological norm after morphine withdrawal.

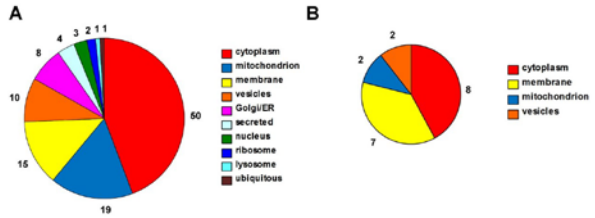

Fig. 1. **Subcellular localization of altered proteins after chronic morphine treatment (A) and 20 days since drug withdrawal (B) identified by MaxLFQ**

This work was supported by the GACR (P207/12/0919) and by RVO:67985823. The authors thank to Karel Harant.

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MECHANISTIC INSIGHTS INTO ASSEMBLY OF EISOSOMES, SPECIALIZED MICROCOMPARTMENTS ASSOCIATED WITH YEAST PLASMA MEMBRANE

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Plasma membrane of all cells is compartmentalized to ensure various physiological functions indispensable for cell living. Membrane compartment of Can1 (MCC) is one of the most studied membrane microdomains in the plasma membrane of yeast *Saccharomyces cerevisiae*. MCC as a membrane microcompartment comprises integral membrane proteins (nutrient transporters and tetraspan proteins of Sur7 and Nce102 family) and is organized from cytosol by cytosolic complex of proteins called eisosome. Eisosomes mainly consist of BAR-domain proteins Pil1 and Lsp1. Due to their crescent shape, Pil1 and Lsp1 shape the membrane of MCC to form an elongated furrow. Pil1 protein, in contrast to Lsp1, is essential for eisosome formation. It´s expression is cell-cycle regulated and new eisosomes are formed only in growing buds; in non-growing cells, eisosomes are extremely stable. Upon Pil1 deletion, all components of MCC are either homogeneously distributed in the plasma membrane or coalesce into so called eisosome remnants.

In this study, we aimed to investigate the mechanism of eisosome formation. We cloned GFP-tagged Pil1 protein under the galactose-inducible promoter, so we could regulate the timing of Pil1 expression. In *pil1*Δ cells, we indeed observed *de novo* formation of eisosomes upon activation of Pil1-GFP expression. Moreover, we observed Sur7-mRFP relocalization from eisosome remnants to the newly-formed eisosomes and investigated the mechanism of Sur7 protein translocation. Interestingly, in wt cells (with already existing eisosomes tagged with Pil1-mRFP) we observed the elongation of existing eisosomes upon activated Pil1-GFP expression. This suggests that it is the level of Pil1 protein that determines the shape and length of eisosomes, not the properties of the plasma membrane. Using FRAP method, we showed that eisosome growth is bidirectional – it grows from both ends of the furrow. Additionally, we examined eisosome formation in growing buds – the only natural situation when eisosomes are formed. We focused on the reorganization of mother cell eisosomes when the bud is starting to form and on the new eisosome site selection in growing bud.

Together, our study provides significant mechanistic insights into the assembly of eisosomes and we propose that this mechanism is conserved throughout the fungi kingdom.

This work was supported by the Czech Science Foundation (project 15-10641S).

THE ROLE OF MICROENVIRONMENT IN THE REGULATION OF B-CELL RECEPTOR SIGNALLING VIA MODULATION OF GAB1 PROTEIN LEVELS

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B-cell chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in the western world. Characteristic feature of CLL is its deep dependence on the tumor microenvironment in lymph nodes, spleen and bone marrow. The microenvironment provides malignant B-cells with supportive signals via adhesion, chemokines and B-cell receptor (BCR) signalling. This is believed to be important for the onset, progression and also incurability of this disease.

We have found that co-culture of CLL cells with bone marrow stromal cells (HS5) upregulates the expression of GRB2-associated adaptor protein GAB1 in malignant B-cells ($P<0.01$). This is of note since we observed that GAB1 protein directly enhances activity of PI3K/AKT signalling. To examine the precise mechanism, we interrogated the regulation of GAB1 levels by various microenvironmental factors such as direct adhesion or soluble factors.

We have shown that direct contact of B-cells with extracellular matrix protein fibronectin contributes to upregulation of GAB1 levels. This could be caused by adhesion-induced down-modulation of microRNA miR-150 ($P<0.05$), which was demonstrated to negatively regulate GAB1 levels[1]. Additionally, culture of B-cell lines in the media supplemented with conditioned media from stromal cells (HS5 cells) leads to similar upregulation of GAB1 as in the direct co-culture. To further investigate the effect, we measured the amount of soluble chemokines in the conditioned media by ELISA and determined that interleukins could be at least partially responsible for GAB1 modulation. *In silico* prediction of binding sites for transcription factors identified STAT3 protein as a possible transcriptional regulator of GAB1. To validate this hypothesis, B-cell lines were treated with various interleukins, which serve as major activators of STAT3. This treatment led to higher expression of GAB1 protein and *vice versa* silencing of STAT3 led to significant down-modulation of GAB1 expression ($P<0.01$).

We have shown a mechanism that enables CLL cells to upregulate GAB1 levels in the context of microenvironment and thus precisely modulate pro-survival signalling via PI3K/AKT pathway.

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ANTIMICROBIAL PEPTIDE HAL-2/39 AS A POSSIBLE REPLACEMENT OF ANTIBIOTICS IN BONE CEMENT

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Bacterial infections represent serious complications in orthopedic surgery. Bacteria colonize the orthopedic implants and adhere to them in the form of biofilms in which they are protected from the immune system and antimicrobials. Therefore, antibiotic-loaded bone cements were implemented, but their common use and benefit is controversial because of increasing bacterial resistance.

In our laboratory we investigate antimicrobial peptides (AMPs) loaded into the model implants made from poly(methylmethacrylate)-based bone cement to prevent the formation of the bacterial biofilm on the surface. Since AMPs kill microbes with different mechanism of action than antibiotics, they might be a promising supplement or substitution for antibiotics, when loaded into the bone cement.

For this study we selected HAL-2/39, an analog of the natural dodecapeptide named HAL-2 which was originally isolated from the venom of wild bee *Halictus sexcinctus*[1]. HAL-2/39 exhibits a strong antimicrobial activity against common pathogens causing the orthopedic implants infections.

To follow the effect of HAL-2/39 for prevention of biofilm formation on the surface of the bone cement we developed an experimental model using commercially available bone cement Palacos® R. We found that HAL-2/39 loaded into this cement partially released from the implants into the media containing various bacteria (S*taphylococcus aureus, Escherichia coli*) and prevented the bacterial colonization and subsequent formation of the bacterial biofilm on the surface. On the contrary, the control implants made from the plain cement were colonized by the bacterial biofilm. In another experimental set-up HAL-2/39 killed bacteria in the pre-grown biofilm on the surface of the bone cement.

The next step is to investigate the mechanism of HAL-2/39 action on the bacteria grown in the biofilm and its synergistic effect with antibiotics.

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CHARACTERIZATION OF GLUTAMATE CARBOXYPEPTIDASE II KNOCK-OUT MICE GENERATED BY TALEN TECHNOLOGY

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Glutamate carboxypeptidase II (GCPII), also known as prostate-specific membrane antigen (PSMA), has been suggested as an important therapeutic and diagnostic target[1]. Overexpression of this transmembrane glycoprotein has been implicated in prostate cancer while inhibition of GCPII activity has been associated with neuroprotection[1]. The physiological function of GCPII has been explored in many ways, including several different attempts to generate GCPII knock-out (KO) mice[2-6]. However, the results of these attempts are rather controversial, ranging from embryonic lethality to viable GCPII KO mice with no obvious phenotype[3-6]. Interestingly, a remaining GCPII-like peptidase activity was detected in GCPII KO mice[3,6]. Here, we present generation of GCPII KO mice by targeting the active site of the enzyme using TALEN technology. While expression of modified GCPII mRNA was observed in our KO mice, no protein expression was detected by Western blot. We also provide preliminary data of GCPII activity analysis in WT vs. KO mice. Our GCPII KO mice are viable, breed normally and do not show any obvious phenotype.

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