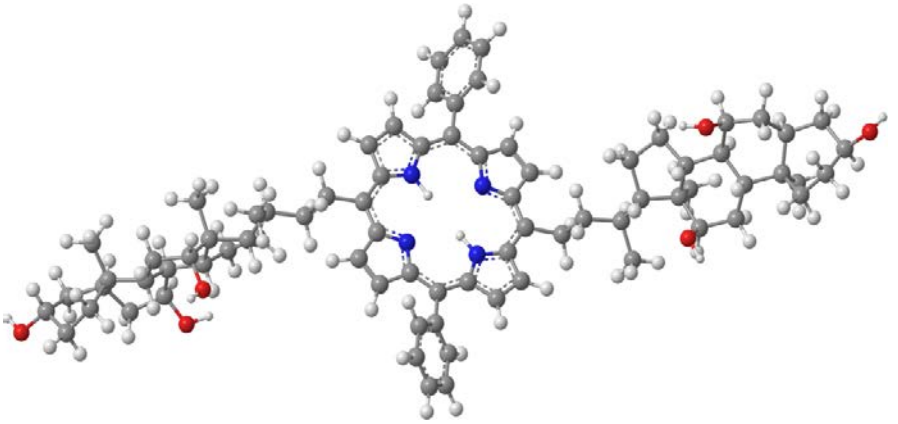

# XX$^{th}$ INTERDISCIPLINARY MEETING OF YOUNG RESEARCHERS AND STUDENTS IN THE FIELD OF CHEMISTRY, BIOCHEMISTRY, MOLECULAR BIOLOGY, AND BIOMATERIALS

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## MASS SPECTROMETRY COUPLED WITH ARTIFICIAL NEURAL NETWORKS FOR MULTIPLE MYELOMA SUBENTITY DISCRIMINATION

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Multiple myeloma (MM) is a heterogeneous disease of malignant plasma cells. Diagnosis of MM is based on bone marrow biopsies and on detection of abnormal immunoglobulin in serum and/or urine[1]. At times, MM may progress into so-called extramedullary multiple myeloma (EM). EM occurs when a subclone of clonal plasma cells migrates out of the bone marrow and infiltrates soft tissues or organs. The diagnosis of EM is made by biopsy and imaging[2]. Plasma cell leukemia (PCL) is a very rare neoplasm of plasma cells, characterized by circulating plasma cells (cPC) in peripheral blood. The diagnosis of PCL is based on presence of more than 20% of cPC in peripheral blood and absolute number of cPC>2x109/l (lit.[3-5]). Since biopsies have a single-site bias, new diagnostic tests and strategies are needed for early detection.

MALDI-TOF MS has become an indispensable research tool, which is used for analysis of biomolecules and various organic molecules. Artificial Neural Networks (ANN) are components of artificial intelligence inspired by biological neural networks. Using ANN, we can model non-linear systems in which the relationship among variables is very complex, as published by our group previously. In our previous study, we recorded mass spectra of MM and healthy donor samples. ANN specifically predicted MM samples with high sensitivity, specificity and accuracy[6].

In this study, we focused on the analysis of low molecular weight molecules in peripheral blood of MM, PCL and EM patients using MALDI-TOF MS to create a diagnostic tool based on prediction by ANN, which should distinguish different groups of diseases. The RStudio was used for statistical analysis, where the data were evaluated using Principal Component Analysis (PCA) and Partial least squares discriminant analysis (PSL-DA). Using MALDI-TOF MS, it was possible to distinguish between samples of MM patients and healthy donors, as well as MM and EM patients. Informative patterns in mass spectra served as inputs for ANN that specifically distinguished between healthy donors and patients.

We demonstrated that using MALDI-TOF MS coupled with ANN is a useful tool that can distinguish between healthy donors and patients, which can be used as a fast alternative to conventional analyses.

*This study was supported by grants of the Ministry of Health of the Czech Republic, grant nr. NV18-08-00299, AZV 17-29343A and Masaryk University (project no. MUNI/A/1421/2019). All rights reserved. LM is junior researcher holder, supported by Faculty of Medicine, Masaryk University (ROZV/28/LF/2020) and supported by MH CZ - DRO (Masaryk Memorial Cancer Institute, 00209805).*

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## UNSCENTED PARTICLE FILTERS FOR ESTIMATION OF GENE EXPRESSION

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This work addresses the problem of estimating gene expression using the unscented particle filter. The response of Gene Regulatory Networks (GRN) to functional requirements in the cell and environmental conditions evolve over time. Dynamic biological processes such as cancer progression and treatment recovery depend on the collected genetic profiles. These processes are behind genetic interactions that rewire over the course of time. The GRN is formulated as a nonlinear/non-Gaussian state-space model. The particle filters (PF) represent the state-of-the-art in the optimal estimation for nonlinear/non-Gaussian dynamical systems. However, the PF is ineffective for high dimensional state-space systems. The unscented particle filters (PF-UKF) able to overcome the problem known as the 'curse of dimensionality'. Therefore, this work presents the PF-UKF to estimate the evolution of gene expression over time from series data considering that the GRN has a high dimensional space. Simulation results on realworld gene expression datasets, shows that the PF-UKF offers effective results compared to three other algorithms.

The biological mechanisms that govern our development are complex and crucial to understand the cellular system. The gene regulatory network (GRN) controls the expression of thousands of proteins and genes in any specific cellular function. However, the biological processes are dynamic and

evolve over time in response to extrinsic factors and various intrinsic, such as cellular development, targeted therapy disease progression and environmental conditions[1]. Understanding these gene regulatory networks can help us significantly enrich our knowledge of health and disease.

The GRN is a set of genes that interact with each other and with other substances to control the functions of the cell[2,3]. In GRN, the state estimation is based on time series data representing by genome expressions[1,4], and it can be represented in multiple methods varying in their degree of sophistication[2]. In addition, the prediction of the evolution of gene expression is defined by a dynamic nonlinear state transition where it has a high dimensional state space model. Different approaches have been proposed to estimate the gene expression time series including, particle filtering[2] and extended Kalman filter[1].

Particle filters (PF) are a sequential Monte Carlo method provides powerful estimation for nonlinear and non-Gaussian state-space scenario[5]. The main idea of PF is to estimate the state optimally by employing a group of random weighted particles. These particles are used to approximate the posterior density of the state[5]. However, it is ineffective in high dimensional spaces where the number of particles needed increases super-exponentially with the dimension of the state[5]. Recently, various approaches have been emerged to ameliorate the performance of PF, where unscented Kalman filter (UKF) or extended Kalman filter (EKF) are used to draw the importance distribution[6]. These approaches called respectively, the Extended Particle Filter (PF-EKF) and Unscented Particle Filter (PF-UKF). It is has been confirmed in many applications that PF-UKF provide robust estimation comparing with PFEKF[7]. Therefore, the main purpose of this work is to use the unscented particle filter to estimate the evolution of gene expression.

Consider a discrete state-space model given by:

$$x_k = f(x_{k-1}) + w_k \quad (1)$$

$$y_k = h(x_{k-1}) + v_k \quad (2)$$

where kx is the state vector and k y is the measurement vector. f and h are nonlinear state and measurement functions, respectively. kw and kv are zero-mean state and measurement white noise sequences with known posterior density functions. From the Bayesian framework, the posterior distribution can be computed using the following prediction and update steps:

$$P(x_k \mid y_{1:k-1}) = \int P(x_k \mid x_{k-1}) P(x_{k-1} \mid y_{1:k-1})\, dx_{k-1} \quad (3)$$

$$P(x_k \mid y_{1:k}) = \frac{P(y_k \mid x_k)\, P(x_k \mid y_{1:k-1})}{P(y_k \mid y_{1:k-1})} \quad (4)$$

For the nonlinear case, it is impossible to use Eqs.(3)-(4) because the introduced integrals are intractable. Particle filters estimate the state using an ensemble of weighted particles $\{x_k^{(i)}, w_k^{(i)}\}_{i=1}^{N}$ (lit.[5]). The approximation of the posterior distribution defined as:

$$P(x_k \mid y_{1:k}) = \sum_{i=1}^{N} w_k^{(i)} \delta\left(x_k - x_k^{(i)}\right) \quad (5)$$

where $\delta$ is the dirac delta function.

The weights are normalized such that: $\sum_{i=1}^{N} w_k^{(i)} = 1$.

Ideally, the particles need to be sampled from the true posterior, which is not available. Therefore, another distribution, referred to as the *proposal distribution*, $q(x_k / x_{k-1}, y_k)$ is used. Practically, the number of particles is finite and the proposal distribution should be chosen to approximate the posterior distribution. The importance weights are given by:

$$w_k^{(i)} = w_{k-1}^{(i)} \frac{p(y_k \mid x_k^{(i)})\; p(x_k^{(i)} \mid x_{k-1}^{(i)})}{q(x_k^{(i)} \mid x_{k-1}^{(i)}, y_{1:k})} \quad (6)$$

Given the discrete approximation to the posterior distribution in (5), the mean of the state at time $k$ is:

$$\hat{x}_k = \sum_{i=1}^{N} w_k^{(i)}\, x_k^{(i)} \quad (7)$$

After few iterations, the weights of particles in PF may are zero or close to zero. This phenomenon is known as 'degeneracy problem'. To address this problem, many approaches have been emerged to overcome this problem such as Extended Kalman Filter (PF-EKF), Unscented Particle Filter (PF-UKF) and Markov Chain Monte Carlo particle filter (PMCMC). All these approaches based mainly on generating the particle from proposal distribution using Metropolis hasting (MH) or gips sampling (GS) in PMCMC, extended Kalman filter (EKF) in PF-EKF, and unscented Kalman filter (UKF) in PF-UKF. It has been shown that the UKF provides a better proposal distribution than the prior transition in that it contains the most recent measurements, that often contain a lot of worthy information for estimating the states spaces[6,7]. Therefore, we used in this work the PF-UKF to estimate the evolution of gene series data.

The discrete state-space model for tracking the gene expression using a measurement data is defined as using Eqs. (1)-(2), where $x_k$ represents the evolution of gene expression at time k. $f$ represents a non-linear function that represents the regulatory relationship between several genes. $h$ represents a nonlinear measurement function. $y_k$ represents the micro-array data. The tracking of genes expressions that are the estimated state at time k represented as:

$$x_k = A\, g_{k-1} + w_k \quad (8)$$

where A represents the matrix coefficient values of the regulatory relationship between various genes. $g_{k-1}$ represents a sigmoid squash function of the latent state estimate. The main idea is to track the evolution of the gene expressions $x_{1:T}$ given an observations data $y_{1:T}$ over time. In this context, we proposed to use each PF-UKF to estimate the evolution of every gene over time. Furthermore, we have reduced the dimensions of the state space model and overcome the problem of 'curse of dimensionality'.

We applied the unscented particle filter to predict the evolution of gene expression from real data. In this work, we used the real data for worm time series, which was presented in [8]. We considered also eight genes for this section. We tracked the eight genes expression using 1000 particles for PF,

PMCMC, PF-UKF, and we implemented 100 Monte Carlo Runs. The coefficients of regulatory relationship that is matrix A are computed using normal distribution.

We computed the root mean square error (RMSE) between the real and estimated state of eight worm time series data. Then, we averaged the RMSE for eight genes: UKF=0.0189, PF=0.4521, PMCMC=0.4799 and PF-UKF=0.0019. Furthermore, PF-UKF offers the best results in terms RMSE while PF, and PMCMC result in large estimation error. As a result, PF-UKF provides powerful solution to the problem of estimating gene regulatory networks.

This work introduced the unscented particle filter for estimation of gene expression. The gene regulatory networks are modelled as a high dimensional problem. The unscented particle filter has been proposed as powerful method to ameliorate the performance of PF in high dimensional state spaces. Simulation results on real-world data showed that the PF-UKF provided a robust estimation for the evolution of gene expression with less RMSE compared to other filters, i.e., UKF, PF and PMCMC. Furthermore, PF-UKF provides an alternative solution to the problem of estimation of gene expression time series data.

*This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic and the European Union (European Structural and Investment Funds—Operational Programme Research, Development and Education) in the frames of the project "Modular platform for autonomous chassis of specialized electric vehicles for freight and equipment transportation", Reg. No. CZ.02.1.01/0.0/0.0/ 16_025/0007293.*

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## THE DISTINCT FUNCTION AND LOCALIZATION OF METTL3/METTL14 AND METTL16 ENZYMES IN CARDIOMYOCYTES

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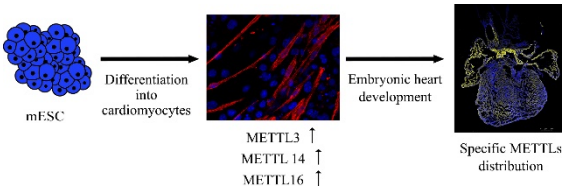
The N6-methyl adenosine (m6A) is the most abundant and influential modification of mRNA that, through its writers (METTL3/METTL14 and METTL16) and erasers (FTO and ALKBH5), shapes the cell state during mouse embryonic stem cell (mESC) differentiation[1], and it is nowadays emerging as a fundamental player in cardiac homeostasis[2]. An understanding of this orchestrated epigenetic regulation will help to pave the way toward the development of new epi-drugs.

In our study[3], the western blot and immunofluorescence analyses showed that the spontaneous differentiation via embryoid bodies is accompanied by a distinct profile of writers for the whole heterogeneously differentiated cell populations (METTL3/METTL 14 decrease and METTL 16 increase) and for the individual differentiated-cardiomyocytes (METTL3/METTL14 and METTL 16 increase). In parallel, the fluorescence analysis revealed that, in various anatomical regions of the mouse embryonic heart, the distribution pattern of the writes METTL3/METTL14 is distinct from that of the METTL16. Moreover, the hypothesis of a link between histone regulation and m6A was explored, revealing a fluctuation of the writers in explanted embryonic hearts treated by three Histone Deacetylase inhibitors (HDACi) and a decreased m6A content in differentiating Histone Deacetylase 1 depleted mESC line (HDAC1 dn mESC).

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## SYNTHESIS OF *N*-PERFLUOROALKYL-3,4-DISUBSTITUTED PYRROLES BY RHODIUM--CATALYSED TRANSANNULATION OF *N*-FLUOROALKYL-1,2,3-TRIAZOLES WITH TERMINAL ALKYNES

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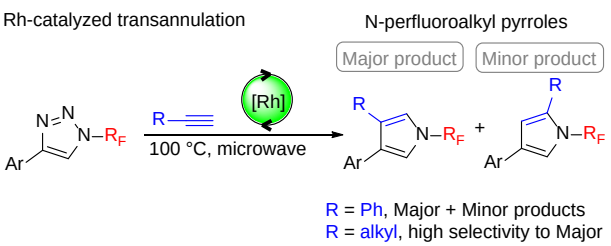
*N*-Sulfonyl 1,2,3-triazoles and *N*-perfluoroalkyl-1,2,3--triazoles have been used as precursors to transition-metal-catalyzed transannulation reactions. In the presence of Rh(II) catalysts, the triazole ring-opening takes place and intermediate highly electrophilic metal-bound iminocarbenes form. These iminocarbenes undergo a variety of intriguing reactions, such as cycloaddition, C-H functionalization, among others, leading mostly to nitrogen heterocycles[1-3].

Rhodium-catalyzed transannulation of *N*-perfluoroalkyl--1,2,3-triazoles with aromatic and aliphatic terminal alkynes under microwave heating conditions afforded *N*-perfluoroalkyl-3,4-disubstituted pyrroles (major products) and *N*-fluoroalkyl-2,4-disubstituted pyrroles (minor products). Observed selectivities in the case of aliphatic alkynes were high.




Scheme 1

*This work was financially supported by the Czech Academy of Sciences (RVO: 61388963) and by the Ministry of Education, Youth and Sports in the program INTER-EXCELLENCE (LTAUSA18037).*

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## STRUCTURED ARCHITECTURES OF BORON-DOPED DIAMOND FOR NEUROSCIENCE

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Novel materials based on porous boron-doped diamond (p-BDD) have attracted lots of research interest due to their enhanced detection ability[1] and biocompatibility[2], favouring them for use in neuroscience.

The present work reports on a comprehensive and comparative study of recently developed p-BDD electrode materials[1], which differ in: (1) utilised deposition templates, *e.g.*, carbon nanotubes, $SiO_2$ nanofibers and their combination, (2) number of deposited layers (*i.e.*, thickness), (3) boron-doping level, and (4) content of $sp^2$-carbon. Fabricated p-BDD materials were tested for sensing of neurotransmitters and their precursors, namely tyrosine, L-DOPA, dopamine, tryptophan, and serotonin, in pH 7.4 media mimicking physiological conditions, such as phosphate and HEPES buffered saline, and Neurobasal-based media commonly used for neuronal cells growth. Besides, p-BDD deposited on $SiO_2$ nanofibers has shown biocompatibility and suitability for neuron cultivation[2].

The above-mentioned alterations in deposition conditions led to fabrication of p-BDD electrodes with diverse: (i) structural and morphological features, investigated by scanning electron microscopy and Raman spectroscopy, and (ii) electrochemical performance, sensitivity, selectivity, and susceptibility to adsorption and fouling when employed for sensing of selected biomolecules, examined by means of voltammetry.

Improved understanding of factors affecting the final characteristics can possibly lead to a rational design of new and highly perspective p-BDD electrode materials, which can be applied in numerous areas including sensors development in biomedical research and neuroscience.

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## ADVANCES IN REGENERATIVE MEDICINE AND TISSUE ENGINEERING: INNOVATION AND TRANSFORMATION OF MEDICINE

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Regenerative medication (RM) is another approach to fix patients other than conventional medication and medical procedure. RM incorporates items from all the mainstays of medical care, for example drugs, biopharmaceuticals, clinical gadgets and cell treatments, to convey clinical results. All around the world, the cell treatment industry is simply developing, and keeping in mind that RM additionally draws upon non-cell-based medicines, undifferentiated organism based items and administrations have probably the most captivating chances and expectations with respect to beforehand serious sicknesses. In this exposition, the emphasis is on undeveloped cell based items and administrations. The fundamental examination question is the way scholastic exploration based advancements happen and can be moved to new organizations and treatments in the RM area. Hypothetically, this exposition expands on advancement frameworks, development related clinical innovation writing, and ability alliance hypothesis. Clinical innovation writing distinguishes parts of clinical innovation advancement development and how its components are conceptualized inside wellbeing advancement frameworks. Skill alliance hypothesis gives a decent clarification to how business develops and what skills are required. This thesis followed a valuable exploration approach and a solitary contextual analysis technique.

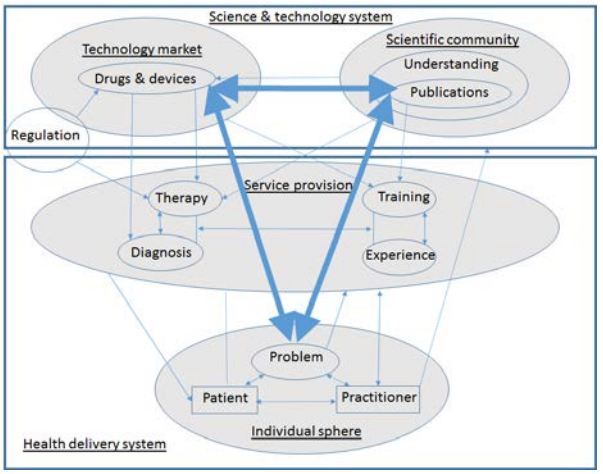


Figure 1. **Health innovation system adapted from Consoli and Mina**

The observational information comprises of 24 interviews and significant auxiliary information (reports, distributions, measurements, and so on) Utilizing observational information and foundation writing, a development was created so as to clarify how development happens at the framework level and to distinguish the entertainers that are included.

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## SELF-IMMOLATIVE PHOSPHATE-BASED PRODRUGS FOR DELIVERY OF ANTIFUNGAL VORICONAZOLE

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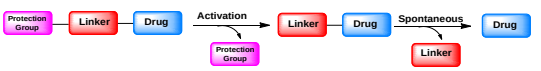
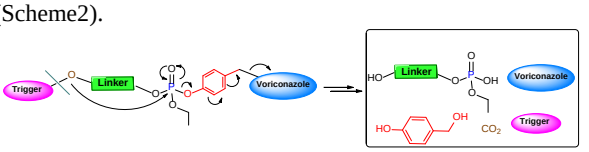
Drug delivery (DD) research aims at the development of novel compounds or carrier systems for effective therapeutic delivery of drugs. The basic idea is to fully control the release of a drug at the target site. Among various methods, self-immolative (SI) linkers play a fantastic role in releasing the drug from its inactive precursor. SI linkers are designed for cascade defragmentation and controlled release of free active drug (Scheme1)[1,2].




Scheme1. **Schematic representation of a SI spacer**

Our research group recently showed that phosphate-based SI linkers have the great potential to perform as effective drug-delivery systems[3]. We are currently focusing on the phosphate-based SI linkers, which would effectively release the antifungal drug voriconazole. Proposed linkers contain a phosphate core, glycol or lactate arms, and *para*-hydroxybenzyl alcohol linkage attaching voriconazole (Scheme2).




Scheme2. **SI linker relying on a cyclization process**

We are currently working on various phosphate-based SI systems, and we are testing their antifungal activities simultaneously. The information on antifungal activity tells us which linker is suitable for further development. The second critical part of this research is trigger selection. The trigger has an essential role in fungi recognition and should be metabolized exclusively by fungi and not by human cells. Choosing an appropriate trigger and linker should lead to targeted delivery of antifungal voriconazole and related azole drugs.

*We thank the Experientia Foundation (O.B., Start-Up grant SG-2018-1), and the Czech Science Foundation (O.B., grant No. 20-25137Y).*

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## IMMUNOCOMPATIBILITY OF AMINE PLASMACHEMICALLY FUNCTIONALIZED SURFACES

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The immune response to biomaterials affects their acceptance and good incorporation into surrounding tissue. Plasma and tissue proteins adsorb to the biomaterial surface immediately after implantation. Immune cells thus react to these adsorbed proteins not only to the pure biomaterial surfaces[1].

In this work, we assess immunocompatibility of amine-rich, plasma-chemically coated biodegradable polycaprolactone (PCL) membranes created by electrospinning, which could potentially serve as vascular prostheses or wound dressings. Toxicity and biocompatibility of amine-coated surfaces has been already tested. It was found out that amine modified surfaces improve cell adhesion. In addition, more cell lines (human skin fibroblasts (LF), vascular smooth muscle cells (VSMC), keratinocytes (HaCaT)) show higher trypsin resistance on these surfaces[2,3]. The logical progression after toxicity and biocompatibility assessment is to evaluate biomaterial immunocompatibility.

Macrophages are the immune cells that affect immune response the most. Macrophages have great polarization capacity and can support pro-inflammatory or pro-regenerative response by directing T lymphocytes to Th1 or Th2 phenotypes, primarily[4]. As T-helper lymphocytes differ to pro-inflammatory Th1 and pro-regenerative Th2 subsets, also macrophages can be divided into M1 and M2 phenotypes[5]. These two macrophage subsets differ in some surface markers, genes, and secreted cytokines. CD86, CD80 and CCR7 are surface markers specific for pro-inflammatory M1 macrophages. Additionally, CD163 and CD206 (MRC-1) are specific for pro-regenerative M2 macrophages. Cytokines, that are produced by M1 macrophages, are IL-1, IL-6, IL-8 and TNF-α. M2 macrophages produce IL-1RA, IL-4 and IL-10, for example[6]. The whole immune response to biomaterials can be predicted by analysis of polarization of macrophages using qRT-PCR, flow cytometry or ELISA. Macrophage polarization must be assessed during longer cultivation, as macrophages can rechange their phenotypes from M1, which promote inflammation, to M2, that support tissue recovery, and vice versa[7].

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## DIRECT ELECTROMEMBRANE EXTRACTION OF ANTHRACYCLINES FROM TISSUE SAMPLES

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Electromembrane extraction (EME) is a hybrid microextraction technique laying between liquid-liquid extraction (LLE) and electrophoresis. The extraction of charged analytes is performed from the aqueous sample through the water immiscible organic supported liquid membrane (SLM) to the acceptor solution. The driving force of the extraction is an electrical potential, which is applied across the SLM. Tissue analysis presents bioanalytical challenge compared to analysis of biological liquids. Firstly, homogenization prior sample treatment is required to ensure both, representative sample and effective sample extraction. Further difficulty is determination of analyte extractability, as the spiked tissue sample or even spiked homogenate do not

exactly mimic drug binding and distribution in intact tissue. The aim of this study was to optimize the EME for isolation of anthracyclines (ANT) from rabbit plasma and to determine the applicability of this method for direct extraction of ANT from tissue samples. The EME conditions primarily optimized for plasma[1] were modified and successfully used for tissue samples. The collected tissues (heart, liver and skeletal muscle) were simply pulverized and homogenized under the liquid nitrogen using the mortar and pestle method prior the extraction and calibration samples were prepared by spiking the pulverized tissue. The optimized EME followed by UHPLC MS/MS method was validated in both plasma and tissues. In addition, for evaluation of sample purification, the level of phospholipids in the EME extracts were examined. Finally, the method was applied for analysis of real plasma (n=12) and tissue samples (n=6) taken after administration of daunorubicin (3 mg/kg, i.v.) to rabbits. In order to verify reliability of the EME, the conventional LLE was used as a referenced extraction method and assayed concentrations in real samples were compared. The difference between the concentrations determined after EME and LLE were lower than 10%. This is the first time, when direct EME of drugs from animal tissues was performed. EME was proved to be simple, effective and reliable microextraction technique for isolation of ANT from tissues.

*The study was supported by the Charles University (SVV 260 547).*

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## BIOAVAILABILITY OF EPOXICONAZOLE AND TEBUCONAZOLE WITH BIOMIMETIC EXTRACTION IN BIOCHAR AMENDED SOIL

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The objective of this study was to describe alterations in the fate and bioavailability of selected conazole fungicides (CF). We studied epoxiconazole (EPC) and tebuconazole (TBC), Fig. 1, in 14 variants generated by high- and low-CF sorbing soils amended with low-, moderate- and high- sorbing biochars at 0.2% and 2% doses on earthworms (*Eisenia andrei*), plants (*Lactuca sativa*)[1] and stir bar solid extraction (SBSE).

Biochar in the soils promoted the dissipation of EPC and TBC with dose, soil, and compound effect less clear of biochar type[2]. Degradation of fungicides followed double first-order kinetic model resulting with low dissipation half-life (DT50) in all variants for EPC and TBC illustrating ending plateau, showing non-degradable or very- or slowly-degradable residues. EPC DT50 ranges (0.2 – 9.0 d) were wider compared to TBC ranges (0.4 – 6.3 d).

Earthworm bioaccumulation, which decreased 0.6 times to 24.7 times with biochar, was higher in low-CF sorbing soil negatively correlating with biochar amended soil total organic carbon. Bioconcentration to lettuce roots, which decreased 0.8 to 55.8 times with biochar, was one to two magnitudes higher than to leaves, which decreased 0.8 to 29.9 times with biochar.

SBSE showed combined differences of earthworm bioaccumulation and lettuce roots and leaves bioconcentration factors using polydimethylsiloxane (PDMS) polymer representing biomimetic extraction[3–5] in Fig. 2. This study explains at some extent effect of biochar application on fate and bioavailability of CF and their effective screening technique from soil.

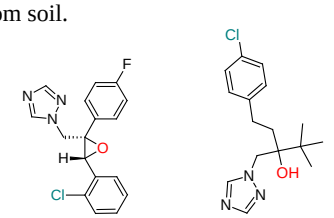


Fig. 1. **Epoxiconazole (left) and tebuconazole (right)**

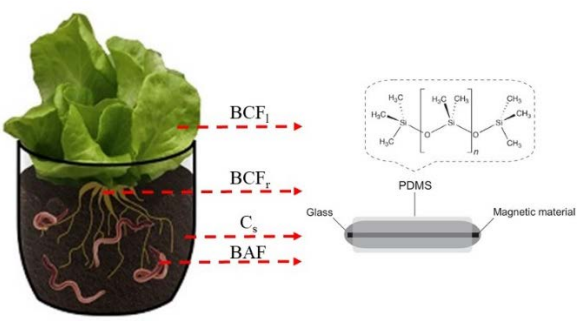


Fig. 2. **Concentration of the substance in the soil ($C_s$), earthworm bioaccumulation factors (BAF), lettuce roots bioconcentration factors ($BCF_r$), lettuce leaves bioconcentration factors ($BCF_l$) and polydimethylsiloxane (PDMS)**

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## FREEZE-DRYING TRANSPARENT HYDROGEL FILMS FROM NATURAL GUM KARAYA MODIFIED WITH OCTENIDINE DIHYDROCHLORIDE FOR INFECTED WOUND HEALING

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Wound healing is a process which requires an appropriate approach for effective and accelerated healing. Variety of materials including natural and synthetic polymers are used for the production of films, gels, hydrogels or sponges. Gum Karaya (GK) is a natural polymer, resin able to form a gel with antimicrobial properties supporting the healing process[1]. Here, GK was modified with synthetic hydrophilic gelling polymers and emollient component to obtain soft, elastic and transparent film via freeze-drying technique. To increase antimicrobial activity an antiseptic agent octenidine dihydrochloride (OCT) was added due to its antimicrobial effect, low absorption to the skin and no systemic toxicity. OCT is widely used antiseptics for topical wounds (acute, chronic) with antimicrobial effect against Gram-positive and Gram-negative bacteria including resistant pathogens.

The main aim of this work was to design and prepare hydrogel dressing with suitable properties for wound healing, such as pro-healing properties, biocompatibility, nontoxicity, antimicrobial activity, transparency and biomechanical properties imitating the skin behaviour. Prepared dressings were transparent, flexible and showed sufficient ability to swell in water and create a suitable environment for wound healing. Hydrogel dressing enriched with OCT showed synergistic antimicrobial activity against many bacteria strains including *Staphylococcus aureus* methicillin-sensitive, *Staphylococcus aureus* methicillin-resistant, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Enterobacter cloaca*, and *Escherichia coli*. Regarding to our results, hydrogel dressings based on Gum Karaya enriched with antimicrobial OCT shown promising activity within a wound closure process in regenerative medicine of chronic wounds.

*This work was supported by the Ministry of Health of the Czech Republic under the project no NU20-05-00166.*

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## GENERATION OF LUNG ORGANOIDS IN CO-CULTURE WITH ENDOTHELIAL CELLS

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Current methods of organoid culture allow creation of complex structures from human pluripotent cells through activation of specific signalling pathways and existing developmental programmes. This allows the organoids to develop through semi-physiological process, giving rise to cellular phenotypes and histological structures resembling the actual organ. However, important component of lung tissue structure is also vasculature.

Our aim is to combine protocols for lung organoid differentiation with methods of *in vitro* vascularization. We embedded endothelial cells into 3D hydrogels and over time assessed the parameters of assembled capillary network. With this approach, we could test several types of hydrogels with unique properties, different types of supporting cells and the effect of initial cellular organization (*e.g.* numbers and ratios of cells used). This allowed us to control the parameters of the final network, such as its density and interconnectedness. In parallel, we generated lung organoids from lung progenitor cells. We characterized specific molecules under which lung organoids undergo extensive branching. Moreover, we show that the development was also affected by other aspects, *e.g.* the nature of the 3D matrix the organoids are cultured in, specific phenotype of progenitor cell population used for the generation of organoids, *etc*.

Finally, we present current advances from our work on intersection of those two approaches to find conditions where both can take place and work in synergy. Such model is especially useful for studying the interaction between the lung epithelium and endothelial cells, and their involvement in morphogenic and developmental process of human lungs. This way, both elements are present in a highly organized form, improving the relevance of such model in comparison to some other, less organized, or even planar cocultures.

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## PSEUDODOMINANCE IN TWO FAMILIES WITH STARGARDT DISEASE HIGHLIGHTS HIGH POPULATION FREQUENCY OF *ABCA4* PATHOGENIC VARIANTS

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Mutations within the retina-specific ATP-binding cassette transporter gene (*ABCA4*) cause a variety of retinal phenotypes, including Stargardt disease (STGD1); the most common autosomal recessive retinal dystrophy with an estimated prevalence of 1 in 10,000. *ABCA4* gene is relatively large (50 exons; 128,313 bp) with total number of reported variants to date 9,132, of these 1,344 are unique[1].

We performed molecular genetic analysis using single-molecule molecular inversion probes (smMIPs) and identified the molecular genetic cause in 66 Czech probands with clinically diagnosed STGD1. The results of this analysis are in part described elsewhere[2]. In two families denying consanguinity one parent was also known to suffer from retinal dystrophy. We have therefore applied exome sequencing and smMIPs to search for the presence of pathogenic variants in them. General population frequency of the detected *ABCA4* variants in both families was searched in gnomAD v2.1.1 comprising 125,748 exomes and 15,708 genomes[3].

The proband from family 1 was found to be compound heterozygote for following alleles c.[5461-10T>C;5603A>T] p.[(Thr1821Valfs*13,Thr1821Aspfs*6);(Asn1868Ile)] and c.5882G>A; p.(Gly1961Glu). Familial segregation showed that the first complex allele was inherited from the father who also had c.6320G>A; p.(Arg2107His) in trans position. The proband from family 2 carried c.2565_2572del; p.(Trp855*) and c.5882G>A; p.(Gly1961Glu) in a compound heterozygous state while her mother had c.2565_2572del; p.(Trp855*) in a homozygous state. Three of the detected pathogenic variants were present in gnomAD with following frequencies 2/282,656 (c.6320G>A), 2/282,194 (c.5461-10T>C), 1291/282,848 (c.5882G>A), while the c.2565_2572del has no frequency in the database. The frequency of c.5603A>T, previously reported as hypomorphic (mild conditional allele), was about 7% in the non-Finnish European population.

Frequency of *ABCA4* pathogenic variants is high in the general population. Based on our dataset the likelihood of an affected Czech patient having a child also suffering from *ABCA4*-associated retinal is more than 3%. This is important and needs reflected in clinical counselling of the affected families.

*This work was supported by NU20-07-00182 and SVV 260367/2017.*

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## DEVELOPMENT OF FULGIDES AS BUILDING BLOCKS FOR PHOTOSWITCHABLE IONIC LIQUIDS

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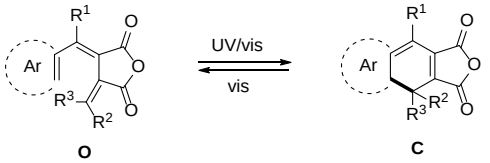
Reversible transformation of bi-stable photochromic compounds has been recently exploited in new polyfunctional materials e.g. for molecular electronics or for optical molecular memories[1,2]. The family of fulgides has received a lot of attention mostly due to their extraordinary thermal stability and fatigue resistance. Irradiation of fulgides leads to an electrocyclic rearrangement giving either an opened **O** or a cyclized **C** form[3] (Scheme 1).




O C

Scheme 1. **Cyclization of fulgides**

We aim to develop a modified fulgide bearing a charged moiety. This should lead to a decrease in melting point and in combination with appropriate counterion an ionic liquid should be formed. The initial studies focus on development of derivatives of 3-indolyl-fulgides bearing a charged trialkylammonium group.

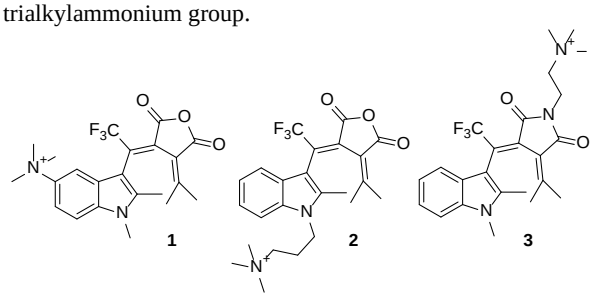



Fig. 1. **Structures of target fulgides**

Fulgide will have the charged group attached either to the aromatic system ***1*** or it would be separated by a linker ***2***.

Furthermore, fulgimide ***3*** is another alternative of attaching the charged group (Figure 1). These prepared compounds will be thoroughly studied revealing their possible usage as a reversible photo-switchable ionic compounds with application as molecular polyfunctional materials.

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## HETEROCYCLIC MODULATORS OF CIRCADIAN RHYTHM

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A circadian rhythm (CR) regulates not only the sleep-wake cycle but also many other physiological processes including metabolism and immunity. CR disruptions have negative consequences on human health. For example, shift workers are highly vulnerable with increased risk of depression, metabolic syndrome or sleep disturbances. Identification of novel chronotherapeutics – drugs for clock-related disorders is a perspective area of research[1,2]. We used U2OS cell line stably expressing Bmal1-Luc reporter cell line to identify circadian rhythm modulators in a library of heterocyclic derivatives. Several derivatives extended period of circadian rhythm without significant toxic effects.

Activity of three of them was further validated using mouse hypothalamus explants[3]. Several compounds are expected to reach CNS after oral administration as indicated by the results of ADME in vitro assays including plasma protein binding, stability in plasma and in the presence of liver microsomal fraction and permeability across model barriers (PAMPA, Caco-2 and MDR1- MDCK cell monolayers).

Such compounds may find use as drugs for jet-lag disorder, advanced sleep phase disorder of familial advanced sleep phase syndrome.

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## MODULAR SYNTHESIS OF DENDRITIC AMPHIPHILES

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Among nanovesicle drug delivery systems, liposomes are the oldest and most widely used vehicles[1]. To overcome some of their drawbacks, analogical supramolecular systems based on different building blocks were developed in two recent decades. One of such new drug delivery systems are dendrimersomes[2]. Dendrimersomes are nanovesicles self-assembled from amphiphilic molecules consisting of at least one dendritic part. Following dendrimersomes design, this project aims at development of modular synthesis of dendritic amphiphiles whose final structure can be easily modified (Fig. 1).

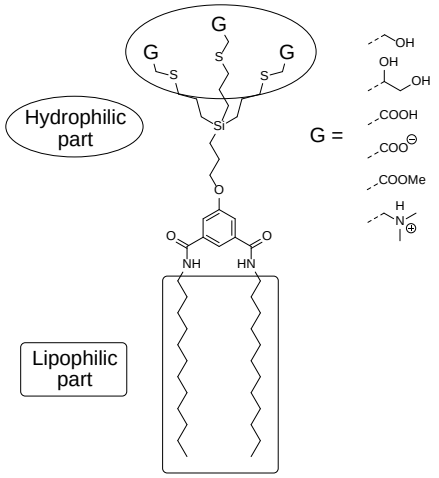


Fig. 1. **Example of prepared dendritic amphiphiles**

In the aqueous solution, these amphiphiles should be able to self-assemble into supramolecular objects which could then serve as nanovehicles for variety of drugs or nucleic acids with therapeutic effects.

The syntheses of such structures start by attaching alkyl chain/s and dendritic wedge/s to the starting molecules. These dendritic wedges are ended by three allyl groups each so that different polar groups can be conjugated to the periphery of the

wedge by so called thiol-ene click reaction. Next to symmetrical structures, a synthesis of unsymmetrical analogues bearing fluorescent tag on one of the alkyl chains will be presented.

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## ENANTIOSEPARATION OF NOVEL PSYCHOACTIVE SUBSTANCES IN SUPERCRITICAL FLUID CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Novel psychoactive substances (NPS) are synthetic compounds that have been designed to produce the physiological and psychological effects of known recreational drugs, while circumventing current drug control laws and scheduling guidelines[1]. Many of these substances, often misidentified as *"research chemicals"* or *"bath salts"*, have not been listed in databases yet, so there is little information about their effectiveness or toxicity. Since many of NPS are chiral, the pharmacological activity and side effects of their enantiomers can differ greatly[2].

Therefore, this work was focused on development and optimization of fast and efficient enantioseparation methods for less-known structurally diverse NPS of various classes, i.e., pyrovalerones, benzofurans and phenidines, in supercritical fluid chromatography (SFC) and high-performance liquid chromatography (HPLC) using vancomycin bonded chiral stationary phases (CSPs) packed with 2.7 μm core shell particles. Results showed the high enantioselective potential of vancomycin-based columns in both chromatographic techniques: 88% of NPS tested were enantioseparated in SFC and 69% of NPS tested were enantioseparated in HPLC. Some complementary enantioseparations on vancomycin-based CSPs in SFC and HPLC were observed. Moreover, the CSPs were found to be suitable for rapid simultaneous enantioseparations of some NPS in both techniques. The developed SFC and HPLC methods can serve as guides for enantioseparation of further upcoming NPS and for checking the enantiomeric composition of real-life samples.

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## SPECIATION OF GERMANIUM IN ENVIRONMENTAL WATERS AT RELEVANT CONCENTRATIONS

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Over last few years, germanium is one of the most interesting elements for many research areas as well as new technologies. In the environment, germanium is present mainly in inorganic form (iGe), $Ge(OH)_4$, and methylated forms: monomethyl germanium (MMGe) and dimethyl germanium (DMGe). The presence of these organic species is most likely attached to the biochemical cycling of the element[1]. Other organic species are rare. Possible different toxic effects of Ge species have been reviewed[2]. Whether Ge may pose a future threat for humans and biota is hard to answer. Knowledge of the biogeochemical cycle of this element is highly recommended in order to study the possible effects of human activities and which of them may have consequences (also from an economical point of view)[3]. However, not much is known about Ge in the environment. This is mainly due to the lack of analytical methods which can deal with the extremely low Ge concentrations found in environmental samples. This is the frame where this work fits.

A method based on the generation of volatile germanes from different germanium species and their separation by cryogenic trap has been combined with ICP-MS/MS for Ge speciation analysis. Due to the ultratrace concentrations of Ge in environmental samples, the blank signals controlling limits of detection (LOD) have been handled with a special care. After a proper optimization of all experimental variables, LODs of 0.015, 0.005 and 0.003 ng $L^{-1}$ for iGe, MMGe and DMGe, respectively, were achieved, corresponding to absolute masses of 15, 5 and 3 fg Ge. Just one mL of sample was needed to perform the analysis. Finally, the method was applied to the determination of Ge species in certified reference materials of unspiked natural waters[4].

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## MULTICOLOR FLUORESCENT GRAPHENE QUANTUM DOTS FOR BIOIMAGING AND BEYOND

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Graphene quantum dots (GQDs) are a fascinating class of nanocarbons that comprise quasi-spherical nanoparticles with sizes below 10 nm[1]. They typically display excitation wavelength dependence, excellent photostability and chemical stability, good biocompatibility, high-water solubility and low toxicity. Furthermore, they can be easily functionalized with biomolecules. GQDs with tunable near-infrared (NIR) fluorescence emission promise an excellent bioapplication potential, especially in bioimaging.

In this work, we report the synthesis of nitrogen-doped GQDs (N-GQDs) from glucose and ethylenediamine, using a one-step and fast microwave-assisted hydrothermal method. Our N-GQDs exhibited fluorescence from NUV to NIR and the synthesis method is unique compared to the previously published approaches that gained NIR-emitting carbon dots or GQDs[2]. Since the origin of NIR fluorescence and its relation to the structure and synthesis conditions is not yet fully understood we concentrated also on the fluorescence mechanism explanation. Structural characterizations with steady-state and time-resolved photoluminescence measurements indicated that band-to-band transitions, size effect and different nitrogen and oxygen functional groups play a role in this multicolor emission. The N-GQDs proved to be highly biocompatible by a cell viability assay using human vascular smooth muscle cells. Together with the wide spectral range emission observed in confocal fluorescence imaging, it demonstrated the potential of the hydrothermally synthesized N-GQDs for *in vitro* bioimaging applications.

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## CAN FLAVIN PHOTOCHEMISTRY FACILITATE AMIDE BOND FORMATION VIA ALDEHYDE AND AMINE COUPLING?

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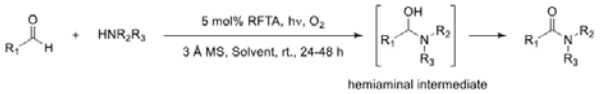
Amides are not only the building block of all-natural peptides but also key functions in the synthesis of polymers and modern pharmaceuticals1[1]. The most common industrial method of amide bond formation is the reaction of activated carboxylic acid derivatives with amines[2]. However, this pathway suffers from inherent drawbacks, such as high costs and the production of stoichiometric waste, and loss of stereochemistry[3,4]. In addition, enzymatic methods involve high isolation costs and can only be used with a limited range of substrates[5]. These issues caused amide formation avoiding poor atom economy reagents to be one of the main challenges for organic chemistry[6]. As an alternative to the traditional methods, the transition-metal-catalyzed coupling of aldehydes with amines and metal-free oxidative amidation of aldehydes have achieved a breakthrough in amide synthesis. Non-toxic aldehydes are readily available and offer the possibility of starting the transformation from substrates other than carboxylic acids. Moreover, many methods starting from aldehydes are efficient and highly chemoselective towards amide formation. Nevertheless, the majority of them involve expensive reagents, toxic solvents, high temperatures, and low tolerance of secondary amines so their use in the industry is rare[7,8]. Consequently, the researchers have directed their attention to the need for highly efficient and benign catalytic strategies.

Considering all of the aforementioned factors and the photooxidation ability of flavin derivatives, we here develop the first flavin-based photocatalytic approach to amide synthesis using riboflavin tetraacetate (RFTA) as a commercially available catalyst. This system involves amide synthesis through the interaction between an aldehyde and a secondary amine using visible light in the presence of oxygen as a terminal oxidant. It has been investigated on the coupling

of *p*-chlorobenzaldehyde and piperidine (2 equiv) as a model reaction using RFTA (5 mol%) in the presence of molecular sieves (3 Å) (Scheme 1). The mechanism of this reaction relies on the formation of hemiaminal intermediate which is oxidized to produce the amide. Overall, we suggest RFTA as a new candidate for the amide bond formation at mild conditions without the need for basic conditions or an $H_2$ acceptor.




Scheme 1. **Oxidative amidation of aldehydes using RFTA**

*This work was supported by the Czech Science Foundation (reg. No. 18-15175S).*

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## NOVEL LIPID NANOPARTICLES FOR NUCLEIC ACID DELIVERY

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Lipid nanoparticles (LNPs) represent a safe and effective tool for nucleic acid delivery. They were studied for possible use in gene therapy and already introduced into the clinics with the first siRNA-LNP medicine called Onpattro[1]. LNP gained global attention recently after introducing the mRNA-LNP vaccine against Severe Acute Respiratory Syndrome Coronavirus 2[2]. These pioneer LNP-based therapeutics are now opening up new possibilities in modern medicine.

To improve the general properties of LNP, we synthesized series of ionizable lipidoids with adamantane core named "XMaNs". Using XMaN LNPs, we delivered different types of nucleic acid (siRNA, mRNA, plasmid DNA, and cyclic dinucleotides) *in vitro* and also *in vivo* with high efficiencies and with none or low cytotoxic effects. The XMaN6 showed the most promising result with 80% entrapment of siRNA or mRNA and up to 95% transfection efficacies. It proved to be suitable for gene therapy in the liver with both siRNA and mRNA cargo without histology changes or organ failure biomarkers alteration.

XMaN LNP can be assembled with various nucleic acid types without further optimisations in LNP composition while maintaining their efficiency, which gives them a significant advantage over other lipids or lipidoids.

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## TRIARYLAMINIUM RADICAL CATION PROMOTED NEW SYNTHETIC TRANSFORMATIONS: FROM C-O BOND CLEAVAGE TO C-C BOND FORMATION

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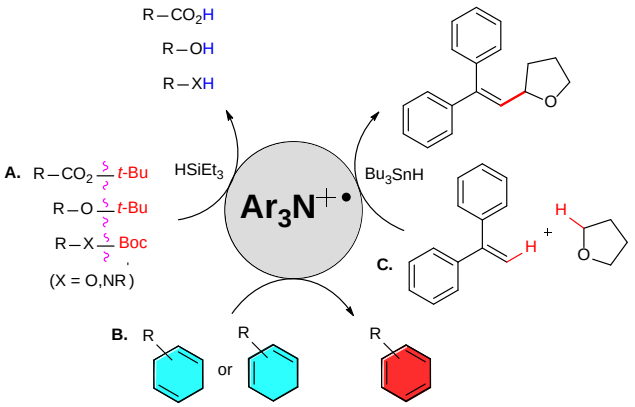
Although a large number of stable and persistent radical cation salts have been prepared and isolated, only a few of these have been used in organic synthesis. Among them, aminium radical cation salts are the most widely employed class of reagents[1–4].

Our research focuses on the stable triarylaminium radical cations for development of novel and efficient synthetic transformations. Recently, we evidenced that triarylaminium cation radical and hydrosilane mediate the facile C-O bond cleavage in O*t*Bu groups in carbamates, carbonates, esters and ethers (Scheme 1, A). This catalytic protocol represents a mild and practical method for de-*tert*-butylation. In view of the importance of aromatic molecules, in particular in material science, we developed a new, simple and efficient aromatization of 1,2-dihydronaphthalenes and dienes using

catalytic amounts of triarylaminium radical cation (Scheme 1, B). Afterwards, the first triarylaminium radical cation-mediated alkenylation of simple ethers to construct allylic ethers was successfully achieved (Scheme 1, C). A notable feature of the developed process is that it does not require dangerous peroxides.




Scheme 1. **Triarylaminium radical cation promoted new synthetic transformations**

*This work was supported by The Czech Science Foundation (reg. No.19-20467Y).*

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## WATER BLOOM CYANOBACTERIA *APHANIZOMENON FLOS-AQUAE* LPS HAS PRO-INFLAMMATORY EFFECTS ON KERATINOCYTES AND MACROPHAGES *IN VITRO*

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Cyanobacteria-dominated harmful algae blooms (HAB) are an important source of various toxins in aquatic environment. Cyanobacterial lipopolysaccharide (LPS), the integral part of the cyanobacterial cell wall, has hardly been studied at all despite the fact that dermatitis is observed after HAB exposure and bacterial LPS is well-known pro-inflammatory agent. Therefore, we focused on *Aphanizomenon flos-aquae* (species commonly found in Czech recreational water bodies) LPS. Axenic culture of *A. flos-aquae* was used to obtain the biomass, LPS was isolated using hot-phenol extraction and its endotoxin activity was determined using Pyrogene assay. Considering, that skin is the first target of the exposure to HAB, HaCaT cells (human keratinocytes) were used to study its biological activity *in vitro*. Surprisingly, the LPS showed significant induction of production of interleukin (IL) 6 and 8, MCP-1 and also CCL-20 despite its very low endotoxin activity. Moreover, it induced migrative activity of HaCaT cells proved by wound-healing assay. It shows significant pro-inflammatory potential of the LPS. Therefore, we decided to study effects on immune cells using RAW 264.7 mouse macrophages. The *A. flos-aquae* LPS activated the macrophages to produce IL-6, tumour necrosis factor α (TNFα), and NO. Whereas the mechanism of *E. coli* LPS activity is known to be via Toll-like receptor (TLR) 4, its inhibitor was used. Surprisingly, inhibition of TLR4 did not play a significant role in *A. flos-aquae* LPS effect but inhibition of TLR2 did.

In conclusion, LPS of *A. flos-aquae* has pro-inflammatory effects on human keratinocytes as well as on immune cells. Its effects are not dependent on TLR4 but rather on TLR2 and its bioactivity is not predictable by routinely used endotoxin test. Therefore, deeper studies of bioactivity of cyanobacterial LPS should be done to evaluate its human health hazards.

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## CHASING CHIRAL HELICENE MACROCYCLES IN SOLUTION AND ON GRAPHITE SURFACE

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Large polyaromatic systems have recently become the subject of intensive research due to their promising applications in material science. Notably, chiral non-planar systems have attracted attention as models for the study of circularly polarized light emission or electron spin filtration.

Motivated by the above applications, we recently prepared a large helicene-based macrocycle ***1*** as an equilibrium mixture of four stereoisomers (Fig. 1). Here, we report on the structural elucidation of the individual stereoisomers using NMR and other spectroscopic techniques.

Kinetics of the chirality inversion was investigated, giving more insight into the mechanism of the macrocycle diastereomerization and providing valuable structural information, inaccessible by spectroscopic methods.

We further studied adsorption of the macrocycles on graphite surface using atomic force microscopy. We found the individual diastereomers self-assemble into aggregates with different morphology. Molecular dynamics simulations of the self-assembly process found a large preference for heterochiral aggregation and showed that the molecules in the aggregates are stabilized by interlocking interactions of their trityl groups.

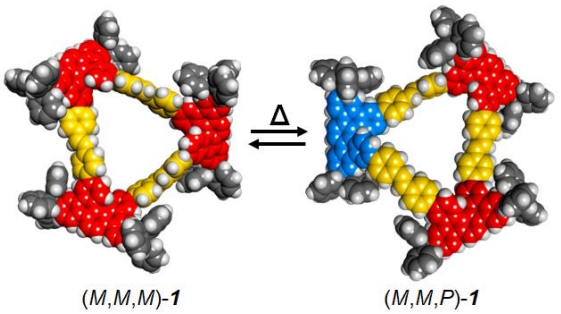


Fig. 1.

*This work was supported by the CSF (Reg. No. 20-23566S), GAUK (Reg. No. 818319), the European Regional Development Fund (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000778.), IOCB CAS (RVO: 61388963), CzechNanoLab Research Infrastructure, Ministry of Education, Youth and Sports of the CR (Reg. No. LM2018110).*

## PROTEIN BINDER (ProBi) AS A NEW CLASS OF STRUCTURALLY ROBUST NON-ANTIBODY PROTEIN SCAFFOLD FOR DIRECTED EVOLUTION

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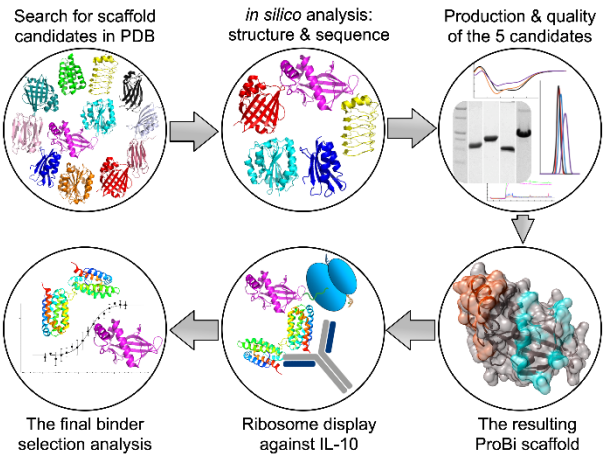
Novel protein binders derived from protein scaffolds are promising tools in protein engineering and are more and more often used as molecules for therapeutics and diagnostics. Their advantages in comparison with classical antibodies are their smaller size and a more robust, single-domain structural framework with a defined binding surface amenable to mutation. The range of applications of these molecules is rapidly expanding, what calls for a more systematic approach in designing new scaffolds with desired characteristics.

In our study, we describe a process based on a detailed analysis of novel suitable monomeric protein structures from the Protein Data Bank. The best candidates were subjected to computational evaluation of the mutability, and to experimental determination of yield in E. coli, solubility, and thermostability; examined were several variants of the tested proteins including wild types and alanine mutants.

We demonstrate the applicability of this procedure by selecting a 16 kDa monomeric single-domain human protein. Our novel protein scaffold called ProBi has a fold that is different from previously known scaffolds. ProBi scaffold contains two independently mutable surface patches. We randomized ten amino acid residues of C-terminus surface patch and utilized the power of in vitro directed evolution to successfully develop binders of the target interleukin-10 cytokine with a nano-molar affinity.




Scheme 1. **The procedure for the development of a novel protein binders derived from scaffold molecule aiming at interleukin-10 cytokine**

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## PLACENTAL EXPRESSION SIGNATURE OF TRYPTOPHAN METABOLISM ASSOCIATED WITH TERM AND SPONTANENOUS PRETERM BIRTH

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Spontaneous preterm birth is a serious medical condition affecting around 10% of pregnant women and a leading cause of perinatal morbidity. Although the etiology remains unclear,

multiple mechanisms have been related to spontaneous preterm delivery, including prenatal infection/inflammation, vascular disorders, breakdown of maternal-fetal tolerance and stress. Notably, both preterm labor with intact membranes (PTL) and preterm premature rupture of the membranes (PPROM) are associated with significant neurological and behavioral challenges in adulthood. Recognizing the hostile fetal environment associated with preterm delivery, it is intuitive to hypothesize that impaired placental functions may contribute to developmental programming of adult mental health. Specifically, in recent years, the placental tryptophan metabolic route has been reported as a plausible mechanistic cause implicated in the fetal programming. To determine the nature and extent of transcriptional alterations in tryptophan homeostasis in pregnancies complicated with preterm delivery, comprehensive gene expression analysis of the pathway was performed in a well-characterized clinical cohort of term (n = 40) and preterm pregnancies (n = 197). Using multivariate analysis, we provide information on the differential gene expression signature between 1) term and preterm deliveries and 2) PTL and PPROM groups. Further, using correlation computations and mediation analysis, we report a putative relationship between maternal inflammation, tryptophan pathway, and pregnancy length in the preterm group. These associations will collectively aid understanding the complex processes linking maternal/intrauterine inflammatory events and poor neurodevelopmental outcomes.

*The study was supported by the Czech Health Research Council [NU20-01-00264]; the Grant Agency of Charles University [SVV 2020/260414]; and the EFSA-CDN project [No. CZ.02.1.01/0.0/0.0/16_019/0000841], which is co-funded by the ERDF.*

## STILBENES ACT AS POTENT ANTIMICROBIAL DRUGS AND ENHANCE ANTIBIOTICS ACTION IN COMBINATION AGAINST *STAPHYLOCOCCUS AUREUS*

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*Staphylococcus aureus* belongs to by far the most severe pathogens of today medicine causing a wide spectrum of infectious diseases e.g. sepsis, blood-stream infections, respiratory problems, or complications due to colonized implants[1]. Its resistance to an alarming amount of antibiotics makes search for possible treatment alternatives of an utmost importance. Such alternative might be natural compounds stilbenes with characteristic structure of two polyhydroxylated benzene circles linked to each other by vinylidene bridge[2]. The most well-known compound is resveratrol (*trans*-3,5,4′--trihydroxystilbene; RES) produced by *Vitis vinifera* possessing antioxidant, antitumor, hypoglycaemic and antimicrobial properties. Similar effects are exerted also by pterostilbene (*trans*-3,5-dimethoxy-4-hydroxystilbene; PTE) from sandal wood or pinosylvin (*trans*-3,5-dihydroxystilben; PIN) from pine trees[3].

In presented work we evaluated the effect of mentioned stilbenes alone and in combination with antibiotics erythromycin, tetracycline (TET) and vancomycin on suspension growth and biofilm formation of four *S. aureus* strains.

PTE was alone the most effective on suspension cells ($MIC_{80}$ = 16 – 45 mg $L^{-1}$) and biofilm formation ($MBIC_{80}$ = 32 – 75 mg $L^{-1}$) of *S. aureus*. RES and PIN were less effective ($MIC_{80}$ greater than 100 mg $L^{-1}$) yet all stilbenes had bactericidal action on *S. aureus* in contrast to antibiotics. PIN acted synergistically with TET (FICi = 0.4, 150 mg $L^{-1}$ PIN with 0.06 mg $L^{-1}$ TET).

All stilbenes were proved to be potent antimicrobial compounds with bactericidal properties. They enhanced antibiotics action thus proving their possible application as supplements to common antibiotic therapy of *S. aureus* infections.

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## DOES AN IDUCTION OF NETOSIS INCREASE THROMBOLYTIC RESISTANCE OF CLOTS: AN *IN VITRO* STUDY

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Ischemic stroke is a globally high-risk cerebrovascular disease, causing nearly 6 million deaths a year worldwide[1]. Currently approved thrombolytic treatment with recombinant tissue plasminogen activator (alteplase) shows limitations. There are several possible mechanisms responsible for such reduced efficacy, including clots composition.

It has been demonstrated that neutrophil extracellular traps (NETs) promote clot formation[2]. NETs are networks of DNA, histones, and proteolytic enzymes produced by activated neutrophils through different mechanisms[2,3]. Recent studies have demonstrated that NETs scaffold is, at least partially, responsible for clots resistance to alteplase treatment[4-7].

In this study *in vitro* static and (patho)physiologically relevant flow models were used to determine whether the induction of NETosis during clotting affects alteplase mediated thrombolysis and recanalization. Red blood cells dominant clots were used for this purpose. We have shown that the induction of NETosis reduced alteplase mediated thrombolysis in the static model. In the flow model we have observed the same level of thrombolysis but reduced recanalization.

This observed phenomenon supports recent findings showing the increased resistance of clots containing NETs and suggests the influence of hydromechanical forces in the process of recanalization.

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## REGULATION OF iPSC-DERIVED CARDIOMYOCYTE PHENOTYPE BY RETINOIC ACID

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Retinoic acid (RA) is important morphogen that plays the key role in mammalian development. It was shown that RA signalling drives development of cardiomyocytes phenotype. RA promotes atrial cardiomyocytes formation through inhibition of gene expression associated with formation of ventricular cardiomyocytes. However, precise mechanism of cardiac specification is still unknown and needs further studies.

Here, we investigate effect of RA on cardiomyocyte phenotype in human induced pluripotent stem cells (hiPSCs) *in vitro*. RA both inhibits development of ventricular and induces development of atrial cardiomyocytes, respectively. This RA-mediated regulation of cardiomyocyte phenotype is dependent on treatment time window (from day 1/3 to day 5/7 of differentiation). RA did not shift the phenotype of cardiomyocytes, but inhibited development of ventricular cardiomyocyte progenitors overall. This observation was unexpected, and its mechanism is unknown.

These results encouraged us to explore the possibility of generating ventricular and atrial cardiomyocyte fluorescence-based iPSC reporter lines by CRISPR-Cas12a technology. Reporter hiPSCs lines might serve as a powerful tool helping to reveal the role of RA in cardiomyocyte specification.

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## SYNTHESIS OF FUNCTIONALIZED β-CYCLODEXTRIN NANOSPONGES

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Cyclodextrins are cyclic oligosaccharides of natural origin, which usually consist of 6, 7 or 8 glucopyranose units. Their inner hydrophobic cavity gives them the ability to form inclusion complexes with a number of hydrophobic molecules.

Cyclodextrin nanosponges are polymers which are composed of highly crosslinked cyclodextrins. Crosslinking enhances the complexation ability, as hydrophobic molecules may be entrapped in the cyclodextrin cavity and hydrophilic molecules in the interstitial space between hydrophilic cyclodextrin surface and crosslinker units. Thanks to the complexation ability and easy tunability of the polymer structure, nanosponges have emerged as a promising material in a number of various applications, including environmental, agricultural, biomedical and pharmaceutical applications[1].

Apropriate functionalization gives nanosponges new properties, desired in specific application. Glycosylation of nanosponges may create a novel nanocarrier, able to bind to specific receptors. This approach finds application in drug delivery, as functionalization may lead to targeting to specific receptors and releasing encapsulated drug in treated tissue[2].

This work is focused on the synthesis of β-cyclodextrin nanosponges functionalized with mannose. Such material finds application as a carrier for targeted drug delivery aiming on the tissue with overexpressed mannose receptors. Another advantage of such carrier is a broad range of drugs, which can be encapsulated in the structure leading to certain versatility in further use.

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## SCAFFOLD HOPPING OF THE SYK AND BTK INHIBITORS BROADLY TARGETING THE BCR SIGNALOSOME

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Chronic or recurrent stimulation of the B-cell receptor (BCR) signalling pathway plays a crucial role in promoting the cell proliferation and survival underlying the progression of many common B-cell malignancies. Spleen tyrosine kinase (SYK) together with Bruton's tyrosine kinase (BTK) and phosphatidylinositol 3-kinases (PI3K), are key mediators of BCR signalling, thus attractive targets in human haematological malignancies, particularly in various lymphoid neoplasms. Isosteric replacement of functional groups or alteration of the heterocyclic core (scaffold hopping) is a commonly used strategy in drug discovery and development. In the case of [6 + 5] nitrogenous heterocycles, imidazo [4,5-b]pyridines and imidazo[4,5-c]pyridines have rarely been studied within the large family of different protein kinase inhibitors, probably due to relatively poor synthetic availability of substituted scaffolds, compared to their purine analogues.

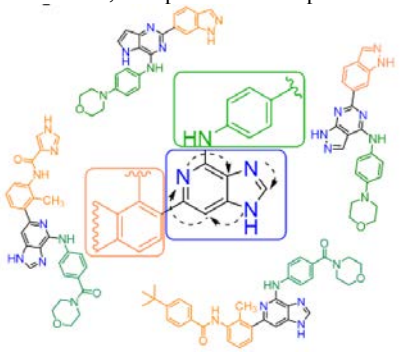

Fig. 1. **Scaffold hopping approach**

Therefore, we recently1,2 developed a synthetic approach that enables the preparation of these compounds in a multistep sequence starting from simple and readily available materials. Inspired by the structural features of recently reported SYK and BTK inhibitors, we applied the scaffold hopping approach (Fig. 1) to design the target compounds bearing different peripheral substituent patterns to further extend our understanding of the structure-activity relationships of the imidazopyridine moieties. The synthetic concept and kinases inhibition properties of target compounds will be reported.

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## SYNTHESIS OF NOVEL (THIA)CALIX[4]DI(HYDRO)QUINONE DERIVATIVES

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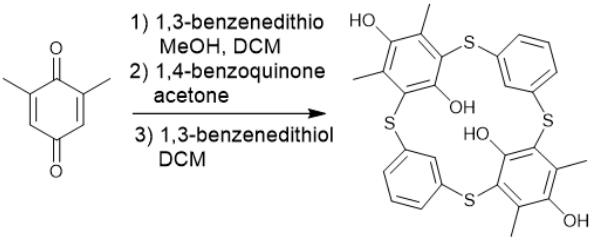
Calix[4]hydroquinones and calix[4]quinones are macrocycles consisting of repeat units of para-quinone and methylene group. Recently, interest in this group of substances has increased mainly due to their active centres capable of reversible electrode reactions[1], which could provide an opportunity to control not only their binding abilities but also their chemical selectivity in the case of complexation properties. Finally, such electrochemical control of the binding properties of the host molecule could lead to otherwise inaccessible applications in both chemical and biochemical reaction systems[2,3].




Scheme 1. **Synthesis of thiacalix[4]dihydroquinone by the sequence of conjugate additions**

The aim of this work is the synthesis of (thia)calixarene derivatives consisting of two *para*-(hydro)quinone units in combination with other arene units and their subsequent modification. To prepare described derivatives different synthetic routes were employed. Interestingly, these derivatives were not prepared by conventional condensation

reactions but by conjugate addition of either 1,3-benzenedithiol or other 1,3-substituted benzene (e.g. 1,3-phenylenediamine) to an appropriately substituted 1,4-benzoquinone (Scheme 1). These reactions offered good to medium yields and therefore they represent an alternative route to this type of substances.

*This work was supported by Czech Science Foundation (reg. No. 20-07833S).*

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## MYCOTOXINS ENNIATINS B AND B1 ALTER VOLATILE FATTY ACIDS PRODUCTION IN THE RUMEN FLUID

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Rumen is the most voluminous and metabolically crucial part of the digestive system of ruminant. The metabolic processes connected with feed digestion depend largely on the microbial composition of the rumen population, which includes protozoa, fungi, bacteria, and archaea. The primary role of the rumen is digestion of carbohydrates, which serve as the primary source of energy for ruminants. Volatile fatty acids (VFA), primally acetic, propionic and butyric, are released during microbial fermentation of carbohydrates. These metabolites are subsequently absorbed through the rumen wall.

Mycotoxins comprise a broad group of fungal secondary metabolites, which can exhibit toxic effects on animals and humans. The fibrous fungi are the main producer of mycotoxins including *Fusarium* species, which contaminate growing crops. Improper harvesting, processing, and storage contribute to their growth. *Fusarium* is responsible for the production of numerous mycotoxins. In our work, we focused on the mycotoxin group enniatins. Even though their levels are not legislatively limited, they are considered toxic, which is connected with their ionophore properties. Enniatins form cation-selective pores in the cell membranes and thus can influence the transmission of ions through the membrane.

The aim of the presented *in vitro* study was to monitor changes in the VFA production caused by presence enniatins in the rumen fluid taken from dairy cows. Also was studied the influence of uses enniatins and core or bulk feed on VFA levels. The rumen fluid was incubated with known amount of mycotoxins and different type of feed. VFA levels were determined by high-performance liquid chromatography. We found that enniatins significantly ($p<0.05$) influenced the VFA levels. These changes in the VFA levels caused changes in the rumen pH, which, in turn, induced changes in the composition and quantities of the microbial community. Our study demonstrated that enniatins present in the cattle feed can have a detrimental effect on fermentation processes in the rumen.

## PHOTOCROSSLINKED MICELLAR HYDROGELS TAILORABLE FOR WOUND HEALING AND CONTROLLED DRUG DELIVERY

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Thermoresponsive block copolymers, which undergo gelation at body temperature, are of high interest in the field of drug delivery. The formed hydrogels protect the encapsulated drug against hydrolysis and enzymatic degradation. Moreover, they could also enable targeted delivery and controlled gradual release resulting in increased therapeutic activity compared to repetitive administrations[1].

In order to enhance the mechanical properties and lifetime of the physical network formed due to the temperature change, a chemical network may be introduced[2]. The amphiphilic triblock copolymer composed of poly(D,L-lactide-*co*-glycolide) PLGA and poly(ethylene glycol) PEG blocks in ratio 2.5 forms a micellar network at body temperature (37 °C) due to hydrophobic interactions. Furthermore, when functionalised by itaconic anhydride, the copolymer *α,ω*-itaconyl-PLGA-PEG-PLGA contains double bonds at the ends of the chains enabling additional chemical crosslinking[3].

The hybrid hydrogel, stabilized with both physical and chemical network, was prepared by irradiating the physically stabilized hydrogel by blue light (430–490 nm) in the presence of a hydrophilic and biocompatible photoinitiator. Photocrosslinking may be carried out *in situ* and in the presence of living cells since it generates minimal heat. The resulting hydrogel is potentially applicable as an injectable drug carrier and as a resorbable wound dressing. The release of the bioactive compound depends on diffusion and degradation of the hydrogel structure. Therefore, it could be adjusted with chemical composition of the copolymer or with the degree of chemical crosslinking[2].

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## PROPERTIES OF $Cu^{2+}$ AND $Ga^{3+}$ NOTA-MONOAMIDE COMPLEXES

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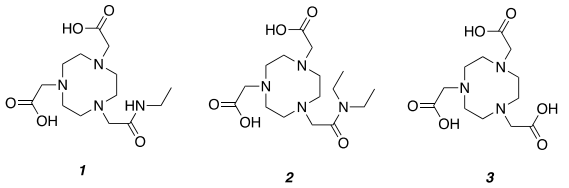
Some metal radioisotopes, e.g. $^{64}Cu^{2+}$ and $^{68}Ga^{3+}$, are key components of promising probes for cancer diagnosis or treatment. The metal ions cannot be used in their aqua complex form because of their non-specific distribution in tissues. Therefore, the metal ions have to be bound in a thermodynamically stable and a kinetic inert complex. The most suitable ligands for these purposes are derivatives of polyazamacrocycles. Their complexes are commonly used as experimental contrast agents but their chemical properties some of them have been poorly investigated.

Currently, NOTA monoamides are the most commonly used NOTA-based ligands in molecular imaging. However, their complexes have not been investigated. Two NOTA amides (***1*** and ***2***) were prepared (Figure). Acid-base and coordination properties were investigated by potentiometry. Replacement of a carboxylic pendant arm by amide group caused a decrease of overall ligand basicity and, consequently, values of stability constants were decreased by several order of magnitude if compared to NOTA[1]. Formation and dissociation kinetics of $Cu^{2+}$ complexes were investigated by stopped-flow UV-VIS spectroscopy. Acid-assisted decomplexations (3M $HClO_4$) of $Cu^{2+}$ complexes were slower comparing to the NOTA complex. The $Ga^{3+}$ complexes were investigated by NMR. Amide bond in the $[GaL(\mathbf{2})]^+$ complex was hydrolysed to [GaL(**3**)] even in slightly basic solution due to coordination of carbonyl group. At pH 4–7, $Ga^{3+}$ in complex $[GaL(\mathbf{1})]^+$ is coordinated in $N_3O_3$ mode. At pH above 7, $[GaL(\mathbf{1})]^+$ deprotonates on the amide nitrogen atom and the deprotonated amide nitrogen atom binds to $Ga^{3+}$ ion. It significantly increases thermodynamic stability of the complex.

Results show that NOTA primary amide complexes are suitable pro *in vivo* utilization unlike those of the secondary amide derivatives.



***1*** ***2*** ***3***

*This work was supported GACR (19-17980S) by INTER-COST LTC20044 (CA18202 – NECTAR).*

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## DEOXYFLUORINATION AS A PROMISING SYNTHETIC APPROACH TO SELECTIVE CARBOHYDRATE-BASED GALECTIN INHIBITORS

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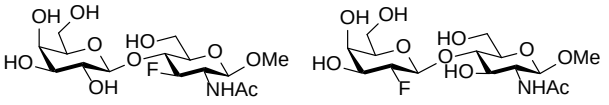
Galectins (gals) are carbohydrate-binding proteins playing key roles in a plethora of physiological processes in a human organism. There are capable of modulating immune responses or participating in processes such as neoplastic transformation via molecular recognition of β-galactoside containing glycans[1]. Therefore, the development of selective gal inhibitors has become a focus of pharmaceutical research. However, the preparation of inhibitors targeting individual galectins remains challenging as 12 human gals expressing similar substrate specificities have been identified. Deeper understanding of subtle differences between individual human gals could provide guidelines for the preparation of novel gal inhibitors. Deoxyfluorinated carbohydrates are established tools for this purpose as they enable to evaluate the importance of each hydroxyl group in the binding event or can be used as $^{19}F$ NMR probes[2].

In this work, a complete series of mono-deoxyfluorinated *N*-acetyllactosamine analogues (6 compounds) have been prepared. The synthesis of each analogue required about 15 synthetic steps, including stereoselective deoxyfluorination of monosaccharide precursors and chemical glycosylation. The binding affinities of the prepared analogues to human gal-1 and gal-3 were determined by ELISA assay and $^{19}F$ NMR $T_2$-filter techniques. These experiments revealed that individual galectins tolerate fluorine at different positions which makes deoxyfluorinated carbohydrates promising galectin inhibitors.




**1** **2**

Scheme 1. **Two examples of the prepared *N*-acetyllactosamine analogues: 3-fluoro LacNAc (1) and 2´-fluoro LacNAc (2)**

*We thank Ministry of Education, Youth and Sport (INTER COST, no. LTC20052) for support of our research.*

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## SINGLE-SPERM PHENOTYPING WITH SUBCELLULAR RESOLUTION VIA MULTIMODAL OPTICAL IMAGING

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The specialized architecture of the spermatozoon plays a critical role in uniting gametes for fertilization. Teratozoospermia resulting from defects in spermatozoa structure(s) represent a spectrum of disorders that can compromise or abolish male fertility[1]. Although the genetic basis for many male infertility disorders has been elucidated[2], to explain their mechanisms remains challenging, in part because we have an incomplete understanding of how sperm structure affects its function. Understanding the structure-function relationship requires tools that can access the interior of a spermatozoon and unveil its anatomical and/or chemical landscape (i.e., composition and distribution of metabolites). Because individual spermatozoa exhibit phenotypic variation within an ejaculate[3], it is essential to visualize and quantify different traits in the same cells.

To achieve this goal, we applied cutting-edge optical imaging tools by combining fluorescence microscopy and focused-ion beam scanning electron microscopy (FIB-SEM) to examine the (ultra)structural organization of spermatozoa at the single-cell level. Additionally, we adapted two-photon fluorescence lifetime imaging microscopy (FLIM) for quantifying metabolic dynamics in living spermatozoa with high spatial and temporal resolution relying on the endogenous fluorescence of NADH metabolite. By comparing normal spermatozoa to teratospermic spermatozoa from semifertile mice, we noted differences in (sub)cellular structures as well as metabolic changes in the healthy versus disease state.

Our results suggest that these imaging modalities are powerful tools to study the structure-function relationship of mammalian spermatozoa and open new avenues for future investigation of the mechanisms and treatments of infertility.

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## SYNTHESIS OF PYRIDOOXA[5]HELICENE

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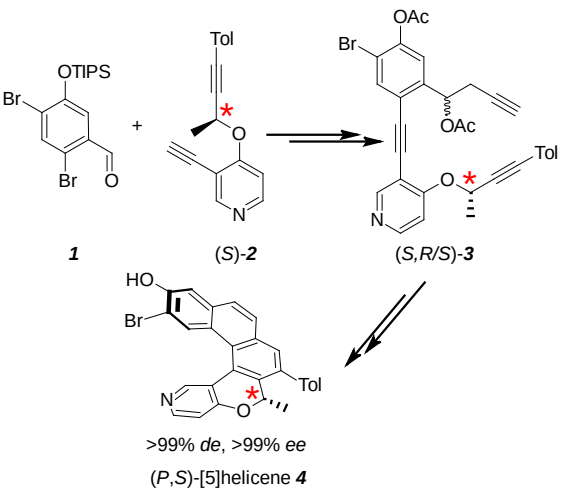
Improving the efficiency of OLEDs and OLETs is a challenging task. The potential solution might be found in developing organic materials showing the chiral induced spin selectivity effect (CISS effect) and exhibiting emission of circularly polarized light[1]. Helicenes are *ortho*-fused aromatics with inherent helical chirality. Molecular materials based on them are promising candidates to possess the mentioned physical properties.




***1*** (*S*)-***2*** (*S,R/S*)-***3***

Scheme 1. **Key intermediates in pyridooxa[5]helicene *4* synthesis**

Here, we report on the synthesis of pyrido-oxa[5]helicene ***4***, a potential precursor of hybrid helacene molecules for use in chiral light emitting devices (Scheme 1). The key triyne (*S,R/S*)-***3*** was prepared by a sequence of Sonogashira cross-coupling reaction of dibromide ***1*** and diyne

(*S*)-***2***, Grignard reaction and protecting groups manipulations. The helicene backbone was assembled by a stereoselective [2+2+2] cyclotrimerization previously developed in our group[2]. Following aromatization and deprotection led to the target (*P*,*S*)-helicene ***4*** with diastereoselectivity *de* >99 % (>99 % *ee*).

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## N-FLUOROALKYLTRIAZOLES: PRECURSORS OF IMINOCARBENES AND AMINOVINYL CATIONS WITH HIGH SYNTHETIC APPLICATION

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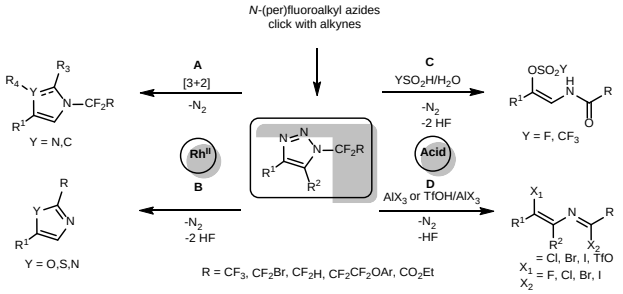
The introduction of the concept of „click chemistry ", a highly reliable ligation reaction was pioneered by the discovery of mild copper-catalyzed azide-alkyne cycloaddition (CuAAC) providing access to 1,2,3-triazoles, privileged heterocyclic scaffolds in life sciences.[1] The large chemical space of accessible 1,2,3-triazoles would make their chemistry even more appealing if there were methods for their further transformations into other valuable heterocycles. However, the stability of the aromatic ring in 1,2,3-triazoles makes such transformations rather difficult and limited in scope.

We have recently described N-fluoroalkyl-1,2,3-triazoles, easily available heterocycles *via* copper(I)-catalyzed azide-alkyne cycloaddition (CuAAc) of safe and stable N-fluoroalkyl azides and alkynes[2,3] as building blocks with a great synthetic potential. N-Fluoroalkyl triazoles undergo rhodium-catalyzed transannulations towards N-fluoroalkyl heterocycles (Scheme A) as well as 2-fluoroalkyl azoles (Scheme B)[4,5]. Additionally, acid-mediated reactions provide access to (*Z*)-β--enamido sulfonates (Scheme C) and N-vinyl substituted imidoyl halides (Scheme D)[6]. In comparison to rhodium catalyzed reactions involving carbene intermediates, acid mediated reactions proceed *via* a new type of reactive intermediate – aminovinyl cation.




Scheme 1. **Overall scheme**

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## FUNCTIONALIZED HELICALLY SHAPED AROMATICS FOR SINGLE-MOLECULE CONDACTANCE MEASURMENTS

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With a development of scanning tunnelling microscopy (STM) it has become possible to display single molecules deposited on a surface by measuring the tunnelling current flowing between a nanosized metallic tip and a substrate-covered surface. In a break-junction (BJ) mode, STM repeatedly establishes a metal-molecule-metal junction (see Fig. 1), that is used to measure single-molecule conductance, a crucial property of organic molecules applicable in future molecular electronics devices.

Among attractive compounds studied for this purpose are helicenes – helically shaped polyaromatic hydrocarbons that have shown for example a piezoelectric and spin-filtering effect both at a molecular level[1,2].

Herein, we report the synthesis of two helicene chloro derivatives designed so that the chlorine atoms are finally displaced by the acetylsulfanyl anchoring groups required for STM-BJ measurements. These compounds were prepared by Sonogashira and Suzuki coupling reactions and nickel mediated alkyne [2+2+2] cyclotrimerization. The conductance of the final acetylsulfanyl helicenes was measured experimentally and calculated theoretically.

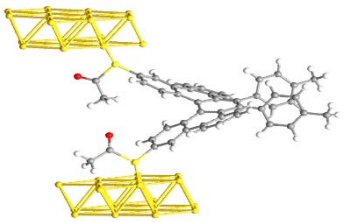

Fig. 1. **Gold-acetylsulfanylhelicene-gold junction**

*This work was supported by the Czech Science Foundation (Reg. No. 20-23566S) and IOCB CAS (RVO: 61388963).*

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## THE ROLE OF NpnNs-RNA AND NUDIX ENZYMES IN CELLULAR REACTION TO STRESS IN EUKARYOTES

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Currently more than 170 RNA modifications are known in prokaryotes and eukaryotes but their role is still poorly understood. 5' termini of the eukaryotic mRNAs and certain viral RNAs are covered with a specific structure, the 7-methyl-guanosine ($m^7G$) cap, involved in transcription, stability and translation of mRNAs. In bacteria the $m^7G$ cap is not present and most mRNAs are simply stabilized by a 5' terminal triphosphate. Recently, in *E. coli*, further non-canonical caps were discovered at the 5' end of RNAs: Nicotine Adenine Nucleotide (NAD), 3'-dephospho-Coenzyme A (CoA) and dinucleoside polyphosphates ($Np_nNs$). Using LC-MS technique we identified nine new $Np_nNs$-RNA caps ($Ap_2A$, $Ap_3A$, $Ap_3G$, etc). In bacteria, the concentration of methylated NpnNs caps increased significantly in later stationary phase in comparison with exponential phase of growth, indicating that bacteria use RNA capping as reaction to stress conditions[1].

In mammalian tissue cell culture under stressed conditions, we observed higher amount of certain $Np_nNs$-RNA caps. Recently, two human enzymes were identified to cleave non-canonical caps such as NAD. Human DXO (decapping exonuclease) can remove the entire NAD moiety from RNA and human Nudt12 (NudiX family) cleaves pyrophosphate backbone of NAD[2]. However, the biological role NAD cap in eukaryotic cells has not been elucidated yet. We studied Nudt12 decapping activity towards $Np_nNs$-RNA in vitro. In addition, we used overexpression and deletion of Nudt12 and DXO in HEK293T cells to study regulation of $Np_nNs$-RNA metabolism.

Finding a connection between human Nudt12 and the production of $Np_nNs$ in eukaryotic cells under stress conditions will help us to understand the role of $Np_nNs$ RNA caps and NudiX enzymes.

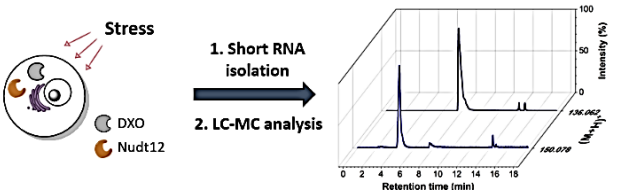


Fig. 1. **$Np_nNs$-RNA isolation from HEK293T cells and LC-MS analysis**

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## ZINC OXIDE COATED COTTON FABRIC WITH ENHANCED COMFORT PROPERTIES

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In this present work, an environmentally friendly and comparatively cheaper method was used to incorporate zinc oxide nanoparticles (ZnO NPs) on cotton fabric and physicochemical impact of ultrasonic rays on surface topography of cotton fabric was investigated. In a one step process, the synthesis and coating of ZnO NPs was successfully achieved. Alambeta and moisture management tester were used for thermal and moisture evaluation. The results of thermophysiological comfort of ZnO coated cotton were evaluated on the basis of thickness and ZnO NPs coated amount. In addition, the achieved results depicted the impact of sonication (pressure gradient) on surface roughness. ZnO NPs coating and surface topography were estimated by inductively coupled plasma atomic emission spectroscopy and

ultrahigh-resolution scanning electron microscopy (UHR-SEM).

Thermophysiological comfort is considered as most demanding and desirable characteristics that is achieved by maintaining heat and mass transfer. Comfort helps the customer to choose a suitable fabric for cold as well as for hot weather. From experimental point of view, thermophysiological and sensorial comfort are two most important and significant categories of clothing comfort. In recent years, many researchers worked with different textiles for thermophysiological comfort and reported interesting results but their results were based on thermophysiological properties of plain non-coated fabrics (nanostructures were not applied on samples). However, there are very limited studies based on nanomaterials coated textiles and their thermophysiological comfort evaluation. In previous studies, thermophysiological evaluation of nano TiO2 coated fabrics were carried out and interesting results were obtained[1].

ZnO is a fascinating material with exceptional physicochemical properties. In a recent work, ZnO NPs with pure wurtzite crystal structure were fabricated and coated on cotton fabric by sonication under optimised conditions[2]. However, comfort properties were not discussed. Based on above discussion, it was concluded that a simultaneous synthesis and coating of ZnO NPs on textiles (single step) via sonication is a novel approach for the investigation of thermophysiological comfort evaluation. This study explicitly demonstrates and elaborates the effects of ultrasonic irradiations as a potential tool to deposit ZnO NPs on cotton fabrics for thermophysiological comfort. The approach delineates here thereby opens up a new avenue towards other textile substrates and coating materials.

Woven fabrics (plain weave) made of 100 % pure cotton threads used in this work.

Table 1, Important Parameters

| Sample ID | ZnO NPs Coated Amount | GSM [gm-2] | Thickness [mm] |
|---|---|---|---|
| $F_1$ | - | 110 | 0.25 |
| $F_2$ | 581 | 115 | 0.31 |
| $F_3$ | 1090 | 118 | 0.38 |
| $F_4$ | - | 224 | 0.66 |
| $F_5$ | 598 | 229 | 0.72 |
| $F_6$ | 1110 | 233 | 0.77 |

Synthesis and coating of ZnO NPs: In a typical method, samples were immersed in a beaker and varying amount of ZnCl2 was added. Distilled water was added into the beaker to adjust 100 mL of total volume. The solution was sonicated for 5 min to prepare homogeneity. The granules of NaOH were added to solution in order to complete reaction. The running solution was sonicated for 1 h with ultrasonic probe homogeniser under optimised conditions. After sonication, samples were removed from their respective solution, squeezed on padder at a pressure of 3 kN with 1 mmin-1 velocity and dried at 60 °C in an oven. The remaining solution of each sample was centrifuged at 4000 rpm to separate the flocculates of ZnO NPs from liquid for XRD analysis. The collected ZnO powders were dried at 80 °C for 1 h in oven to eliminate impurities and excess amount of moisture[3,4]. A schematic explanation of the proposed method is presented in Figure 1.

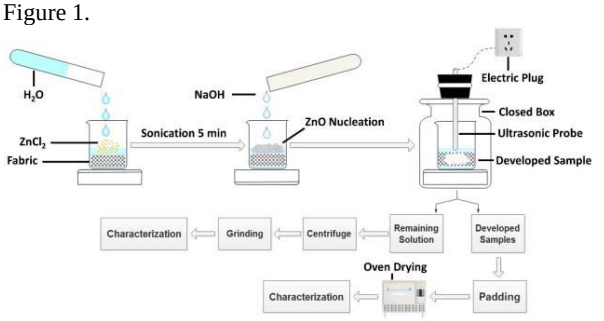


Figure 1 **Graphical representation of proposed system and experimental study**

Thermophysiological Comfort Properties: Alambeta instrument was utilised for the evaluation of thermal conductivity coefficient (λ) [Wm-1K-1]. The coefficient of thermal conductivity is estimated by:

$$\lambda=(Q\times h)/(A\times t\times \Delta T)$$

Moisture management tester (MMT) was used for the measurement of overall moisture management capacity (OMMC). AATCC 195-2009 method was performed to determine OMMC. This property calculates the ability of textiles to deal with moisture.

The results regarding morphology and surface topography of all coded samples are illustrated in Figure 2. SEM images were carried out at 5.0k and 10.0k magnifications for cotton samples (F1 (untreated), F3 (treated)). A smooth and very clean surface of untreated sample can be observed in Figure 2 (a). Higher magnification was taken to visually guess estimated quantity (higher deposition or lower deposition) of ZnO NPs on treated fabrics. A homogenous distribution with quasi-spherical shape of ZnO NPs was detected for cotton as depicted in Figure 2 (b-c). The impact of sonication was dominantly appeared in case of cotton as the surface was fully covered with ZnO NPs.

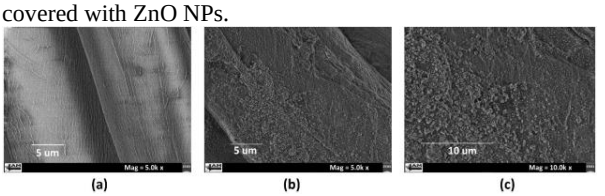


Fig. 2 **SEM images (a) untreated (b) sample F3 (c) sample F3 with higher magnification of cotton fabric.**

Thermal conductivity is a significant and important approach to evaluate thermal comfort of any textiles. Thermal conductivity results are described in Figure 3. The results were higher for all treated samples of cotton (F2, F3, F5, F6) than untreated. The results depict that ZnO coating on fabrics by sonication impart positive effects on porosity by covering the pores on fabric surface. ZnO NPs coating covered many empty spaces on fabric surface and reduced air entrapped inside fibre

volume, and increased thermal conductivity. Moreover, higher ZnO NPs deposition increased fabric thickness and reduced the air portion in treated samples and eventually provided higher thermal conductivity. Figure 3 (b) explains the results of thermal conductivity as a function of thickness. The trendline shows increasing tendency for thermal conductivity as thickness increased. Regression equation and R2 coefficient statically explain thermal conductivity dependency on sample thickness. A strong positive linear relationship and a strong dependency trend between fabric thickness and thermal conductivity was observed. Therefore, the developed products are a perfect option for summer wear.

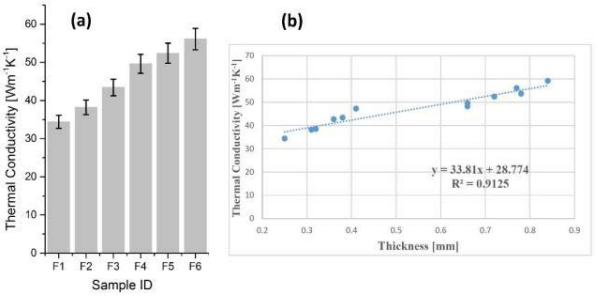


Fig. 3 (a) **Thermal conductivity of cotton samples (F1 to F6) and (b) Thermal conductivity as a function of thickness**

OMMC is another important parameter and influential indicator for thermophysiological comfort evaluation. OMMC describes the capacity of a textile substrate to transfer liquid in all three dimensions. The OMMC values ranges from (0 to 1) whereas a value closer to 1 indicates better moisture management properties of a substrate. OMMC results were higher for all treated samples of cotton (F2, F3, F5, F6) than untreated samples i.e., F1, F4 for cotton. The results show that the deposition of ZnO NPs through sonication induced positive and significant effects on the moisture management properties. In a previous study, the advantages of using sonication as an economical, user friendly and robust tool during the synthesis of nanomaterials and functional textiles is explained in details. Fluid flow acceleration occurs inside fibre internal structure during sonication, and textile substrate swelling is achieved by acoustic cavitation phenomenon. These two factors resulted in better moisture management properties.

The motivation of this work was to evaluate comfort properties of ZnO NPs coated fabrics with varying thickness. The following conclusions were drawn that significantly based on heat and moisture transportation and air permeability.

- Fabric thickness is an effective variable that affects comfort properties particularly thermal conductivity. Furthermore, the results between thermal conductivity and thickness were statistically significant with R2 value 0.9125. By keeping comfort feeling in mind, the result illustrated that the deposition of ZnO NPs by sonication improved thermal conductivity significantly.
- Structure and morphology of textile materials play a major role in the evaluation of fabric comfort. Moisture transportation phenomenon significantly depends on porosity.

*This work was supported by the Ministry of Education, Youth and Sports CR and the EU (European Structural and Investment Funds - Operational Programme Research, Development and Education) in the frames of the project "Modular platform for autonomous chassis of specialized electric vehicles for freight and equipment transportation", Reg. No. CZ.02.1.01/0.0/0.0/16_025/0007293.*

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## DESIGN AND SYNTHESIS OF PRODRUGS OF 6-DIAZO-5-OXO-L-NORLEUCINE; POTENTIAL TREATMENT OF GLIOBLASTOMA

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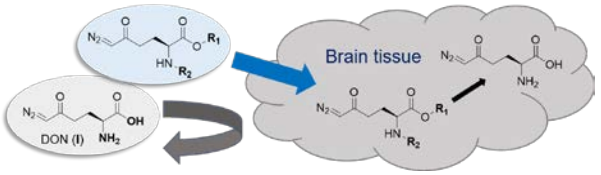
6-Diazo-5-oxo-L-norleucine (DON, **I**) is a non-standard amino acid with proven antitumor activity found in soil bacteria of the genus *Streptomyces*. However, due to the considerable systemic toxicity manifested mainly in the gastrointestinal tract, DON alone is not a suitable clinical candidate for the treatment of cancer. One of the ways to solve the problem of its toxicity is the reversible structural modification of this molecule by protecting both its amino group and carboxyl functional group, by preparing the so-called prodrug of DON. The prepared prodrug may suitably alter the distribution of DON in the body and at the same time increase its permeability to brain tissue. Due to this structural modification, its side effects can be eliminated and a substance for the treatment of different types of tumors can potentially be formed.

We designed and prepared new prodrugs with potential ability to be either capable of spontaneous penetration across the blood-brain barrier (BBB) or of being a substrate for one of its influx transporters. At the same time, these prodrugs should be stable in other metabolically active organs and blood plasma in order to sufficiently reduce the already mentioned systemic toxicity of DON. Substituents on both the amino and carboxyl groups of DON in the prepared prodrugs should then be enzymatically easily cleaved in brain cells to release their own effective chemotherapeutic - unsubstituted DON.




Scheme 1. **Penetration of DON prodrugs across the BBB**

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## HYDROGEN BONDING OF METHYLATED NUCLEOBASES ANALOGUES STUDIED BY NMR SPECTROSCOPY

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The DNA methylation is one of the most important epigenetic mechanism in genome of organisms and it is observed in connection with physiological or pathological processes in their body[1]. The methylated nucleobases can be successfully studied by low-temperature NMR spectroscopy because two methylamino group rotamers are observed as two sets of signals in NMR spectra. 2-(methylamino)adenine ***1*** was used as a model compound. Its rotational equilibrium is dependent on temperature, solvent and binding partner concentration. Each rotamer, shown in Figure 1, has different hydrogen-bonding pattern and, therefore, different ability to interact with binding partner, e.g. thymine. Rotamer ***1a*** can form hydrogen-bonded complex with a thymine via three hydrogen bonds (modified Watson-Crick type), on the other hand, rotamer ***1b*** can form only a Hoogsteen type complex.

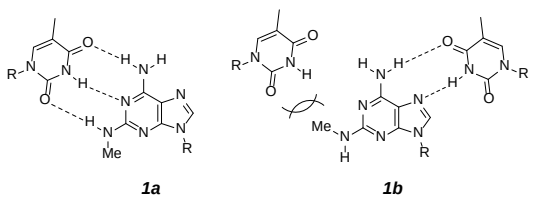


Fig. 1. **Two observed rotamers of compound *1* and their binding ability**

Using low-temperature NMR spectroscopy in conjunction with DFT calculations, the free-energy changes associated with hydrogen-bonded complex formation and complexes' geometries were determined. The free-energy changes associated with hydrogen-bonded complex formation can be calculated from rotamer-ratio changes[2] or by our newly developed methodology based on the concentration dependence of chemical shifts.

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## INSULIN RECEPTOR ISOFORM SPECIFIC INSULINS. A DIFFICULT TASK

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Insulin is a hormone that has a key role in glucose metabolism and energy homeostasis. Insulin elicits its functions through binding to the insulin receptor (IR), which exists in two isoforms, IR-A and IR-B, resulting from the alternative splicing of the IR gene[1]. The only difference between the isoforms is the 12-amino acid insert (IR-B plus and IR-A minus 12 amino acids) at the C-terminus of the extracellular α-subunit, called the α-CT peptide (Fig. 1). Both IR isoforms have different tissue distribution with the longer IR-B being the largely predominant form in adult humans in hepatocytes (more than 90 %), skeletal muscle and subcutaneous fat (both about 70 % IR-B)[2], while the shorter IR-A is almost exclusively expressed in the brain, lymphatic tissues, or embryo[3].

An insulin analogue with a preferential binding to one of the IR isoforms could target a specific tissue and find application in treatment of diabetes or neurological disorders. We are systematically mutating human insulin at different positions that can have impact on IR isoform binding with the aim to shift insulin binding specificity in favour of IR-A or IR-B. This is an extremely difficult task because the structure of IR-B:insulin complex is not available. We are using a competitive binding assay with radioactively labelled insulin in cell cultures which specifically express IR-A or IR-B to assess the selectivity of our insulin analogues. The recent results of this effort will be presented.

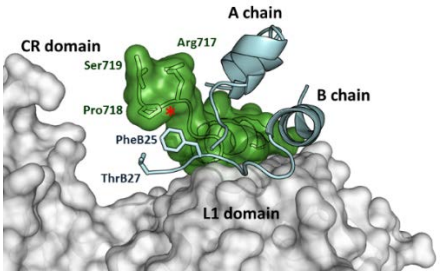


Fig 1. **Insulin (A and B chains in cyan) interaction with L1 and CR domains (both in grey) and α-CT-A peptide (in green) of the insulin receptor (IR-A isoform). The red asterisk shows the insertion site of extra 12 amino acids in IR-B[4]**

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## CYCLAM DERIVATIVES WITH PHOSPHORUS PENDANT ARMS FOR COMPLEXATION OF COPPER RADIOISOTOPES

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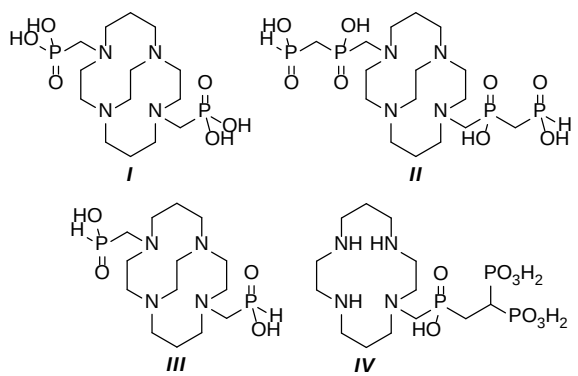
This project aimed to preparation of the new effective carriers of copper radioisotopes for Positron Emission Tomography. The cross-bridged cyclam derivatives bearing two phosphonate (***I***), bis(phosphinate) (***II***) or phosphinate (***III***) pendant arms and non-bridged cyclam derivative with one monophosphinato-bis(phosphonate) (***IV***) pendant were synthesized and studied (Scheme 1). Their thermodynamic and kinetic properties were studied by potentiometric titration and UV-Vis spectroscopy. The study of acid-base properties showed a high macrocycle basicity of the investigated ligands ($pK_1 > 13$).




Scheme 1. **Studied macrocyclic ligands**

The thermodynamic measurements showed that the Cu(II) complexes are highly stable compounds with values of stability constants log*K* in the range of 20–26. The formation kinetics of the complexes with the cross-bridged derivatives ***I***, ***II*** and ***IV*** is very fast and quantitative formation is achieved within 1 s at pH ~ 6. All studied ligands are characterized by very high kinetic inertness. The dissociation half-lives of the bridged macrocyclic copper complexes ***I*** and ***III*** are 120 h and 111 h, respectively in 1 M $HClO_4$ at 90 °C. As a part of a possible targeted therapy, the cyclam derivative ***IV*** has been studied for the affinity to bone tissue. Model tests on hydroxyapatite showed high affinity and rapid sorption. These findings suggest that the investigated ligands are promising carriers of copper radioisotopes for further research towards clinical application.

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## BLACK PHOSPHORUS IN MALDI TOF MS ANALYSIS OF BIO-MOLECULES

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Biological samples, such as cell extracts, are amazingly rich in various compounds (bio-molecules) with different composition, structure, and behaviour. Analytical perspective is a possibility to analyse whole range of bio-molecules with high sensitivity, selectivity, and repeatability. MALDI TOF MS might be an option. Matrix choice can then improve ionisation efficiency, decrease analyte fragmentation, and provide the high repeatability of whole mass range of compounds. Commonly used matrices (organic acids) are well established, but they might suffer by low ionisation efficiency and reproducibility when applied on complex biological samples.

Currently, there is high interest in use of inorganic materials, such as metal nano-particles and metal layers as a new group of matrices. These inorganic materials give minimal background in mass spectra (low fragmentation) and they improve ionisation of bio-molecules.

In this work, black phosphorus (BP) in combination with commonly used matrices was used for systematic MS analysis of selected biomolecules (amino acids, peptides, and proteins) and cells extracts. Aim of this work is to show how BP improves ionisation of these bio-molecules and to show possibility of use BP for cell classifications.

Efficiency of ionisation in BP-enriched matrices was significantly increased and the final intensities in mass spectra were ~10 times higher than without BP. Matrices enhanced with BP were also demonstrated to improve intact cell MALDI TOF MS analysis. Obtained mass spectra of intact cells were pre-processed, peaks were detected (based on S/N ratio), and their intensities were used for multivariate statistical analysis. Cluster analysis shows improvement in cell discrimination

when BP is used. In summary, BP has promising properties improving the outputs of wide range of applications in MALDI TOF MS.

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## CLOTS STRUCTURE AFFECTS THROMBOLYTIC RESISTANCE: AN IN VITRO STUDY

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Stroke is one of the leading causes of death and disability worldwide and gives rise to a serious socio-economic burden[1]. The most common treatment of ischemic stroke is intravenous thrombolysis with alteplase (rt-PA)[2]. The clot composition is considered to be a relevant factor responsible for the limitation of rt-PA efficacy[3–5]. The understanding of the cause of lytic resistance of clots is essential for the improvement of thrombolytic treatment[3].

The aim of this work is to show whether the composition of clots prepared in vitro affects the lytic efficacy of rt-PA.

In this study four types of clots were prepared: (I) red blood cells (RBCs) dominant clots, (II) fibrin dominant clots, (III) platelet-rich plasma clots, and (Iva-c) semi-synthetic clots (SSCs) differing in the content of RBCs. The rate of lysis by rt-PA was determined by two methods: relative clot weight loss and RBCs released into the incubation media. The histological analysis was done with Hematoxylin-Eosin and Mallory-Azan staining. Selected clots were analysed by scanning electron microscopy.

The results showed statistically significantly higher lytic resistance of clots prepared from platelet-rich plasma compared to other types of clots ($p<0.05$), the histological analysis indicated that these clots are highly compact and their composition did not change even after lysis by rt-PA. SSCs (RBCs 2.50×1012 cells/L) showed statistically significantly higher lytic resistance compared to other SSCs ($p<0.05$), the histological analysis revealed densely packed RBCs.

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## IMIQUIMOD-LOADED NANOCAPSULES BASED ON OLEIC ACID FOR DERMAL APPLICATION

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Imiquimod (IMQ) is an immunostimulating drug used in the treatment of basal cell carcinoma (one of the most diagnosed skin cancer types around the world) and actinic keratosis (pathological precancerous skin condition). Due to its low solubility in most pharmaceutical excipients and poor skin bioavailability, the dermal formulation of IMQ remains highly challenging[1].

Improved IMQ skin penetration can be achieved by encapsulating it into lipid nanocapsules[2]. Here, we describe a new nanocapsule system based on oleic acid, the best solvent for IMQ.

Our nanocapsular system was prepared by the combination of two methods: high-shear and high-pressure homogenization. Dynamic light scattering revealed a hydrodynamic diameter and polydispersity index of around 200 nm and 0.2, respectively. Transmission electron microscopy confirmed that the nanocapsules had the desired spherical shape. An encapsulation efficiency of 99% was achieved and the nanocapsules remained stable in an aqueous medium for 20 days.

Compared with a commercial 5% IMQ cream, our 2% IMQ nanoparticles accumulated twice as high IMQ in the porcine skin with lower IMQ permeation through the skin.

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## THE ANTIVIRAL POTENTIAL OF SYNTHETIC STING AGONISTS AND THEIR PRODRUGS AGAINST CHRONIC HEPATITIS B

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Hepatitis B is a viral infection of hepatocytes caused by the hepatitis B virus (HBV). The majority of infections are acute, with only short-term symptoms and full recovery. The chronic hepatitis B (CHB) develops only in less than 5 % of infected adults, whereas in 20 – 30 % of infected children aged 1 – 5 years and in up to 95 % of neonates. Currently available vaccine is an effective prevention against HBV infection, yet not therapeutics. Chronic HBV continuously damages the liver tissue leading to the development of cirrhosis or hepatocellular carcinoma. With approximately 250 million of cases (WHO - 2015), CHB presents a world-wide health problem[1]. The currently available treatment options, the nucleotide/nucleoside analogues and pegylated interferon alpha (PEG-IFNα), are insufficient. The nucleotide/nucleoside analogues typically do not result in complete cure of the patients and the life-long treatment is necessary. PEG-IFNα successfully cures only limited percentage of patients and is often accompanied by side effects among others flu-like symptoms, neutropenia and/or thrombocytopenia, and depression[2]. Therefore, the universal and effective cure of CHB remains to be discovered.

One of the problems of CHB is the inability of the host immune system to clear the infection from liver cells due to its exhaustion[1]. The recent studies on immunomodulatory approach towards CHB cure show that the innate immunity activation by small molecules (e.g. toll like receptor agonists) leads to the production of interferons and cytokines with anti-HBV activity. Moreover, their balanced mixture inhibits HBV infection more than IFNα (or PEG-IFNα) alone[3,4].

Our laboratory focuses on the cGAS-STING (cyclic GMP-AMP synthase - stimulator of interferon genes) innate immunity pathway. This signalling is crucial for the recognition of intracellular pathogens and intrinsic cell damage. The interaction of double-stranded DNA with cGAS in cytoplasm activates the enzyme to produce a cyclic dinucleotide (CDN), 2',3'-cGAMP (cyclic GMP-AMP), which binds to STING. STING then activates downstream proteins of the signalling pathway resulting in the type I interferon and pro-inflammatory cytokine expression with antiviral potential[5]. Most importantly, STING can be directly activated by various CDNs of eukaryotic, bacterial[5] or synthetic origin[6]. We have shown that some of the synthetic CDNs are superior to natural CDNs in the cGAS-STING pathway activation both in reporter cell-based assays and more physiologically relevant cell-based assay in peripheral blood mononuclear cells (PBMC) isolated from blood of healthy human donors[6]. Therefore, we further explored the most potent synthetic CDNs as candidate compounds for (pre)clinical testing. We enhanced the CDN ability to pass through lipid cell membrane by preparing a series of novel CDN-derived lipophilic prodrugs, thus improving their activity up to 1000 times, and lowering their effective dose required. In addition, both CDNs and their prodrugs induced expression of antiviral cytokines in PBMC.

With these promising results, we tested the antiviral effect of the lead prodrug and its parent CDN on HBV-infected primary human hepatocytes. We demonstrated that the cytokine-conditioned medium from stimulated PBMC had strong anti-HBV effect.

Next, we studied the liver-resident immune system stimulation with STING agonists as the targeted delivery of CDNs to liver tissue should be at the forefront of the research interest. To achieve this goal, we optimized the multiparametric immunophenotyping flow cytometry-based analyses as well as multiparametric cytokine profiling of produced cytokines. We isolated the complex non-parenchymal liver cell population, which contains liver-resident immune cells, from liver tissue obtained from our unique non-infectious mouse model of CHB, and showed that the lead CDN and its prodrug *ex vivo* activated these cells and induced production of antiviral cytokines. Moreover, we observed no significant difference in the activation of the liver-resident immune cells from control and CHB mice suggesting that the HBV chronicity does not affect the liver immune system sensitivity to STING agonists. In summary, the CDN/prodrug treatment of CHB represents a viable option as a universal targeted therapy regardless the chronicity.

*The work was supported by Gilead Sciences, Inc. and OP RDE Project "Chemical biology for drugging undruggable targets" Cheb-BioDrug. No. CZ.02.1.01/0.0/0.0/16_019/0000729.*

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## RESIDUES FLANKING THE ARK$^{met}$T/S MOTIF ALLOW BINDING OF DIVERSE TARGETS TO THE HP1 CHROMODOMAIN: INSIGHTS FROM MOLECULAR DYNAMICS SIMULATIONS

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Molecular dynamics (MD) simulations enable studies of structural dynamics with a detailed spatial and temporal resolution, unreachable for any experimental technique. Here, I have used MD technique to unravel the key role of structural dynamics in biomolecular recognition mediated by Heterochoomatin protein 1 (HP1). HP1 is a multifunctional hub protein found at chromosomes and a well-known reader of specific regions of lysine-methylated histone tails. The ARK$^{met}$S amino-acid sequence motif was originally proposed to be recognized and bound by HP1 but not all sequences bearing this motif bind while histone tail segment with unusual ATK$^{met}$A motif was later confirmed as binder as well. My MD simulations of HP1 bound to various peptides show that a larger segment, up to 11 amino-acids, is read by HP1. We suggest that very different sequences can bind to the HP1 chromodomain in a very similar manner thanks to the ability of different amino-acid positions to form mutually replaceable interactions and thus substitute for each other. This mutual amino-acid replaceability allows for biding of the ATK$^{met}$A motif if embedded in a suitable sequence context, while it also increases sequence flexibility of the read histone tail segments[1].

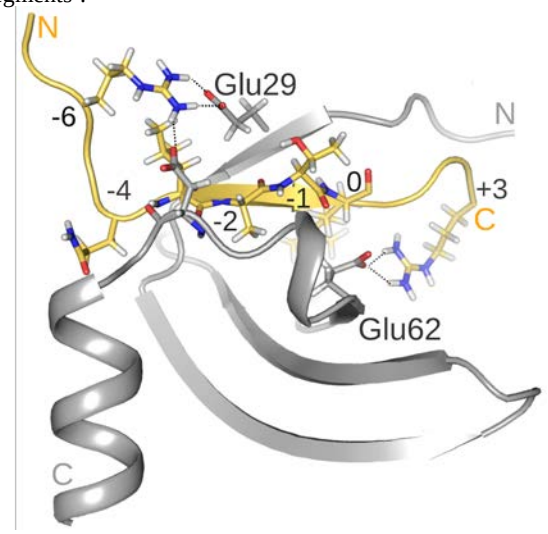


Fig. 1. **Proposed interaction network between HP1 chromodomain and H3K23$^{met}$ peptide containing unusual ATK$^{met}$A motif.** Key interactions with HP1 seen in HP1 complexes with other peptides with experimentally resolved structures are preserved despite being mediated by different amino-acids of the bound peptide. Numbers mark amino-acid positions of the bound peptide relatively to K$^{met}$

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## DELIVERING LIPIDS INTO CELL MEMBRANES USING BODIPY-BASED PHOTOCAGES

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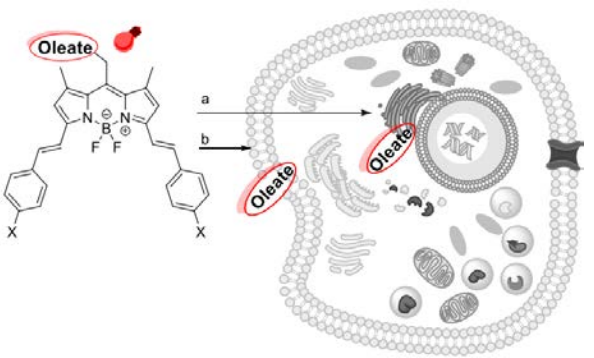
Photocages[1] (PhC) are highly desirable compounds due to their potential applications in drug delivery systems. The most crucial factors limiting their use are short wavelengths of light needed for the photorelease or low quantum yields of this process. Low stability or solubility also often belong among them. BODIPY-based PhC are promising candidates as the reactivity of BODIPY scaffold enables wide range of tuning of all abovementioned parameters by a proper design.[2-3]




Scheme 1. **Red light induced photorelease of oleic acid to: a) a plasma membrane; b) internal membranes of cells**

In this study we focused on development of water soluble BODIPY-based PhC which, based on their substitution, were used to deliver a cargo (oleic acid) either to a plasma membrane or to internal membranes of cells (Scheme 1). Design, synthesis and studies of the photorelease mechanism was studied in detail and the developed molecules were used in vitro for photoinduced activation of oleic acid-dependent $Ca^{2+}$ signalling.

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## LYNX: AN INTERACTIVE BIOINFORMATIC TOOL FOR CAPTURE-BASED NEXT-GENERATION SEQUENCING DATA ANALYSIS IN LYMPHOID MALIGNANCIES

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Next-generation sequencing (NGS) represents one of the fastest evolving technologies used in both biomedical research and clinical practice. NGS library preparation using hybridization probes (so-called capture-based NGS) allows the analysis of a large number of markers in one reaction. However, the analysis and interpretation of the obtained data remains a challenge for diagnostic laboratories.

In our laboratory, we introduced a comprehensive capture-based NGS panel, which enables simultaneous analysis of mutations in selected genes, copy number variations, translocations, and rearrangements of antigen receptors in the most common lymphoid malignancies. For data analysis, we aimed to develop a modular bioinformatic tool LYNX (LYmphoid NGS) with an interactive user interface (UI) making a computational analysis accessible for laboratory personnel without deeper bioinformatic skills.

Our tool allows a user to upload data straight from a sequencing machine, organise samples into projects, run the analysis and explore results via interactive visualizations - all in a simple and user-friendly UI. Thanks to its modularity, a user can choose independent steps of the required analysis or execute them separately. On top of that, the analytical part of LYNX is ready and open for possible future extensions with new computational modules based on other diagnostic applications or needs of a user. The result visualization part of LYNX was designed in collaboration with molecular geneticists to meet all the requirements of routine diagnostics. All processed data are presented to a user in interactive tables interconnected with dynamic visualizations and follow the flow of analytic procedure. With all its features, LYNX is ready to improve and simplify the data analysis in lymphoid malignancies for dozens of diagnosticians and researchers.

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## PROTEIN C-MYB: A NEW PROGNOSTIC FACTOR IN OSTEOSARCOMA

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Aberrant activation of the transcription factor c-Myb promotes cell proliferation and survival, thereby contributing to malignant transformation. Recent studies described its role in bone development and growth as well as its expression in osteosarcoma cell lines but the relevance of c-Myb in control of osteosarcoma development, progression and chemoresistance remained unknown[1,2].

We prepared derivatives of osteosarcoma cells SAOS-2 LM5 deficient in c-Myb expression. Then we analysed the impact of c-Myb deficiency on their chemosensitivity and metastatic potential. The absence of c-Myb in SAOS-2 LM5 cells increased their sensitivity to doxorubicin and methotrexate and markedly impaired their ability to form lung metastases in immunodeficient mice.

We also performed retrospective immunohistochemical study on 93 osteosarcoma archival tissues from patients diagnosed and treated in Masaryk Memorial Cancer Institute, Brno and Department of Paediatric Oncology, University Hospital Brno in the period 2007–2020. The analysis showed significant heterogeneity in c-Myb expression in patients' tissue. High c-Myb expression was associated with poor overall survival in this cohort.

Our results revealed the importance of c-Myb in regulation of osteosarcoma cell line chemosensitivity and metastatic activity and disclosed c-Myb as a negative prognostic factor for osteosarcoma patients.

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## UNCOVERING OF THE STRUCTURE AND REACTIVITY CORRELATION OF CO-PHOTOREACTIONS OF 3-HYDROXYFLAVONE-BASED ACID-BASE FORMS

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The well-established toxicity of carbon monoxide (CO) appears contradictory with its possible therapeutical function. Indeed, it has recently been discovered that CO is produced endogenously. Studies of the effects of CO have demonstrated its potential to produce a variety of beneficial health outcomes, including anti-inflammatory, anti-bacterial effects, and antiproliferative effects on cancer.[1] Therefore, carbon monoxide-releasing molecules (CORMs), biologically compatible agents allowing for defined administration of carbon monoxide into living organisms to circumvent its acute toxicity, are of special interest.[2] A precise spatial and temporal control over the CO release can be achieved via activation of the CORM by light (photoCORMs).

A good photoCORM should be stable under ambient conditions and soluble in aerobic aqueous environments. It should release CO using light at wavelengths that do not have the potential to impart cellular damage and may exhibit fluorescence to enable tracking in the cell. Understanding the mechanism is a key step for designing new derivatives with improved properties for biological applications, such as water solubility, higher quantum yield and the absorption spectra in the visible light region.

The detailed mechanism of the photochemically induced CO release from 3-Hydroxy-2-phenyl-benzo[g]chromen-4-one (**1**) has been studied in our group.[3]

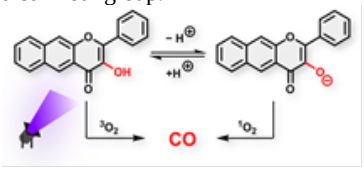


Fig. 1. **CO releasing mechanism of 1**

For a deeper comprehending, an uncovered study of the structure and reactivity correlation of CO-photoreactions of this family of compound will be presented.

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## ELECTROCHEMICAL BIOSENSOR FOR THE ANALYSIS OF DNA POINT MUTATIONS IN COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the most common malignancies in the Czech republic with eight thousand cases each year[1]. It has been reported that mutations in *KRAS*, *BRAF* and *p53* genes, microsatellite instability, as well as epigenetic alterations could play a pivotal role in CRC development[2]. Currently, the diagnostics of CRC involve detection of mutations in *KRAS* gene by using direct sequencing method. However, this method is expensive, time consuming and laborious, and often challenging due to heterogeneity of a tumor. Therefore, we are trying to develop electrochemical biosensor as an easy to perform, cost effective and highly sensitive alternative. To overcome heterogeneity in a tumor (i.e. presence of both non-mutated and mutated *KRAS*) we employed PCR clamping during the amplification. The PCR clamp is oligonucleotide with 3'end modification which blocks amplification for wild type gene[3]. By this approach, we expect to amplify mutated amplicons and suppress wild type amplification in tumor samples. We also added digoxigenin-dUTP into amplification reaction to be incorporated into PCR product for sensitive recognition by antidigoxigenin antibody at screen printed carbon electrodes[4]. Our preliminary results show that we were able to detect *KRAS* mutation in selected CRC cell lines i.e HT-29 cell lines (wild type), DLD-1 (G35T mutation) and SW-480 cell lines (G38A mutation). After optimization steps, we will validate the method for detection of the *KRAS* mutations in tumor tissues and eventually in liquid biopsy format from blood of patients.

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**SMALL-MOLECULE MODULATORS AFFECTING PROTEASOME ACTIVITY AND HEAT SHOCK RESPONSE FOR TREATMENT OF NEURODEGENERATIVE DISORDERS**

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The 26S proteasome is a large enzymatic complex that selectively degrades polyubiquitinated substrates, thereby ensuring of protein homeostasis in the cell. It comprises of at least 33 precisely positioned subunits, each of which differ in function and structure, and cannot substitute one another. Owing to its complicated structure, the biogenesis of the 26S proteasome is strictly regulated at transcription, translation, and molecular assembly levels. Intracellular accumulation of abnormal proteins or another defects in protein homeostasis cause pathological stages such as proteotoxic stress. This phenomenon is in parallel with declined proteasome activity. When the accumulation of redundant proteins exceeds their degradation, undesirable signaling and/or aggregation occurs which are the hallmarks of neurodegenerative diseases. NRF1 (encoded by *NFE2L1*) is a transcription factor that upregulates the expression of all proteasome subunits in a concerted manner, especially during stress conditions. In normal conditions, it is embedded in the membrane of endoplasmic reticulum, retro-translocated by VCP/p97 complex to cytosol, deglycosylated, ubiquitinated and degraded by the proteasome. However, when cell proteostasis is impaired, NRF1 is cleaved by the DDI2 protease, and as an active transcription factor switches on the expression of proteasome genes. Therefore, increasing proteasome activity has been recognized as a new approach to delay the onset or ameliorate the symptoms of neurodegenerative and other disorders with disturbed proteostasis. Enhancement of proteasome activity has many therapeutic potentials, but is still a relatively unexplored field. It was shown that loss of NRF1 leads to the impairment of the proteasome function and following accumulation of misfolded proteins. Particular compounds could switch on the NRF1-dependent proteasome synthesis and heat shock response. Our compounds do not affect proteostasis and do not cause oxidative stress in cells. Therefore, they might be of high importance as a new approach to delay the onset or ameliorate the symptoms of neurodegenerative and other disorders linked with proteotoxic stress.

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**ROLE OF Np$_n$Ns CAPS IN RNA STABILITY AND CELLULAR REACTION TO STRESS**

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Recently a new class of 5´-RNA caps with structure of dinucleoside polyphosphates (Np$_n$N), was discovered in bacteria[1]. Np$_n$Ns are present in all organisms and as their concentration increases under the stress conditions they are often called alarmones. We showed that Np$_n$Ns are substrates for RNA polymerases during the RNA transcription and that they are incorporated into RNA as non-canonical initiating nucleotides [2]. We found that some Np$_n$N caps were methylated and that the methylation protected them from the cleavage by some decapping enzymes[1]. Moreover, the concentration of Np$_n$N capped RNAs increased in the late stationary phase. Nevertheless, it is not clear, whether the incorporation of Np$_n$Ns into RNA is regulated solely by increased concentration of free Np$_n$Ns or whether any capping enzymes exist in bacteria. To address this question, we study the cellular composition of free Np$_n$Ns in exponential and late stationary phase of bacterial growth. We isolate the fraction of small molecules from *E. coli* and analyse and quantify the Np$_n$Ns by triple-quadrupole LC-MS.

In the second part of the project, we focus on identification of methyltransferases responsible for the methylation of Np$_n$Ns RNA caps. We have identified five different tRNA methyltransferases (Am and m$^6$A) that might be also responsible for the methylation of RNA caps. We isolate RNA from the methyltransferase knockout *E. coli* strains and analyse it using LC-MS. The comparison of isolated RNA from wild type and knockout samples will help us to identify the methyltransferase responsible for the methylation of Np$_n$Ns.

Our work should help us to understand the role of Np$_n$Ns RNA caps in RNA stability and cellular reaction to stress.

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**STRUCTURE-PROPERTIES RELATIONSHIPS OF CROCONAINE DYES**

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Hemi-croconaines **1** and croconaines **2** are class of polymethine fluorescent dyes (Figure 1)[1]. They are analogs of squaraines with five-membered central cycle in the fluorophore and already found application, e.g. in material sciences[2] and imaging[3]. We prepared a small library of these dyes by condensation of croconic acid with aromatic heterocycles with exocyclic methyl[4]. They possess strong absorption in the visible and NIR region and **2** show emission in the so called tissue-transparent window (ttw, 650-950 nm).

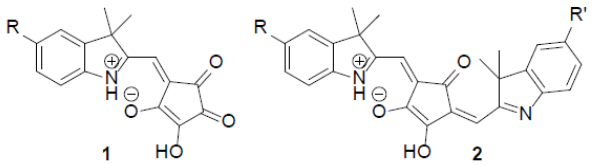


Figure 1. **Hemicroconaine 1 and croconaine 2 dyes**

We studied photophysical properties of the dyes in our library and their stability and photostability in polar protic solvents, which are relevant to biological conditions. Time-resolved spectroscopy experiments provided insight into the nature and fate of their excited states and stability determination provided some inputs for the assessment of their application potential. With regard to electronic properties of attached substituents (R, R') we suggest the structure-properties relationships.

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## ENDOCRINE DISRUPTORS, OBESITY, AND CYTOKINES - HOW RELEVANT ARE THEY TO PCOS?

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Polycystic ovary syndrome (PCOS), a complex and heterogeneous disorder with a prevalence of approximately 5–10 % of premenopausal women, is considered one of the leading endocrinopathy in women. PCOS is usually accompanied by metabolic, reproductive, and neuroendocrine derangements. Its underlying causes remain uncertain, but they are likely to be both genetic and environmental/nutritional, which makes its diagnostics difficult. The aim of the study was to analyze already known markers along with the new potent ones that may help in the diagnostics. A group of selected PCOS women was carefully defined by two experts in the field independently, as a correct diagnosis is often a pitfall of clinical studies.

This study consisted of nine women forming a 'normal-weight PCOS' group, ten women forming a 'obese PCOS' group and twenty healthy controls. The levels of endocrine disruptors, steroid hormones, cytokines, and metabolic parameters were analyzed. Differences between the groups were assessed using the Mann-Whitney U test. Spearman correlation coefficients were calculated for the relationship of the individual parameters.

Significantly higher levels of BPA, Anti-Müllerain hormone, lutropin, lutropin/folitropin ratio, testosterone, androstenedione, 7β-OH-epiandrosterone, and cytokines (IL-6, VEGF, PDGF-bb) were found in normal-weight PCOS women compared to controls. In PCOS women concerning the weight, there were no differences in hormonal, but in metabolic levels. Obese PCOS women had significantly higher insulin resistance, fatty-liver index, triglycerides, cytokines (IL-2, IL-13, IFN-γ). No differences were observed in the paraben exposure.

The assumption that obesity worsens the symptoms of the disease and leads to its worse metabolic manifestations has been confirmed. This study is the first to point out the possibility of using 7β-OH-epiandrosterone as a new diagnostic marker. While the correlation of increased levels of BPA in PCOS women was confirmed, parabens were excluded from the spectra of potential markers of the disease[1].

*Supported by Ministry of Health CR, RVO (Institute of Endocrinology - EU, 00023761), and Specific university research - grant A2-FPBT_2020_017.*

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## ELUCIDATING THE BIOLOGICAL ROLE OF ADAR1 IN THE INNATE IMMUNITY RESPONSE

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One of the most common and best studied type of RNA editing in higher eukaryotes is the hydrolytic deamination of

adenosine to inosine within double-stranded RNAs (dsRNAs), by the enzyme family adenosine deaminases acting on RNA (ADAR). As inosine base-pairs with cytidine (C), it is translated and reverse-transcribed as a guanosine (G), changing the sequence of an RNA.

Three members of the ADAR gene family (ADAR 1-3) have been identified in vertebrates. In addition, two isoforms of ADAR 1 are synthesized by translation initiation at alternative start codons, an interferon-inducible, cytoplasmic 150-kDa protein (p150) and a constitutive, nuclear 110-kDa protein (p110). ADAR1 edits cellular dsRNA to prevent aberrant activation of cytoplasmic antiviral dsRNA sensors and missense mutations, that change ADAR1 residues and reduce RNA editing activity, cause Aicardi-Goutières Syndrome, a childhood encephalitis and interferonopathy. ADAR2 is most highly expressed in brain and it is primarily required for site-specific editing of glutamate receptor transcripts. Mutations in ADAR2 could contribute to excitability syndromes such as epilepsy, to seizures and to diseases involving neuronal plasticity defects. Vertebrate-specific ADAR3 is almost exclusively expressed in the nervous system but its functional significance is unknown.

Mice deficient in Adar1 show embryonic lethality by embryonic day E12.5 1,2 with a type I interferon (IFN) signature similar to that observed in the AGS patients. The most prominent cellular phenotypes of these embryos are: fetal liver disintegration, failed hematopoiesis and widespread apoptosis, overexpression of IFN and IFN-stimulated genes (ISGs) 3,4,5. This immune response is initiated by the MDA5/RIG-I/MAVS pathway which indicates that endogenous unedited transcripts are recognized as foreign by the cells.

We generated Adar1;Mavs double mutant mice that lack the essential adapter protein required for interferon induction signaling from both RIG-I and Mda5. These Adar1;Mavs double mutant mice survived till 20 days after birth, showing that the embryonic lethality is due to an aberrant innate immune response of RLRs to unedited dsRNA.

The principal aim of my project is to characterize the immune signaling pathway aberrantly activated in the absence of Adar1. The cause why the Adar1;Mavs mice are dying is still unknown and during my project I will test if the knockout of specific proteins involved in the apoptotic pathway or in the immune signaling pathway could rescue the mutant lethality. Mice Adar1;Mavs show a mild inflammation in the brain, an increase in the apoptotic level in the intestine and a disrupted spleen morphology.

The second aim of my project is to investigate, by LC-MS/MS, if the lack of inosine in the mutant murine models is affecting the equilibrium of other main RNA modifications. mRNA analysis of Adar2 -/- brains and of mice with no editing activity (Adar1 and Adar2 null mice) shows an increase in m6A level and also confirms that ADAR2 is the main effector in the brain.

## THE EFFECT OF EXCITED STATE ANTIAROMATICITY ON PHOTOCHEMISTRY OF SIMPLE (HETERO)ARENES

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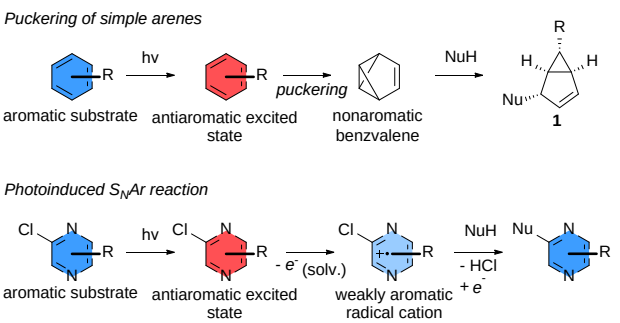
In analogy to Hückel's rule, Baird's rule defines the (anti)aromatic character of the lowest ππ* excited singlet ($S_1$) and triplet ($T_1$) states of [4*n*]- and [4*n*+2]annulenes[1]. The (anti)aromaticity (ES(A)A) of the ground and excited state plays important role in altering physicochemical properties and photoreactivity of the (hetero)annulenes. We investigated two photochemical processes in the optics of the ES(A)A alteration: puckering of simple arenes[2] (benzene, toluene, TMS-benzene) and photo-$S_NAr$ reaction of pharmaceutically relevant pyrazine derivatives (Scheme 1)[3].

We found out that the build-up of ESAA upon single or double photon excitation of Hückel aromatic molecules leads to high tendency to alleviate the antiaromatic character.[4] This is translated into puckering followed by benzvalene formation and solvolysis in the case of simple arenes. The electron rich pyrazine analogues do not pucker, but they ionize and form solvated electrons and corresponding radical cations which undergo photo-$S_NAr$ reaction.




Scheme 1. **Effect of ESAA on the photochemical reactivity of arenes**

*This research was supported by the program INTER-EXCELLENCE, subprogram INTER-ACTION of the Ministry of Education, Youth and Sports CR, reg. No. LTAIN19166 and by the Czech Science Foundation (reg. No. 19-20467Y).*

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## PREPARATION OF PHOTOSENSITIVE AXIALLY CHIRAL DOPANTS FOR LIQUID CRYSTALLINE MATRICES

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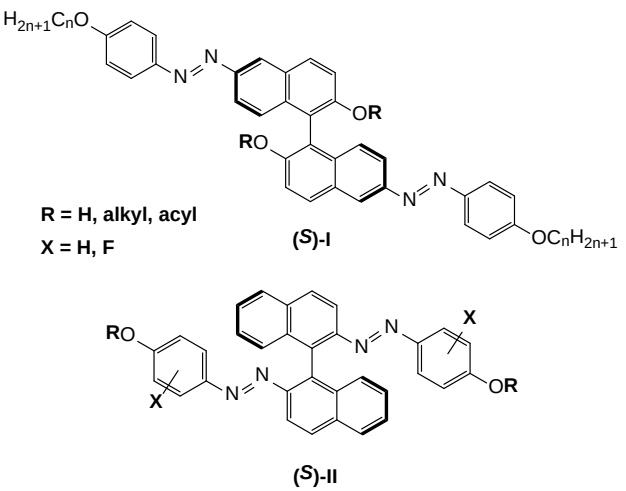
Chiral liquid crystals (LC) have attracted considerable attention due to their unique self-assembling properties, which can be driven by external stimuli such as temperature, pressure or electric field. We focus on preparation of chiral dopants, which amplify macroscopic chirality in achiral LC matrices[1]. We introduce new series of photosensitive axially chiral dopants based on the (*S*)-1,1'-binaphtalene core with various substituents. Due to photosensitive azo linkages in the structure (Scheme 1), it is possible to change the properties of developed cholesteric mesophases dynamically by external light source[2]. We have varied the molecular structure of the presented materials in several ways to establish the structure-properties relationship. We intend to use the best performing materials for decorating magnetic nanoparticles, which will enable us the introduction of an additional functionality into liquid-crystalline composites.




Scheme 1. **General structures of final materials (*S*)-I/II**

*The financial support by Czech Science Foundation (projects 19-03564S) is gratefully acknowledged.*

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## DISCOVERY OF POTENT FXR ANTAGONISTS/GPBAR1 AGONISTS

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Bile acids (BAs) are key signalling steroids that regulate glucose, lipid and energy homeostasis via interactions with farnesoid X receptor (FXR) and G-protein bile acid receptor 1 (GPBAR1). Extensive medicinal chemistry modifications of BA scaffold led to the discovery of potent selective or dual FXR and GPBAR1 agonists. We identified the first-in-class potent steroidal compounds with combined FXR antagonistic and GPBAR1 agonistic activities. We discovered a novel FXR antagonist /GPBAR1 agonist with no off-target activation representing the most efficient GPBAR1 activation by a steroidal compound so far.

Extensive *in vitro* and *in vivo* investigations suggest that compounds 2a may be prospective therapy of type II diabetes as dual modulation of GPBAR1 and FXR has been supposed to be effective in synergistic regulation of glucose homeostasis in the intestine.

*Study was supported by GAUK 170/50/85006 and SVV 260 549.*

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## EXPLORING TAUTOMERISM AND HYDROGEN BONDING OF GUANINE ANALOGUES VIA NMR SPECTROSCOPY

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Tautomerism of nucleobases plays an important role in ensuring that the replication and transcription of genetic information within cells happens correctly. Uncommon tautomers of nucleobases are suggested to be participating components of several processes catalysed by nucleic acid enzymes and responsible for some nucleic acid mutations. Guanine in particular has a large number of possible tautomers

with several different hydrogen-bonding patterns[1]. Based on previous research of isocytosine, which can be thought of as a structural fragment of guanine, we became interested in studying guanine and its structural analogues through variable temperature NMR spectroscopy and DFT calculations[2].

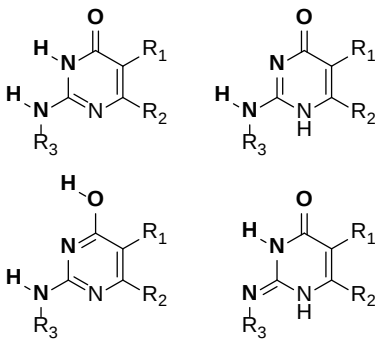

Fig. 1. **Various tautomeric forms of substituted isocytosine with different hydrogen bonding patterns**

Based on extensive computational studies backed by experimental evidence, we present a detailed analysis of the effects responsible for the remarkable stability of the canonical guanine *3H*-ketoamino tautomer and also the possibilities of rare tautomer propagation.

*This work was supported by.the Czech Science Foundation (grants No. 18-11851S and 20-01472S).*

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## FIBROBLASTS ARE MECHANICAL ACTUATORS OF MAMMARY BRANCHING MORPHOGENESIS

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Mammary gland morphogenesis is regulated by reciprocal signalling between epithelium and surrounding stromal tissue. Fibroblasts, the principal component of mammary stroma, provide signaling molecules and extracellular matrix into epithelial microenvironment to support healthy development, and if deregulated, promote mammary neoplasia formation. However, our understanding of the mechanism by which fibroblasts promote epithelial morphogenesis is very limited.

In our study, we employed advanced 3D co-cultures of primary mammary organoids with fibroblasts, that we previously described as a model of epithelial-stromal interaction in branching morphogenesis, together with genetic mouse models and state-of-the-art microscopy techniques to tackle the question of how fibroblasts promote mammary morphogenesis.

First, we discovered a necessity of direct contact between epithelium and fibroblasts to facilitate organoid morphogenesis. Next, we screened cytoskeleton proteins and regulators by pharmacological and genetic inhibition, and we identified fibroblast contractility as a novel factor regulating mammary morphogenesis. Moreover, we revealed a connection of fibroblast contractility with activation of major intercellular signaling hub ERK1/2 and subsequent epithelial activation of YAP signaling pathway. Finely, we found contractile fibroblast sub-population in vivo, in close relation to mammary terminal end buds, stem cell containing structures that lead mammary morphogenesis.

Taken together our discoveries change the perception of stromal tissue in organogenesis, adding a new, yet undescribed, way of interaction between stromal fibroblasts and stem cells contacting epithelium.

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## APPLICATION OF STILLE'S TYPE CROSSCOUPLING REACTIONS IN CALIXARENE CHEMISTRY

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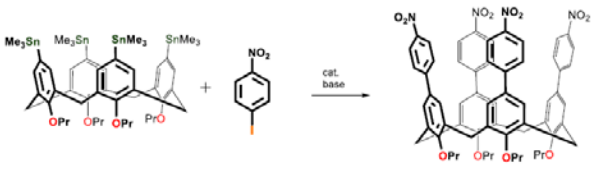
Calixarenes1 are cup-shaped supramolecules, which are widely used as a building blocks for the synthesis of molecules with different properties. The cross-coupling reactions is one of the options for the expansion and derivatization of a basic calix[4]arene skeleton. The typical method consists of the synthesis of organometallic derivative and a subsequent crosscoupling with aryl halides. Using calix[4]aryl boronic acid or calix[4]aryl zinc chlorides in Suzuki and Negishi coupling, respectively, were applied during previous research[1]. The first part of this work is focused on the preparation of stable stannio derivatives of calix[4]arene in the cone, 1,3-alternate, partial cone and 1,2-alternate conformations prepared from the halogenated calixarene derivatives.



Scheme 1. **Stille's type cross-coupling reactions**

In the second part, these substrates were used to study Stille's type cross-coupling reactions using different aryl

halides and a variety of catalysts and bases (Scheme 1). As shown in this work, the products of these reactions can be applied for construction of more complex receptors.

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## ONE-POT SYNTHESIS OF 1-ARYL-2-AMINO ALCOHOLS FROM THE CORRESPONDING AMIDES OR NITRILES

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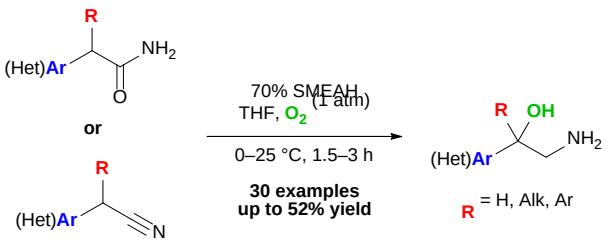


Scheme 1. **Developed one-pot method for the arylaminoalkanol synthesis**

In connection with our interest in the development of enantioselective organocatalysts, we have been continuously compelled to explore novel chiral auxiliaries for the catalyst design. As part of the above synthetic efforts, we have prepared many chiral non-racemic amine scaffolds by reductions of the corresponding enantiopure amides[1–3]. For such transformations, we normally utilized sodium bis(2-methoxyethoxy)aluminum hydride (SMEAH) as a reductant.

However, (*S*)-naproxamide subjected to the above-mentioned reduction gave a curious product, which was then, to our surprise, identified as 1,2-amino alcohol derivative[4].

Accordingly, we have demonstrated that amides and nitriles of aryl-alkanoic acids bearing hydrogen atoms at benzylic α-carbon exhibit a unique reactivity towards SMEAH under $O_2$ atmosphere. The substrate scope of this process was examined on 30 entries and, although the respective products were provided in moderate yields only, the above simple protocol may serve as a direct and powerful entry to the sterically congested 1,2-amino alcohols that are difficult to prepare by other routes.

The plausible mechanistic proposal for the observed results was given based on the isolation and identification of the stable intermediates and stereochemical evidence.

The present method may open up attractive prospective routes for future developments in $^{18}O$-labeling strategies or stereoselective synthesis thereof. The reaction was applied to a synthesis of a potentially bioactive target.

*Support was provided by the project: LM2015043 (MEYS CZ).*

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## ISL1 CONDITIONAL DELETION IN MOUSE INNER EAR CAUSES SENSONEURAL HEARING LOSS

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The mammalian inner ear is the sensory organ responsible for hearing and balance. It contains sensory hair cells detecting sound and movement, and neurons transducing sensory inputs as electrical signals to the central auditory nervous system. The neurons of the inner ear are located in the spiral and vestibular ganglia which innervate the auditory organ (cochlea) and the vestibular system, respectively[1]. Any failure during the development or damage to the spiral ganglion is permanent and leads to sensoneural hearing loss[2]. Comprehensive understanding of cellular and molecular development of the spiral ganglion can help to improve therapeutic procedures, such as regenerative medicine or gene therapy.

Inner ear development is governed by a complex network of transcription factors, growth factors etc. We focus on ISL1, a LIM-homeodomain transcription factor expressed in both neural and hair cell precursors of the inner ear. We generate a new mouse model (*Isl1CKO*) using the *Neurod1-cre* driver for conditional deletion of *Isl1* specifically in neurons of the inner ear. To evaluate the morphological alteration during the development of the inner ear, we use immunohistochemistry. To reveal changes in transcriptional profile caused by *Isl1* deletion, we exploit *tdTomato* reporter mice to sort and collect the inner ear neuronal cells and perform molecular analyses using RNA sequencing. We also perform hearing tests on adult mice.

Our *Isl1CKO* immunohistochemistry results show significant changes in inner ear development, such as shortened cochlea, disorganized spiral and vestibular ganglia and disorganized neuronal axon projections. Besides impairments in the inner ear, we also detect smaller cochlear nuclei in the central auditory nervous system. These morphological changes in *Isl1CKO* cause deterioration of hearing function.

In conclusion, ISL1 has a key role in development and maintenance of the auditory system. Understanding the function

of ISL1 in the development and survival of auditory neurons is crucial for future more effective hearing loss treatment strategies.

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## AZURIN: A MODEL PROTEIN TO STUDY AN ELECTRON TRANSFER PROCESS

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Countless electron transport (ET) processes occur in living organisms every day. Their study is a crucial field of modern structural and functional proteomics. In many cases model proteins like azurin from *P. aeruginosa* are utilized similarly like in our experiments. This small cupredoxine exhibits absorbance maximum at 630 nm ($A_{630}$) in 2+ redox state of the central Cu atom. During its reduction to 1+ state the $A_{630}$ value decreases allowing UV-VIS detection of ET reaction. Additionally, azurin is known to bind other metals in its active site (e.g. Zn-azurin is non-reducible). Oligomerization of this protein in solution was also reported with dimers observed at >70μM concentration.

Recombinant expression and purification protocols for metal-pure forms of apo-, Cu- and Zn-azurins containing 100% Met were optimized and basic native electrophoresis has revealed an intriguing behavioral discrepancy between those forms which may put the occurring oligomerization processes into greater context. While in apo-azurin a multitude of azurin bands with varying electrophoretic mobility was observed, metallized azurins did not exhibit such behavior. More than that, partial metallization of apo-azurin has led to significant decrease in described oligomerization.

To study the ET processes and azurin oligomerization we have introduced a structural photoinducible analogue of canonical amino acid Met – L-2-amino-5,5-azi-hexanoic acid (photo-Met) – into azurin structure. Using previously optimized protocols for recombinant expression in *E. coli* B834 we have inserted photo-Met into two types of azurin: wild-type (WT) azurin and Az2W mutant where two adjacent W residues with confirmed role in electron hopping across protein-protein interface are present. The incorporation percentage of photo-Met in analyzed samples was determined *via* MALDI-TOF MS and reached values in range of 10–20% for Az2W mutant and 40–50% for WT, respectively. Four different concentrations of protein with Cu/Zn mixed metallization (in range of 5–180 μM) were analyzed and after exposure to intense UV light the results were evaluated *via* UV-VIS spectroscopy and SDS-PAGE. The UV-VIS revealed that $A_{630}$ value decreased for both studied proteins (confirmation of ET process) and an additional protein band was observed on SDS-PAGE corresponding to covalent dimer (cross-linking study – XL).

We have observed higher dimerization yield in Az2W mutant compared to wild-type azurin and our findings support the role of two additional W residues on the interacting surface (formed by a β-sheet close to $Cu^{1+/2+}$ center) not only during ET hopping but also in azurin oligomerization. The described photo-Met-dependent XL and ET experimental approaches should be further employed to study individual metal-pure or apo- azurin forms.

*The project was supported by Charles University (GAUK n. 1538119) and Grant agency of Czech Republic (20-28126S).*

## ALBUMIN IN PH-DEPENDENT DRUG DELIVERY SYSTEM FOR MAGNETIC RESONANCE ANGIOGRAPHY

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(Human) serum albumin (HSA) is the most abundant protein in plasma. It is utilized in many processes such as maintaining oncotic pressure and transportation of hydrophobic molecules. The latter property of HSA has been widely used in a delayed release of administered drugs[1]. The hearth-shaped molecule of HSA contains two main binding sites in which lipophilic parts of molecules can be non-covalently anchored. This interaction is also used in magnetic resonance (MR) angiography[2] visualizing blood vessels of patient. Mostly, the HSA serves only as a drug carrier with a non-specific release and it may lead to additional issues, *e.g.*, too long clearance of not-stable-enough drug[2]. The surface of the HSA main binding sites is covered with positively charged amino acid residues. Thus, non-covalent interaction of drugs with HSA may be affected by a change of a charge (*e.g.*, through protonation state nearby of the binging site). As plasma pH slightly varies due to the glomerular filtration in kidneys or ongoing pathological processes such as cancer and inflammation, alternation of protonation state may lead to a controlled HSA interaction and drug biodistribution (*e.g.*, to localize cancerous cells with MR angiography).

We have synthetized and characterized several MR contrast agents based on $DO3AP^R$ moiety (Fig. 1) which complexes are stable and suitable for *in vivo* applications. The complexes have lipophilic pendant arms capable of a non-covalent interaction with HSA[3]. The lipophilic groups are attached to a protonable pendant amino group and its $pK_A$ is tuneable through the *N*-substituents. In this work, we explored the interaction of differently protonated complexes with HSA which led to changes in affinity of complexes to HSA and, thus, to change of their relaxometric properties towards MR angiography.

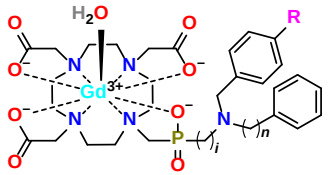

Fig. 1. **The structure of complexes with lipophilic pendant groups**

*The work was supported by LTC20044 and UNCE/SCI014.*

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## NON-INFECTIOUS MURINE MODEL OF CHRONIC HEPATITIS B AND ITS CHARACTERIZATION

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Chronic hepatitis B (CHB) is worldwide disease caused by Hepatitis B virus which targets hepatocytes. Later staged CHB increases the risk of liver failure, cirrhosis or hepatocellular carcinoma. HBV infected new-borns or children younger than 5 years old often develop the chronic condition, for which no effective and long-lasting treatment without side effects is available. The use of antiviral agents such as nucleoside/ nucleotide analogues requires treatment during the entire life[1,2]. Immunomodulation with PEGylated interferon α allows HBV clearance, though only limited percentage of patients is cured[1,3].

To develop innovative therapy based on the activation of the host immune system, a suitable *in vivo* model is of urgent need. Here, we present complex establishment of a unique non-infectious mouse model of CHB. We performed the hydrodynamic induction (HDI) to selectively deliver HBV genome-encoding plasmid with mutation in start codon for HBV polymerase into liver hepatocytes. Furthermore, we developed a set of rapid screening methods allowing us to monitor different viral markers in mouse plasma and liver hepatocytes. We also focused on the liver resident immune cell population as the liver immunity is considered to be the key point in CHB therapy. To study the liver-associated immune cell millieu in the chronic as well as normal state, we optimized conditions for isolation of various liver cell populations. PBS-perfused liver was homogenized combining enzymatic degradation and mechanical dissociation to obtain a single cell suspension, which is an essential prerequisite for further immunophenotyping. Using multiparametric flow cytometry along with detailed gating strategy, we were able to differentiate among liver endothelial cells and populations of lymphocytes as well as granulocytes; namely T/B/NK/NKT/dendritic/Kupffer cells, monocytes, neutrophils and macrophages.

Taken all together, we present a stable CHB non-infectious murine model based on HDI delivery, accompanied with comprehensive liver immunity characterization, all of which might be of pharmaceutic interest.

*The work was supported by Gilead Sciences, Inc. and OP RDE project No. CZ.02.1.01/0.0/0.0/16_019/0000729.*

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## CELL-PENETRATING PEPTIDES ENHANCE NUCLEAR DELIVERY OF MOUSE POLYOMAVIRUS-DERIVED PARTICLES

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Nowadays, research is focused on developing nanoparticles that can selectively enter target cells and deliver there their cargo. However, various nanoparticles face a common challenge - after the uptake via endocytosis, they must escape endocytic vesicles before being degraded in lysosomes and often fail to do so[1]. To overcome this issue, we utilized model nanoparticles derived from the mouse polyomavirus and modified them by cell-penetrating peptides (CPPs) which are short peptides that can cross the membranes and help to transfer other agents inside various cellular compartments. CPPs were attached to the viral nanoparticles that contained the luciferase reporter gene either by simple co-incubation or by genetic modification of the viral capsid protein. By measuring the luciferase signal, we found out that the co-incubation of the particles with histidine-rich designed peptide LAH4[2], oligohistidine peptide KH27K and octaarginine peptide R8 increased the efficiency of particle nuclear delivery. When these CPPs were genetically attached

to particles, increased transduction was observed but to a much lower extent (max 7-fold) compared to mere co-incubation with the most efficient CPP, LAH4 (40-fold). The results suggest that CPPs provide an interesting potential in improving the efficiency of nanoparticle delivery into cells. Further research could help to reveal the detailed mechanism of CPP-mediated membrane translocation.

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## PHOTOREDOX BEHAVIOUR OF HEXAPHENYLETHANE-BASED SWITCHES

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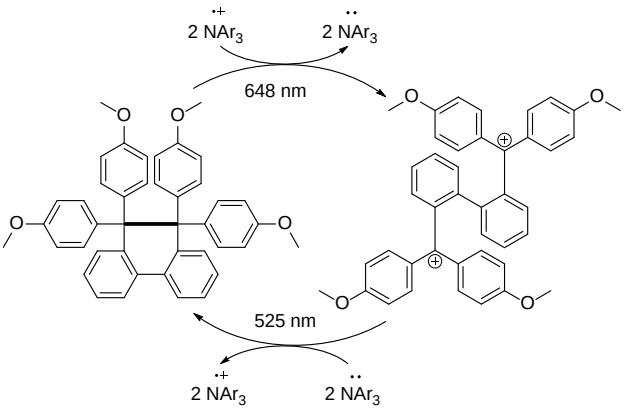
Organic switches capable of reversible covalent bond formation have potential applications in molecular electronics[1,2] and motors[1]. Whereas the chemical and electrochemical switching of these compounds has been described[3-5], no studies about their photochemical behaviour have been conducted so far.




Scheme 1. **Photoswitching of a redox switch**

The photophysical properties of the hexaphenylethane-based redox switches such as UV/Vis absorption, emission spectra and excited state potentials were examined. The light-mediated reduction and oxidation of the switches was tested with the scope of classical sacrificial electron donors and acceptors, as well as triarylamminium radical cations. The system of a switch and a triarylamine capable of both opening and closing of the switch was discovered. As the products of the photoredox reaction are thermodynamically stable, this transformation can be further utilized in systems for photocontrollable reversible manipulation with electric charges of the respective components.

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## INTERACTIONS OF HEME SENSOR PROTEINS WITH A HEME MOLECULE AS A POTENTIAL TOOL FOR BIOLOGICAL DOSIMETRY

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Biological dosimetry focuses on the determination of ionising radiation dose based on the specific biomarkers that occur in the organism after irradiation. Although there has been designed techniques suitable for this purpose, none of them would be easily applicable in case of a nuclear incident or accident as they require at least two days for the samples to be prepared[1]. Therefore, we suggest that heme sensor proteins and their interaction with heme may serve as a potential biomarker of stress caused by ionising radiation.

We focus our research on the interaction of heme sensor proteins with heme molecules. Specifically, we are interested in the determination of the number of heme molecules that selectively interacts with the sensors and oxidoreductive state of heme iron during the process. In addition to that, we aim to describe the structural changes and modulation of heme sensor proteins activity as a result of their interaction with heme. Therefore, we selected two model eukaryotic heme sensor proteins for detailed characterisation, namely (i) tumor suppressor p53 and (ii) heme-regulated inhibitor (HRI). With the physiological function of the regulator of the cell cycle and gene transcription, p53 has been increasingly rising of interest in the field of biological dosimetry. Recently, there has been published a preliminary study discussing its ability to interact selectively with heme molecules[2]. Moreover, depending on heme concentration, HRI is able to phosphorylate eucaryotic

initiation factor 2α. When in interaction with heme, HRI kinase activity is inhibited[3].

The possibility of applying heme sensor proteins as potential biomarkers of cellular stress induced by ionising radiation will be discussed. The results of the study will broaden our knowledge about heme sensor proteins and contribute to further development of biological dosimetry as well.

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## INTERACTIONS OF PROTEIN AND LIGAND IN THE STING BINDING SITE PROBED BY ARTIFICIALLY INTRODUCED SINGLE POINT MUTATIONS

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STING protein (Stimulator of Interferon Genes) plays an important role in the innate immune system as part of cyclic GMP-AMP synthase (cGAS)-STING pathway where serves for early detection of invading pathogens and activation of the cellular defence of the host[1]. During the activation STING binds cyclic dinucleotide 2′,3′-cGAMP, which is produced by cGAS after detection of dsDNA in cytosol[2]. Apart from eukaryotic 2′,3′-cGAMP, STING be activated by bacterial cyclic dinucleotides too. As result of pathway activation STING recruits and activates a TANK binding kinase (TBK1) and inhibitory kappa B kinases (IKK) which phosphorylates transcription factors Interferon regulatory factor 3 (IRF3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). Activation of whole pathway results in induced secretion of type I interferons and proinflammatory cytokines, which essential for the host's defence and potentially for induction of an antitumoral immunity too[4]. Several potent compounds regulating its activity have been reported, mainly derivatives of cyclic dinucleotides (CDNs) – natural STING agonists.

Here we focus on delivering complementary information to large-scale "ligand-profiling" studies by probing the importance of protein-ligand (STING:CDN) interactions on the protein side. We examined in detail six CDNs (mammalian, bacterial and synthetic) each in complex with 14 rationally devised mutated STING variants. Nine of them artificially designed S162A, S162T, Y167F, R232K, A233L, A233I, R238K, T263A, T263S. And four prepared considering naturally occurred allelic forms: G230A, R232H, R293Q and double mutation G230A/R293Q. The mutations affected various types of protein-ligand interactions π- π stacking, hydrogen bonding, ionic pairing and non-polar contacts. We correlated experimental data obtained by differential scanning fluorimetry, X-ray crystallography, and isothermal titration calorimetry with theoretical calculations. This enabled us to provide a mechanistic interpretation of the differences in the binding of representative CDNs to STING.

We found out that the mutations R238K and Y167F led to an unmitigated loss of stabilization (ligand binding), whereas G230A mutation radically increased thermal stability of the protein-ligand complex indicating an improved ligand binding. The effects of the other mutations depended on the type of ligand (CDN) and varied, to some extent. A very good correlation ($r^2$= 0.6) between the experimental binding affinities and interaction energies computed by quantum chemical methods enabled us to explain the effect of the studied mutations in detail and evaluate specific interactions quantitatively.

Understanding both structural variations of ligands and the role of amino acid residues participating in the ligand binding on the side protein is crucial to the discovery of efficient agonists. Thus, we believe our work may inspire development of new high-affinity ligands against the common STING haplotypes by targeting the right protein-ligand interactions.

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## COPPER COMPLEXES AS PROMISING CANCER THERAPEUTICS AND THEIR EFFECTS ON ENDOPLASMIC RETICULUM

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Endoplasmic reticulum (ER) is a key cellular organelle involved in protein synthesis, folding transport and posttranslational modification, and also in lipid synthesis and calcium storage.[1] Disruption of the ER homeostasis (ER stress) by increased protein synthesis or expression by abnormal proteins can occur during cell differentiation or as a result of DNA damage. Usually, ER stress induces a complex signaling pathway called the unfolded protein response (UPR). An adaptive branch of UPR directly reduces the amount of misfolded proteins in the ER lumen. However, excessive and extended ER stress can induce the cell death, typically by apoptosis.[2] Many compounds, including copper complexes, with cytostatic effect induce DNA damage, however their effect on other cellular structures, including ER and UPR remain unclear.

Previously, we demonstrated that mixed copper Cu(II)–phenanthroline complexes induce cell death by ER-dependent mechanism in ovarian cancer cell.[3] Here we investigated effects of Cu(II)-phenanthroline complex bound to tauroursodeoxycholic acid (TUDCA) in ovarian and pancreatic cancer cells. We found out that Cu(II)-phenanthroline-TUDCA induce cell death of cancer cells and shows distinct biochemical effects than Cu(II)-phenanthroline or TUDCA alone.

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## PHOTOCHEMICAL VAPOR GENERATION – A NOVEL METHOD FOR ULTRATRACE ANALYSIS OF TRANSITION METALS?

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The ever-increasing importance of transition metals in modern technologies is affecting the processes associated with their natural environmental cycle and their exposure to the biosphere. Therefore, there is an increasing need for ultrasensitive analytical methodology capable of monitoring transition metals in various matrices. Atomic spectrometry is typically the first analytical method of choice. However, sample introduction is the Achilles´ heel of atomic spectrometry methods. Photochemical vapor generation (PVG) is nowadays an emerging alternative sample introduction technique which employs a source of UV-radiation that irradiates a low molecular weight organic acid medium with an analyte. Generated highly reducing radicals and aquated electrons convert the analyte into its volatile species which is separated from the liquid matrix and transported into a detector with the efficiency significantly higher than that of conventional pneumatic nebulization. Since its first introduction in 2003[1], the coverage of PVG has expanded to many elements including Hg, hydride forming elements (e.g. Se, As), halogens and transition metals (e.g. Ni, Fe, Co)[2].

This presentation will focus on the novel PVG of Mo, W, Re and Ru that was achieved for the first time. Efficient PVG was accomplished in a flow-through photoreactor using formic acid-based reaction media. Generated volatile products (metal carbonyls) were directed by an argon carrier gas to a gas-liquid separator and introduced into an inductively coupled plasma mass spectrometer for detection. Details on optimization of generation conditions and influence of various metal sensitizers (additives) will be presented. Particular attention will be paid to the determination of PVG efficiency, which is the parameter that describes the best overall yield of PVG and is directly reflected in achieved sensitivity. The accuracy and feasibility of this sensitive methodology (limits of detection in pg $ml^{-1}$) will be demonstrated by the results from analyses of Certified Reference Materials and real samples covering a variety of sample matrices. Mechanistic aspects of PVG of these transition metals will be also pointed out and discussed.

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## BENZO[D]THIAZOLE-2-SULFOAMIDES – ENFANT TERRIBLE OF SULFONAMIDE FAMILY

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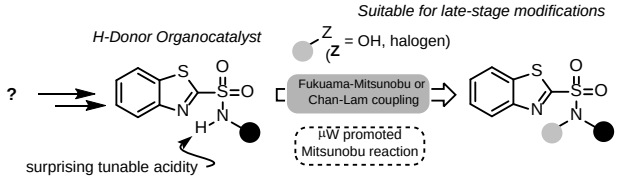
The sulfonamide moiety has been considered as very important pharmacophore since early 1930s, and its structural pattern has found its place in several pharmaceuticals[1]. For long time, sulfonamide moiety has been regarded only as a terminal group in organic synthesis. However, the view has changed in recent years leading to a development of novel synthetic methods that use sulfonamides as synthetic intermediates[2]. The unique properties of sulfonamide functional group resulted in a fast development of several novel synthetic methods (photochemical reactions[3]) or increased the scope of already well-established methods (coupling reactions[4]).

The goal of our project is to find a short and convenient synthetic route to previously hard-to-get sulfonamides – sulfonamides bearing electron poor heterocycles (Scheme 1). Such type of sulfonamides is virtually impossible to prepare using the standard sulfonamide synthetic methods[5]. In our study we have decided to use benzothiazole heterocycle as a typical "troublemaker" heterocycle representative, Our latest achievements in our rush to get a general synthetic method allowing us to prepare "enfant terrible" of sulfonamide family as well as their use of such unique structures as proton donor/nucleophile will be presented in our contribution.




Scheme 1. **Preparation of BT-sulfonamides and their modifications**

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## BABA-INDUCED RESISTANCE TO PATHOGENS: THE MULTI-OMICS ANALYSIS OF BABA-TREATED TOMATO

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Plants, compared to animals are confined to one location and they are regularly exposed to many stressful situations e.g., changing of environmental condition, and interactions with numerous pests and pathogenic microorganisms, without the possibility of escape. To defend against pathogens, plants have developed a sophisticated innate immunity. Nevertheless, extensive infections of crops by plant pathogens have repeatedly caused significant economic and social problems worldwide[1]. Nowadays, there are efforts to replace pesticides with agents able to prime plants for enhanced defence. This primed state is characterized by a faster and more reliable response of a plant to a stimulus. Even though the term defence priming was proposed during the 1930s, the molecular mechanisms underlying this phenomenon were partially elucidated recently, particularly in the model plant Arabidopsis thaliana[2]. One of the most effective and the most promising defence priming chemical agents is a non-proteinaceous amino acid, beta-aminobutyric acid (BABA)[3].

We have investigated the molecular basis of BABA-induced resistance in tomato, one of the major crops with a worldwide production of over 180 million tons in 2019. Transcriptome and proteome analysis of tomato plants after treatment with BABA was done. The application of BABA led to a change in the expression of approx. 6% of transcripts. Most transcripts were induced and related to general stress response and defence. The proteomic analysis also showed massive upregulation of proteins responsible for response to stimulus and stress. In addition, analysis of amino acid changes and phytohormones revealed changes in homeostasis and the main role of ethylene and JA for the defence reaction of tomato plant.

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## EXPLORING CLONAL EVOLUTION AND CAUSES OF THERAPY FAILURE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Oncological diseases with highly heterogeneous clinical course represent an intriguing research challenge in the field of cancer biology[1]. One of such examples is chronic lymphocytic leukemia (CLL) – the most frequent form of adult leukemia in the Western world characterized by proliferation and accumulation of tumorous B lymphocytes.

The overall survival of patients with CLL varies from months to decades depending on a need for treatment, therapy response and duration of disease remission. There are also patients who survive for years without a need for therapy administration. This clinical heterogeneity is associated with a variable genetic background[2]. Recent discoveries using next generation sequencing technologies have revealed a high number of recurrent but also nonrecurrent gene mutations rather than a universal genetic event. Subclones of CLL cells harbour a broad spectrum of somatic mutations and show different proliferation capacity and sensitivity to treatment[3,4]. Thus, it is still challenging to stratify patients for a particular therapy accurately, which results in a high economic and emotional burden. Considering the enormous genomic complexity of CLL, sophisticated models and external validation are required for reliable prognostication and precision medicine[5]. Revealing the clonal architecture and mutational profile of leukemic cells may contribute to improvement of the therapy tailoring.

Within the present project, we aimed to identify molecular genetic factors affecting the clonal evolution of CLL using the whole exome sequencing method. We investigated samples from 52 CLL patients with known clinical course and different scenarios of *TP53* gene mutation expansions. We took *TP53* mutation status into account because the gene encodes an important tumour suppressor, and its aberrations represent the strongest predictive marker of unfavourable disease course and therapy resistance in CLL. Our cohort included patients treated with standard chemoimmunotherapy, but also with modern targeted therapies. We assessed somatic mutations by analysing tumour DNA isolated from CLL cells against normal DNA from buccal swabs or separated T lymphocytes. For every patient we analysed samples before and after particular therapies. The median time between the first and last sample collection was 51 months (range 11 – 132). Finally, we compared mutation profiles and their evolution during the disease course for all tested patients.

We identified mutations in genes associated with CLL, such as *SF3B1*, *ATM*, *RPS15*, *MED12*, *NOTCH1* or *NFKBIE*, which expanded or diminished differently after specific types of therapy and also in relation to the *TP53* mutation profiles. The classification, annotation and co-occurrence of detected mutations was set into context of therapy response and aggressiveness of the disease.

Furthermore, we assigned mutated genes into molecular pathways and calculated the so-called pathway mutation scores for each patient. Using advanced statistical methods, we defined groups of patients with similar pathway mutation profiles. We examined whether the respective groups differed in clinical course and identified abnormal molecular processes specific to each group.

Our results aid the understanding of molecular grounds of the clonal evolution in CLL, which is necessary for the accurate use of available treatment regimens and for design of suitable diagnostic panels.

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## UNDERSTANDING PHYSICAL EFFECTS OF CHEMICAL MODIFICATION IN RNA CONTEXT WITH COMPUTATIONAL METHODS

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Phosphorothioates (PTs) are important chemical modifications of the RNA backbone where a single non-bridging oxygen of the phosphate is replaced with a sulphur atom. PT can stabilize RNAs by protecting them from hydrolysis and is commonly used as tool to explore their function. It is, however, unclear what basic physical effects PT has on RNA stability and electronic structure. Neomycin-sensing riboswitch (NSR) is the smallest biologically functional riboswitch consisted of only 27 nucleotides. As shown in Figure 1, NSR has well-defined structure, two discontinuous helices separated by a three-nucleotide buldge, stabilized by a U-turn motif. Three of the signature interactions of the U-turn, signature H-bonds, an anion-π interaction and a potassium binding site, are formed by RNA phosphates, contributing to the stability of the U-turn. The simplicity of the NSR model and the variety of the phosphate-involved

interactions in the NSR makes it an ideal RNA model to study the physical effects of PT in RNA context. Considering the time consumption and the difficulty of purifying NSR with single PT site, we turned to apply computational methods to investigate PT structural effects on the NSR.

Here, we present how Molecular Dynamics (MD) simulations and quantum mechanical (QM) calculations unveiled the effects of PT modifications in the structural context of the NSR. PT modifications on different phosphate sites of the NSR U-turn affect the signature interactions by larger van der Waals radius compared with non-bridging oxygen, while the structural stability is remained. By comparing with high-level QM calculations, we reveal the distinct physical properties of the individual interactions facilitated by the PT. The sulphur substitution, besides weakening the direct H-bond interaction, reduces the directionality of H-bonding while increasing its dispersion and induction components. It also reduces the induction and increases dispersion component of the anion-π stacking. The sulphur force-field parameters commonly employed in the literature do not reflect these distinctions, leading to unsatisfactory description of PT in simulations of the NSR. We show that it is not possible to accurately describe the PT interactions using one universal set of van der Waals sulphur parameters and provide suggestions for improving the force-field performance.

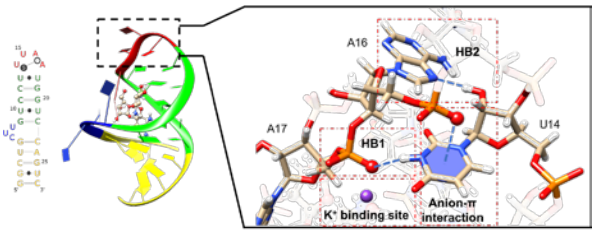


Fig. 1 **Secondary structure of the NSR and its 3D structure with the bound ribostamycin shown in CPK representation.** The A-RNA helical segments are shown in green and yellow, respectively, separated by the flexible bulge in blue. The U-turn loop is in red. Its two signature H-bonds (termed as HB1 and HB2, respectively), the anion-π interaction, and the potassium binding site are shown in detail. The interaction involved oxygen atoms of the A16 and A17 phosphate groups are highlighted as spheres.

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**REMARKS AND NOTES**

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