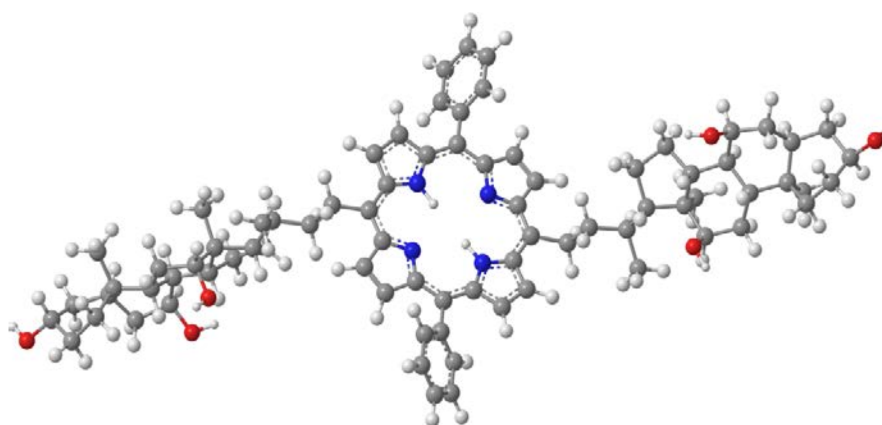
Czech Chem. Soc. Symp. Ser. *20*, 1-52 (2022). “Amerika” 21st

MERCK

IOCB Tech

Chemické listy



XXIst INTERDISCIPLINARY MEETING OF YOUNG RESEARCHERS AND STUDENTS IN THE FIELD OF CHEMISTRY, BIOCHEMISTRY, MOLECULAR BIOLOGY, AND BIOMATERIALS

May 16 – May 19, 2022

Edited by
Radmila Řápková, Martin Fusek, Pavel Drašar

i&i Prague

inventions | investments

Corresponding authors are fully responsible for the content of their communications, the editors in some cases corrected the format of articles that were not submitted in accordance with the CCSSS journal rules.

FUNCTIONAL STUDY OF GH3-MEDIATED HORMONE HOMEOSTASIS IN PLANTS

<u>ANITA AMENT</u>, FEDERICA BRUNONI, ONDŘEJ NOVÁK

Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany of the Czech Academy of Sciences, Šlechtitelů 27, 78371 Olomouc, The Czech Republic
anita.ament@upol.cz

The Gretchen Hagen (GH3) family of acyl-acid-amido synthetases catalyse the formation of amino acid conjugates to modulate levels of indole-3-acetic acid (IAA), jasmonic acid (JA) and salicylic acid (SA). Depending on the amino acid attached to the molecule, the reaction can lead to the formation of either active or inactive forms of plant hormones. In the case of IAA, conjugation with aspartate or glutamate targets the molecule for degradation, while reaction with other amino acids (*e.g.* alanine, valine) produces storage forms ready to be hydrolysed back to the active form. Despite their relevance in fine-tuning homeostatic processes, the underlying mechanisms by which these proteins exert their functions at the subcellular level have not been explored yet.

This research focuses on unveiling how GH3 enzymes regulate intracellular auxin homeostasis by mapping the Arabidopsis GH3 protein subcellular localization and the distribution of amide-linked conjugates. To study localization, fluorescently tagged GH3 proteins were transiently expressed in protoplast derived from Arabidopsis root suspension culture or transgenes were delivered into tobacco leaves by Agrobacterium infiltration. Preliminary results raised a question of possible nuclear and cytosolic localization that will be confirmed by generating Arabidopsis GH3-GFP stable transgenic lines. To understand how the balance of auxin is maintained in different cell compartments, the transport activity of previously characterized intracellular auxin transporters will be explored with radiolabelled IAA metabolites. Additionally, organelle-enriched fractions will be isolated and profiled for auxin and auxin amide conjugates from mutants with different genetic backgrounds.

Transport assay is funded by grant "Development of Plant Hormones Transport Assay" (reg. no. DSGC-2021-0171) under the OPIE project "Improvement of Doctoral Student Grant Competition Schemes and their Pilot Implementation" (reg. no. CZ.02.2.69/0.0/0.0/19_073/0016713).

UNRAVELING THE FUNCTION OF HAT3.1, A REPRESENTATIVE MEMBER OF THE PHD-HD PROTEINS

<u>LUCIA BAĎUROVÁ</u>[a]*, JAN SKALÁK[a], JIŘÍ RUDOLF[a,b], GABRIELA LOCHMANOVÁ[c], ZBYNĚK ZDRÁHAL[c], JAN HEJÁTKO[a]

[a]Department of Functional Genomics and Proteomics of Plants, CEITEC Masaryk University, Kamenice 5, 625 00 Brno; [b]Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno; [c]Proteomics Core Facility, CEITEC Masaryk University, Kamenice 5, 625 00 Brno
lucia.badurova@ceitec.muni.cz

Domain modularity is an important feature of transcription factors (TFs), enabling their functional diversification during evolution. HAT3.1 is an example of a protein with a unique plant-specific domain combination: a homeodomain (HD) and a plant homeodomain (PHD)[1]. The HD is a conserved DNA-binding domain often found within proteins regulating developmental processes[2]. The PHD functions as an epigenetic reader, having the capacity to modulate chromatin landscape[3]. The presence of the HD and PHD within HAT3.1 may imply its involvement in the developmental processes via epigenetically mediated transcriptional regulation.

To address the relevance of the spatiotemporal specificity of the *HAT3.1* activity, we analyzed its expression pattern in different vegetative and generative plant tissues. Albeit its expression was seemingly ubiquitous, it showed a considerable increase in flowers and early developmental stages of siliques.

Using *in silico* prediction tools and available ChIP-Seq data, the binding sites of type-B *Arabidopsis* response regulators, downstream mediators of the cytokinin signaling, were found in the *HAT3.1* promoter, adding an interesting regulatory loop mediated by phytohormones. Moreover, we showed that exogenous treatment with cytokinins resulted in the induction of the *HAT3.1* expression.

Assuming that presence of the PHD domain may link the HAT3.1 function to epigenetic regulations, we analyzed the global pattern of histone post-translational modifications by LC-MS/MS. Our preliminary data revealed moderate organ-specific changes in the relative abundance of the modified histone forms in the *hat3.1* mutant line, which will be further validated.

Uncovering the function of HAT3.1 might help us to better understand the evolutionary role of the PHD-HD TFs, as well as the molecular crosstalks governing plant development.

This work was supported by „SINGING PLANT“ (No. CZ.02.1.01/0.0/0.0/16_026/0008446).

REFERENCES

1. Schindler U., Beckmann H., Cashmore A. R.: Plant J. *4*, 137 (1993).
2. Mukherjee K., Brocchieri L., Bürglin T. R.: Mol. Biol. Evol. *26*, 2775 (2009).
3. Sanchez R., Zhou M. M.: Trends Biochem. Sci. *36*, 364 (2011).

ELECTROMEMBRANE EXTRACTION: A NOVEL AND EFFECTIVE MICROEXTRACTION TECHNIQUE IN BIOANALYSIS

HANA BAVLOVIČ PISKÁČKOVÁ, RADIM KUČERA, PETRA ŠTĚRBOVÁ-KOVAŘÍKOVÁ

Faculty of Pharmacy in Hradec Králové, Charles University, Akademika Heyrovského 1203,
Hradec Králové, 500 05, Czech Republic
piskacha@faf.cuni.cz

Electromembrane extraction (EME) is a hybrid microextraction technique laying between liquid-liquid extraction (LLE) and electrophoresis. The technique was firstly introduced in 2006 by Pedersen-Bjergaard and Rasmussen under the name electromembrane isolation[1]. The EME of charged analytes is performed from the aqueous sample through the water-immiscible supported liquid membrane (SLM) to the aqueous acceptor solution. The driving force of the extraction is an electrical potential, which is applied across the SLM.

One of the biggest challenges in the development of bioanalytical methods is the sample preparation, since the co-eluting endogenous matrix components may negatively affect the obtained results. For example phospholipids, which are eluted over a wide range of retention times, have been recognized as a major cause of deleterious matrix effects.

Our studies focused on use of EME for isolation of analytes from various biological matrices. EME efficiency was monitored not only in plasma[2] but also in very complex and complicated matrices namely tissues (liver, heart and skeletal muscle)[3] and breast milk[4]. Among the analytes were amphetamines, synthetic cathinones and polar anthracyclines with zwitterionic nature.

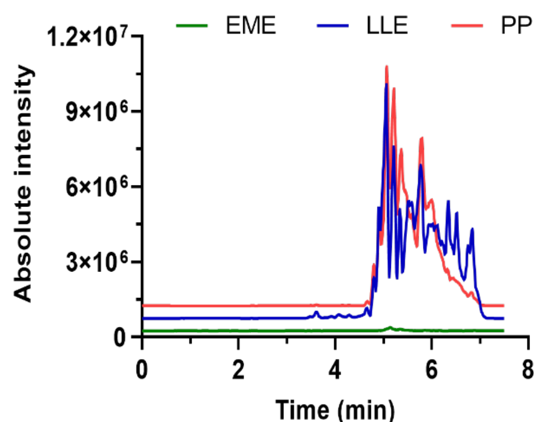


Fig. 1. **Phospholipid profiles. LC-MS chromatograms of detected phospholipids in extracted plasma samples by EME (green), LLE (blue) and PP (red)[2]**

The optimized EME procedures were compared with other extraction methods (e.g. LLE, protein precipitation, and salting out assisted liquid-liquid extraction). Both, good recoveries (higher than 70 %) and excellent sample clean-up (matrix effects maximally 11 %), were achieved from all matrices with EME. Furthermore, EME provided outstanding sample purification from phospholipids in comparison to other extraction techniques (Figure 1). The optimized EMEs in all matrices, followed by UHPLC-MS/MS, were successfully validated.

In conclusion, EME proved to be simple, reliable, effective, and repeatable microextraction technique. Additionally, it enables direct enrichment of the sample, provides efficient sample clean-up and shows also a great potential for high-throughput analysis of clinical samples.

REFERENCES

1. Pedersen-Bjergaard S., Rasmussen K. E.: J. Chromatogr. A *1109*, 183 (2006).
2. Bavlovič Piskáčková H., Oiestad E. L., Váňova N., Lengvarská J., Štěrbová-Kovaříková P., Pedersen-Bjergaard S.: Talanta *223*, 121748 (2021).
3. Bavlovič Piskáčková H., Kollarová-Brazdová P., Kučera R., Macháček M., Pedersen-Bjergaard S., Štěrbová-Kovaříková P.: Anal. Chim. Acta *2021*, 1177.
4. Bavlovič Piskáčková H., Nemeškalová A., Kučera R., Pedersen-Bjergaard S., Najmanová V., Štěrbová-Kovaříková P., Kuchař M., Sýkora D.: J. Pharm. Biomed. Anal. *210*, 114549 (2022).

ACCUCALC: A PYTHON-BASED GWAS TO GENES TOOL ENABLES IDENTIFICATION OF CAUSATIVE MUTATIONS IN SOYBEAN AND OTHER ORGANISMS

JANA BIOVÁ[a], MÁRIA ŠKRABIŠOVÁ[a], KRISTIN D. BILYEU[b]

[a]Department of Biochemistry, Faculty of Science, Palacky University, Olomouc, 78371, Czech Republic
[b]Plant Genetics Research Unit, United States Department of Agriculture-Agricultural Research Service, University of Missouri, Columbia, MO, 65211, USA
jana.biova@upol.cz

Soybean serves as a food source for both human and livestock around the world. This fact makes soybean one of the most economically important crops. It is not surpassing that breading of new variants with better agronomical traits is of high importance for broader community[1]. Recent progress in bioinformatics and decreasing cost of sequencing methods brings a lot of advantages but also disadvantages. Classical breeding with usage of molecular markers identifies associated loci but rarely leads to causative mutation (CM) discovery. GWAS (Genome-wide association study) reveals links between the genotype and phenotype[2]. When utilizing whole-genome-sequencing (WGS) genotype, GWAS can lead to the CM identification. However, because of the still high cost of resequencing, researchers tend to analyse rather smaller data sets that leads to potential weakening of the analysis power. To overcome the burden of resequencing, low-density DNA chips are used as a source of genotype. No matter how dense

a genotype information is, GWAS identifies a phenotype associated region with a haplotype tagging marker. Unfortunately, a haplotype tagging marker often does not equal to CM, neither is necessarily in a close vicinity of the CM. This is, however, widely used practise[2]. We developed a strategy that overcomes the obstacles caused by limited genotype quality and low number of known phenotypes. This strategy leverages information of both low-density genotyped accessions with known phenotypes and resequenced data sets with missing phenotype. The strategy consists of two novel concepts: Synthetic phenotype and Accuracy as an additional GWAS evaluation criterion. We tested the strategy on important soybean agronomical and nutritional traits and proved, that the strategy improves GWAS-driven CM identification. Finally, we developed a Python-based AccuCalc GWAS to Genes tool that enables and simplifies the whole CM identification pipeline. This work aims to demonstrate utilization of the AccuCalc for soybean and other organisms.

REFERENCES:

1. Pagano M. C., Miransari M. *The importance of soybean production worldwide*, Academic press, 2016.
2. Gondro C., van der Werf J., Hayes B.: *Genome-Wide Association Studies and Genomic Prediction*, Humana Press, 2017.
3. Yano K., Yamamoto E., Aya K., Takeuchi H., Lo P. C., Hu L., Yamasaki M., Yoshida S., Kitano H., Hirano K., Matsuoka M.: Nat. Genet. *48*, 927 (2016).
4. https://www.soybase.org/ [downloaded Jan. 31, 2022].

VARIOUS APPROACHES TO DETECTION OF ANTI-GLYCAN ANTIBODIES PRESENT IN CANCER

ANNA BLŠÁKOVÁ, FILIP KVĚTOŇ, LENKA LORENCOVÁ, JÁN TKÁČ*

Institute of Chemistry, Slovak academy of Sciences, Dúbravská cesta 9, Bratislava-Karlova Ves, 841 04 Slovak Republic
chemabls@savba.sk

Cancer is one of the world's most prevalent diseases. In 2020 there were 19.3 million new cancer cases and almost 10 million cancer deaths[1]. The earlier this pathological condition in an organism can be diagnosed, the sooner it is possible to intervene against the disease.

Glycans are an essential component of all living organisms. The presence of aberrant glycans (Tn antigen, sTn antigen, T antigen) has been demonstrated in many types of cancer (prostate, stomach, colon, lungs, esophagus…), to which the immune system responds by producing antibodies circulating in the blood [2-4].

We prepared a biosensor with graphene surface and we focused on optimizing the conditions for the preparation of a glycan biosensor sensitive to anti-Tn antibody and lectin *Dolichos Biflorus Agglutinin* (DBA). The main electrochemical method for determining glycan-protein interactions was differential pulse voltammetry (DPV). The developed biosensor detected an analyte with high selectivity and sensitivity up to the atomolar level. We monitored the electrochemical behavior on variously treated surfaces of screen-printed graphene electrodes by cyclic voltammetry (CV) [5]. Subsequently, we detected antibodies by creating a more commonly used test – the standard ELISA and modified ELISA assay - SUspension Magnetic-Bead-based Assay (SUMBA). The SUMBA method was extensively optimised for signal enhancement. With all steps optimised, we were able to detect antibodies ultrasensitively with a limit of detection of 0.45 pM. Moreover, antibodies could be detected in serum samples with a recovery index in the range of 98% - 104% (ref.[6]).

The work has been supported by APVV 17-0300 and this publication is the result of the project implementation CEMBAM – Centre for Medical Bio-Additive Manufacturing and Research, ITMS2014+: 313011V358 supported by the Operational Programme Integrated Infrastructure funded by the European Regional Development Fund.

REFERENCES

1. Sung H., Ferlay J., Siegel R. L., Laversanne M., Soerjomataram I., Jemal A., Bray F. C. A.: Cancer J. Clin. *71*, 209 (2021).
2. Ju T., Lanneau G. S., Gautam T., Wang Y., Xia B., Stowell S. R., Willard M. T., Wang W., Xia J. Y., Zuna R. E., Laszik Z., Benbrook D. M., Hanigan M. H., Cummings R. D.: Cancer Res. *68*, 1636 (2008).
3. Dai L., Tsay J. C., Li J., Yie T. A., Munger J. S., Pass H., Rom W. N., Zhang Y., Tan E. M., Zhang J. Y.: Lung Cancer *99*, 172 (2016).
4. Zaenker P., Gray E. S., Ziman M. R.: Autoimmun. Rev. *15*, 477 (2016).
5. Kveton F., Blsakova A., Lorencova L., Jerigova M., Velic D., Blixt O., Jansson B., Kasak P., Tkac J.: Sensors *19*, 5409 (2019).
6. Blšákova A., Květoň F., Lorencová L., Blixt O., Vikartovská A., Kasak P., Tkac J.: Anal. Chim. Acta *1195*, 339444 (2022).

INTERVENCIN, NEW LINCOSAMIDE ANTIBIOTIC WITH 4-METHYL-L-PROLINE MOIETY

LADA BRÁZDOVÁ[a,b], ZDENĚK KAMENÍK[a], JUAN DAVID GUZMAN[c], JIŘÍ JANATA[a], JAN MASÁK[b]

[a]Institute of Microbiology of the CAS, v. v. i., Vídeňská 1083, 142 20 Prague 4; [b]Department of Biotechnology, UCT Prague, Technická 5, 166 28 Prague 6, [c]Faculty of Agricultural Sciences, Universität Giessen, Goethestrasse 58, 353 90 Giessen
lada.brazdova@biomed.cas.cz

Microorganisms are a rich source of specialized metabolites with a range of biological activities and a wide variety of applications in medicine. Specifically, actinomycetes produce a wealth of structurally diverse natural

compounds. 4-alkyl-L-proline derivatives (APDs) represent specialized metabolites with an unusual building moiety. The APD moiety biosynthesises in a specialized metabolic pathway from L-tyrosine (L-tyrosine-derived APDs) or L-leucine (L-leucine-derived APDs). APD moiety is incorporated into structurally and functionally diverse groups of natural compounds (lincosamides[1], pyrrolobenzo-diazepines[2], hormaomycin[3], griselimycin[4], and others).

This study focuses on a small, but significant group of clinically used antibiotics, lincosamides (Fig. 1). The mechanism of action is binding of the molecule to a 50S ribosomal subunit and inhibiting protein biosynthesis[5]. Lincomycin and its semi-synthetic derivative clindamycin are used in clinical practice for the treatment of infections caused by Gram-positive staphylococci and streptococci[1]. Lincosamides contain two structural moieties, an unusual amino thio-octose unit, which binds to an amino acid unit via an amide bond. Until recently, the amino acid unit has represented L-tyrosine-derived APD or L-proline[1].

Intervencin (Fig. 1) is a new lincosamide antimicrobial, which was discovered in our laboratory. It contains APD moiety (4-methyl-L-proline) derived from L-leucine. Based on the analysis of genomic DNA, we identified homologous genes encoding the formation of 4-methyl-L-proline in the biosynthesis of griselimycin. This discovery not only extends the group of lincosamides as such, but it also extends their structural and biosynthetic diversity.

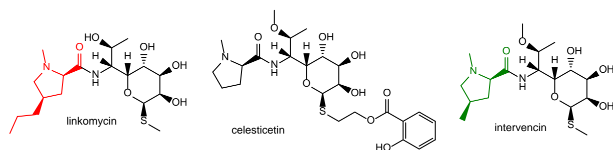


Fig. 1. **Lincosamides with colour-highlighted APD moieties. APD moiety – 4-prolyl-L-proline in red. APD moiety – 4-methyl-L-proline in green**

The work was supported by the Lumina Quaeruntur Program No. LQ200202002 from the Czech Academy of Sciences, by Project No. 20-09811Y from the Czech Science Foundation.

REFERENCES

1. Spizek J., Rezanka T.: Biochem. Pharmacol., *133*, 20 (2017).
2. Gerratana B.: Med. Res. Rev. *254*, 32 (2012).
3. Omura S., Mamada H., Wang N. J., Imamura N., Oiwa R., Iwai Y., Muto N.: J. Antibiot. *700*, 37 (1984).
4. Holzgrabe U.: Chem. Biol. *22*, 8 (2015).
5. Schlünzen F., Zarivach R., Harms J., Bashan A., Tocilj A., Albrecht R., Yonath A., Franceschi F.: Nature *413*, 841 (2001).

CONSTRUCTING A SYNTHETIC *PSEUDOMONAS PUTIDA* CONSORTIUM FOR UTILISATION OF WASTE PLANT BIOMASS OLIGOSACCHARIDES

BARBORA BURÝŠKOVÁ, BARBORA BŘENKOVÁ, PAVEL DVOŘÁK

Microbial Bioengineering Laboratory, Department of Experimental Biology (Section Microbiology), Faculty of Science, Masaryk University, 62500 Brno – Bohunice
bburyskova@sci.muni.cz

Lignocellulosic biomass is gaining increased attention as a cheap and renewable carbon source for sustainable biotechnologies. The building blocks of waste plant biomass can be fed to industrial strains of bacteria or yeast to produce valuable compounds in a carbon-neutral fashion.

However, pretreatment of plant residues is expensive, and its efficiency is limited. It generates a mix of monosaccharides (mostly glucose, xylose, and arabinose) and oligosaccharides, together with aromatic compounds derived from lignin that are often inhibitory for downstream fermentation processes. Employing genetic engineering, the depolymerisation of lignocellulose could be partially assigned to microorganisms[1]. *Pseudomonas putida* is a robust soil-dweller with native pathways for the degradation of aromatic compounds and it can be engineered to utilise non-native sugar substrates[2].

In this study, we prepared a synthetic *Pseudomonas putida* consortium metabolically engineered to consume two disaccharides – xylobiose and cellobiose. We tested the expression of genes encoding for β-xylosidase Xyl43A and β-glucosidase BglC (both from the actinobacterium *Thermobifida fusca*) in *P. putida* and confirmed their activity in degrading xylobiose and cellobiose to xylose and glucose, respectively. Moreover, our data indirectly revealed the presence of native oligosaccharide transporters in *P. putida* KT2440. Based on these findings, a consortium with a synergistic relationship was constructed. The consortium consists of two *P. putida* strains: a glucose consuming strain producing β-xylosidase and a xylose consuming strain producing β-glucosidase. This setup establishes a mutual dependence of the two consortium strains via carbohydrate cross-feeding (Figure 1).

Our study showcases a bioengineering approach inspired by natural microbial communities, applied here for waste plant biomass repurposing. The oligosaccharide catabolism could enable more complete utilisation of lignocellulose-derived carbohydrates, and also help bypass carbon catabolite repression – a big challenge for microbial biotechnology.

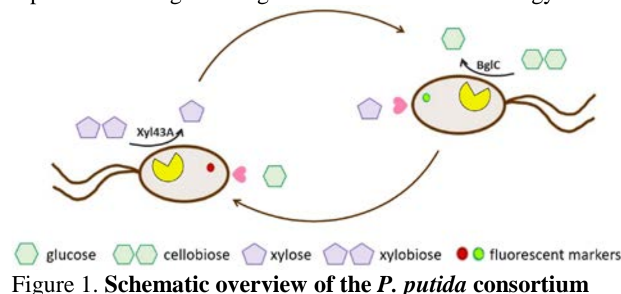


Figure 1. **Schematic overview of the *P. putida* consortium**

This work was supported by FEBS Summer Fellowship, Brno Ph.D. talent, MUNI/A/1425/2020, and the Czech Science Foundation grant GA22-12505S.

REFERENCES

1. de Paula R. G., Antoniêto A. C. C., Ribeiro L. F. C., Srivastava N., O'Donovan A., Mishra P. K., Gupta V. K., Silva R. N.: Biotech. Adv. *37*, 107347 (2019).
2. Dvořák P., de Lorenzo V.: Met. Eng. *48*, 94 (2018).

TRANSPARENT, COST-EFFECTIVE HYDROGEL DRESSING FOR THE TREATMENT OF SKIN WOUNDS: FABRICATION AND CHARACTERIZATION

EVA ČERNÁ[a], VILÉM NEDĚLA[b], EVA TIHLAŘÍKOVÁ[b], JANA DORAZILOVÁ[a], PETR POLÁČEK[a], LUCY VOJTOVÁ[a]

[a]CEITEC, University of Technology, Advanced Biomaterials Group, Purkyňova 656/123, Brno; [b]Institute of Scientifc Instruments CAS, Environmental Electron Microscopy Group, Královopolská 147, 612 00 Brno
Eva.Cerna1@ceitec.vutbr.cz

Effective and affordable wound treatment is a worldwide challenge due to the cost and problems associated with wound healing, including infections, prolonged recovery, or chronic wound creation. The development of new transparent materials to monitor the wound site can reduce the occurrence of infection by monitoring the wound. Wound management demands multifunctional materials with a pro-healing effect for healing acceleration and support scarless healing[1]. Natural polysaccharide gum Karaya (GK) is a gel-forming polymer with high swelling and water retention used for its abundance, easy processing, and, more importantly, for its naturally occurring antimicrobial activity[2]. The addition of synthetic biopolymer poly(vinyl alcohol) (PVA) increases the mechanical strength of the final hydrogel dressing by creating a stable porous, 3D network, and its combination with glycerol and GK form a transparent hydrogel dressing suitable for wound treatment[3].

The main aim of this work is the development, fabrication, and characterization of a multifunctional hybrid hydrogel with desirable wound healing properties. Physicochemical characterization shows trends in GK/PVA ratio, which strongly determine hydrogel swelling and increase of GK (250 – 600% from the original weight), on the other hand, an increase of PVA influence porosity and mechanical strength (148% elongation), which is empowered by glycerol presence (plasticizer). Additionally, a higher concentration of glycerol in the hydrogel matrix increases hydrogel transparency and reduces pores size, creating micro/nanopores in the hydrogel. Concerning the results, hydrogels based on gum Karaya show desirable properties as a dressing for long-term wound healing of acute and chronic wounds for wound closure. They can also serve as a matrix for drug enrichment to amplify the healing process.

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

REFERENCES

1. Lipový B., Holoubek J., Vacek L., Růžička F., Nedomová E., Poštulková H., Vojtová L.: Cesko-Slov. Epidemiol. Mikrobiol. Imunol. *67*, 166 (2018).
2. Singh B., Pal L.: Eur. Polym. J. *44*, 3222 (2008).
3. Francesko A., Petkova P., Tzanov T.: Curr. Med. Chem. *25*, 5782 (2019).

DESIGN AND SYNTHESIS OD EASILY TUNABLE OPTICALLY PURE SPIROPYRAN PHOTOSWITCHES

TEREZA ČERNÁ, JAKUB DÁVID MALINA, JIŘÍ TŮMA, MICHAL KOHOUT

Department of Organic Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague
cernag@vscht.cz

Smart organic materials capable of quick and fully reversible response to the external stimuli have become a very powerful tool of modern organic chemistry. Amongst such compounds, chiral photoswitches have gained very high attention due to their numerous envisioned applications, *e.g.* in data storage[1].

Spiropyran-based molecular photoswitches are of particular interest because of the nature of their photoswitching, which involves both the change in molecular shape as well as the overall polarity of the compound. However, synthesis of optically pure spiropyrans is a very challenging task as their switching also causes their spontaneous racemization[2].

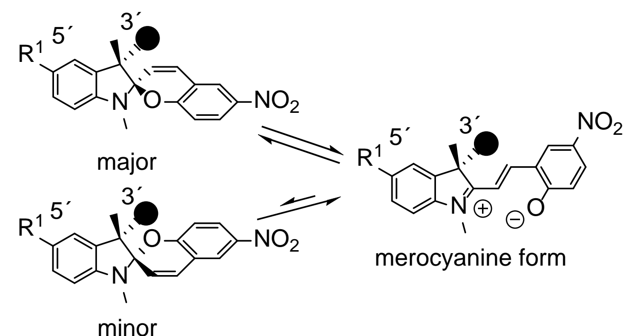


Fig. 1. **Thermodynamical equilibrium of targeted spiropyrans**

In the presented study, we propose a systematic method for synthesis of optically pure spiropyrans by introducing a second element of chirality, causing energy differentiation

between the two formed diastereomers, giving rise to the partial or full dominance of one diastereomer over the other (Fig. 1).

We prepared a series of spiropyrans bearing various bulky aromatic units in the position 3´ and studied their diastereomeric ratio by NMR spectroscopy. Furthermore, we prepared a model compound with propargyloxy group in the position 5´, to increase the diastereomeric ratio. The terminal alkynyl group also allows us to incorporate the spiropyran moiety into any type of desired functional material via *click*-chemistry. As a proof of concept, preliminary tests using model organic azides have been performed.

This work was supported by CSF (reg. No. 21-31139J).

REFERENCES

1. Sharifian M.: Langmuir *33*, 8023 (2017).
2. Feringa B. L.: *Molecular Switches.* Wiley Somerset, 2012.

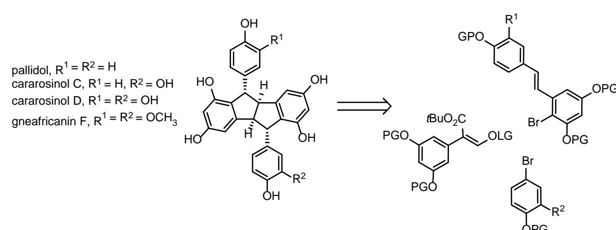
TOTAL SYNTHESIS OF STILBENE DIMERS

<u>VÁCLAV CHMELA</u>, ULLRICH JAHN

Institute of Organic Chemistry and Biochemistry, CAS, Flemingovo nám. 542/2, 160 00 Prague 6
vaclav.chmela@uochb.cas.cz

Stilbene dimers with indane skeleton are a growing class of polyphenolic natural products. Although the members of this class of polyketides exhibit a wide range of biological activities such as selective quenching of oxygen radicals[1], inhibition of human colon tumorigenic cells[2], inhibition of pancreatic α-amylase[3], antioxidant activity[4], and activity against HIV-1[5], the studies of biological activities are limited by their scarce amounts available by extraction from Nature. Therefore, practical methods that allow rapid access to larger quantities of these natural products and their analogues are demanded.

We report a novel approach to stilbene dimers with indane skeleton exploiting two consecutive 1,4-additions and subsequent oxidation reactions in one pot to furnish the tetracyclic core of indane stilbene dimers.



Scheme 1. **Retrosynthetic analysis of naturally occurring stilbene dimers**

We thank the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences and the Grant Agency of Czech Republic (reg. No. 19-22085S) for funding.

REFERENCES

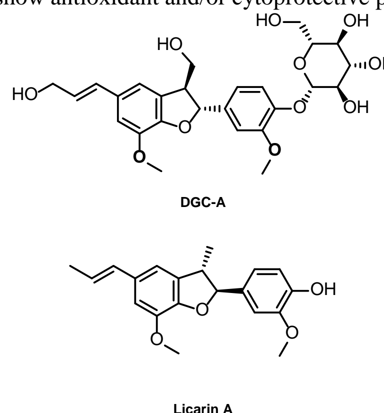
1. Jiang L., He S., Sun C., Pan Y.: Phytochemistry *77*, 294 (2012).
2. González-Sarrías A., Gromek S., Niesen D., Seeram N. P., Henry G. E.: J. Agric. Food Chem. *59*, 8632 (2011).
3. Mattio L. M., Marengo M., Parravicini C., Eberini I., Dallavale S., Bonomi F., Iametti S., Pinto A.: Molecules *24*, 3225 (2019).
4. Iliya I., Tanaka T., Iinuma M., Ali Z., Furasawa M., Nakaya K., Matsuura N., Ubukata M.: Heterocycles *57*, 1507 (2002).
5. Yang G., Zhou J., Li A., Hu C.: Planta Med. *71*, 569 (2005).

DUAL ORGANOCATALYSIS-BASED APPROACH TO 2,3-DIHYDROBENZOFURAN SKELETON

<u>DANIEL CHRENKO</u>[a], JIŘÍ POSPÍŠIL*[,a,b,c]

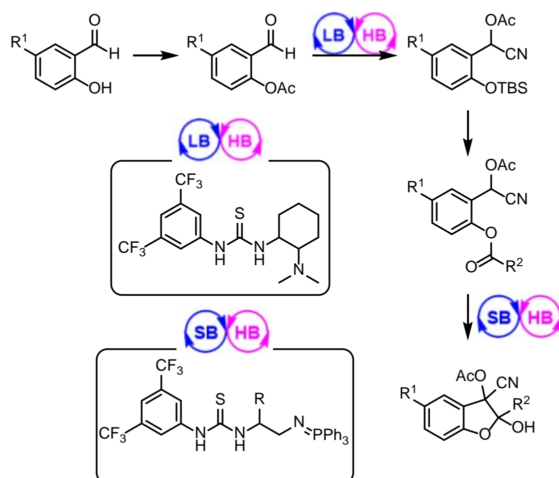
[a]Department of Chemical Biology and [b]Laboratory of Growth Regulators, Palacky University & Institute of Experimental Botany AS CR, Šlechtitelů 27, CZ-783 71 Olomouc; [c]Department of Organic Chemistry, Faculty of Science, Palacky University, tř. 17. listopadu 1192/12, Olomouc CZ-771 46, Czech Republic
daniel.chrenko01@upol.cz

Neolignans are secondary metabolites of plants that originate from the shikimic acid biosynthetic pathway[1]. From the structural point of view, neolignans are dimers of phenylpropanoids that originate from the metabolism of L-phenylalanine. Phenylpropanoids further undergo homodimerization, and the dimers then undergo countless (non)enzymatic transformations such as acid-catalyzed cyclization, methylation and oxidation[2,3]. Targeted 2,3-dihydrobenzofurans (DHB) are then the prominent member of the neolignan family[4]. DHB is present as a core structure in wide range of biologically active natural such as DGC-A and Licarin A (**Scheme 1**) as well as synthetic compounds possessing antibacterial[5] and antifungal[6] to anticancer[7], antitubercular[8] and antimalarial activity[9]. Other DHB natural products show antioxidant and/or cytoprotective properties[10].



Scheme 1. **Structures of neolignans DGC-A and Licarin A**

In our contribution we wish to report several novel approaches to DHB and DHB-related (un)natural products that are based on both radical (homo)coupling reactions and organocatalytic reactions (**Scheme 2**). In addition, the biological evaluation of the prepared compounds against model systems of human, plant, and animal parasites (antileishmanial and anthelmintic activity) will also be present.



Scheme 2. **Proposed approach to benzofurane core**

This work was supported by the European Regional Development Fund-Project "Centre for Experimental Plant Biology" (no. CZ.02.1.01/0.0/0.0/16_019/0000738).

REFERENCES

1. Vogt T.: Mol. Plant. *3*, 2 (2010).
2. Zálešák F., Bon D. J. Y. D., Pospíšil. J.: Pharm. Res. *146*, 104284 (2019).
3. Radadiya A., Shah A.: Eur. J. Med. Chem. *97*, 356 (2015).
4. Miao Y. H., Hu Y. H., Yang J., Liu T., Sun J., Wang X. J.: RSC Adv. *9*, 27510 (2019).
5. Park J. Y., Hwan Lim S., Ram Kim B., Jae Jeong H., Kwon H. J., Song G. Y., Bae Ryu Y., Song Lee W. S.: Bioorg. Med. Chem. Lett. *27*, 3060 (2017).
6. de Lima Moreira D., de Paiva R. A., Marques A. M., Borges R. M., Barreto A. L. S., da Rocha Curvelo J. A., Cavalcanti J. F., Romanos M. T. V., de Araújo Soares R. M., Kaplan M. A. C.: Rec. Nat. Prod. *10*, 472 (2016).
7. Wang Y., Li J., Ma H. R., Numonov S. R., Aisa H. A.: Chem. Nat. Compd. *52*, 396 (2016).
8. Huang H. Y., Ishikawa T., Peng C. F., Tsai I. L., Chen I. S.: J. Nat. Prod. *71*, 1146 (2008).
9. Zhang H. J., Li W. F., Fong H. H. S., Soejarto D. D.: Molecules *21*, 1448 (2016).
10. Chin Y. W., Chai H. B., Keller W. J., Kinghorn A. D.: J. Agric. Food Chem. *56*, 7759 (2008).

ELEMENTS' BIOACCESSIBILITY AND OXIDATIVE POTENTIAL OF URBAN AEROSOL IN THREE SIMULATED LUNG FLUIDS

HANA CIGÁNKOVÁ*, PAVEL MIKUŠKA

Institute of Analytical Chemistry of the Czech Academy of Sciences, Veveří 967/97, 602 00 Brno
cigankova@iach.cz

Air pollution is a significant public health problem. Increased concentration of atmospheric particulate matter (PM) that enter human bodies through inhalation exposure can impact health[1]. The ability of aerosol to produce reactive oxygen species (ROS) is one of the widely proposed mechanisms related to the negative effects of aerosol on human health. Oxidative stress, caused by the production of ROS, is associated with several cardiovascular and respiratory diseases. The capacity of aerosol to produce ROS is also called oxidative potential (OP). Although ROS generation capacity of PM exhibits a good correlation with PM mass concentration, it may depend more on physico-chemical properties of PM[2,3]. Elements bound to PM play a significant role in contributing to the OP of aerosol. Exposure to elements was linked with several health issues (e.g. respiratory and cardiovascular diseases, asthma, inflammatory effects, lung cancer)[4,5].

The aim of this work was to determine the bioaccessibility of 21 elements bound to PM1 and PM2.5 urban aerosol and OP of both studied aerosol size fractions in three simulated lung fluids: deionised water, simulated alveoli fluid (SAF) and Gamble's solution. It was found that the bioaccessibility of elements depends on their chemical compound, aerosol size fraction and the chemical composition of the SLF.

The OP of urban PM was studied as a relevant metric for health effects using the dithiothreitol assay. The difference of OP value in deionised water to SAF and Gamble's solution indicated the crucial effect of individual SLFs' chemical composition on the OP. The complexation of the elements with different ligands present in the solution can influence OP and, therefore, the potential health effects of inhaled aerosol.

This work was supported by project of the Ministry of Education, Youth and Sport (FCH-S-21-7398), and by the Grant Agency of the Czech Republic (503/20/02203S).

REFERENCES

1. Gao P., Guo H., Zhang Z., Ou C., Hang J., Fan Q., He C., Wu B., Feng Y., Xing B.: Environ. Pollut. *242*, 1669 (2018).
2. Patel A., Rastogi N.: Atmospheric Environ. *175*, 127 (2018).
3. Wong J. P. S., Tsagkaraki M., Tsiodra I., Mihalopoulos N., Violaki K., Kanakidou M., Sciare J., Nenes A., Weber R. J.: Environ. Sci. Technol. *53*, 12 (2019).
4. Huang H., Jiang Y., Xu X., Cao X.: Sci. Total Environ. *610-611*, 546 (2018).
5. Liu X., Ouyang W., Shu Y., Tian Y., Feng Y., Zhang T., Chen W.: Environ. Pollut. *254*, 113113 (2019).

WHAT MINING HEAPS HIDE

<u>MICHAELA CIMERMANOVÁ</u>, MÁRIA PIKNOVÁ, PETER PRISTAŠ

Institute of Biology and Ecology, P. J. Safarik University, Kosice, Slovakia
michaela.cimermanova@student.upjs.sk

While being a threat to the environment and human health, mining heaps characterised by high levels of heavy metal contamination are also an extreme habitat where new bacterial species with unique properties can be found[1]. For nearly last 100 years, bacteria of the genus *Streptomyces* have been widely recognized as a valuable source of antibiotics and other bioactive compounds[2]. Due to their metabolic versatility as key matter cycle decomposers, low pathogenicity and endurance to unfavorable environmental conditions, *Streptomyces* are also considered a promising biotechnological tool for bioremediaton[3]. Demand for new bacterial species producing active metabolites for medicine and industry applications is still increasing nowadays[4].

In our work we analysed mining heaps as a potential source of unique *Streptomyces* species. All heaps included in the study were characterised by high content of lead and zinc. Several *Streptomyces*-like isolates were identified, their resistance to heavy metals was examined followed by a preliminary evaluation of their biosynthetic potential.

Phylogenetic analysis of 28 isolates revealed that mining heaps are dominated by streptomycete isolates related to *S. pratensis, S. microflavus,* and *S. globisporus* species. Based on the data obtained, 3 isolates might represent new streptomycete species. Most of isolates showed increased metal resistance and capacity of bioaccumulation, what are interesting properties in terms of bioremediation applications. However, further experiments must be performed to confirm absorption and accumulation abilities of these strains. Most isolates tested produced industrially interesting enzymes. To conclude, our results confirm that despite being harsh, extreme environments are an unexplored source of novel microorganisms with a huge potential for biotechnology.

This work was supported by VEGA Grant Agency (grant number 1/0779/21), APVV Grant Agency (grant number DS-FR-19-0008) and VVGS-pf-2021-1766 grant.

REFERENCES

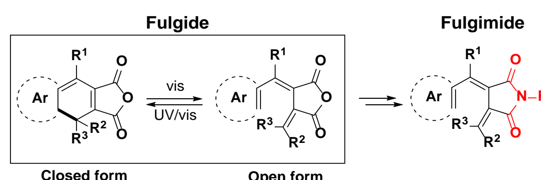
1. Sayed A.M., Hassan M.H.A., Alhadrami H.A., Hassan H.M., Goodfellow M., Rateb M.E.: J. Appl. Microbiol. *128*, 630 (2019).
2. Kämpfer P., Glaeser S.P., Parkes L., van Keulen G., Dyson P.: *The family Streptomycetaceae* v knize: *The Prokaryotes: Actinobacteria* (Rosenberg E., DeLong E. F., Lory S., Stackebrandt E., Thompson F. eds). str. 889–1010, Springer Berlin Heidelberg 2014.
3. El Baz S., Baz M., Barakate M., Hassani L., El Gharmali A., Imziln B.: Sci. World J. *2015*, 761834.
4. Law J.W.-F., Chan K.-G., He Y.-W., Khan T.M., Ab Mutalib N.-S., Goh B.-H., Lee L.-H.: Sci. Rep. *9*, 15262 (2019).

FULGIMIDES AS BUILDING BLOCKS FOR PHOTOSWITCHABLE IONIC LIQUIDS

<u>JAKUB COPKO</u>, TOMÁŠ SLANINA*

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nám. 2, 16610, Prague 6, Czech Republic
jakub.copko@uochb.cas.cz

Photochromic compounds are slowly emerging as building blocks for smart materials. Their reversible transformation has been more and more exploited in the devices that we use in our everyday lives, e.g., for molecular electronics or optical data storage[1,2]. Out of many photochromic motives, the family of **fulgide**s is particularly interesting as its structure can be easily exploited. Transformation of anhydride moiety to an imide gives a **fulgimide** (Scheme 1)[3]. This easy conversion opens endless possibilities as almost any functional group can be introduced in the very last step of the synthesis.



Scheme 1. **Cyclization of fulgides (left), structure of fulgimide (right)**

We aim to exploit this inherent reactivity of fulgides as we want to introduce a chargeable moiety into the structure. A careful combination of a proper linker, chargeable group and counter anion should influence the melting point and give rise to an ionic liquid. The initial studies focus on understanding the influence of various chargeable groups on photo-switching properties of indolyl-fulgimides. The most promising compounds will be further studied, revealing their possible usage as a reversible photo-switchable ionic compound with an application as polyfunctional molecular materials.

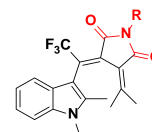


Fig. 1. **Indolyl-fulgimide**

This research was supported by the program INTER-EXCELLENCE, subprogram INTER-ACTION of the Ministry of Education, Youth and Sports CR, grant No. LTAIN19166.

REFERENCES

1. Zhang J., Zou Q., Tian H.: Adv. Mater. 25, 378 (2013).
2. Seibold M., Handsduch M., Port H., Wolf H. C.: J. Lumin. 72-74, 454 (1997).
3. Hart R. J., Heller H. G., Salisbury K.: Chem. Comm. 1968, 1927.

4-QUINOLONE-BASED DC-SIGN INHIBITORS

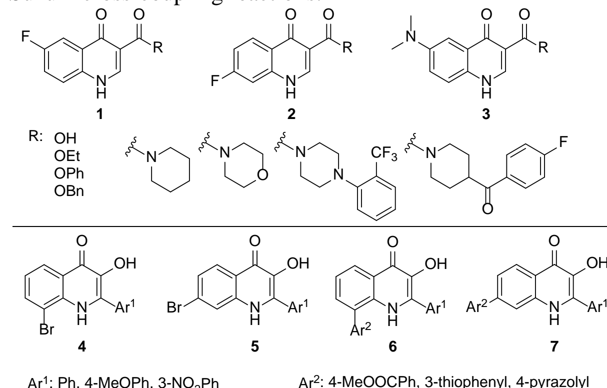
ONDŘEJ DANĚK, PETRA MÉNOVÁ*

Department of Organic Chemistry, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague 6
ondrej.danek@vscht.cz, menovap@vscht.cz

DC-SIGN receptor is present on the cell membrane of macrophages and dendritic cells and has a high affinity for mannose and fucose-rich *N*-glycans typical for pathogens. Interaction of DC-SIGN with HIV was identified as a critical step in trans-infection of T-cells and thus DC-SIGN was proposed to be a viable medicinal chemistry target.

So far, no drug-like inhibitor of DC-SIGN has been developed, mainly due to the shallow and hydrophilic nature of the carbohydrate binding site of DC-SIGN. However, in 2017, Aretz et al. identified multiple secondary binding sites in a fragment-based drug discovery (FBDD) screening, opening the option of allosteric inhibition[1].

Two small libraries of substituted derivatives of 4-quinolone-3-carboxylic acid (**1**, **2**, **3**) and 2-phenyl-3-hydroxy-4-quinolone (**6**, **7**) were synthesized; **1**–**3** *via* a modified Gould–Jacobs reaction and coupling reactions and **6**–**7** by carboxylate alkylation, acid catalyzed cyclization and Suzuki cross-coupling reactions.



Scheme 1. **Structures of the synthesized substituted 4-quinolones**

Structure–activity relationship study was done using chemical shift perturbation NMR and saturation transfer difference reporter displacement assay to characterize the binding affinities of the synthesized quinolones.

Acknowledgements: Ing. Petra Ménová, Ph.D. for supervision, Hengxi Zhang for determining the binding affinities.

REFERENCE

1. Aretz J., Baukmann H., Shanina E., Hanske J., Wawrzinek R., Zapol'skii V. A., Seeberger P. H., Kaufmann D. E., Rademacher C.: Angew. Chem. Int. Ed. *56* (25), 7292 (2017).

URINE-DERIVED STEM CELLS - AN EASILY HARVESTED CELLS WITH IMMENSE IMMUNOMODULATORY POTENTIAL

MICHAELA DEBREOVA[a], MARTINA CULENOVA[a,b], ANDREAS NICODEMOU[a,b], ZUZANA VARCHULOVA NOVAKOVA[a,b], LUBOS DANISOVIC[a,b]

[a]*National Institute of Rheumatic Diseases, Nabrezie I. Krasku 4, 921 12 Piestany, Slovakia;* [b]*Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia*
mdebreova@gmail.com

In tissue engineering, mesenchymal stem cells (MSCs) are frequently used due to their unique biological behavior. However, the methods of MSCs harvesting are often invasive. Urine-derived stem cells (UDSCs) show similar activity as MSCs and can be easily obtained via non-invasive spontaneous voiding. They represent an attractive candidates for the cell therapy, thus we studied their biology. Morphological analyzes revealed that two different cell types can be distinguished. The first one with a rice-grain shape and the second one exhibited a polyhedral shape (Fig. 1).

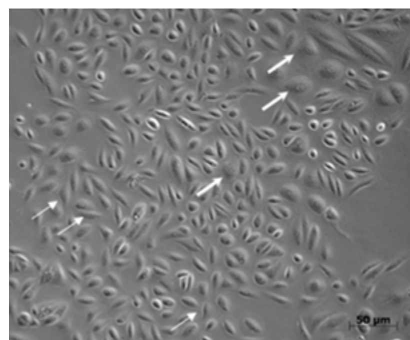


Fig. 1. **UDSCs morphology: rice-grain shaped (thin arrows) and polyhedral-shaped (thick arrows) cells**

Likewise, the ultra-structural analysis applying TEM confirmed the presence of two different cell populations. The first type had the typical ultrastructure of stem cells – nucleus rich in euchromatin with multiple indentations and numerous cytoplasm organelles. These could be also detected in other types of the stem cells[1]. The second cell type of UDSCs showed only few cytoplasmic organelles together with euchromatic spheroid shaped nucleus without indentations. This morphology is typical for basal urothelial cells derived from the urinary bladder[2]. UDSCs showed high expression of typical MSC-like surface markers, CD73, CD90 and CD105. We have also successfully induced UDSCs into several cell lineages. Moreover, the UDSCs secretome analysis revealed a significantly higher secretion of IL-8, IL-6 and MCP-1 and GM-CSF confirming their immunomodulatory activity. Our results demonstrate that UDSCs may possess immense potential in the regeneration of damaged structures.

The study was supported by the CEMBAM—Centre for Medical Bio-Additive Manufacturing and Research, ITMS2014+: 313011V358 supported by the Operational Programme Integrated Infrastructure funded by the European Regional Development Fund.

REFERENCES
1. Miko M., Danišovič L., Majidi A., Varga I.: Eur. J. Histochem *59*, 2528 (2015).
2. Polák Š., Žiaran S., Mištinová, J., Bevízová K., Danišovič L., Varga I.: Biologia *67*, 1018 (2012).

A COUPLING OF MICROCHIP ISOTACHOPHORESIS WITH ION MOBILITY SPECTROMETRY FOR FOOD, CLINICAL AND PHARMACEUTICAL ANALYSIS

MARTA ĎURIŠ, JASNA HRADSKI, ROMAN SZUCS, MARIÁN MASÁR

Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 84215 Bratislava, Slovakia
duris48@uniba.sk

In the context of green analytical chemistry, miniaturization, automation, reduction in the use of toxic solvents, and multi-component analysis, play a key role in the development of modern analytical techniques[1]. Miniaturized separation techniques can reduce the financial cost of analysis, speed up the analytical process, reduce the amount of the analyzed sample as well as the amount of waste produced. Microchip electrophoresis is used to analyze a wide range of compounds, e.g., organic and inorganic ions, amino acids, drugs and proteins[2]. In this work, microchip isotachophoresis (µITP) was used as a suitable miniaturized electrophoretic technique for coupling with ion mobility spectrometry (IMS) for the analysis of complex samples. IMS is a sensitive and fast separation and identification technique, which was applied to the analysis of food, environmental and biological samples[3].

µITP-IMS combination integrates preconcentration power of the µITP followed by the unambiguous identification of the analytes by IMS. Short-chain carboxylic acids, used as model analytes, were successfully separated and identified by the µITP-IMS method. The practical applicability of the developed approach was demonstrated by the analysis of food (apple vinegar, wine, fish sauce), pharmaceutical (ear drops) and biological (saliva) samples, in which the studied acids are naturally present. Acetic acid was expected in the studied food samples, as a result of fermentation, and in ear drops, where it was present as an active ingredient. Presence of acetic acid was confirmed in all studied samples and its concentration levels were determined by the standard addition method. In addition, propionic acid was identified and quantified in saliva sample. The rest of the carboxylic acids were not present in the studied samples above the limits of detection, however, their reduced ion mobilities in the complex samples were confirmed by the standard addition method. The developed approach can be beneficial for the separation and unambiguous identification of various analytically important analytes present in complex samples of food, pharmaceutical and biological origin.

This research was funded by the Slovak Research and Development Agency (APVV-17-0318).

REFERENCES
1. Gałuszka A., Migaszewski Z., Namieśnik J.: TrAC Trends Anal. Chem. *50*, 78 (2013).
2. Nuchtavorn N., Suntornsuk W., Lunte S. M., Suntornsuk L.: J. Pharm. Biomed. Anal. *113*, 72 (2015).
3. Chouinard C. D., Wei M. S., Beekman C. R., Kemperman R. H. J., Yost R. A.: Clin. Chem. *62*, 124 (2016).

MONITORING THE EXPRESSION OF GENES OF THE BIOSYNTHETIC PATHWAY OF IBOTENIC ACID FROM *Amanita muscaria*

KLEVINDA FILI[a], TEREZA LEONHARDT[b]

[a]Institute of Physiology CAS, 142 20, Vídeňská 1083, 142 00 Praha 4; [b]University of Chemistry and Technology UCT, Technická 5, Dejvice, 166 28 Praha 6.
klevinda.fili@fgu.cas.cz

Amanita muscaria despite being acknowledged as poisonous, has been shown to have broad pharmacological uses. Two of its most important compounds, ibotenic acid and muscimol, have been known for their hallucinogenic and sedative effects[1,2]. Considering these effects, the presence of these compounds in psychotherapy can have added value in the future. The synthetic pathway of ibotenic acid has been described only recently by The Institute of Pharmaceutical Sciences and the University of Freiburg[3]. However, this finding was not directly confirmed in *A. muscaria*; rather a synthetic construct of *IboH*, codon-optimized for expression in *E. coli*, was used to prove the enzyme’s ability to hydroxylate glutamate.

In this study, we have successfully designed primers that were used to isolate a large part of the *IboH* gene which is coding for the enzyme used in the first step of the biosynthesis of ibotenic acid, hydroxylation of glutamate. The *IboH* gene sequence was successfully isolated with minor differences between the original sequence from *A. muscaria* genome and the sequence isolated form *A. muscaria* mycelial strain. Moreover, we have designated two different media in order to increase the yield of the ibotenic acid that can be considered for further research in the future. The evaluation of the results was done based on the appearance of the growing mycelia.

REFERENCES
1. Benkherouf, A. Y., Taina R., Meera P., Aalto J., Li X., Soini S., Wallner M., Uusi-Oukari M.: J. Neurochem. *149*, 41 (2019).

2 Zinkand W., Moore C., Thompson C., Salama A., Patel J.: Mol. Chem. Neuropathol. *16*, 1 (1992).
3. Obermaier, S. and Müller, M.: Angew. Chem., Int. Ed. *59*, 12432 (2020).

IFITM PROTEINS REGULATE SENSITIVITY OF CERVICAL CANCER CELLS TO CISPLATIN

NELA FRIEDLOVÁ[a,b]*, MICHAELA VAŠINOVÁ GALIOVÁ[c], KRISTÝNA BILAVČÍKOVÁ[c], PAVEL COUFALÍK[d], LENKA HERNYCHOVÁ[a], VLASTIMIL TICHÝ[a], TED HUPP[a,e], BOŘIVOJ VOJTĚŠEK[a], MARTA NEKULOVÁ[a]

[a]Research Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, 656 53 Brno; [b]Department of Experimental Biology, Faculty of Science, Masaryk University, 625 00 Brno; [c]Institute of Chemistry and Technology of Environmental Protection, Faculty of Chemistry, 612 00 Brno; [d]Institute of Analytical Chemistry CAS, 602 00 Brno; [e]University of Edinburgh, Institute of Genetics and Molecular Medicine, Crewe Road South, EH4 2XR Edinburgh, UK
nela.friedlova@mou.cz

Tumor therapy resistance is a frequent limiting factor to achieve cure of cancer patients. Therefore, an effort to elucidate mechanisms of this phenomenon is essential to make cancer treatment more effective. Upregulation of interferon-related DNA damage resistance signature (IRDS) genes was described in different radio- and chemotherapy resistant tumors. IRDS genes ensure prolonged viral and DNA damage resistance bringing tumors a selective advantage[1]. Interferon-induced transmembrane proteins (IFITMs), as a part of IRDS, provide cellular defense against broad range of viruses, while contributing to tumor progression[2].

During our investigation focused on IFITM protein functions in tumors we described a linkage between these proteins and cellular response to chemotherapeutic drugs. Using cervical cancer cells, we proved enhanced cisplatin sensitivity of single IFITM1 or IFITM3 knock-out (KO) derivatives. Interestingly, double IFITM1/3 KO clones exert exclusive resistance to cisplatin. Therefore, we further investigate causes of the observed phenomenon. Using inductively coupled plasma mass spectrometry (ICP-MS) technique, we analyzed the cisplatin uptake and efflux rates revealing limited cisplatin influx by IFITM1/3 KO cells. Based on the differentially expressed proteins in IFITM1/3 KO cells after cisplatin treatment identified by pulse SILAC approach we focused our research also on the process of autophagy and vesicle trafficking. We continue to investigate the exact mechanism of IFITM proteins involvement in the chemoresistance of cancer cells.

This work was supported by the project OP VVV ENOCH CZ.02.1.01/0.0/0.0/16_019/0000868, the program NVV MOÚ PPV 1/2021 and the project of Ministry of Education, Youth and Sports of the Czech Republic FCH-S-21-7398.

REFERENCES

1. Padariya M., Sznarkowska A., Kote S., Gómez-Herranz M., Mikac S., Pilch M., Alfaro J., Fahraeus R., Hupp T., Kalathiya U.: Biomolecules *11*, 622 (2021).
2. Liang R., Li X., Zhu X.: Mol. Diagn. Ther. *24*, 433 (2020).

SYNTHESIS OF (UN)NATURAL GIBBERELLINS

MARKÉTA FUKSOVÁ, JIŘÍ POSPÍŠIL*

Department of Chemical Biology, and Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Šlechtitelů 27, 78371 Olomouc
marketa.fuksova@upol.cz

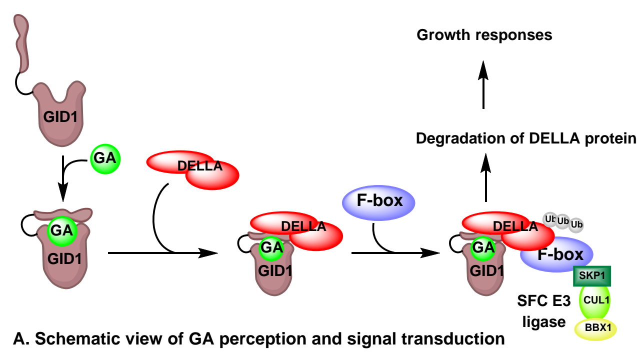
Gibberellins (GAs) are one of the most important plant hormones responsible for the regulation of many growths and developmental processes in plants. Among other things, GAs promote seed germination, flower transition, and flower and fruit development[1]. The biological activity of GAs (control of plant growth) can be controlled in two ways, through the addition of exogenic GAs (an increase in endogenic levels) or by applying growth retarders (a decrease in endogenic GAs). Not surprisingly, the use of GAs to control and induce flower and fruit development in modern horticulture becomes a common practice[2]. Unfortunately, their extensive use in modern agriculture is hampered due to the cost of the application. In addition, several GA derivatives were recently identified as promising lead compounds with antitumor activity[3].

In our group, we are interested in the synthesis of novel GA derivatives that structurally originate from commercially available gibberellin A_3, A4 and A_7. Our work has two main objectives: (A) develop new derivatives of GA capable of controlling the development of flowers and fruits in tomatoes and (B) to develop a new generation of “anti-gibberellins”, efficient competitive antagonists of bioactive GA.

In the first objective, the tomato *stamenless* mutant (a male sterile) is used as a model plant, and the goal is to find an unnatural GA derivative that would be able to revert its male sterility. At the same time, it should promote the development of parthenocarpic fruits. Thus, control of fertility restoration and fruit production should be achieved.

In the second goal, we should generate a GA derivative molecule that will have a stronger affinity for the GA-receptor (GID1) in plants than natural GAs. Our derivative should bind specifically to this active site and at the same time, its structural modification should disable the activation of GID1 as a host site for the DELLA protein (repressors of plant development). Using this approach, the growth and development of the plant will be efficiently stopped. On the other hand, newly developed competitive gibberellin has to be able to leave the site within the defined period to allow us to restore plant growth. Such an approach should allow us to modulate plant growth and favor the formation of fruits.

The latest achievements of both projects will be presented.



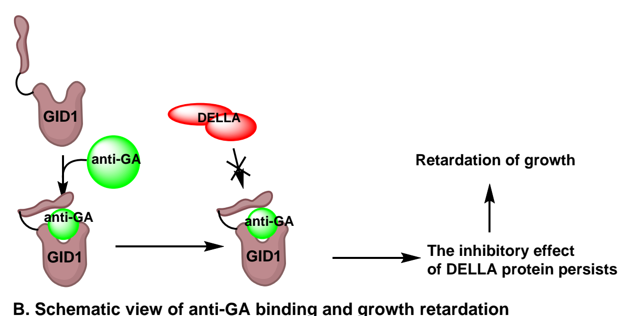


Fig. 2. **(A) Binding of GA (e.g. GA_4) results in a conformational change in the GID1 receptor that promotes interaction with DELLA proteins. Further binding of the F-box protein initiates DELLA ubiquitination by the SFC E3 ubiquitin ligase that triggers DELLA proteasomal degradation. DELLA degradation relieves growth repression and suppresses other DELLA-mediated responses. (B) Anti-GA blocks the active side of GID1 that disables its activation. No new surface for binding to the DELLA protein is created. The inhibitory effect of DELLA protein persists and growth retardation occurs**

REFERENCES
1. Brian P. W.: Biol. Rev. *34*, 37 (1959).
2. Mander L.: Nat. Prod. Rep. *20*, 50 (2003).
3. Tian H., Xu Y., Liu S., Jin D., Zhang J., Duan L., Tan W.: Molecules *22*, 694 (2017).
4. Swinson J.: PharmaChem *4*, 26 (2005).
5. Shimada A., Ueguchi-Tanaka M., Nakatsu T., Nakajima M., Naoe Y., Ohmiya H., Kato H., Matsuoka M.: Nature *456*, 520 (2008).
6. Ueguchi-Tanaka M., Ashikari M., Nakajima M., Itoh H., Katoh E., Kobayashi M., Chow T. Y., Hsing Y. I. C., Kitano H., Yamaguchi I., Matsuoka M.: Nature *437*, 693 (2005).
7. Inoue M., Sumii Y., Shibata N.: ACS Omega *5*, 10633 (2020).

NOVEL 1,3,5-TRIAZINYL AMINOBENZENESULFONAMIDES AS POTENT ANTI-VRE AGENTS, AND CARBONIC ANHYDRASES I, II, VII, IX, AND XII INHIBITORS

EVA HAVRÁNKOVÁ[a]*, JOSEF JAMPÍLEK[b], VLADIMÍR GARAJ[c], CLAUDIU T. SUPURAN[d]

[a]Dept Chem. Drugs, Faculty of Pharmacy, Masaryk University, 612 42 Brno; [b]Dept Chem. Biol., Faculty of Science, Palacky University, 783 71 Olomouc; [c]Dept Pharm. Chem., Faculty of Pharmacy, Comenius University, 832 32 Bratislava, SK; [d]Section Pharm. Neutraceut. Sci., Neurofarba Department, University of Florence, 50019 Sesto Fiorentino, IT
havrankovae@pharm.muni.cz

Carbonic anhydrases (CA, EC 4.2.1.1) are metallo-enzymes present in various organisms[1].

In pathogenic bacteria, CAs play an important role in growth and survival[2]. Inhibition of bacterial CAs leads to growth defects, growth retardation and makes bacterias vulnerable to host defense mechanisms. Bacterial CAs are therefore a very promising target in the search for new antibiotics.

A series of 1,3,5-triazinyl aminobenzenesulfonamides substituted by aminoalcohol, aminostilbene, and aminochalcone structural motifs were synthesized as potential bacterial CAs inhibitors. The compounds were tested against vancomycin-resistant *Enterococcus faecalis* (VRE) isolates. A great number of the tested compounds exhibit a significant inhibitory activity against VRE, for example compound ***1*** with MIC = 26.33 μM against all tested strains[3].

To evaluate the selectivity of the compounds against bacterial CAs towards human CAs (hCA) the inhibitory activity of compounds against tumor-associated hCA IX and hCA XII, hCA VII isoenzyme present in the brain, and physiologically important hCA I and hCA II were determined. While tested compounds had only a negligible effect on physiologically important isoenzymes, many of the studied compounds significantly affected the hCA XII isoenzyme, including compound ***1*** with K_{IS} = 5.9 nM.

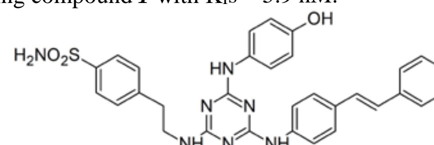


Fig. 1. **The structure of compound *1***

In conclusion, newly prepared compounds have a great potential as antibacterial agents with high activity and at the same time with high selectivity for bacterial CA in comparison with metabolically important hCA isoenzymes (e.g. hCA I, hCA II) found in the human body.

This research was supported by INGA MU (MUNI/A/1202/2020).

REFERENCES

1. Angeli A., Carta F., Nocentini A., Winum J.-Y., Zalubovskis R., Akdemir A., Onnis V., Eldehna W. M., Capasso C., Simone G. D., Monti S. M., Carradori S., Donald W. A., Dedhar S., Supuran C. T.: Metabolites *10*, 412 (2020).
2. Flaherty P., Seleem D. M. N., Supuran C. T.: Future Med. Chem. *13*, 1619 (2021).
3. Havránková E., Garaj V., Mascaretti Š., Angeli A., Soldánová Z., Kemka M., Motyčka J., Brázdová M., Csöllei J., Jampílek J., Supuran C. T.: Int. J. Mol. Sci. *23*, 231 (2022).

ORGANIC CAGE TRIARYLAMINES: FROM SYNTHESIS TO FUNCTION

DENISA HIDASOVÁ, TOMÁŠ SLANINA*

Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo nám. 542/2, 160 00 Prague 6
denisa.hidasova@uochb.cas.cz

The research on organic cage compounds offers a variety of opportunities relevant for catalysis, supramolecular interactions, and radical ion stabilization. Purely organic cage compounds are usually charge-neutral, and their structure is formed by a covalent bond which is translated into their high stability. Only a few examples of cage compounds based on triarylamines can be found in literature[1-4].

Herein we present the synthesis and complete characterization of purely organic covalent cages composed of a triarylamine attached to an aryl unit through ester bonds (Fig. 1). The triarylamine unit enables single-electron oxidation of the cage molecule forming a stable radical cation. We have investigated the properties of the synthesized organic cages, especially their specific interaction with other smaller molecules (guests) binding inside the cage. The host-guest interaction will be modulated by redox switching between neutral amine and its radical cationic form.

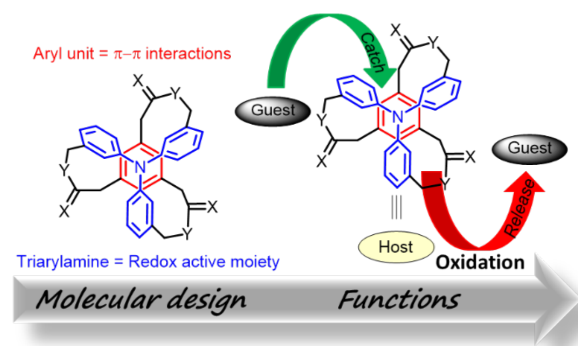


Fig. 1. **Organic cage triarylamines**

This work was supported by MEYS, INTER-COST program (reg. No. LTC20076).

REFERENCES

1. Sathyamoorthi S., Mague J. T., Pascal R. A.: Org. Lett. *14*, 3427 (2012).
2. Yokoyama Y., Sakamaki D., Ito A., Tanaka K., Shiro M.: Angew. Chem. Int. Ed. *51*, 9403 (2012).
3. Mondal B., Acharyya K., Howlader P., Mukherjee P. S.: J. Am. Chem. Soc. *138*, 1709 (2016).
4. Míguez-Lago S., Gliemann B. D., Kívala M., Cid M. M.: Chem. Eur. J. *27*, 13352 (2021).

HETEROCYCLIC SULFONAMIDES AS A NEW TUNABLE H-BOND CATALYST FOR THE DEVELOPMENT OF BIFUNCTIONAL ORGANOCATALYSIS AND THEIR APPLICATION IN THE SYNTHESIS OF NATURAL PRODUCTS

VLADIMÍR HORKA, JIŘÍ POSPÍŠIL*

Department of Organic Chemistry, Faculty of Science, Palacký University, 17. listopadu 1192, CZ-779 00, Olomouc
vladimir.horka01@upol.cz, j.pospisil@upol.cz

The development and application of bifunctional catalysts in stereoselective synthesis is a new rapidly growing field of organic synthesis. The power of such catalysts is their ability to activate/promote either two independent subsequent reactions, or more interestingly, to activate at the same time both reacting partners. In the latter case, often the catalytic sites that are responsible for the catalysis oppose in their reactivity, because one is there to e.g. activate nucleophile/nucleophilic site, while the second activates the electrophile/electrophilic site during the transformation[1-3]. In the catalyst, often small organic molecule, are thus embodied two reactive sites with opposite reactivity. The stereo-selectivity of such transformations is then driven by a chiral scaffold that includes those sites. As a consequence, many new previously impossible transformations can be achieved[1].

In our group, we are interested in the development of synthetic methods leading to previously inaccessible heteroaryl sulfonamides. One of the reasons for our synthetic efforts is the Brønsted acidity of such molecules[4]. Indeed, the pKa of simple N-alkylated heteroaryl sulfonamides is similar to acetic acid (pKa ~ 4). Taking into account the acidity of such simple sulfonamides, a new generation of bifunctional catalysts would embody a nucleophilic partner (N-heterocyclic carbene) and an H-bond donor (BT-sulfonamide) ***1***. Catalyst ***1*** should then serve as a urea-based catalyst ***2*** alternative that would allow us to fine-tune the reactivity/power of coordination of the H-bond donor part.

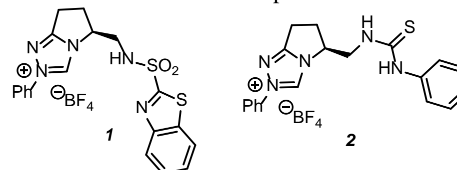
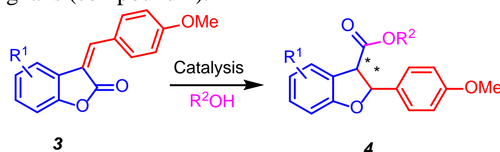


Fig. 1. **Bifunctional organocatalysts: our tunable catalyst *1* and the previously used thiourea-based alternative *2***

Having established a new catalyst, new types of transformation could be discovered. Our aim is to focus on novel approaches to biologically active substances, mainly phenylpropanoid-based plant secondary metabolites such as neolignans (compound ***4***).



Scheme 1. **Stereoselective rearrangement of compound *3* to neolignan-like compound *4***

REFERENCES

1. Lu L.-Q., An X.-L., Chen J.-R., Xiao W.-J.: Synlett, *2012*, 490.
2. Nawaz F., Zaghouani M., Bonne D., Chuzel O., Rodriguez J., Coquerel Y.: Eur. J. Org. Chem *2013*, 8253.
3. Brand, J. P., Osuna Siles J. I., Waser J.: Synlett *2010*, 881.
4. Zálešák, F., Kováč O., Lachetová E., Šťastná N., Pospíšil J.: J. Org. Chem, *86*, 11291 (2021).

LIQUID BIOPSY IN CANCER RESEARCH – DEVELOPMENT OF A NEW 3D PRINTED DEVICE FOR SAMPLE PRETREATMENT

HELENA HRUŠKOVÁ[a,b], **ROMAN ŘEMÍNEK**[a], **FRANTIŠEK FORET**[a]

[a]*Institute of Analytical Chemistry of the Czech Academy of Sciences, Veveří 967, 60200, Brno;* [b]*Masaryk University, Faculty of Science, Department of Chemistry, Kotlářská 2, 602 00, Brno*
HelenaHruskova@email.cz

Liquid biopsy techniques are a relatively new scientific field, where cancer can be detected by analyzing the free circulating tumor DNA fragments from biological fluids like blood plasma. These short DNA fragments can provide significant information about the occurrence and the stage of the disease. In addition, such diagnostic tool is low invasive because it requires only venous blood sampling[1,2].

However, liquid biopsy requires special demands on the sample composition. In the blood sample, the presence of larger DNA fragments can significantly reduce the quality of the obtained diagnostic data. For this reason, the development of new techniques for sample pretreatment is crucial[2].

In the presented study, we developed a preparative device for the separation of short DNA fragments with less than 1000 base pairs. This precleaning should result in higher specificity and sensitivity of the subsequent DNA analyses. The design of the device enables the parallel separation of multiple samples. For the fabrication, 3D printing was used, providing fast and low-cost manufacturing of the product. The device is based on electrodriven separation methods. These techniques enable the partition of DNA fragments in an external electric field. The separation itself is mediated by the sieving effect of the separation media and/or difference in the analyte's migration velocities. At the end of the separation process, the precleaned fraction can be collected from the device, and DNA analyses performed.

This ALIQUID project is co-financed with the state support of the Technology Agency of the Czech Republic and the Ministry of Industry and Trade under the TREND2 Programme (FW02020209).

REFERENCES

1. Benesova L., Belsanova B., Suchanek S., Kopeckova M., Minarikova P., Lipska L., Levy M., Visokai V., Zavoral M., Minarik M.: Anal. Biochem. *433*, 227 (2013).
2. Underwood J. J., Quadri R. S., Kalva S. P., Shah H., Sanjeevaiah A. R., Beg M. S., Sutphin P. D.: Radiology *294*, 5 (2020).

COMPARTMENTALIZATION OF CYTOKININ METABOLISM IN ARABIDOPSIS THALIANA AND SUBCELLULAR TRANSPORT OF CYTOKININS

MARTIN HUDEČEK, VLADIMÍR SKALICKÝ, ONDŘEJ NOVÁK, ONDŘEJ PLÍHAL

Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, 783 71 Olomouc
martin.hudecek02@upol.cz

During the last decades, we have gained considerable knowledge on many aspects of cytokinins, such as their biosynthesis, perception and signaling. Yet, their transport is poorly characterized. Recently, a model of the cytokinin acropetal (shootward) long-distance distribution has been introduced, with Arabidopsis ABCG14 transporter playing a key role[1]. In line with some previous results on the cytokinin cellular transport, it would not be surprising, if some members of the equilibrative nucleoside transporter (ENT) family also played a role in long-distance cytokinin transport.

Combining quantitative analysis of plant hormones as well as using molecular biology approaches, we have characterized a novel cytokinin transporter, ENT1. Characterization of the knock-out line *ent1* revealed a cytokinin-related phenotype that manifests primarily in the shoot. We are currently generating Arabidopsis overexpression and reporter lines of ENT1 fused to a green fluorescent protein (GFP). For the first time, we were able to confirm *in planta* ENT1 localization to the tonoplast, in line with previous data from a proteomic study[2]. In addition, the treatment of Arabidopsis protoplasts with series of isotopically labeled cytokinins helped us to reveal the substrate preference of the cytokinin ENT1 transporter. The reduced content of cytokinins (of the isopentenyladenine-type) in Arabidopsis leaves suggests possible involvement of ENT1 in long-distance cytokinin transport, in accordance with the fact that *ENT1* was reported to be expressed mainly in the root tissues[3].

These results suggest an interesting, yet still not fully characterized involvement of ENT1 in cytokinin transport. Further investigations are needed to integrate the role of ENT family proteins with other known cytokinin transporters and to understand their role in long-distance cytokinin transport.

This work was financed from the funds of the Operational Program Research, Development and Education under the project "Improving schematics of Doctoral student grant competition and their pilot implementation", Reg. No. CZ.02.2.69/0.0/0.0/19_073/0016713.

REFERENCES
1. Zhao J., Ding B., Zhu E., Deng X., Zhang P., Wang L., Dai Y., Xiao S., Zhang C., Liu C. J., Zhang K.: Plant Physiol. *186*, 2111 (2021).
2. Jaquinod M., Villiers F., Kieffer-Jaquinod S., Hugouvieux V., Bruley C., Garin J., Bourguignon J. A.: Mol. Cell Proteomics *6*, 394 (2007).
3. Bernard C., Traub M., Kunz H.-H., Hach S., Trentmann, O., Möhlmann T.: J. Exp. Bot. *62*, 4627 (2011).

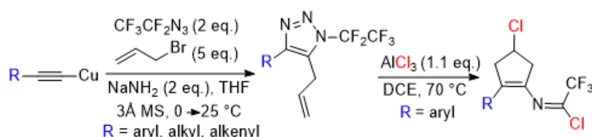
SYNTHESIS OF 4-HALO-1,2-DISUBSTITUTED CYCLOPENTENES BY LEWIS ACID-MEDIATED TRANSFORMATION OF 5-ALLYL-*N*-FLUORO-ALKYL-1,2,3-TRIAZOLES

LUKÁŠ JANECKÝ[a,b], ATHANASIOS MARKOS[a], PETR BEIER[a]*

[a]Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 2, 160 00 Prague 6; [b]Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 2030/8, 128 43 Prague
lukas.janecky@uochb.cas.cz

N-Fluoroalkyl-1,2,3-triazoles available by CuAAC from azides offer an easy access to (*Z*)-*β*-enamido triflates and fluorosulfonates under Brønsted acid conditions[1]. Lewis acids such as $AlCl_3$, $AlBr_3$ or AlI_3 are suitable reagents for the transformation leading to the corresponding haloalkenyl imidoyl halides;[2] in the case of $BF_3{\cdot}OEt_2$, the stereoselective formation of (*Z*)-*β*-enamido fluorides was observed[3].

A novel transformation leading to 1,4,5-trisubstituted 1-fluoroalkyl-1,2,3-triazoles was described (Scheme 1). The triazoles undergo a reaction with $AlCl_3$ to generate 4-chloro-1,2-disubstituted cyclopentenes via a vinyl cation intermediate. The modification of the imidoyl halide structure moiety or the introducing of another halogen atoms will be also subject of discussion. The presented methodology not only shows a new approach to *N*-alkenyl compounds, but also provides a view on vinyl cation generation and its reactivity.



Scheme 2. **$AlCl_3$-mediated transformation of 5-allyl-*N*-fluoroalkyl-1,2,3-triazoles**

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).

REFERENCES
1. Markos A., Voltrová S., Motornov V., Tichý D., Klepetářová B., Beier P.: Chem. Eur. J. *25*, 7640 (2019).
2. Markos A., Janecký L., Chvojka T., Martinek T., Martinez H., Klepetářová B., Beier P.: Adv. Synth. Catal. *363*, 3258 (2021).
3. Markos A., Janecký L., Klepetářová B., Pohl R., Beier P.: Org. Lett. *23*, 4224 (2021).

EXTRACELLULAR DNA IS HIGH IN PLASMA OF MICE WITH HEMOLYTIC UREMIC SYNDROME

LUBICA JANOVICOVA*, ALEXANDRA LESAYOVA, JAKUB JANKO, MICHAL PASTOREK, PETER CELEC

Institute of Molecular Biomedicine, Sasinkova 4, 81106 Bratislava, Slovakia
lubica.janovicova@gmail.com

Hemolytic uremic syndrome (HUS) is a condition commonly caused by Shiga toxin-producing *E. coli* that presents as hemolytic anemia, renal failure and thrombocytopenia. Extracellular DNA (ecDNA) is DNA found outside of cells and it is proinflammatory[1]. Deoxyribonucleases (DNases) are enzymes that cleave DNA[2]. Since activation of immune system is key in HUS, we aimed to describe molecules contributing to the inflammation. We hypothesized that ecDNA concentration will be higher in mice with HUS.

HUS was induced by injection of Shiga toxin 2 in 18 mice. Mice were sacrificed after 48 h. Blood was centrifuged twice to obtain plasma for isolation of ecDNA. EcDNA concentration was quantified using a fluorometric method and real-time PCR.

Total ecDNA concentration and nuclear DNA (ncDNA) were higher in plasma of mice with HUS (Fig. 1A+B). No difference was observed in the concentration of mitochondrial DNA (mtDNA) (Fig. 1C). DNase activity was higher in plasma of mice with HUS too (Fig. 1D). Despite high neutrophil counts in blood in mice with HUS the formation of neutrophil extracellular traps displayed high variability with inconclusive results. These results suggest that ecDNA may contribute to the already present inflammation in HUS. However, high ecDNA concentration is also a result of the tissue damage and it should be cleared by enzymes, macrophages, or other mechanisms.

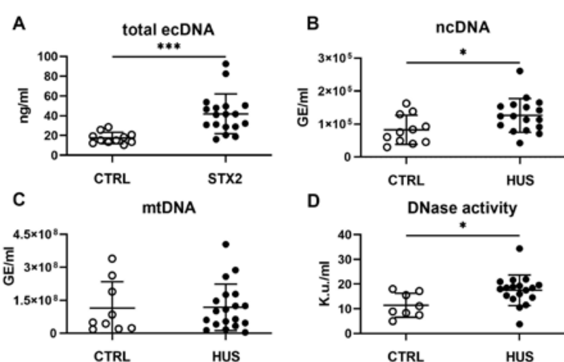


Fig. 1. **Concentration of A) total ecDNA, B) nuclear DNA (ncDNA), C) mitochondrial DNA (mtDNA) and D) DNase activity in plasma of mice with hemolytic uremic syndrome (HUS)**

REFERENCES

1. Marsman G., Zeerleder S., Luken B. M.: Cell Death Dis. *7*, 12 (2016).
2. Fujihara J., Yasuda T., Ueki M., Iida R., Takeshita H.: Comp. Biochem. Physiol. B, Biochem. Mol. Biol. *163*, 263 (2012).

ALTERNATIVE BACKDOOR PATHWAY OF ANDROGEN PRODUCTION AND ITS RELATION TO AUTISM SPECTRUM DISORDERS

KATARÍNA JANŠÁKOVÁ[a]*, MARTIN HILL[b], DIANA ČELÁROVÁ[a], HANA CELUŠÁKOVÁ[a], MARIE BIČÍKOVÁ[b], GABRIELA REPISKÁ[a], LUDMILA MÁČOVÁ[b], DANIELA OSTATNÍKOVÁ[a]

[a]*Institute of Physiology, Faculty of Medicine, Comenius University, Sasinkova 2, 813 72 Bratislava, Slovakia,*
[b]*Department of Steroid Hormones and Proteohormones, Institute of Endocrinology, Národní 8, 116 94 Prague*
katarina.jansakova@*fmed.uniba.sk*

Autism spectrum disorders (ASD) affect more males than females in ratio 4:1. This fact raises a question if sex hormones are involved in the etiopathogenesis of ASD[1]. Androgens are formed by two pathways, the classic frontdoor and also alternative backdoor pathway. We aimed to analyse hormones of both these pathways in prepubertal boys with ASD and neurotypical controls (CTRL).

A total number of 62 boys diagnosed with ASD (4.4±1.1) and 24 controls (4.29±0.95) were recruited. Diagnostic scales ADOS-2 and ADI-R were used for ASD diagnostics. Steroid hormones were analysed in plasma using gas chromatography/mass spectrometry.

Significantly lower level of pregnenolone sulfate (p=0.04) and progesterone (p=0.007) was observed in ASD group. No significant differences between the groups regarding the other hormones of frontdoor pathway were observed. A trend towards lower concentrations of dehydroepiandrosterone (p=0.088), or androstenediol (p=0.056) in ASD was found. Significantly lower concentration of several hormones involved in the backdoor pathway like conjugated epipregnanolone (p=0.048); sulfates of several hormones such as androsterone (p=0.008), epiandrosterone (p=0.012), etiocholanolone (p=0.011) and epietiocholanolone (p<0.001) or a trend toward a lower level of isopregnanolone sulfate (p=0.087) was observed in ASD.

We found differences in several hormones in a row in a particular part of the steroidogenesis, specifically alternative backdoor pathway. These results are in line with previous studies pointing to involvement of sex hormones in the etiopathogenesis of ASD. However, it could be considered as a breakthrough since only main intermediates were believed to play a role in autistic phenotype[2].

This study was supported by grants APVV 15-0045, APVV 15-0085, APVV-20-0070 and VEGA 1/0068/21.

REFERENCES

1. Baron-Cohen S., Lombardo M.V., Auyeung B., Ashwin E., Chakrabarti B., Knickmeyer R.: Plos Biol. *9*, e1001081 (2011).
2. Jansakova K., Hill M., Celarova D., Celusakova H., Repiska G., Bicikova M., Macova L., Ostatnikova D.: Transl. Psych. *10*, 340 (2020).

RETINAL ORGANOIDS ON CHIP – OPTIMIZATION FOR LONG TERM CULTIVATION

VINCENT A. JONGEN[a], MÁRIO KANDRA[a,b], TOMÁŠ BÁRTA[a], ALEŠ HAMPL[a,b], JOSEF JAROŠ[a,b]*

[a]*Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 126/3, 625 00 Brno;*
[b]*International Clinical Research Center (ICRC) of St. Anne's University Hospital Brno, Pekařská 664/53, 656 91 Brno*
jaros@med.muni.cz

Organoids are self-organizing stem-cell derived 3D constructs that mimic *in vivo* structure and function. Since the development of the first retinal organoids roughly one decade ago, methods for retinal organoid cultivation have been modified to give rise to all major cell types of the neuroretina, including mature photoreceptors. However, several challenges remain, such as heterogeneity between organoids, poor photoreceptor maturation and the degradation of retinal ganglion cells in long term cultures. Microfluidic platforms have the potential to help overcome some of these challenges by allowing for automation and precise control of the microenvironment.

For this work we designed a microfluidic device for the differentiation of human pluripotent stem cells towards retinal organoids. We induced organoid formation within a device capable of continuous medium perfusion and compared them with organoids grown in static conditions by analysis of morphology, proliferation, cell viability and cell differentiation.

Organoids were formed within the device using an array of microwells specifically designed for quick aggregation by varying the shape of the wells as well as application of a non-adherent coating. The size of the microwells was adapted to allow for prolonged organoid cultivation. Continuous medium perfusion was used to improve oxygen and nutrient supply without the need for agitation. Retinal organoids cultivated within the chip developed at a timescale representative of the *in vivo* situation and these were evaluated by the expression of eye field transcription markers and retinal progenitor cell markers.

This work was funded by the European Regional Development Fund (project INBIO CZ.02.1.01/0.0/0.0/16_026/0008451), Internal Grant Agency of Masaryk University (project MUNI/IGA/1297/2021 and MUNI/A/1398/2021), Brno city municipality (Brno Ph.D. Talent Scholarship), Ministry of Health of the Czech Republic (NU21-08-00561).

NEDD4-2 BINDING TO 14-3-3 MODULATES THE ACCESSIBILITY OF ITS CATALYTIC SITE AND WW DOMAINS

ROHIT JOSHI[a,b], TOMAS OBSIL[a,b], VERONIKA OBSILOVA[a]

[a]*Department of Structural Biology of Signaling Proteins, Division BIOCEV, Institute of Physiology CAS, 252 50 Vestec;* [b]*Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, 12843, Prague*
Rohit.Joshi@fgu.cas.cz

Neural precursor cells expressed developmentally downregulated protein 4-2 (Nedd4-2), a homologous to the E6-AP Carboxyl Terminus (HECT) ubiquitin ligase, triggers the endocytosis and degradation of its downstream target molecules by regulating signal transduction through interactions with other targets, including 14-3-3 proteins. In our previous study, we found that 14- 3-3 binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains. Here, we used time-resolved fluorescence intensity and anisotropy decay measurements together with fluorescence quenching and mass spectrometry to further characterize interactions between Nedd4-2 and 14-3-3 proteins. The results showed that 14-3-3 binding affects the emission properties of AEDANS-labelled WW3, WW4 and, to a lesser extent, WW2 domains and reduces their mobility, but not those of the WW1 domain, which remains mobile. In contrast, 14-3-3 binding has the opposite effect on the active site of the HECT domain, which is more solvent exposed and mobile in the complexed form than in the apo-form of Nedd4-2. Overall, our results suggest that steric hindrance of the WW3 and WW4 domains combined with conformational changes in the catalytic domain may account for the 14-3-3 binding-mediated regulation of Nedd4-2.

This study was supported by the Czech Science Foundation (Projects 20-00058S), the Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology) and by Grant Agency of Charles University (Project No.348421).

REFERENCES

1. Manning J. A., Kumar S.: Trends Biochem. Sci. *43*, 635 (2018).
2. Ichimura T., Isobe T.: J. Biol. Chem. *280*, 13187 (2005).
3. Pohl P., Joshi R., Petrvalska O., Obsil T., Obsilova V.: Commun. Biol. *22*, 899 (2021).

MUCOADHESIVE INJECTABLE HYDROGEL CARRIERS ENRICHED WITH ENCAPSULATED PRO-HEALING PROTEIN FOR HARD-TO-HEAL ORAL WOUNDS

ZUZANA KADLECOVÁ, LUCY VOJTOVÁ

CEITEC - Central European Institute of Technology, Brno University of Technology, Advanced Biomaterials Group, Purkyňova 656/123, Brno, CZ
Zuzana.Kadlecova@ceitec.vutbr.cz

More extensive wounds at the oral mucosa site formed after surgical or dental resections are hard-to-heal. It was shown that mucosa wounds close in a mechanism different from skin wounds and tend to shrink during the healing process. This results in distortion of the face shape or it can lead to speech impediment of the patient[1]. Such wounds can be treated by filling the cavity with a mucoadhesive injectable material to prevent shrinking and support cell proliferation and wound closure at the same time.

This work aims to prepare an injectable thermosensitive drug delivery system enriched by biologically active substances to enhance healing. The mucoadhesive matrix was formed by an FDA-approved biocompatible, non-toxic synthetic polymer based on poly(lactide-*co*-glycolide) and poly(ethylene glycol) (PLGA-PEG-PLGA) copolymer functionalized with itaconic anhydride (ITA) patented by BUT[2]. This polymer system is body thermo-responsive and is suggested for delivering a pro-healing protein – stable fibroblast growth factor-2 (FGF2-STAB® from Enantis L.t.d.), which stimulates re-epithelization and thus promotes overall tissue regeneration.

The FGF2-STAB® was encapsulated into liposomes involving the “heat-method” technique to prolong the half-life and reduce the matrix-protein interaction. Prepared liposomes with diameters of around 200 nm exhibited encapsulation efficiencies around 30 % and were stable regarding the zeta potential value. Due to the unique properties, PLGA-PEG-PLGA/ITA hydrogels enriched with liposomal FGF2-STAB® seem to be a promising candidate for the intended application in tissue healing enhancement. The release of FGF2-STAB® from the bioresorbable hydrogel is currently being evaluated.

This work was supported by the ProfiBONE project (TO01000309) that benefits from the EEA Grants and the TACR within the KAPPA Programme and within the project Quality

Internal Grants of BUT (KInG BUT), Reg. No. CZ.02.2.69/0.0/ 0.0/ 19_ 073 / 0016948, which is financed from the OP RDE.

REFERENCES
1. Mistry R. C., Qureshi S. S., Kumaran C.: J. Surgical Oncol. *91*, 131 (2005).
2. Vojtová L., Michlovská L., Jančář J.: EP 3097134 B1 (2017).

ELECTROCHEMICAL DETECTION OF PROTEIN BIOMARKERS USING METAL NANOPARTICLES

GYLXHANE KASTRATI, LUCIE KORECKA, ZUZANA BILKOVA

Institution, Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice
gylxhane.kastrati@student.upce.cz

In recent years, immunoassays are used for the detection of proteins biomarkers that are the essential objective of the diagnosis, treatment, and prognosis of many genetic, metabolic, infectious diseases, and biowarfare agents. The huge progress is received in the development of immunoassays, especially in nanoscale electrochemical biosensors for clinical significance[1,2]. Among the most important challenges in biosensors is the selection of suitable metal nanoparticles and the achievement of high sensitivity. Thanks to its unique advantages on the fabrication of electroactive species with aid of nanotechnology, is available biofunctionalization of metal nanomaterials that are used as a powerful label. The investigations showed that nanomaterials could generate electrical signals, amplify them by lowering detection potentials due to higher surface area, smaller current density, due to electronic, chemical, and mechanical properties, since biomolecules are unable to communicate with electrodes directly[3,4]. In this immunoassay, bioconjugate was labeled with electroactive metal nanoparticles and combined with mesoporous silica nanoparticles, which enabled the conjugation with ligands (specific antibodies) and kept the ability to interact with the targeted protein. Evaluation of the binding efficiency of prepared bioconjugate was done by SDS-PAGE, while the outcome signal was analyzed with square wave voltammetry, known as an analytical tool, that allows direct analyses at the ppb or at ppt when is used in a stripping mode.

REFERENCES
1. Korecká L., Vytřas K., Bílková Z.: Curr. Med. Chem. *25*, 1 (2018).
2. Zhang C. C., Du X.: Front. Chem. *8*, 651 (2020)
3. Wang J., Liu G., Jan R.: J. Am. Chem. Soc. *126*, 3010 (2004).
4. Lim S.A., Ahmed M.U.: RSC Adv. *6*, 14995 (2016).

PredictSNP^{ONCO}: A TOOL FOR RAPID ANALYSIS OF CANCER ASSOCIATED MUTATIONS AND SUBSEQUENT TREATMENT SELECTION

RAYYAN TARIQ KHAN[a,b], JAN STOURAC[a,b], SIMEON BORKO[a], PETRA POKORNA[c], ADAM DOBIAS[a], GASPAR PINTO[a,b], JAROSLAV STERBA[d], ONDREJ SLABY[c], DAVID BEDNAR[a,b], JIRI DAMBORSKY[a,b]

[a]Loschmidt Laboratories, Dept Exp. Biology & RECETOX, Masaryk Univ., 625 00 Brno; [b]Intl Clin. Res. Centre, St. Anne's Univ. Hospital Brno, 656 91 Brno; [c]Central European Inst. Technol., Masaryk Univ., 621 00 Brno, & Dept Biology, Faculty of Medicine, Masaryk Univ., 625 00 Brno; [d]Dept Paediatric Oncology, University Hospital Brno, 613 00 Brno, & Faculty of Medicine, Masaryk University, 625 00 Brno
Rayyan.Tariq.Khan@gmail.com

Overall, 19 292 789 cancer cases were diagnosed in 2020 with a projected load of 28.4 million cases in 2040 (ref.[1]). The development of novel cancer therapies is generally driven by the availability of new drugs. However, their approval is time-demanding, and clinical trials do not focus on interpatient heterogeneity within the same cancer type. One of the most promising approaches is personalised medicine[2]. The feasibility of this approach has been demonstrated by programmes, which successfully employ combinations of whole exome, genome, or transcriptome sequencing to identify driver mutations and other actionable alterations in the biology of individual tumor[3]. Large-scale molecular profiling generates a great amount of data, however, its interpretation still remains challenging. Determination of the potential oncogenic effect of the variants of uncertain significance identified by DNA sequencing, on a protein level, especially missense single nucleotide variants, is unclear. To fill this gap, we designed a novel web server - PredictSNP^{ONCO}, which compares a wild-type protein with its mutated variant, from structural and sequential perspectives. It includes evaluation of stability change by Rosetta and FoldX, structural changes in catalytic pockets and tunnels analysed by CAVER and P2Rank, and prediction of the effect on protein function by PredictSNP and HOPE. The most important analysis is the assessment of the changes in binding of potential inhibitors from FDA/EMA approved drugs by AutoDock Vina. Calculated results are quickly available in an interactive and easy-to-use format. The functionality of PredictSNP^{ONCO} was validated using a list of 829 mutations with a known deleterious or likely deleterious impact on cancer progression and 309 mutations with known benign or likely benign impact as a control. The service is running in the Czech national supercomputing centre and will be maintained using funding from the Czech Clinical Research Infrastructure Network. The service is provided free of charge at: https://loschmidt.chemi.muni.cz/predictsnp-onco/.

REFERENCES
1. Sung H., Ferlay J., Siegel RL., Laversanne M., Soerjomataram I., Jemal A., Bray F.: Ca-Cancer J. Clin. *71*, 209 (2021).

2. Chin L., Andersen J. N., Futreal P.A.: Nat. Med. *17*, 297 (2011).
3. Pinto G., Hendrikse N., Stourac .J, Damborsky J., Bednar D.: Semin. Cancer Biol. *in press* (2021); https://doi.org/10.1016/j.semcancer.2021.07.012.

GLYCOPROFILING OF FIXED LUNG TISSUE

KRISTÍNA KIANIČKOVÁ[a], LUCIA PAŽITNÁ[a], PARAS KUNDALIA[a], PETER BARÁTH[a], ZUZANA PAKANOVÁ[a], MAREK NEMČOVIČ[a], FILIP KVĚTOŇ[a], FILIP PANČÍK[a], PAVOL JANEGA[b], JAROSLAV KATRLÍK[a]

[a]Institute of Chemistry, SAS, Dubravska cesta 9, 84538, Bratislava; [b]Institute of Pathological Anatomy, Comenius University in Bratislava and University Hospital Bratislava, Sasinkova 4, 811 08 Bratislava
chemkian@savba.sk

The human lungs weigh around 1 kg and their role are change of gases between airways and blood through alveoli. Within the lungs, there are about 300 million of alveoli with 90 m^2 of surface exchange area[1]. Formalin-fixed paraffin-embedded (FFPE) sample is a way for preservation of tissues for later research. FFPE tissues are often used in immunohistochemistry – tissues are mounted onto microscopic slide and incubated with specific antibodies[2]. Another type of fixation is alcohol fixation. Ethanol can denature proteins due to its ability to replace water in tissue[3]. Our aim was to develop protocols for protein isolation from fixed tissues into “mild” extraction buffers suitable for mass spectrometry (MS) and lectin-based microarray, that are used in glycomics[4], and apply them for glycoprofiling of proteins extracted from lung tissue samples. We tested commercially available kits for FFPE tissues, optimized our own protocols for protein isolation from ethanol fixed tissues, and evaluated their efficiency for subsequent glycomic/proteomic analyses. Our next step will be the glycoprofiling of fixed tissue samples of patients succumbed to the disease COVID-19. In case of lung tissue, there are some reports about histopathological features in which was mentioned diffuse alveolar damage as main histopathological finding[5]. We want to know if there are some changes in glycans of COVID-19 lungs.

This work was supported by the grants VEGA 2/0120/22, APVV-17-0239 and COST CA18132. This publication is the result of the project implementation CEMBAM – Centre for Medical Bio-Additive Manufacturing and Research, ITMS2014+: 313011V358 supported by the Operational Programme Integrated Infrastructure funded by the European Regional Development Fund.

REFERENCES

1. Effros R. M.: GI Motility online, 2006, DOI: 10.1038/gimo73.
2. https://www.biochain.com/general/what-is-ffpe-tissue/ [downloaded Jan. 27, 2022].
3. https://www.leicabiosystems.com/knowledge-pathway/fixation-and-fixatives-3-fixing-agents-other-than-the-common-aldehydes/ [downloaded Jan. 27, 2022].
4. Gupta G., Surolia A., Sampathkumar S. G.: OMICS J. Integr. Biol. *14*, 419 (2010).
5. Montero-Fernandez M. A., Pardo-Garcia M.: Diagn. Histopathol. *27*, 123 (2021).

HARD TISSUES ON A CHIP – DEVELOPMENT OF A PLATFORM FOR GENERATING 3D BONE AND CARTILAGE CULTURES

MICHAEL KILLINGER[a,b], TEREZA SZOTKOWSKÁ[b,c], TOMÁŠ VÁCLAVEK[a,b], MARCELA BUCHTOVÁ[b,c], KAREL KLEPÁRNÍK[a]

[a]Institute of Analytical Chemistry CAS, Department of Bioanalytical Instrumentation, Veveří 967, 60200, Brno; [b] Masaryk University, Faculty of Science, Kamenice 5, 625 00, Brno; [c]Institute of Animal Physiology and Genetics, Laboratory of Molecular Morphogenesis, Veveří 967, 60200, Brno
killinger@iach.cz

Animal models have been used for decades in biomedical research and drug development, representing an indispensable step in pre-clinical studies. Such models are a key for bringing substantial knowledge of the mechanisms initiating diseases as well as for testing their potential therapy. Nevertheless, recently extensive effort is undertaken to develop alternatives aiming to the reduction, refinement, and replacement (3Rs) of animal usage for research or for pre-clinical studies.

Novel methods of three-dimensional (3D) cell cultures became frequently utilised in many research fields due to their enhanced biological functions as compared to conventional two-dimensional (2D) cultures. 3D cell spheroids or organoids can replicate tissue functions, which enables their use both as *in vitro* models or as necessary intermediate step in tissue biofabrication approaches. The most promising tool for generating and analysis of 3D cell structure is a recent application of microfluidic chips. Microfluidic technology allows controlled conditions, automatization, reduced amount of reagents and cells, and mainly dynamic conditions for continuous perfusion of nutrients and removal of the metabolic waste of the cells.

In this study, we developed a polydimethylsiloxane (PDMS) microfluidic platform suitable for 3D bone and cartilaginous cells cultivation. The proper fabrication and preparation led to the development of homogenous 3D cell cultures of MC3T3-E1 osteoblast cells. Moreover, the system was used to study primary chondrocyte differentiation while using WNT inhibitors. These protocols were compared to standard cultivation methods such as hanging drops to reflect the advantages of microfluidic platforms. In addition, the PDMS microfluidic can be connected with the syringe pump to mimic *in vivo* perfusion conditions and increase the value of 3D cell cultivation.

The research was supported by the Czech Science Foundation (GA CR project 20–00726S). M.K. is Brno Ph.D. Talent Scholarship Holder funded by the Brno City Municipality.

VALIDATION OF *IN VITRO* FLOW MODEL AND *IN VIVO* RAT MODEL FOR USE IN TRANSLATIONAL RESEARCH

PATRÍCIA KITTOVÁ[a,b,c], PETER SCHEER[a,e], JANA HLOŽKOVÁ[a,e], ELIŠKA BRHELOVÁ[a], SANDRA THALEROVÁ[a,b], MICHAELA PEŠKOVÁ[b], JAN VÍTEČEK[a,b], LUKÁŠ KUBALA[b,d], ROBERT MIKULÍK[a]

[a]International Clinical Research Center, St. Anne’s University Hospital Brno, Pekařská 53, 656 91 Brno; [b]Institute of Biophysics CAS, Královopolská 135, 612 65 Brno; [c] Department of Biochemistry, Faculty of Medicine and [d]Department of Biochemistry, Faculty of Science and [e]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Masaryk University, Kamenice 5, 625 00 Brno
451736@mail.muni.cz

Acute ischemic stroke (AIS) is one of the leading causes of death and disability worldwide. The only approved pharmacological treatment of AIS is intravenous thrombolysis with alteplase[1]. However, alteplase is of limited efficacy in some cases[2]. Therefore, a number of new thrombolytics have been developed in recent years[3]. Their testing requires the implementation of reliable methods and models for translating the results into clinical practice. Such models should provide high-throughput screening as well as sufficient clinical relevance.

This study presents cross-validation of two thrombolytic models, both *in vitro* and small animal *in vivo,* using clinically approved alteplase and recently introduced thrombolytic tenecteplase. *In vitro* flow model and *in vivo* systemic embolization rat model were used to determine the efficacy of alteplase and tenecteplase. Semi-synthetic clots were used *in vitro,* and synthetic clots were used *in vivo*. Both alteplase and tenecteplase were applied at a dose or concentration clinically relevant to the treatment of AIS. In both models, determination of efficacy was performed by real-time measuring of the clot size change during 60-minutes incubation/thrombolytic infusion.

In *in vitro* (n=36 – alteplase, n=10 – tenecteplase) and *in vivo* (n=28 alteplase, n=4 – tenecteplase) experiments, alteplase-treated clots showed relative clot reduction *in vitro* 54±41% vs *in vivo* 52±29% (p=0.803). Tenecteplase-treated clots showed relative clot reduction *in vitro* 52±43% vs *in vivo* 82±15% (p=0.079). *In vitro*, alteplase and tenecteplase induced the same relative clot reduction (p=0.920), whereas, *in vivo*, tenecteplase induced higher clot reduction compared to alteplase (p=0.005).

Introduced ischemic stroke models consistently showed a high level of resemblance in thrombolysis rate of alteplase, also congruent with clinical data from the literature (clot size reduction of 41% in humans)[4]. However, tenecteplase showed an increased thrombolysis rate in the *in vivo* model. It could be due to the prolonged half-life of tenecteplase *in vivo*. In summary, the data indicate that our models could be suitable for the prediction of thrombolytic efficacy in clinical practice.

Supported by the European Regional Development Fund – Project INBIO (No. CZ.02.1.01/0.0/0.0/16_026/0008451).

REFERENCES

1. Powers W. J., Rabinstein A. A., Ackerson T., Adeoye O. M.: Stroke *50*, 344 (2019).
2. Ma H., Campbell B. C. V., Parsons M. W., Churilov L.: N. Engl. J. Med. *384*, 13 (2021).
3. Warach S. J., Dula A. N., Milling T. J. Jr.: Stroke *51*,11 (2020).
4. Kim Y. D., Nam H. S., Kim S. H., Kim E. Y. Song D.: Stroke *46*, 7 (2015).

SELF-ASSEMBLING HUMAN HEART ORGANOIDS FOR THE MODELLING OF HEART INJURY AND REGENERATION

ELIŠKA KOHOUTKOVÁ[a,b], WOUTER DERKS[a], OLAF BERGMANN[a]

[a]Center for Regenerative Therapies Dresden, TU Dresden, Germany; [b]Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
kohoutkovaeliska@email.cz

Cardiovascular diseases (CVDs) are the leading cause of death worldwide. The main hallmark of most CVDs is a loss and insufficient regeneration of cardiomyocytes, the heart muscle cells.

There have been many efforts to develop models of cardiac regeneration and disease, where the induced pluripotent stem cells (iPSCs) serve as a great tool. iPSCs enable us to produce cardiac cells *in vitro*, however currently used cell models are still far away from the human heart organ as they lack higher structural and cellular complexity. Although, in recent times the protocols for the generation of human heart organoids (HHOs) have been developed. Due to their 3D structure, they can serve as a more faithful *in vitro* model of the human heart.

We have optimized a protocol for the generation of self-assembling HHOs from human induced pluripotent stem cells that we use as an *in vitro* model of human heart disease and regeneration.

We characterized the multi-lineage complexity of cell types and their organization within HHOs. Moreover, we used FUCCI human iPSC-derived organoids to track the cell-cycle status of cardiomyocytes.

For further investigation of human heart regeneration, we performed cryoinjury of HHOs to mimic damage of the heart tissue during heart disease. The cryoinjured organoids will be further used as an *in vitro* model of human heart regeneration assisted by iPSC-derived macrophages to study the role of macrophages in the human heart regeneration.

HETEROCYCLIC SULPHONAMIDES AS NEW TUNABLE CHIRAL H-BOND DONORS AND BRØNSTED ACIDS IN ORGANOCATALYSIS AND THEIR APPLICATIONS IN THE SYNTHESIS OF NATURAL PRODUCTS

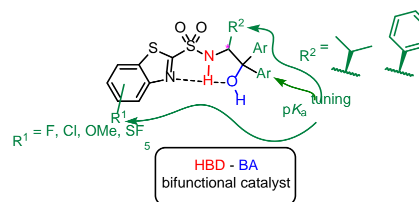
<u>JOZEF KRISTEK</u>, JIŘÍ POSPÍŠIL*

Department of Organic Chemistry, Faculty of Science, Palacký University, 17. listopadu 1192, CZ-779 00, Olomouc
jozef.kristek01@upol.cz, j.pospisil@upol.cz

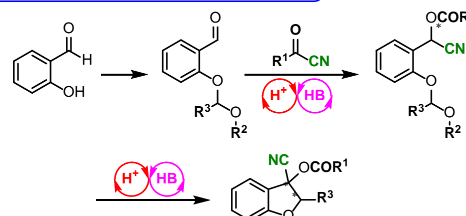
The onset of the 21st century is highlighted by tremendous progress in the field of asymmetric catalysis. The emerging field of asymmetric catalysis, hand in hand with endless efforts connected with the development of safer and non-toxic means, led to the birth of a new field of research, organocatalysis. This emerging concept is based on the use of small chiral organic molecules as a precatalyst for organic reactions[1,2]. Over the past 20 years, organocatalysis underwent rapid development and led to the discovery of numerous new, so far unknown, synthetic transformations and the development of novel concepts. One of the new areas covers the development of novel so-called bifunctional catalysts. In this approach, one catalyst simultaneously activates two functional groups/reaction partners that further react together and form a new chemical entity[3]. In general, thus the catalyst at the same time activates the nucleophile and electrophile reaction entity. Thus, the catalyst itself embodies an electrophile site for activating nucleophiles and a nucleophilic site designed for electrophile activation. Such two orthogonal reactivity sites then simultaneously affect both reaction partners (in general, the LUMO energy of the electrophile is lowered and the HOMO energy of the nucleophile is increased) but do not interact between themselves.

The goal of our project is to develop a new type of bifunctional chiral organocatalysts that would embody the H-bond donor site (HBD) and Brønsted acid (BA)[3,4]. The strength of such a catalyst should be in the possibility of catalyzing new types of transformation, that would allow the activation of Lewis base sites (lone pairs in heteroatoms) by the HBD activation/coordination site and, at the same time, the activation of even stronger electrophiles (e.g. carbonyl group, formation of oxonium) with Brønsted acid catalysis (Scheme 1A). The strength of our catalyst is the possibility of Brønsted acid pK_a modification that should allow us to fine-tune the desired transformations[5]. The enantioselectivity of the whole process is then ensured by the chiral scaffold that can be traced back to simple α-amino acids. The application of such novel organocatalysts is primarily focused on the domain of natural substances, mainly phenylpropanoids – secondary plant metabolites such as neolignans (Scheme 1B).

A. Design of novel bifunctional organocatalysts



B. Two step approach to neolignan-like molecules



Scheme 1. **(A) Bifunctional organocatalyst with the tuneable pK_a. (B) Synthesis of neolignan-like molecules using the bifunctional HBD/BA catalysis concept**

REFERENCES

1. List B., R. Lerner A., Barbas C. F.: J. Am. Chem. Soc. *122*, 2395 (2000).
2. MacMillan D. W. C., Ahrendt K. A., Borths C. J.: J. Am. Chem. Soc. *122*, 4243 (2000).
3. Lu L.-Q., An X.-L., Chen J.-R., Xiao W.-J.: Synlett *23*, 490 (2012).
4. Min C., Seidel D.: Chem. Soc. Rev. *46*, 5889 (2017).
5. Zálešák F., Kováč O., Lachetová E., Šťastná N., Pospíšil J.: J. Org. Chem. *86*, 11291 (2021).

SYNTHESIS AND DERIVATIZATION OF OXIDIZED THIACALIXARENES

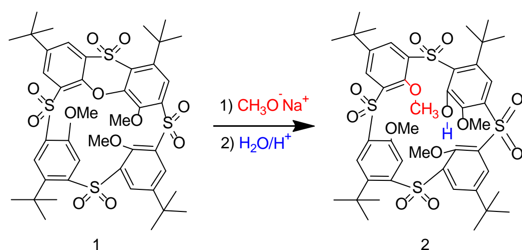
<u>KAMIL MAMLEEV</u>, PAVEL LHOTÁK*

VŠCHT Praha, Technická 5, 166 28 Praha 6 – Dejvice
mamleevk@vscht.cz

Calixarenes are macrocyclic oligomers that consist of four or more phenols connected by methylene bridges. The main advantages of calixarenes are straightforward preparation from cheap and available starting materials, easy derivatization of the upper and lower rims and derivatization of the cavity's basic structure. Novel receptors can be formed due to the excellent complexation properties of calixarenes[1,2]. One of the essential types of calixarenes is thiacalixarene. Unlike classical calixarenes, phenyl groups in thiacalixarenes are connected by sulfur atoms. This leads to significant changes in chemical and complexing properties. One of the most important properties of thiacalixarenes is the oxidation of sulfide bridges, which changes both the complexation properties and the overall reactivity of the molecule. However, oxidation takes place not only on the sulfur atoms. The reaction of thiacalixarenes with chloramine-T forms spiro

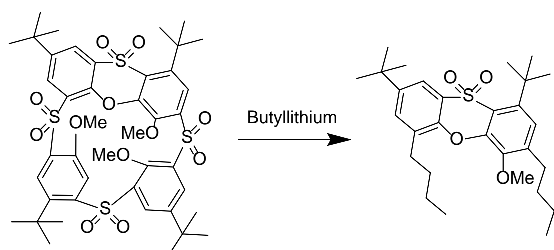
derivatives. Furthermore, subsequent treatment with hydrochloric acid produces a phenoxathiin structure[3,4].

Previous studies have shown that *O*-nucleophile attack cleaves the Ar-O-Ar bond in the middle of the phenoxathiin cycle. The reaction of sodium methoxide with phenoxathiin derivative ***1*** formed new product ***2*** (Scheme 1)[5].



Scheme 1. ***O*-nucleophile attack**

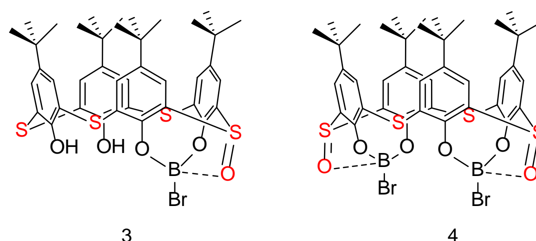
It was expected to find similar properties in the *C*-nucleophile attack. Nevertheless, the use of butyllithium as a *C*-nucleophile splits the calixarene structure unexpectedly (Scheme 2). Hence, our project aims at the cleavage of the calixarene structure of a phenoxathiin derivative under the influence of *C*-nucleophiles. In addition, it would be interesting to investigate the influence of the nucleophile type on the reaction outcome and its mechanism.



Scheme 2. **The cleavage of the calixarene structure under the influence of *C*-nucleophile**

Our second project has described the synthesis of spiro- and phenoxathiin structures based on the thiacalixarene molecule without any substituents. Only one spiro- and subsequently phenoxathiine structure is formed during the oxidation of the basic structure of thiacalixarene without substituents. Our primary goal was to prepare monooxidized thiacalixarene as a starting structure for further oxidation. However, a new unexpected complex ***3*** was isolated and analyzed (Scheme 3) in the preparation process using boron tribromide as the demethylating agent.

The discovery of this new complex paves the way to the generation of new receptors. Interestingly, complex ***3*** is synthesized under very mild reaction conditions in good yields. In the past, the synthesis of polynuclear complexes based on the thiacalixarene molecule using triethyl borate proceeded by a different mechanism under energy-intensive reaction conditions. These polynuclear complexes have the potential to form new molecules based on thiacalixarenes, so our next goal is to create polynuclear complex ***4*** and study the properties of these molecules.



Scheme 3. **Boron complexes *3* and *4***

REFERENCES

1. Gutsche C. D. in: *Calixarenes An Introduction 2nd Edition*; The Royal Society of Chemistry: Cambridge 2008.
2. Zajícová M., Eigner V., Budka J., Lhotak P.: Tetrahedron Letters *2015*, 5529.
3. Mikšátko J., Eigner V., Dvorakova H., Lhotak P.: Tetrahedron Letters *2016*, 57.
4. Landovský T., Dvořáková H., Eigner V., Babor M., Krupička M., Kohout M., Lhoták P.: New J. Chem. *2018*, 20074.
5. Landovský T., Eigner V., Babor M., Tichotová M., Dvořáková H., Lhoták P.: Chem. Commun. *2020*, 78.

REVERSE TRANSCRIPTASE FINGERPRINT METHOD AS A SELECTIVE PROFILING TECHNIQUE FOR THE IDENTIFICATION OF Np*n*N-RNAs

FLAMINIA MANCINI, PAVEL VOPÁLENSKÝ, LENKA GAHUROVÁ, NIKOLAS TOLAR, HANA CAHOVÁ

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 2, 16610, Prague 6
cahova@uochb.cas.cz

Recently, entirely new class of 5' RNA caps was discovered in *E. Coli*. These caps have structure of dinucleoside polyphosphates (Np*n*N)[1]. Free Np*n*N were discovered 50 years ago in all types of cells[2]. It is known that their concentrations increase under the stress. Nevertheless, the intracellular role of Np*n*N is still enigmatic.

Preliminary data from our laboratory, show that Np*n*N-RNAs are also present in mammalian tissue cell culture. However, nothing is known about the types of RNA bearing Np*n*N caps and how they influence cellular reactions to stress.

Therefore, it is essential to develop a selective capturing/profiling technique that allows for identification of RNA types capped with Np*n*Ns.

It is known that reverse transcription of RNA templates containing RNA modifications, leads to synthesis of cDNA containing information on the modification in the form of misincorporation, arrest, or nucleotide skipping events. A collection of such events from multiple cDNAs coming from different reverse transcriptases (RT) represents an RT-signature (fingerprint) that is typical for a given modification[3].

Recently, machine learning of reverse transcriptase signatures was used to distinguish internal RNA modifications, such as m^6G (6-methylguanosine)[3]. Because nothing is known about the reading and recognition of these Np*n*N caps by RTs, we want to exploit this RT fingerprint method for the preparation of RNA-seq libraries. The idea is to test model Np*n*N-capped RNAs with a combination of commerically available RTs (e.g. SuperScript™ III, SuperScript® IV, TGIRT™-III, AMV) assuming behavior similar to fingerprint. Model RNA bearing different 5` RNA caps (NAD, $Ap_{2-6}A$, $Gp_{2-4}G$, etc.) will be used as substrates for different RTs. Then, we will prepare RNA-seq library suitable for Oxford Nanopore Technology, established in our laboratory. If we observe significantly different behaviour of various RT with our Np*n*N RNA caps by bioinformatic analysis (i.e. machine learning), the method will be applied on RNA from real samples (*E. coli* or human tissue cells).

REFERENCES

1. Hudeček O., Benoni R., Reyes-Gutierrez P. E., Culka M., Šanderová H., Hubálek M., Rulíšek L., Cvačka J., Krásný L., Cahová H.: Nature Commun. *11*, 1 (2020).
2. Vanbogelen R. A., Kelley P. M., Neidhardt F. C.: J. Bacteriol. *169*, 26 (1987).
3. Werner S., Schmidt L., Marchand V., Kemmer T., Falschlunger C., Sednev M. V., Bec G., Ennifar E., Höbartner C., Micura R., Motorin Y., Hildebrandt A., Helm M.: Nucl. Acids Res. *48*, 3734 (2020).

BIOLOGICAL ACTIVITY OF MIR-29B

JAN MANDRLA, ZDENĚK DOSTÁL, JANA BUCHTÍKOVÁ, MARTIN MODRIANSKÝ

Department of medical chemistry and biochemistry, medical faculty, Palacký University, Hněvotínská 3, 775 15 Olomouc
IanMandrla@gmail.com

Growing mean life expectancy in combination with unhealthy lifestyle and polluted environment results in higher incidence of oncological diseases. This fact poses a big challenge – search for new therapeutic methods. MicroRNA is one of the discussed options in this field. MicroRNAs represent 22 nucleotides long molecules of non-coding RNA, which possess ability to influence expression of up to hundred different proteins. Thanks to this effect on cells, certain microRNA molecules could be used in therapy of cancer.

The aim of this research was to verify whether the effect of miR-29b is unique to HeLa cell line[1] or can be applied to other cell models. The next goal was to investigate the mechanism of action in selected cell cultures by using siRNA directed against miR-29b target molecules[2].

Experiments have shown increased cytotoxicity of etoposide in all tumorous cell lines transfected with precursors of miR-29b. Transfection using anti-Mcl-1 siRNA increased etoposide cytotoxicity in Hep G2 cell line whilst Caco-2 cell line was practically immune to this effect. Pre-miR-29b transfected Caco-2 cell line demonstrated increased cytotoxicity. That led us to evaluate Bcl-2 protein level in the transfected Caco-2 culture. Bcl-2 protein expression was decreased. Transfecting Caco-2 cell line with anti-Bcl-2 siRNA resulted in 40% higher etoposide cytotoxicity in comparison to negative control. Conducted experiments verified that used siRNA molecules are effective.
Collected data show, that miR-29b enhanced cytotoxicity of etoposide in Hep G2 and HeLa cell lines correlates to lower expression of Mcl-1 protein. In case of Caco-2 cell line, effect of miR-29b is based on reduced Bcl-2 protein expression.

This study was supported by IGA_LF_2021_011.

REFERENCES

1. Kollinerová S., Dostál Z., Modrianský M.: Toxicol. in Vitro *40*, 289 (2017).
2. Jiang H., Zhang G., Wu J., Jiang C.: Oncol. Rep. *31*, 1509 (2014).
3. Hande K. R.: Eur. J. Cancer *34*, 1514 (1998).

IN-HOUSE BUILT CAPILLARY ELECTROPHORESIS DEVICE FOR ANALYSIS OF PLANT SAP

NATÁLIA MELICHEROVÁ[a,b], FRANTIŠEK FORET[a]

[a]Institute of Analytical Chemistry of the Czech Academy of Sciences, Veveří 967/97, 602 00 Brno; [b]Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno
melicherova@iach.cz

The CE analytical method was chosen to meet the requirements of the task to analyze changes in plant sap composition during their development or stress response without disturbing the plant environment. The CE method provides many advantages highlighting short analysis time, high separation efficiency, sensitivity, and very low volumes regarding analyzed samples[1]. The last-mentioned one is a big benefit in the case of analyzing living plants, where the sample is often limited to sub microliter volume[2].

Our work focused on developing instrumentation for the CE analysis of various ionogenic species in plant sap while using a semi-invasive sampling method. The developed device shown in Fig.1 utilizes two types of detection, i.e., UV detector and Capacitively-Coupled Contactless Conductivity Detector (C4D). The base plate of the device is mounted to the top of the UV detector with two vessels for the background electrolyte. A swinging arm on the inlet side is used to fix and position the sampling end of the capillary. On the outlet side, there is a unit designed to flush the capillary after each analysis by using a vacuum.

Various methods for separating inorganic ions and plant hormones – auxins were successfully applied using this device on mixtures of standards. The next step in our work is to measure chosen analytes in real sap samples within the *Brassicaceae* family, mainly *B. napus* and *B. rapa* (oilseed rape), and other crops from this family.

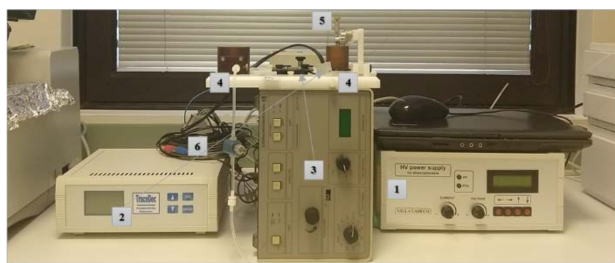


Fig. 1. **In-house built CE device 1 – HV power supply; 2 - C4D; 3 – UV detector; 4 – vials + electrodes; 5 – fused silica capillary; 6 – DAQ**

The work has been supported by from European Regional Development Fund-Project “SINGING PLANT” (No.CZ.02.1.01/0.0/0.0/16_026/0008446).

REFERENCES

1. Kubáň P., Kalberg B., Kubáň P., Kubáň V.: J. Chromatogr. A *964*, 227 (2002).
2. Melicherová N., Řemínek R., Foret F.: J. Sep. Sci. *43*, 271 (2020).

ENDOPLASMIC RETICULUM STRESS ALTERS THE MORPHOLOGY AND CELLULAR FUNCTIONS OF THE OVARIAN SURFACE EPITHELIUM IN OVARIAN TISSUE EXPLANTS

LUKÁŠ MORÁŇ[a,b], LUKÁŠ PEČINKA[c], VERONIKA SEDLÁKOVÁ[a],LENKA KREJČÍ[a], TEREZA VESSELÁ[a], VIKTORIE GABRIELOVÁ[a], VOLODYMYR POROKH[a], ALEŠ HAMPL[a], PETR VAŇHARA[a]

[a]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 3, 625 00 Brno; [b]Research Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Žlutý kopec 7, 656 53 Brno; [c]Faculty of Science, Masaryk University, 611 37 Brno
lukasmoran@gmail.com

Ovarian surface epithelium (OSE) is a superficial layer of cubic cells covering the ovaries. OSE actively participates in the ovulatory cycle and regularly undergoes through physiological cycles of wound and repair. OSE is also constantly exposed to a number of stress factors coming from both the inside of the ovary and extrinsically from the peritoneal cavity. Consequently, understanding the morphological changes of OSE cells and their response to stress induced during aging or incessant ovulation may clarify histopathological background of ovarian dysfunction, infertility, and cancer. The endoplasmic reticulum (ER) is an essential cellular organelle responsible for protein synthesis, posttranslational modifications, membrane biosynthesis, and calcium ions management. ER is also an important signalling hub integrating various forms of stress and organizing the signalling response. ER stress can be triggered by a variety of factors and has been demonstrated to play a role in many pathologies.

In our work we study the morphological changes of OSE evoked by ER stress. To tackle these questions, we have studied whole mouse ovarian explants by electron, confocal and light microscopy, and advanced bioanalytical methods. We revealed that OSE exposed to ER stress shift the epithelial morphology towards mesenchymal, reduce the number of microvilli on the cell surface, change the intracellular levels of the main regulators of the ER stress response and their distribution within ovarian structures. Cells with deregulated ER stress response show the altered proliferation rate, propensity to senescence and also distinct molecular signature revealed by mass spectrometry fingerprinting.

Our findings can help to clarify key aspects of carcinogenesis, regeneration or functions. In addition, the identification of biologically relevant links between the ER molecular machinery and ovarian biology can extend the portfolio of molecular targets for the treatment in the female reproductive system.

This study was supported by Masaryk University (MUNI/A/1398/2021), by financial resources of the Medical Faculty of MU of the Junior Researcher project (Lukáš Moráň, ROZV / 28 / LF / 2020), LM is supported by MH CZ – DRO (MMCI, 00209805). LP is supported by Masaryk University (MUNI/A/1421/2021).

ELECTROCHEMICAL LAMP-BASED ASSAY FOR DETECTION OF RNA BIOMARKERS IN PROSTATE CANCER

LUDMILA MORÁŇOVÁ[a], MICHAL STANÍK[b], ROMAN HRSTKA[a], MARTIN BARTOŠÍK[a]

[a]Masaryk Memorial Cancer Institute, Research Centre for Applied Molecular Oncology (RECAMO), Žlutý kopec 7, 656 53 Brno; [b]Department of Urologic Oncology, Masaryk Memorial Cancer Institute, Žlutý kopec 7, 656 53 Brno
ludmila.moranova@mou.cz

Current molecular diagnostics of a prostate cancer is based on monitoring elevated levels of prostate-specific antigen (PSA) protein in serum. Relying on this single biomarker, however, can lead to unnecessary biopsies, because its increased levels occurs also within non-malignant diseases, such as prostate inflammation. Due to this, PSA has relatively low specificity for determining prostate cancer. Clinically approved long non-coding RNA (lncRNA) biomarker, prostate cancer antigen 3 (PCA3), combined with PSA determination, offers a better distinction between prostate cancer and non-malignant prostate diseases[1]. PCA3 levels are determined from prostate cells which are released in urine after digital rectal examination and compared to PSA mRNA levels[2].

We utilized this clinical feature to develop an electrochemical assay for the determination of PSA and PCA3 on RNA level from urine. We combined LAMP reaction,

which is an isothermal amplification technique showing high sensitivities at constant temperatures and shorter reaction times, with hybridization at magnetic beads and chronoamperometric detection at carbon electrode chips. We reached good sensitivity and specificity for both biomarkers in prostate cancer cell lines. We validated our approach in clinical samples, i.e., urine samples from 11 prostate cancer patients and 7 healthy controls, where we measured both biomarker levels. Their ratios (PCA3/PSA) showed up excellent correlation with clinical data[3].

The work has been supported by Czech Health Research Council, AZV No. NU21-08-00057, MH CZ – DRO [grant number MMCI, 00209805]; BBMRI-CZ no. LM2018125.

REFERENCES

1. Gittelman M.C., Hertzman B., Bailen J., Williams T., Koziol I., Henderson R.J., Efros M., Bidair M., Ward J.F.: J. Urol. *190*, 64 (2013).
2. Groskopf J., Aubin S.M., Deras I.L., Blase A., Bodrug S., Clark C., Brentano S., Mathis J., Pham J., Meyer T., Cass M., Hodge P., Macairan M.L., Marks L.S., Rittenhouse H.: Clin. Chem. *52*, 1089 (2006).
3. Moráňová L., Staník M., Hrstka R., Campuzano S., Bartošík M.: Talanta *238*, 123064 (2022).

HYPERSALINE ENVIRONMENT AS A SOURCE OF NOVEL SPECIES OF HALOPHILIC SULFUR-OXIDIZING BACTERIA

LEA NOSÁĽOVÁ[a], MÁRIA PIKNOVÁ[a], PETER PRISTAŠ[a,b]

[a]Department of microbiology, Institute of Biology and Ecology, Faculty of Science, University of P. J. Šafárik in Košice, Šrobárova 2, 04154 Košice, Slovakia; [b]Institute of Animal Physiology, Centre of Biosciences, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia
lea.nosalova@student.upjs.sk

The sulfur cycle participated significantly in life evolution but even some of modern bacteria specialize in obtaining energy from sulfur oxidation[1]. Sulfur-oxidizing bacteria are confined to sulphide rich environments, usually freshwater springs or deep-sea sediments. Among the chemolithotrophic microorganisms, sulfur oxidizers adapted the most successfully to extreme conditions, such as high salt habitats, because of high energy yields from oxidation of reduced sulfur compounds[2]. In our experiments focused on characterization of halophilic bacteria from salt brine from former salt-mine in Solivar (Prešov, East Slovakia), unexpectedly high diversity of cultivable bacteria was observed. Surprisingly, about 20% of isolates were found to be able of chemolithotrophic growth using sulfur oxidation on thiosulfate medium with 5% of NaCl. During growth on thiosulfate, the isolates formed thin, translucent colonies, some of which produced visible elementary sulfur (nano)particles. A combination of ARDRA-based molecular fingerprinting with 16S rRNA gene sequencing was used for dereplication and subsequent identification of isolates. The studied halophilic isolates were found to belong to four groups of *Gammaproteobacteria*, being related to *Marinobacter, Halomonas, Hydrogenovibiro,* and *Guyparkeria* genera. Low 16S rRNA gene sequence similarity indicated that seven obligately halophilic bacteria identified represent novel species/ genera of sulfur-oxidizing bacteria. Three of these isolates showed the highest 16S rRNA gene sequence similarity (~98%) to a typical halophilic genus *Halomonas*. Three genetically highly similar vibrio-shaped isolates were obtained in pure cultures with *Hydrogenovibrio crunogenus* being identified as their closest relative. The last identified isolate formed a distinct, well-separated branch in phylogenetic tree (Figure 1) with 16S sequence similarity as low as 97 % to bacteria of the genus *Guyparkeria.* Overall, the results clearly indicate that oligotrophic hypersaline habitats harbor a novel and diverse world of extremophilic sulfur-oxidizing bacteria.

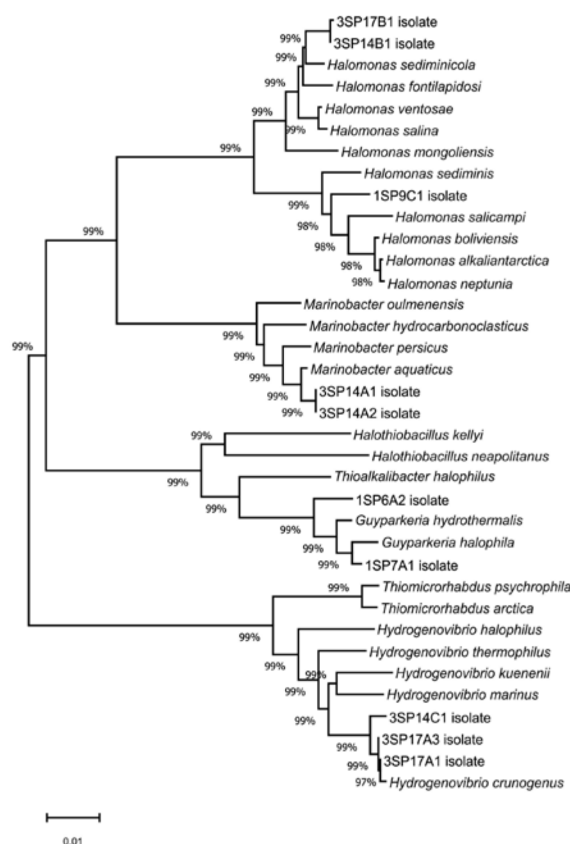


Fig. 3. **Neighbour-joining phylogenetic tree showing relationship of 16S rRNA gene sequences obtained through this study with other closely related sulfur-oxidizing bacteria. Numbers at nodes indicate bootstrap values after 1000 repetitions**

This work was supported by Pavol Jozef Safarik University in Kosice [VVGS-PF-2021-1745].

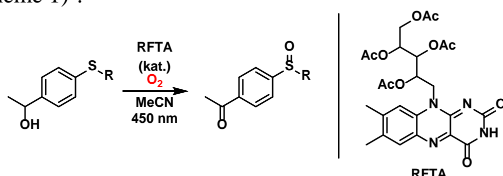
REFERENCES
1. Burini R. C., Kano H. T., Yu Y.: in: *Glutathione in Health and Disease* ch. 2, IntechOpen London, 2018.
2. Sorokin D. Y. in: *Microbial sulfur metabolism*, p. 225. Springer Berlin, 2008.

CHEMOSELECTIVE ANAEROBIC PHOTOOXIDATIONS CATALYZED BY 5-DEAZAFLAVINIUM SALTS

<u>RÓBERT OBERTÍK</u>, RADEK CIBULKA

Department of Organic Chemistry, University of Chemistry and Technology, Prague, Technická 5, Prague 6,166 28
obertiko@vscht.cz

Procedures in modern organic chemistry are focused on highly chemoselective approaches.[1] One of the basic procedures is oxidation based on stoichiometric agents like transition metals, hypervalent iodine complexes etc.[1] Unfortunately, these reactions provide various side products with low chemoselectivity. One of the alternative approaches, aerobic photooxidation catalyzed by flavins is characterized by overoxidation and low tolerance to other functionalities (Scheme 1)[2].

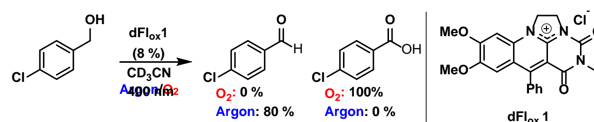


Scheme 1. **Aerobic photooxidation by flavins**

Usually, catalytic photooxidations use oxygen which serves both as a source of oxygen atom and as an agent for photocatalyst regeneration. However, under irradiation, singlet oxygen as well as other reactive particles are formed causing side-products formation[2].

For the first time, analogs of flavins – deazaflavinium salts **dFl$_{ox}$** have been successfully tested on aerobic photooxidations of benzyl alcohols, but also these reactions provided side product of overoxidations (Scheme 1).

Surprisingly, dehydrogenation of benzyl alcohols catalyzed by **dFl$_{ox}$1** have been highly effective also in the absence of oxygen (Scheme 2). The question is: How does deazaflavinium salt regenerate under anaerobic conditions? In the presentation, we introduce novel mechanism of anaerobic photooxidations based on deazaflavinium salt **dFl$_{ox}$1** using suitable solvent as an electron acceptor for regeneration of catalysts. This approach leads chemoselectively to aldehydes. Other application of new system will be discussed as well.



Scheme 2. **Aerobic and anaerobic photooxidations**

We thank, Czech Science Foundation (reg. No. 19-09064S) for their great funding.

REFERENCES
1. Bäckvall J.-E.: *Modern Oxidation Methods.* 2nd ed.; Wiley-VCH Weinheim, 2010.
2. Hassan Tolba A., Krupicka M., Chudoba J., Cibulka R.: Org. Lett. *23*, 6825 (2021).

DESIGN AND SYNTHESIS OF *N*-HYDROXY-CINNAMAMIDE DERIVATES AS NOVEL HDAC INHIBITORS: EVALUATION OF BIOLOGICAL ACTIVITY IN CANCER CELLS

<u>MAGDALÉNA ONUŠČÁKOVÁ</u>[a], HANA PÍŽOVÁ[a], TEREZA KAUEROVÁ[b], MARIE JANKŮJOVÁ[c], DAVID BEDNÁŘ[c], PETER KOLLÁR[b], PAVEL BOBÁĽ[a]

[a]Department of Chemical Drugs, and [b]Department of Pharmacology and Toxicology. Faculty of Pharmacy, Masaryk University, Palackého třída 1946/1, 612 00 Brno; [c]Molecular Modelling and Bioinformatics-Lochmidt Laboratories of Protein Engineering-RECETOX, Faculty of Science, Kamenice 5, 625 00 Brno
onuscakovam@pharm.muni.cz

Histone deacetylases (HDACs) are enzymes involved in the regulation of fundamental cellular processes, such as cell cycle progression, differentiation, and tumorigenesis[1]. The abnormal function of HDACs can induce various and severe human diseases, including cancer, pulmonary disease, and neurodegenerative disorders[1]. HDAC inhibitors (HDACi), which form a complex with the Zn^{2+} ion in the catalytic pocket of enzymes, have considerable anticancer activity[2].

On the basis of the common three-motif pharmacophore model of HDACi, we have designed a series of the *N*-hydroxycinnamamide derivates. We have synthesized compounds with variously substituted anilides as a capping group and hydroxamic acids as a zinc binding group (Fig.1). The antiproliferative activity of the series was investigated in the monocytic leukemia cell line THP-1 and evaluated by WTS-1 analysis. The first registered HDACi, Vorinostat®, was used as a positive control for the detection. The most potent inhibitors are compounds that contain a methyl substituent on the aromatic ring of the anilide with a value of IC_{50} <2 µmol/L. The docking study was performed using AutoDock Vina 1.2.0. program[3]. The predicted binding affinity for HDAC enzymes suggests the high potential of the compounds tested. Biological tests confirmed the results of molecular docking. We hypothesized that the tested substances may share the ability of Vorinostat® to inhibit the activity of

class I and II HDAC enzymes. Therefore, the analysis of changes in the enzymatic activity of HDACs induced by new hydroxamic acid derivates or Vorinostat® was performed using a fluorimetric assay kit.

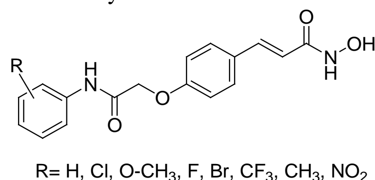


Fig. 1. **Structure of *N*-hydroxycinnamamide derivates**

The study was supported by grants MUNI/IGA/1339/2021 and MUNI/A/1598/2020.

REFERENCES

1. Ho T. C. S., Chan A. H. Y., Ganesan A.: J. Med. Chem. *63*, 12460 (2020).
2. Suk-Youl P., Jeong-Sung K.: Exp. Mol. Med. *52*, 204 (2020).
3. Eberhardt, J., Santos-Martins, D., Tillack F. A., Forli S.: J. Chem. Inf. Model. *61*, 3891 (2021).

NEW WAYS FOR STUDYING FRIZZLED-DISHEVELLED DYNAMIC INTERACTIONS

PETRA PACLIKOVA[a], MIREK MICKA, HANNES ScHIHADA[c], GUNNAR SCHULTE[c], VITEZSLAV BRYJA[a,b]*

[a]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno; [b]Institute of Biophysics, The Czech Academy of Sciences, Brno; [c]Department of Physiology and Pharmacology, Section of Receptor Biology and Signaling, Karolinska Institutet Stockholm, Sweden 394465@mail.muni.cz

The Wnt signalling system includes essential signalling cascades involved in virtually all aspects of embryogenesis and homeostasis in the adult organism, modulating fundamental processes such as proliferation, differentiation, migration and establishment of the cell polarity. The Wnt signalling network comprises of two major distinct modes of signalling: the Wnt/β-catenin pathway and β-catenin independent, so called noncanonical Wnt signalling. Dishevelled (DVL) proteins are key mediators of Wnt-induced pathways and mediate signal processing from receptor-coreceptor complexes (including Frizzled-class receptors-FZDs) to downstream effectors[1].

Despite our growing understanding of DVL proteins, numerous aspects of their biology remain elusive including the main challenge- molecular mechanisms underlying Wnt-FZD-DVL transduction, including ligand recognition, signal initiation and signal selection. In our study we wanted to address this issue by combining two innovative tools: (i) BLItz (BLI-Bio-Layer Interferometry optical technique)[2] and (ii) circularly permuted GFP (cpGFP)[3].

The BLItz approach enabled us to inspect the FZD-DVL interaction following Wnt activation, an event that remains poorly understood despite being essential for Wnt signalling. Moreover, by leveraging a cpGFP sensor-based strategy and live-cell approach, we should be able to map DVL structural rearrangements under various conditions and link them to distinct functional outcomes in living cells. Detailed knowledge of DVL conformation and FZD-DVL interactions will help us to better understand unexplored biology of Wnt signal transduction.

In summary, we would like to present these two innovative methods that can be used for studying dynamic changes and interactions during various biological events in our case the interaction between Frizzled receptors and DVL proteins. We strongly believe that combining both techniques is particularly powerful to detect subtle changes and dynamics that are essential for signal transduction.

This study was supported by the Czech Science Foundation (15-21789S, GA17-16680S), by Masaryk University (MUNI/G/1100/2016), EMBO Short-Term Fellowship 8868.

REFERENCES

1. MacDonald B. T., Tamai K., He X.: Dev. Cell, *17*, 9 (2009).
2. Verzijl D., Riedl T., Parren P., Gerritsen A.: Biosens. Bioelectron. *87*, 388 (2016).
3. Kostyuk A. I., Demidovich A. D., Kotova D. A., Belousov V. V., Bilan D. S.: Int. J. Mol. Sci. *20*, 4200 (2019).

FINDING A RECEPTOR ISOFORM SELECTIVE AND THERMALLY STABLE INSULIN ANALOGUE FOR A BETTER LIFE COMFORT OF DIABETIC PATIENTS

TEREZIE PÁNÍKOVÁ, KATARÍNA MITROVÁ, LENKA ŽÁKOVÁ, JIŘÍ JIRÁČEK*

Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo nám. 2, 166 10 Praha 6 terezie.panikova@uochb.cas.cz, jiri.jiracek@uochb.cas.cz

Insulin elicits its functions through binding to the insulin receptor (IR), which exists in two isoforms. The longer IR-B is predominant in adult hepatocytes (more than 90 %), skeletal muscle and subcutaneous fat (both about 70 % IR-B) 1, while the shorter IR-A is almost exclusively expressed in the brain, lymphatic tissues, or embryo 2. We are aiming to design insulin analogues, which could increase comfort of diabetic patients. First, such an insulin analogue should bind preferentially to IR-B and therefore enable a more physiological control of internal glucose metabolism. Second, thermally more stable insulin could eliminate a constant need for refrigeration of insulin. We systematically designed dozens of insulin analogues based on the known structures of the insulin – receptor complexes. We assessed binding specificities of all analogues through a competitive binding assay with radioactively labelled insulin in cell cultures which

only express IR-A or IR-B. We have chosen several analogues with increased selectivity for IR-B to assess their thermal stability. From these we have selected one analogue for blood glucose lowering test in mice 3. We have filed a patent application for this most promising analogue (PCT/CZ2021/050123).

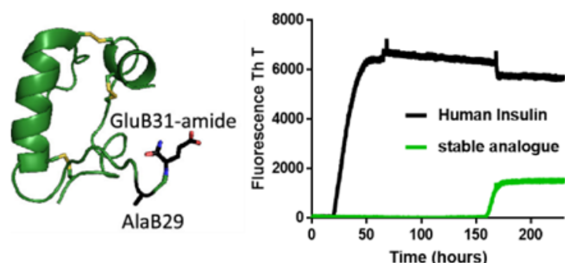


Fig 1. **Left:Structural model of the most promising insulin analogue based on 6SOF structure adjusted with Pymol 3. Right: Example of thermal stability measurement using Thioflavin T (Th T). When insulin precipitates and forms fibrils Th T binds them and emits fluorescence**

REFERENCES

1. Vienberg S. G., Bouman S. D., Sorensen H., Stidsen C. E., Kjeldsen T., Glendorf T., Sorensen A. R., Olsen G. S., Andersen B., Nishimura E.: Biochem. J. *440*, 301 (2011).
2. Westermeier F., Saez T., Arroyo P., Toledo F., Gutierrez J., Sanhueza C., Pardo F., Leiva A., Sobrevia L.: Diabet. Metab. Res. *32*, 350 (2016).
3. Páníková T., Mitrová K., Halamová T., Mrzílková K., Pícha J., Chrudinová M., Kurochka A., Selicharová I., Žáková L, Jiráček J.: J. Med. Chem. *64*, 14848 (2021).

A MILLIFLUIDIC CHIP FOR CULTIVATION OF FISH EMBRYOS AND TOXICITY TESTING FABRICATED BY 3D PRINTING TECHNOLOGY

PETR PANUŠKA[a], ZUZANA NEJEDLÁ [a], JIŘÍ SMEJKAL [a], PETR AUBRECHT [a], MICHAELA LIEGERTOVÁ [a], MARCEL ŠTOFIK [a], JAROMÍR HAVLICA[b,c], JAN MALÝ[a]

[a]Department of Biology and [b]Department of Chemistry, Faculty of Science, University of J.E. Purkynje, 400 96 Usti nad Labem; [c]Czech Academy of Sciences, Institute of Chemical Process Fundamentals, 165 02 Prague petr.panuska@ujep.cz

Fish embryo test (FET) is a very effective toxicity screening method, using the embryos of *Danio rerio* for 96 hour exposure in microtiter plates. Over the last years there has been an effort to develop various systems for a high-throughput zebrafish embryo cultivation and FET testing. We have created a novel simple design of a millifluidic system fabricated mostly with DLP (digital light processing) 3D printing technology and evaluated its functional properties for these purposes. The development and the optimization of the millifluidic chip was performed by CAD (computer-aided design) modelling, based on CFD (computational fluid dynamics) simulations and real experiments. The embryos were reliably trapped inside the system by free sedimentation by gravity. An equal flow around all embryos through two separated channels has been achieved with a single liquid inlet. We developed a mechanism for removal of single selected embryo during cultivation experiments, which was tested as a proof-of-concept. The chips were manufactured by DLP 3D printer, which allowed quick fabrication directly from the CAD and has proven the capability of printing complex inner channel structures with sufficient resolution. The transparency of the printed material was limited, which was solved by the fabrication of the device as a two-component system with transparent foil laminated to the bottom part of the chip. Long-term cultivation experiments have shown normal development of the embryos in the chip. Model toxicity tests were also performed with diluted ethanol. Compared to the standard FET assays, which were executed in parallel, an increased toxic effect of the ethanol was observed. We conclude that our FET chip is suitable for long-term zebrafish embryos cultivations and toxicity testing and has potential to become an alternatve to the standard FET, with further development.

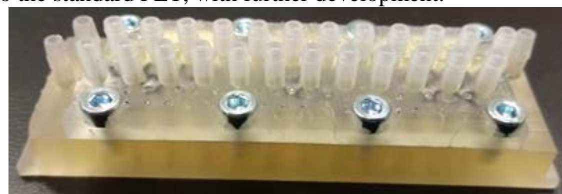


Fig. 1. **Assembled fish embryo test chip with individual embryo removal add-on (without tubing)**

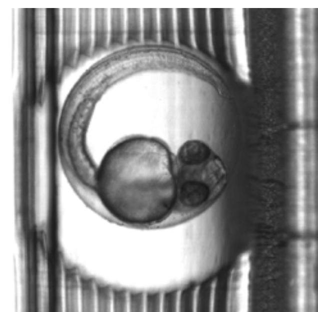


Fig. 2. **Developing fish embryo in one of the cultivation wells of the chip observed with inverse microscope**

Acknowledgements to Research Infrastructure NanoEnviCz (Project No. LM2018124) supported by the Ministry of Education, Youth and Sports CR, the ERDF/ESF project "UniQSurf – Centre of biointerfaces and hybrid functional materials" (No. CZ.02.1.01/0.0/0.0/17_048/0007411), the project No. TJ01000077 of the Technology Agency CR, the project No. UJEP-IGA-TC-2019-53-01-2; UJEP-SGS-2018-53-005-3 and UJEP-SGS-2020-53-006-3.

REFERENCES
1. OECD: *Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2.* OECD (2013). doi:10.1787/9789264203709-en
2. Macdonald N. P., Zhu F., Hall C. J., Reboud J., Crosier P. S., Patton E. E., Wlodkowic D., Cooper J. M.: Lab on a Chip *16*, 291 (2016).
3. Zhu Z., Geng Y., Yuan Z., Ren S., Liu M., Meng Z., Pan D.: Micromachines *10*, 168 (2019).
4. Nejedlá Z., David P., Regina H., Miechaela L., Marcel Š., Jiří S., Šícha V., Pavel K., Jan M.: RSC Adv. *11*, 16252 (2021).

ONE STEP CHEMICAL DERIVATIZATION OF SECRETED CELL METABOLITES FOR SENSITIVE MASS SPECTROMETRY DETECTION

LUKÁŠ PEČINKA[a,b], LUKÁŠ MORÁŇ[c,d], VOLODYMYR POROKH[b,c], MARTINA MARCHETTI-DESCHMANN[e], JOSEF HAVEL[a,b], ALEŠ HAMPL[b,c], PETR VAŇHARA[a,c]*

[a]Faculty of Science, Masaryk University, Brno; [b]International Clinical Research Center, St. Anne's University Hospital Brno, Brno; [c]Faculty of Medicine, Masaryk University, Brno; [d]Research Centre for Applied Molecular Oncology (RECAMO), Masaryk Memorial Cancer Institute, Brno; [e]Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria
436922@mail.muni.cz

Metabolomics studies for biomarker discovery consist of a complete set of metabolites in complex biological systems (databases contain ~ 115 000 metabolites). Metabolome is much smaller than proteome (1 800 000 different proteins) making it relatively simple for data analysis. On the other hand, metabolites are chemically diverse compounds that occur at vide range of concentrations (ten orders of magnitude) which makes metabolomics analysis challenging.

Various chemical derivatization procedures have been extensively studied to enhance ionization efficacy, retention time (chromatography), and shift molecular mass to a higher value (mass spectrometry). Functional groups including aldehyde, primary amines, and carboxylic acid have been targeted using different derivatization reagents. Around 80% of a total number of described metabolites can be derivatized and detected by mass spectrometry using *i.e.* Girard´s reagent T – GT (aldehyde groups), Coniferyl aldehyde – CA (primary amines), and 2-picolylamine – 2-PA (carboxylic acids).

Metabolomics methods are highly used to analyze large-scale phenotypic changes in the cell from intracellular (cell lysate) or extracellular (cell secretome) biological matrices. Cell cultivation process is accompanied by secretion of a broad range of metabolites into the cultivation media (i.e., autocrine and paracrine factors such as immune receptors, cytokines, and hormones) which are characteristic for individual cell types.

Herein, the method for derivatization of various classes of small molecules has been optimized and compared to the detection sensitivity of non-derivatized molecules. The secreted metabolites profile of human embryonic stem cells (hESCs) has been analyzed through multiple derivatized procedures coupled with metabolomics with the aim of a better understanding of hESCs therapeutical effects.

Supported by the Masaryk University: project no. MUNI/A/-1421/2021, MUNI/IGA/1208/2021, MUNI/A/1398/2021, MUNI/IGA/1127/2021 and MUNI/11/ACC/3/2022.

REFERENCE
1. Dueñas M. E., Larson E. A., Lee Y. J.: Front. Plant Sci. *10*, 860 (2019).

INVESTIGATION ON NANOPARTICLE-BASED FORMULATIONS FOR DERMAL DELIVERY OF IMIQUIMOD

ELIŠKA PETROVÁ[a], STANISLAV CHVÍLA[b], MARTIN BALOUCH[b], JARMILA ZBYTOVSKÁ[a]

[a]Department of Organic Technology and [b]Department of Chemical Engineering, UCT Prague, Technická 5, 16628 Prague
Eliska.Petrova@vscht.cz

The immunostimulating agent imiquimod (IMQ) plays a key role in (pre)cancerous skin treatment[1]. Unfortunately, the bioavailability of IMQ delivered via standard formulations (e.g., cream, gel) to deeper skin layers is highly problematic. Therefore, this study focuses on investigation of two novel nanoparticle systems (lipid nanocapsules, nanocrystals) to improve the penetration of IMQ into the skin. Generally, small nanocapsules (up to 1000 nm) with a large surface area allow very close contact with the upper skin layer, the *stratum corneum,* and facilitate the drug absorption into the skin[2]. Increased drug solubility associated with colloidal nanoparticulate systems also significantly improves skin delivery[3].

The nanocrystals were prepared by wet beat milling and nanocapsules by two homogenization steps (shear and high-pressure homogenization). Dynamic light scattering revealed a volume size of 500 and 170 nm for nanocrystals and nanocapsules, respectively. The size and shape of the nanoparticles were proved by transmission electron microscopy. Encapsulation of 99% was achieved for nanocapsules and was confirmed by FTIR[4]. The final systems were integrated into a HPMA-based hydrogel to prolong the drug release and its accumulation in the skin tissue.

In conclusion, the efficiency of the nanosystems was determined in an extensive *in vitro study* on porcine skin against commercial cream.

This work was supported by OP RDE no.: CZ.02.69/0.0/0.0/19_073/0016928, founded by ESF and the Czech Science Foundation (project 19-09600S).

REFERENCES
1. Haque T., Rahman K. M., Thurston D. E., Hadgraft J., Lane M. E.: Eur. J. Pharm. Sci. *77*, 279 (2015).
2. Alkilani A. Z., McCrudden M. T. C., Donnelly R. F.: Pharmaceutics. *7*, 4 (2015).
3. Zeb A., Arif S.T., Malik M., Shah F.A., Din F.U., Qureshi O.S., Lee E.S., Lee G.Y., Kim J.K.: J. Pharm. Investig. *49*, 485 (2019).
4. Pandita D., Kumar S., Poonia N., Lather V.: Food Res. Int. *62*, 1165 (2014).

SPECIALIZED METABOLITES OF ACTINOBACTERIA: NEW BIOLOGIC ACTIVE COMPOUNDS IN TIME OF ANTIBIOTIC CRISIS

MICHAELA PLECHATÁ[a,b], ZDENĚK KAMENÍK[a], JIŘÍ JANATA[a], JAN MASÁK[b]

[a]Institute of Microbiology of the CAS, v. v. i., Vídeňská 1083, 142 20 Prague 4; [b]Department of Biotechnology, UCT Prague, Technická 5, 166 258 Prague 6
michaela.plechata@biomed.cas.cz

An increase in antimicrobial resistance is a global public health threat. It is estimated that antimicrobial resistance is a cause of at least half a million deaths per year worldwide. This number is still growing and predictions say that about ten million deaths will be caused due to antimicrobial resistance in 2050 if this issue is not addressed adequately. WHO (World Health Organisation) classified multidrug-resistant bacteria into three priority groups according to the species and the type of resistance: critical, high, and medium. The most dangerous are carbapenem-resistant pathogenic bacteria including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* according to WHO[1].

Specialized metabolites produced by Actinobacteria have many possible uses. From the discovery of actinomycin, many other antibiotics produced by Actinobacteria were identified, especially from the genus *Streptomyces*. During recent years, researchers focused on the production of new biologically active compounds by Actinobacteria[2]. Literature shows that many specialized metabolites from Actinobacteria were successfully used against some of the antibiotic-resistant bacteria[3,4].

Our goal was to find new biologically active compounds with antimicrobial properties produced by Actinobacteria. The samples of Actinobacteria are from soil collected in various places in Europe. We prepared forty crude extracts from culture medium supernatant using two types of solid-phase extraction techniques to cover as many microbial metabolites as possible. Additional crude extracts were obtained from Thailand: 167 crude extracts from marine Actinobacteria and twelve samples from Actinobacteria grown on plants. Extracts were tested *in vitro* against seven different types of resistant pathogens: *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococci* spp. The 20 active extracts were fractionated by preparative HPLC, and the resulting fractions were tested against the resistant bacteria to reveal the antimicrobial compound among all produced metabolites. LC-MS analysis and GNPS metabolomics platform was used to avoid tedious identification of already known substances. Our main focus was to find a compound active against Gram-negative resistant pathogens and the bioassays showed several soil extracts and eleven Thailand extracts as effective against *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococci* spp.

The work was supported by the SEA-Europe Joint Funding Scheme 4th Joint Call for Proposals (Innovation) in the Thematic Area of Infectious Diseases (including Antimicrobial Resistance [AMR]) Project No. SEAEUROPEJFS19IN-080, by the Lumina Quaeruntur Program No. LQ200202002 from the Czech Academy of Sciences.

REFERENCES
1. WHO: *Antimicrobial Resistance: Global Report On Surveillance*. World Health Organization, Geneva 2014.
2. Anandan R., Dharumadurai D., Manogaran G. P., v knize: *Actinobacteria-Basics and Biotechnological Applications*. Intechopen London, 2016.
3. Mast Y., Stegmann E.: Antibiotics *8*, 105 (2019).
4. Valli S., Suvathi S. S., Aysha O. S., Nirmala P., Vinoth K. P., Reena A.: Asian Pac. J. Trop. Biomed. *2*, 469 (2012).

EXPLORATION OF A MACROMOLECULAR BIOLUMINESCENCE RESONANCE ENERGY TRANSFER COMPLEX BY STRUCTURAL AND KINETIC ANALYSES

DANIEL PLUSKAL[a], ANNA SMRČKOVÁ[a], JIŘÍ DAMBORSKÝ[a,b], ZBYNĚK PROKOP[a,b], MARTIN MAREK[a,b]

[a]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Kamenice 5/C13, 625 00 Brno
[b]International Clinical Research Center, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno
pluskal.daniel@gmail.com

Bioluminescence, the emission of light by a living organism, is a fascinating phenomenon, which finds wide use in numerous biomedical and biotechnological applications. Luciferase from *Renilla reniformis* (RLuc) is one of the most utilized bioluminescent enzymes, notably in ultrasensitive optical bioassays and gene reporter systems[1,2]. RLuc catalyzes the oxidation of its substrate, coelenterazine, leading to an emission of a blue (~480 nm) photon. However, the presence of green fluorescent protein from *R. reniformis* (RGFP) shifts the emission maximum towards green color (~509 nm) while simultaneously increasing the quantum yield of the bioluminescent reaction. A bioluminescence resonance energy

transfer (BRET) from RLuc to RGFP is deduced to be responsible for this effect, but its understanding in molecular terms remains elusive[3].

To fill in this gap, we biochemically reconstituted, crystallized, and solved an RGFP-RLuc complex structure, revealing molecular details of the protein-protein interaction. Specifically, the RGFP controls luciferase partner's active-loop dynamics, suggesting its impact on catalytic efficiency. Indeed, our kinetic measurements showed that the binding of RGFP significantly improves RLuc catalytic parameters and decreases product inhibition. Furthermore, microscale thermophoresis titration was used for the determination of the binding affinity between RGFP and RLuc and showed that RGFP interacts with RLuc in a substrate-independent manner.

Collectively, we report the first structure of the macromolecular BRET complex and its comprehensive biochemical and kinetic analysis. Our findings offer new research directions in bioluminescence research and will help to engineer next-generation BRET systems for both science and society.

This work was supported by the Czech Science Foundation (22-09853S) and GAMU (MUNI/H/1561/2018).

REFERENCES

1. Sadikot R.T., Blackwell T.S.: Proc. Am. Thorac. Soc. *2*, 537 (2005).
2. Tsuboi S., Jin T.: RCS Adv. *9*, 34964 (2019).
3. Ward W.W., Cormier M.J.: Ann. Rev. Biochem. *80*, 2289 (1976).

STRUCTURAL GLIMPSE INTO 14-3-3 PROTEIN DEPENDENT REGULATION OF UBIQUITIN LIGASE NEDD4-2

PAVEL POHL[a], TOMAS OBSIL[b], VERONIKA OBSILOVA[a]*

[a]*Dept. of Structural Biology of Signalling Proteins, Division BIOCEV, Institute of Physiology of the Czech Academy of Sciences, Prumyslova 595, 252 50 Vestec*
[b]*Dept. of Physical and Macromolecular Chemistry, Faculty of Science,Hlavova 8, 128 40 Prague 2*
pavel.pohl@fgu.cas.cz

Neural precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is an E3 ubiquitin ligase that targets proteins for ubiquitination and endocytosis, thereby regulating numerous ion channels, membrane receptors and tumour suppressors. Nedd4-2 activity is regulated by autoinhibition, calcium binding, oxidative stress, substrate binding, phosphorylation and 14-3-3 proteins[1-3]. However, the structural basis of 14-3-3-mediated Nedd4-2 regulation remains poorly understood. Here, we combined several techniques of integrative structural biology to characterize Nedd4-2 and its complex with 14-3-3. We demonstrate that phosphorylated Ser^{342} and Ser^{448} are the key residues that facilitate 14-3-3 protein binding to Nedd4-2 and that 14-3-3 protein binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains. Overall, our findings provide the structural glimpse into the 14-3-3-mediated Nedd4-2 regulation[4] and highlight the potential of the Nedd4-2:14-3-3 complex as a pharmacological target for Nedd4-2-associated diseases such as hypertension, epilepsy, kidney disease and cancer.

This study was supported by the Czech Science Foundation (Project 20-00058S).

REFERENCES

1. Goel P., Manning J. A., Kumar S.: Gene *557*, 1 (2015).
2. He H., Huang C., Chen Z., Huang H., Wang X., Chen J.: Biomed. Pharmacother. *125*, 109983 (2020).
3. Manning J. A., Kumar S., Trends Biochem. Sci. *43*, 635 (2018).
4. Pohl P., Joshi R., Petrvalska O., Obsil T., Obsilova V.: Commun. Biol. *22*, 899 (2021).

CKB IN OSTEOSARCOMA PROGRESSION

JANA POKLUDOVÁ[a], KAMILA ŘÍHOVÁ[a,b], LUCIA KNOPFOVÁ[a,b], PETR BENEŠ[a,b]

[a]*Department of Experimental Biology, Faculty of Science, Masaryk University, 62500 Brno;* [b]*International Clinical Research Centre, St. Anne´s University Hospital, 65691 Brno*
473926@mail.muni.cz

The *CKB* gene encodes one of two cytosolic isoforms of creatine kinase (CK). This enzyme plays a key role in the cell energy metabolism. It is required for immune response and cell cycle regulation. CKB is predominantly expressed in the brain, but its deregulation has been observed in various cancer types including breast cancer and metastatic melanoma. On the other hand, lower CKB expression has been found in colorectal cancer. The expression and functional relevance of this enzyme has never been studied in osteosarcoma, the most common primary bone cancer in children and adolescents.

The aim of this study is to clarify the function of CKB in osteosarcoma progression. As a model system we use human non-metastatic/metastatic osteosarcoma cell line pair SAOS2/SAOS2-LM5. Using CRISPR/Cas9 approach we derived SAOS2-LM5 cells with depleted expression of *CKB*. *In vitro* and *in vivo* experiments were conducted with control and CKB KO cells to identify the effect of CKB on osteosarcoma cell growth, metastasis and sensitivity to chemotherapeutics.

Our results showed higher expression of CKB in metastatic SAOS2-LM5 variant compared to non-metastatic SAOS2 cells. Furthermore, expression of CKB was altered in chemotherapy-resistant SAOS2-LM5 cells. CKB expression correlates with expression of transcription factor c-Myb, recently identified pro-metastatic regulator in osteosarcoma[1]. Depletion of CKB does not affect a proliferation rate of SAOS2-LM5 cells.

Further experiments are ongoing to analyse the effect of CKB depletion on SAOS2-LM5 chemosensitivity and metastatic activity.

Supported by Ministry of Health of the Czech Republic grant NV18-07-00073 and by the European Regional Development Fund - Project ENOCH (CZ.02.1.01/0.0/0.0/16_019/0000868).

REFERENCE

1. Říhová K., Dúcka M., Staniczková Zambo I., Vymětalová L., Šrámek M., Trčka F., Verner J., Drápela S., Fedr R., Suchánková T., Pavlatovská B., Ondroušková E., Kubelková I., Zapletalová D., Tuček Š., Múdry P., Adámková Krákorová D., Knopfová L., Šmarda J., Souček K., Borsig L., Beneš P.: Clin. Exp. Metastasis, in print, DOI: 10.1007/s10585-021-10145-4.

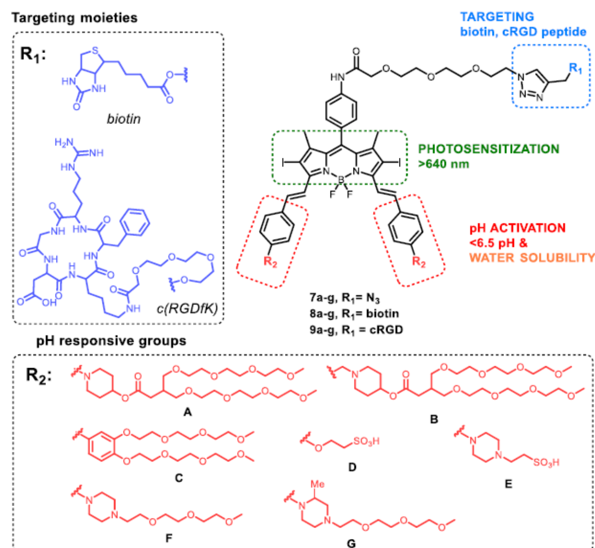
TARGETED AND pH-ACTIVATABLE BODIPY PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

MARTIN PORUBSKÝ[a], JIŘÍ HODOŇ[a], JARMILA STANKOVÁ[b], PETR DŽUBÁK[b], JAN HLAVÁČ[a]

[a]*Department of Organic Chemistry, Faculty of Science, Palacky University, 17. listopadu 1192, 771 46 Olomouc;*
[b]*Institute of Molecular and Translation Medicine, Faculty of Medicine and Dentistry, Palacky University, Hněvotínská 5, 779 00 Olomouc*
martin.porubsky@upol.cz

Photodynamic therapy (PDT) is a clinically-approved cancer treatment that is based on production of cytotoxic reactive oxygen species to induce cell death. However, its efficiency depends on distribution of photosensitizer (PS) and depth of light penetration across tissues[1]. Together with the fact that pathological cancer tissues tend to have lower pH than healthy tissues inspired us to explore dual-targeted pH-activatable photosensitizers based on tunable near-infrared (NIR) boron-dipyrromethene (BODIPY) dyes. Our BODIPY PSs (Scheme 1) were designed to carry three main attributes: (i) biotin or cRGD peptide as cancer cells targeting unit, (ii) piperidyl moiety that is protonated in acidic (pH <6.5) conditions suited for pH-activation of the PS inside cancer cells and (iii) hydrophilic groups enhancing the water solubility of otherwise hydrophobic BODIPY dyes.

Amino moieties can be protonated at lower pH and thus activate PSs for PDT. Piperidyl and piperazinyl groups have the most suitable pKa's for pH activation of the PS inside the cancer cells or tissues which are often more acidic than healthy tissues (Figure 1). Generation of reactive oxygen species is then characterized by triplet state quantum yield (ΦΔ) and was determined with use of 1,3-diphenylisobenzofuran (DPBF). PSs with pH sensitive moieties showed ~2-3 times higher ΦΔ in acidic buffer compared to neutral pH (Table 1). Illumination of such system with suitable LED light source (>640nm) allowed for increased toxicity on HCT116 ($\alpha_v\beta_3$ integrin and biotin positive)[2,3] cells in comparison with A549 ($\alpha_v\beta_3$ integrin negative, biotin positive)[4,5] cancer cells that showed higher IC_{50}. Moreover, tested compounds exhibited no significant dark toxicity compared to the reference PS methylene blue (MB).



Scheme 1. **Targeted and pH-activatable BODIPY photosensitizers**

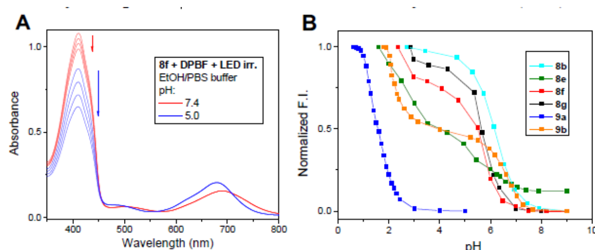


Fig. 1. **(a) Determination of singlet oxygen quantum yield (ΦΔ) of compound 8f by DPBF method and (b) determination of pKa values by fluorescence titration in HEPES buffer**

Table 1. **Triplet quantum yields (Φ_Δ) of selected compounds. As a reference methylene blue was used ($\Phi_{\Delta R}$ = 0.52 in acetonitrile)**

Compound	ΦΔ pH 7.4	ΦΔ pH 5.0
7a	0.5	0.45
7f	0.08	0.17
8a	0.09	0.08
8c	0.44	0.43
8f	0.08	0.2
8g	0.08	0.17
9a	0.09	0.09
9c	0.5	0.49
9f	0.08	0.17
9g	0.1	0.18
MB	0.52	0.52

Authors are grateful for the financial support from Czech Ministry of Education, Youth, and Sports (projects IGA_PrF_2021_024, IGA_LF_2021-038).

REFERENCES

1. Callaghan S.: Photochem. Photobiol. Sci. *17*, 1490 (2018).
2. Jinushi M., Chiba S., Baghdadi M., Kinoshita I., Dosaka-Akita H., Ito K., Yoshiyama H., Yagita H., Uede T., Takaoka A.: Cancer Res. *72*, 56 (2012).
3. Babak M. V., Plazuk D., Meier S. M., Arabshahi H. J., Reynisson J., Rychlik B., Błauz A., Szulc K., Hanif M., Strobl S., Roller A., Keppler B. K., Hartinger C. G.: Chem. Eur. J. *21*, 5110 (2015).
4. Shaw S. K., Schreiber C. L., Roland F. M., Battles P. M., Brennan S. P., Padanilam S. J., Smith B. D.: Bioorg. Med. Chem. *176*, 139 (2017).
5. Jung D., Sukhendu M., Lee J. H., Lee J. H., Kim J. S.: Chem. Commun. *50*, 3044 (2014).

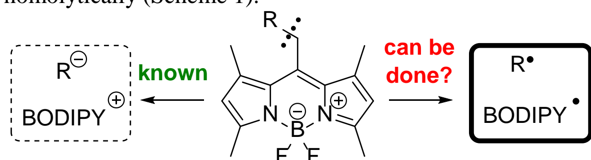
IS IT POSSIBLE TO RELEASE RADICAL SPECIES FROM BODIPY SCAFFOLDS?

ANNA PORYVAI, TOMÁŠ SLANINA*

IOCB CAS Prague, Flemingovo nám. 2, 160 00 Prague
anna.poryvai@uochb.cas.cz

BODIPY dyes are known for their application in imaging or as photocatalysts. Furthermore, they can be used as photoremovable protecting groups (cages). It has been previously shown that the process of uncaging from BODIPY scaffolds occurs through light-induced S_N1 reaction[1,2].

In this presentation, I would like to discuss whether the bond between cargo and a BODIPY cage can also be cleaved homolytically (Scheme 1).



Scheme 1. **Light-induced photorelease from a BODIPY scaffold**

I will present the evidence of the previously non-described radical pathway of uncaging, its efficiency, and required conditions. Furthermore, I will present the utilisation of this mode of reactivity in material science and fluorescent labelling.

The authors acknowledge Dr Ján Tarábek for EPR measurements and the Czech Science Foundation, project Nr. 22-20319S.

REFERENCES

1. Slanina T., Shrestha P., Palao E., Kand D., Peterson J. A., Dutton A. S., Rubinstein N., Weinstain R., Winter A. H., Klán P.: J. Am. Chem. Soc. *139*, 15168 (2017).
2. Peterson J. A., Wijesooriya C., Gehrmann E. J., Mahoney K. M., Goswami P. P., Albright T. R., Syed A., Dutton A. S., Smith E. A., Winter A. H.: J. Am. Chem. Soc. *140*, 7343 (2018).

MICROFLUIDIC TISSUE BARRIERS

DAVID POUSTKA, PETR PANUŠKA, JAN MALÝ, MARCEL ŠTOFIK

Jan Evangelista Purkyně University in Ústí nad Labem, Pasteurova 3544/1, 400 96 Ústí nad Labem
david.poustka@ujep.cz

As current drug testing methods are related to both technical and ethical difficulties, ever growing demand for faster and more effective drug evaluating processes comes with the need for a different approach. Functional tissue models or whole organs-on-chips seem to be promising alternatives to currently widely used animal models[1,2].

The key aspect of creating mentioned substitutes lies in specific cell cultivations taking place in precisely controlled microenvironments achieved via microfluidics. Namely the development of so-called tissue barriers is of great importance, because they represent a place where drugs and active agents pass through endothelium from a bloodstream into the tissues[1]. Mimicking these tissue barriers is crucial for the advancement in tissue models and improved drug testing.

We are working on two approaches to creating an artificial, microfluidic endothelium tissue barrier. In the first approach, a polymeric platform is 3D printed from photosensitive resin E-Shell 300 via stereolithography and nanofibers combining modified chitosan, gelatine and polyethylene oxide are electrospun straight onto its surface. Created nanofibrous membrane separates two channels of a chip and acts as a matrix for growth of cells introduced after final assembly. In the second approach, we utilize standard lithographic techniques to create a system of microchannels from off-stoichiometry thiol-ene chemistry (OSTE). Prepared channels are then partially filled by hydrogel that both creates a structural body of a tissue barrier and supports cell growth.

Our goal is to create a functional platform that will be utilized for the future development of tissue models and organs-on-chips.

Project UJEP-SGS-2020-53-006-3 was supported by a grant within the Student grant competition at UJEP.

REFERENCES

1. Polini A., Prodanov L., Bhise N. S., Manoharan V., Dokmeci M. R., Khademhosseini A.: Expert Opin. Drug Discov. *9*, 335 (2014).
2. Carvalho M. R., Lima D., Reis R. L., Correlo V. M. Oliveira J. M.: Trends Biotechnol. *33*, 667 (2015).

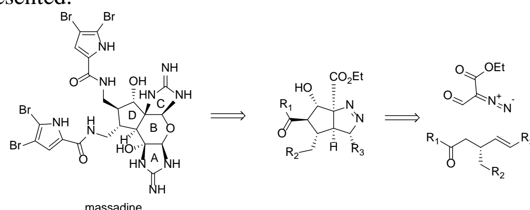
TOWARDS THE SYNTHESIS OF MASSADINE

LADISLAV PRENER, ULLRICH JAHN

Institute of Organic Chemistry and Biochemistry, CAS, Flemingovo nám. 542/2, 160 00 Prague 6
ladislav.@uochb.cas.cz

The pyrrole-imidazole family of alkaloids is a diverse class of over 200 marine natural products with a wide spectrum of biological activities[1]. The structural complexity of these nitrogen- and halogen-rich alkaloids is forged from a single biogenic precursor, oroidin, by various cyclization, dimerization and tetramerization modes. Massadine, a hexacyclic member of the [3+2] dimeric subgroup of pyrrole-imidazole alkaloids with a dense array of functionalites and stereocenters, displays potent antifungal and neurotoxic activity[2,3]. Thus, pracitical methods that allow rapid access to massadine and its analogues are required.

The shortest synthesis of the carbocyclic core of massadine is reported, which exploits an unprecedented tandem aldolization/[3+2] cycloaddition yielding bicyclic pyrazolines. Moreover, the conversion of a model bicyclic pyrazoline to the C,D-ring spirocyclic subunit of massadine is presented.



Scheme 1. **Retrosynthetic analysis of massadine**

We thank the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences for funding.

REFERENCES

1. Lindel T., in: *The Alkaloids: Chemistry and Biology*, chapter 3, p. 117, Academic Press, Cambridge 2017.
2. Nishimura S., Matsunaga S., Shibazaki M., Suzuki K., Furihata K., van Soest R. W. M., Fusetani N.: Org. Lett. *5*, 2255 (2003).
3. Bickmeyer U., Grube A., Klings K.-W., Köck M.: Toxicon *50*, 490 (2007).

PROSTAT, GLIOBLASTOMA AND MAMMARY CARCINOMA CELLS DERIVED EXOSOMES: THEIR ISOLATION, CHARACTERIZATION AND LOADING WITH DOXORUBICIN

MICHAELA PRŮŠOVÁ, OLGA ŠEBESTOVÁ JANOUŠKOVÁ, MICHAELA KOCHOLATÁ

Jan Evangelista Purkyně University in Ústí nad Labem, Faculty of Science, Purkyňova 15, Ústí nad Labem, 400 96
michaela.prusova@ujep.cz

Exosomes (EXs) derived from the plasmatic membranes of cells are important indicators of physiological and pathological processes of the organism as well as individual tissues and cells. EXs can occur in various of body fluids and are significant for transport of informatory and regulatory molecules such as protein, lipids microRNAs or they can function as vectors in pathogenesis. Because of that EXs are considered an additional mechanism for intercellular communication and can fundamentally influence the development of metastases in cancer or the progression of degenerative changes, modulation of immune responses. At the same time, EXs are widely studied for their ability to transport embedded regulatory molecules or drugs and can be considered as an alternative to traditional nanoparticle approaches in drug delivery systems. EXs can cross the blood-brain barrier and are therefore interesting transporters for central nervous system (CNS) therapies. The production and composition of EXs depending on therapeutic approaches is still a little studied area that can provide essential information about intercellular communication during therapy. An unknown topic is also the modulation of EXs after interaction with nanomaterials, the transfer of nanomaterials through EXs, and also the possibility of transporting EXs with nanomaterials to the CNS across the blood-brain barrier. Given the widespread field of nanomedicine and the possibilities that nanomaterials offer, such as targeted drug delivery, minimizing the side effects of drugs themselves, or optimizing drug circulation in the body, the transport of nanomaterials via EXs is an important experimental goal[1-3].

Here we present three types of EXs which were isolated from medium of MCF7, PC3 and U87 human cell lines. First, EXs was succesfully isolated using ultracentrifugation. The particle sizes and count of EXs was charaterized by Nanoparticle tracking analysis and Dynamic light scattering. Amount of total and surface protein contain in EXs was measured by BCA assay. Last but not least, protein markers occuring in EXs (CD81, HsP70 etc.) was detected using Western blotting. Second, EXs were loaded with doxorubicin using a simple incubation method. The succes of doxorubicin loading by EXs was measured using High performance liquid chromatography.

The isolation and characterization of EXs from various sources open the potential to future loading of exosomes and their application as natural based nanotherapeutic for the treatment of brain cancer.

REFERENCES

1. Julien S.-P., Gosselet F., Duban-Deweer S., Pottiez G., Karamanos A. Y.: Cells *9*, 1 (2020).
2. Druzhkova T. A., Yakovlev A. A.: Neurochem. J. *12*, 195 (2018).
3. Théry C., Amigorena S., Raposo G., Clayton A.: Curr. Protoc. Cell Biol. *30*, Chapter 3, Unit 3.22 (2006).

c-MYB-DRIVEN TRANSCRIPTIONAL PROGRAM MODULATES OSTEOSARCOMA PROGRESSION

KAMILA ŘÍHOVÁ[a], FILIP TRČKA[a,b], LUCIA KNOPFOVÁ[a,b], PETR BENEŠ[a,b]

[a]*Department of Experimental Biology, Faculty of Science, Masaryk University, 62500 Brno;* [b]*International Clinical Research Centre, St. Anne's University Hospital, 65691 Brno*
rihova.kam@mail.muni.cz

The transcription factor c-Myb is an oncoprotein that contributes to malignant transformation by promoting cell proliferation and survival when aberrantly activated/expressed. Its overexpression has been found in leukemias, breast, colon and adenoid cystic carcinoma. Recently, we elucidated the role of c-Myb in experimental metastatic process of osteosarcoma (OSA) cell lines and identified this protein as a negative prognostic factor for high-grade OSA patients as well[1]. Next, we aimed to clarify the c-Myb-directed transcriptional program involved in OSA progression.

We performed RNA sequencing of control and *MYB* KO SAOS-2 LM5 and 143B OSA cell lines. We identified 149 down- and 103 upregulated genes common for both *MYB* KO cell lines and characterized them functionally using Over Representation Analysis. The common set of c-Myb-relevant targets was enriched for genes involved in cellular stress response, protein localization, cell adhesion, endoplasmic reticulum-associated protein degradation (ERAD) and Wnt signalling. Moreover, we investigated c-Myb-relevant network interrelations between upstream regulators and downstream effects by Ingenuity Pathway Analysis.

As analysis of transcriptomic data suggested possible regulation of Wnt pathway and protein degradation by c-Myb in OSA, we performed *in vitro* validation using highly aggressive 143B OSA cell line. We found that c-Myb KO inhibits and c-Myb overexpression stimulates Wnt signalling, thus confirming the important relationship between these pathways in OSA. Although we detected higher level of BiP protein (an ERAD marker) in *MYB* KO cells, substrate-degradation analyses did not reveal any important modulatory effect of c-Myb on ERAD in OSA cells.

We conclude that transcriptomic analysis and identification of c-Myb-Wnt signalling axis contributed to a more detailed annotation of the c-Myb-driven OSA progression mechanism.

Supported by Ministry of Health of the Czech Republic grant NV18-07-00073 and by the European Regional Development Fund - Project ENOCH (CZ.02.1.01/0.0/0.0/16_019/0000868).

REFERENCE

1. Říhová K., Dúcka M., Staniczková Zambo I., Vymětalová L., Šrámek M., Trčka F., Verner J., Drápela S., Fedr R., Suchánková T., Pavlatovská B., Ondroušková E., Kubelková I., Zapletalová D., Tuček Š., Múdry P., Adámková Krákorová D., Knopfová L., Šmarda J., Souček K., Borsig L., Beneš P.: Clin. Exp. Metastasis, in print, DOI: 10.1007/s10585-021-10145-4.

THE ROLE OF CYTOKININ SIGNALING PATHWAY IN THE REGULATION OF EPIGENETIC PROCESSES IN PLANTS

JIŘÍ RUDOLF[a,b], LUCIA BAĎUROVÁ[b], JAN SKALÁK[b], JAN HEJÁTKO[b]

[a]*Department of Biochemistry, Faculty of Science and*
[b]*CEITEC, Masaryk University, Kamenice 753/5, 625 00 Brno*
484194@mail.muni.cz

Cytokinins are key plant hormones that affect the growth of a plant ranging from germination to seed. Furthermore, cytokinins enable precise adaptation of a plant to ever-changing environment. This process is a very complex phenomenon that is manifested on many levels involving changes in cytokinin-mediated gene expression[1] and crosstalk with epigenetic regulations[2].

Chromatin remodelling is one of the most important epigenetic processes which regulates chromatin accessibility to promote gene expression. The main effectors of these processes are DNA modifications and posttranslational modifications of histones, especially methylation and acetylation[3].

The aim of this project is the characterization of potential reader of histone methylation code in *Arabidopsis thaliana*, homeobox protein *HAT3.1*, and its putative regulation by cytokinin signalling pathway.

HAT3.1 transcription was shown to be upregulated after exogenous application of cytokinins. This induction was shown to form a response wave, which was correlated with the overexpression of cytokinin signalling genes. To decipher the mechanism of cytokinin-mediated control over *HAT3.1* expression, collection of *Arabidopsis* mutant lines in *type-B ARABIDOPSIS RESPONSE REGULATORS* (*ARRs-B*) responsible for cytokinin signal transduction and regulation of gene expression was screened.

HAT3.1 was also predicted to directly interact with *ARRs* on a protein level. Thus, the putative interactions between HAT3.1 and ARRs were tested using Y2H assay. Our data will be used to establish a mechanistic model of cytokinin-mediated regulation of epigenetic reader *HAT3.1*.

This work was supported by a grant for an excellent diploma thesis (Proposal MUNI/C/0051/2022) and from European Regional Development Fund-Project „SINGING PLANT“ (No. CZ.02.1.01/0.0/0.0/16_026/0008446).

REFERENCES

1. Kieber J. J., Schaller G. E.: Development *145*, dev149344 (2018).
2. Potter K. C., Wang, J., Schaller G. E., Kieber J. J.: Nat. Plants *4*, 1102 (2018).
3. Pikaard C. S., Mittelsten Scheid O.: Cold Spring Harbor Perspect. Biol. *6*, a019315 (2014).

GALECTIN-3 AS A POTENTIAL TARGET FOR REVERSING VASCULAR REMODELING IN HYPOXIC PULMONARY HYPERTENSION

ANTONIN SEDLAR[a], KATERINA POKORNA[a], JANA MUSILKOVA[a], VLADIMIR KREN[b], FRANTISEK KOLAR[a], LUCIE BACAKOVA[a]

[a]Institute of Physiology of the Czech Academy of Sciences and [b]Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, 142 20 Praha 4 – Krc
antonin.sedlar@fgu.cas.cz

Pulmonary hypertension is a life-threatening disease, which affects the pulmonary blood vessels and the heart. Its pathophysiology is characterized by progressively increasing pulmonary vascular resistance and remodeling, leading to right ventricular hypertrophy and eventually to heart failure. Chronic hypoxia is an important pathogenetic factor in the development and progression of pulmonary hypertension. A long-term and/or repeated hypoxia leads to remodeling of blood vessels, i.e. thickening and increased muscularity of their walls[1,2].

Galectin-3 (Gal-3) is a β-galactosyl-binding protein that interacts specifically with carbohydrate ligands on other molecules[3]. This pleiotropic molecule has a wide range of positive and negative effects *in vitro* and *in vivo*. It is also involved in the onset and progression of cardiovascular diseases, namely heart failure, atherosclerosis, and systemic and pulmonary hypertension[4,5]. Moreover, Gal-3 has been shown al-3 to be a strong and independent prognostic biomarker of pulmonary hypertension regardless of etiology[6,7]. However, the available data regarding its potential role in this devastating disease are limited.

Therefore, the aim of our project is to elucidate the effect of Gal-3 and hypoxia on vascular smooth muscle cell (VSMC) behaviour *in vitro*. Our results show that recombinant galectin-3 supresses VSMC growth and triggers apoptosis. The apoptosis is inhibitable with oligosaccharide Gal-3 ligands. Interestingly, long-term hypoxia induces the expression of Gal-3 and specific markers of VSMCs. Further experiments will focus on the development of novel potent Gal-3 inhibitors with ability to attenuate the biological effect of Gal-3.

This work is supported by the Grant Agency of the Czech Republic (Grant no. 22-00317S).

REFERENCES

1. Bacakova L., Travnickova M.: *Muscle Cell and Tissue*, Kunihiro, S., Ed. IntechOpen: London, 2018.
2. Wu Z., Geng J.: Pulm. Circ. *10*, 2045894020974919 (2020).
3. Johannes L., Jacob R., Leffler H.: J. Cell Sci. *131*, jcs208884 (2018).
4. Barman S. A., Li X., Haigh S., Kondrikov D., Mahboubi K., Bordan Z., Stepp D. W., Zhou J., Wang Y., Weintraub D. S., Traber P., Snider W., Jonigk D., Sullivan J., Crislip G. R., Butcher J. T., Thompson J., Su Y., Chen F., Fulton D. J. R.: Am. J. Physiol. Lung Cell. Mol. Physiol. *316*, l784 (2019).
5. Hatano Y., Okada H.: Biomolecules *10*, 1277 (2020).
6. Tian L., Chen K., Cao J., Han Z., Wang Y., Gao L., Fan Y., Wang C.: Mol. Med. Rep. *15*, 3840 (2017).
7. Mazurek J.A., Horne B.D., Saeed W., Sardar M. R., Zolty R.: Heart Lung Circ. *26*, 1208 (2017).

STEROID PROFILING IN HUMAN PLASMA, PROSTATE AND TESTICULAR TISSUE

MARKÉTA ŠIMKOVÁ[a,b], LUCIE KOLÁTOROVÁ[b], PAVEL DRAŠAR[a], JANA VÍTKŮ[b]

[a]University of Chemistry and Technology, Technická 5, 166 28 Prague; [b]Institute of Endocrinology, Národní 8, 116 94 Prague
simkovam@vscht.cz

The development of a robust and rapid method with simple sample preparation for the analysis of steroids of C_{18}-, C_{19}-, C_{21}- families is of interest for many research groups. Regarding mapping of steroid hormones in the prostate and testes in connection to the pathophysiological condition, it is known that their circulating levels may not fully reflect the situation in the tissue. Therefore, the measurement of intraprostatic and intratesticular steroid concentrations is of crucial importance.

We developed and validated a novel LC-MS/MS method for the simultaneous quantification of 32 steroid hormones in human plasma and optimized this method for the analysis in prostate and testicular tissue. This method allows the quantification of 22 steroids without any derivatization and another 10 steroids derivatized with 2-fluoro-1-methyl-pyridinium *p*-toluenesulfonate. As both analyses are performed under the same spectrometric and chromatographic conditions, simultaneous profiling in a single analytical run is possible. The separation of steroids is conducted using a C18 column with a gradient elution consisting of methanol and water with the addition of 0.1% formic acid. The mass spectrometer is operated in positive ESI mode. Using this method, it is possible to analyse two C_{18}- steroids (estrone, estradiol), nineteen C_{19}- steroids (testosterone, epitestosterone, dihydrotestosterone, 11-ketodihydrotestosterone, 11β-hydroxyandrostenedione, 11β-hydroxytestosterone, 11-ketotestosterone, dehydroepiandrosterone (DHEA), 7α-hydroxyDHEA, 7β-hydroxyDHEA, 7-ketoDHEA, androsterone, epiandrosterone, androstenedione, androstenediol, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5β-androstane-3α,17β-diol, 5β-androstane-3β,17β-diol), and eleven C_{21}- steroids (cortisol, 21-deoxycortisol, 11-deoxycortisol, cortisone, corticosterone, 11-deoxycorticosterone, pregnenolone, 17-hydroxypregnenolone, progesterone, 17-hydroxyprogesterone, 5α-dihydroprogesterone). The accuracy, intra- and inter-day precision values, as well as stability tests, were in accordance with FDA Guidelines. The lower limits of quantification in plasma range from 0.017 to 7.32 ng/mL. The method was optimized for the analysis of steroids in prostate and testicular tissues. Different

tissue homogenization approaches and steroids extraction were tested, the optimized methodology will be presented.

This method will be a useful tool in investigating the mechanisms of steroid-related diseases.

Supported by projects NU21J-01-00040 from Czech Ministry of Health (MH) and by MH CZ – DRO, EÚ, 00023761.

SOLID-STATE VIBRATIONAL CIRCULAR DICHROISM FOR IDENTIFYING COCRYSTAL STRUCTURES

ADAM SKLENÁŘ[a], LUCIE RŮŽIČKOVÁ[c], JAKUB KAMINSKÝ[b], PETR BOUŘ[b]

[a]Dept. of Analytical Chemistry, University of Chemistry and Technology, Prague; [b]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Science; [c]Imperial College London
adam.sklenar@uochb.cas.cz

To ensure consistent quality of medicaments, various analytical methods are utilized to characterize them. Most of drugs are currently manufactured as solids[1], and detailed knowledge and control of a particular structural form is needed to ensure desired pharmacokinetic and pharmacodynamic properties.

Techniques, that are commonly used to obtain information about solids, are often destructive or provide incomplete structural description. X-ray diffraction is capable to determine the atomic and molecular structure of solids, but it is costly, time-consuming, and requires a considerable degree of expertise. Therefore, we investigate the potential of vibrational circular dichroism (VCD) spectroscopy in solid-state. It appears that it overcomes some of the drawbacks, while also being highly sensitive to small changes in conformation and molecular packing in the solid phase[2].

In particular, we use VCD to distinguish between materials composed of two different chiral compounds (cocrystals) and, thereby, identify their unique crystalline structures. We prepared three cocrystals of L-malic and L-tartaric acid as described before[3]. Their structures were confirmed by differential scanning calorimetry (DSC), solid-state NMR, and powder X-ray diffraction. High-quality and reproducible VCD spectra of all three cocrystals were then recorded, simultaneously with corresponding IR spectra (Fig. 1). We also simulated the observed spectral features by quantum chemical methods. The calculated results were analysed to understand mutual interactions among molecules in the unit cell and across the whole crystal.

While IR spectra indicate no significant differences among the three forms, the cocrystals may be better distinguished using VCD (Fig. 2). Our results therefore suggest that solid-state VCD represents a cost-effective and easy-to-use structural probe for the identification of crystalline structures with a potential use in supramolecular chemistry or pharmaceutical development.

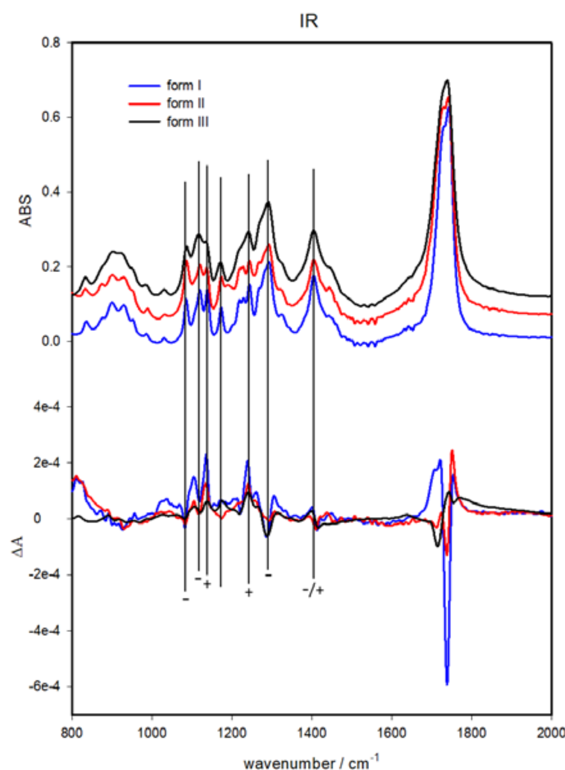


Fig. 1. **Experimental IR (top) and VCD (bottom) spectra of three studied cocrystals**

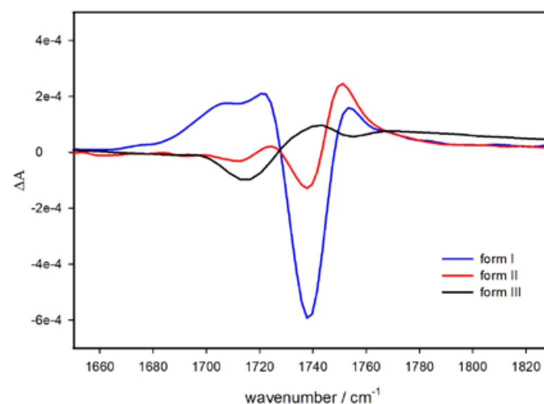


Fig. 2. **Experimental VCD spectra of three studied cocrystals, the C=O stretching region only**

The work is supported by the Czech Ministry of Education, Youth and Sports (grant No. LTAUSA18085).

REFERENCES

1. Karpinski P.H.: Chem. Eng. Technol. *29*, 233 (2006).
2. Rode J.E., Lyczko K., Kosińska K., Metalińska J., Dyniewicz J., Misicka A., Dobrowolski J.C. Lipiński P.F.J.: Spectrochim. Acta A *269*, 120761 (2022)
3. Eddleston D. M., Arhangelskis M., Friščić T, Jones W.: Chem. Commun. *48*, 11340 (2012).

DETECTION OF CANCER BIOMARKERS USING IMMUNOASSAYS WITH UPCONVERSION NANOPARTICLES AND MAGNETIC PRECONCENTRATION

DOROTA SKLENÁROVÁ[a,b], EKATERINA MAKHNEVA[a], JULIAN C. BRANDMEIER[a], HANSHEINER GORRIS[a], ZDENĚK FARKA[a,b]

[a]Department of Biochemistry, Faculty of Science and [b]CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno
sklenarova@mail.muni.cz

Prostate cancer is the major cause of death in the male population caused by oncologic diseases[1]. Consequently, sensitive detection of disease biomarkers, e.g., prostatespecific antigen (PSA), is essential for early-stage diagnosis and effective treatment[2]. In clinical analysis, immunochemical methods such as enzyme-linked immunosorbent assay (ELISA) are widely used[3]. However, conventional ELISA is often not sensitive enough for earlystage diagnosis, resulting in the need for alternative approaches.

Photon-upconversion nanoparticles (UCNPs) are unique for their anti-Stokes luminescence, because NIR excitation results in the emission of UV/vis light without optical background interference due to autofluorescence. Furthermore, the surface of UCNPs can be modified with a variety of biomolecules such as streptavidin or antibodies. These labels can be used in various immunoassay formats such as the upconversion-linked immunosorbent assay (ULISA)[3]. However, to improve the sensitivity even further, it is necessary to search for new methods. Magnetic microparticles (beads) are a promising alternative for microtiter plate-based immunoassays. Their superparamagnetic properties allow for analyte preconcentration, thus improving the assay sensitivity.

We have developed an upconversion-based sandwich immunoassay using magnetic preconcentration for the detection of PSA. Our recent results have reached a limit of detection in the order of 1 pg/mL, which is approximately 20-fold better compared to an ELISA using the same immunoreagents. The higher sensitivity demonstrates the potential of using magnetic microparticles combined with UCNPs for the immunochemical detection of cancer biomarkers.

REFERENCES

1. Mickert M. J., Farka Z., Kostiv U., Hlaváček A., Horák D., Skládal P., Gorris H. H.: Anal. Chem. *91*, 9435 (2019).
2. Farka Z., Mickert M. J., Pastucha M., Mikušová Z., Skládal P., Gorris H. H.: Angew. Chem. Int. Ed. *59*, 10746 (2020).
3. Brandmeier J.C., Raiko K., Farka Z., Peltomaa R., Mickert M. J., Hlaváček A., Skládal P., Soukka T., Gorris H. H.: Adv. Healthc. Mater. *10*, 2100506 (2021).

POLYPYRROLE-DERIVED LOW MOLECULAR WEIGHT SUBSTANCES INDUCE NEUROGENESIS IN EMRYONIC STEM CELLS

KATEŘINA SKOPALOVÁ[a], KATARZYNA ANNA RADASZKIEWICZ[b], JIŘÍ PACHERNÍK[b], JAROSLAV STEJSKAL[c], VĚRA KAŠPÁRKOVÁ[a,d], PATRYCJA BOBER[c], ZDENKA CAPÁKOVÁ[a], MARIÁN LEHOCKÝ[a,d], PETR HUMPOLÍČEK[a,d]

[a] Centre of Polymer Systems, Tomas Bata University, 760 01 Zlín; [b]Department of Experimental Biology, Faculty of Science, Masaryk University, 625 00 Brno; [c]Institute of Macromolecular Chemistry ASCR, 162 06 Prague 6; [d]Faculty of Technology, Tomas Bata University, 760 01 Zlín
skopalova@utb.cz

Conductive polymers (CP) are used as biomaterials in tissue engineering due to their conductivity, biocompatibility, relatively easy of synthesis and their low cost[1]. An advantageous aspect of CP is the combination of electronic and ionic conductivity. The ability to convert electrical signals to ionic (and vice versa) plays a crucial role in creating an active biointerface[2]. The effect of CP conductivity is interesting in connection with the stimulation of electrosensitive tissues such as nervous or cardiac.

Polypyrrole (PPy) is one of the most discussed representatives of CP in tissue engineering. There are many studies where PPy in combination with electric current affects the behavior of cells[3,4]. However, the PPy precursor, heterocycle pyrrole, also has very interesting properties, as the pyrrole molecule is present in many therapeutic agents. Pyrrole derivatives serve as pharmacologically active substances and clearly influencing cellular behavior[5].

The effect of PPy extracts on embryonic stem cell (ESC) (line ES R1) neurogenesis was investigated here. Specifically, ESC-derived embryoid bodies (EB) were cultured in the presence of different concentrations of PPy extract. EB formed in the presence of 5% extract created the most nerve processes (Figure 1).

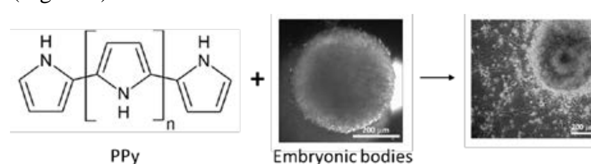


Fig.1. **Formation of EB in the presence of PPy extract**

Furthermore, the expression of genes associated with early neurogenesis, namely Pax6, Sox1, and MASH1, was measured in these EB. In all cases, higher expression was measured in EB formed with PPy extract than in the reference EBs. Similarly, the expressions of neurospecific proteins (specifically Ncadherin, β-III-tubulin, N-CAM, doublecortin) increased in comparison with the reference.

Neurogenesis in EB is probably related to the presence of PPy oligomers in the extracts. The presence of low molecular weight substances was verified by mass

spectrometry. Cellular behavior is therefore influenced by these compounds, but determining exactly which substances are involved will be the subject of further studies.

This work was by the internal grant agency of Tomas Bata University in Zlín (IGA/CPS/2022/001) and by the Ministry of Education, Youth and Sports of the Czech Republic-DKRVO (RP/CPS/2022/001).

REFERENCES

1. Lakard B.: Appl. Sci. *10*, 6614 (2020).
2. Humpolicek P., Kasparkova V., Pachernik J., Stejskal J., Bober P., Capakova Z., Radaszkiewicz K. A., Junkar I., Lehocky M.: Mater. Sci. Eng. C, Mat. Biol. Appl. *91*, 303 (2018).
3. Gilmore K. J., Kita M., Han Y., Gelmi A., Higgins M. J., Moulton S. E., Clark G. M., Kapsa R., Wallace G. G.: Biomaterials *30*, 5292 (2009).
4. Richardson R. T., Thompson B., Moulton S. E., Newbold C., Lum M. G., Cameron A., Wallace G. G., Kapsa R., Clark G., O'Leary S.: Biomaterials *28*, 513 (2007).
5. Ahmad S., Alam O., Naim M. J., Shaquiquzzaman M., Alam M. M., Iqbal M.: J. Med. Chem. *157*, 527 (2018).

REVERSIBILITY IN PHOTOCHEMISTRY: THERE AND BACK AGAIN

TOMÁŠ SLANINA

Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 524/2, 166 10 Praha 6, Czech Republic
slanina@uochb.cas.cz

Compounds that can be interconverted between two forms, **A** and **B**, reach a chemical equilibrium dictated by the relative stabilities of the respective forms. Chemists have always been fascinated by the possibility to fight this equilibrium using external triggers to quantitatively convert **A** to **B** and vice versa. Our approach uses light of different wavelengths to switch a molecule between two states (Fig. 1).

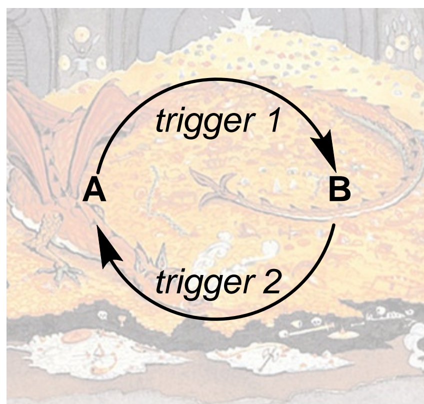


Fig. 1. **Reversible switching between two molecules by external triggers**

We optimise photochemical switching properties of existing diarylethene[1] and fulgimide[2] systems and develop water-soluble photoswitches that can be applied in photopharmacology to reversibly alter the properties of biologically active substances. In addition, we develop entirely new reversible systems based on photoinduced electron transfer ("electron ping-pong") and reversible formation of *ortho*-quinone methides ("catch-and-release"). These approaches broaden the scope of reversible photochemical systems and study their optogenetics and material chemistry applications.

This research was supported by the MEYS (LTAIN19166) and by the Czech Science Foundation (22-20319S).

REFERENCES

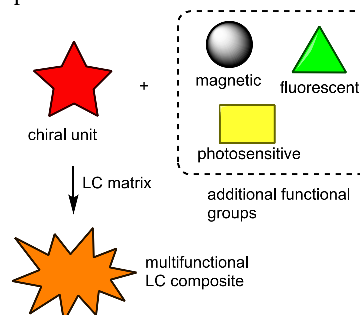
1. Kim Y., Hellmuth T. J., Sysoiev D., Pauly F., Pietsch T., Wolf J., Erbe A., Huhn T., Groth U., Steiner U. E., Scheer E.: Nano Lett. *12*, 3736 (2012).
2. Lachmann D., Lahmy R., König B.: Eur. J. Org. Chem. *31*, 5018 (2019).

PREPARATION OF CHIRAL DOPANTS FOR TUNABLE LIQUID CRYSTALLINE MIXTURES

MICHAL ŠMAHEL[a], VLADIMÍRA NOVOTNÁ[b], MICHAL KOHOUT[a]

[a]*Department of Organic Chemistry, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague 6;*
[b]*Institute of Physics, Czech Academy of Sciences, Na Slovance 1999/2, 182 21 Prague 8*
smahelm@vscht.cz

Chirality is a highly fascinating phenomenon essentially connected with many fields of modern science as well as with everyday life. We focus on preparation of novel chiral composites based on the combination of achiral liquid crystalline (LC) matrices and axially chiral dopants. Resulting mixtures exhibit chiral mesophases that have attracted considerable attention due to their unique helical structure, which can be driven by external stimuli such as temperature, pressure or electric field. It predetermines them for many applications, *e.g.*, in non-linear optics, issue thermometers or organic compounds sensors.



Scheme 1. **Approach for preparation of novel composites**

Amplification of long-range chiral order in the achiral matrix, has many advantages in comparison with preparation of optically pure mesogens. Simple combination of different types of matrices, dopants and their ratios is very versatile and offers high variability of final materials and possibility how to easily enhance their properties. In addition, advanced dopants can bring some extra functionalities (Schema 1.), such as sensitivity to light[1] or magnetic field or capability of circularly polarized luminescence[2] (CPL).

The financial support by Czech Science Foundation (projects 22-16499S) is gratefully acknowledged.

REFERENCES
1. Qin L., Gu W., Chen Y., Wei J., Yu Y.: RSC Adv. *8*, 38935 (2018).
2. Li J., Bisoyi H. K., Lin S., Guo J., Li Q.: Angew. Chem. Int. Ed. *58*, 16052 (2019).

CURRENT METHODS FOR ISOLATION AND DETECTION OF SMALL NUCLEIC ACIDS

DENISA SMĚLÁ, ZUZANA BÍLKOVÁ

Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Studentská 95, 53210 Pardubice, Czech Republic
Denisa.Smela@upce.cz

About 90% of human genome is translated from DNA to RNA, although only about 1-2% of those transcripts are then translated into functional proteins. Most of the RNA are non-coding RNAs and have regulatory functions. They can be divided into long non-coding RNAs with size >200 nucleotides (nt) or small non-coding RNAs (sncRNAs) with size <200 nt (ref.[1]).

MicroRNAs (miRNA) belong to scnRNAs and are approximately 23 nt long. They are important in gene expression regulation and therefore in various cellular processes such as cell cycle, differentiation or apoptosis[2]. Thanks to their association with several pathological processes, miRNAs are considered diagnostic and prognostic biomarkers[3]. However, isolation and detection of miRNAs can be difficult due to their small size, low concentrations (femtomol) and high homology in sequences[4].

Commonly used methods for miRNA isolation are phenol-chloroform extraction and solid-phase extraction. Methods used for miRNA quantification are real-time PCR, digital PCR, microarray, and high-throughput small RNA-sequencing[1].

Currently, there are also efforts to develop new approaches using various micro- and nanomaterials for isolation of miRNA. Among others, it was described, that materials based on TiO_2 have an affinity to nucleic acids thanks to their strong interactions with phosphate backbone. It was also suggested, that the adsorption of DNA to TiO_2 is caused by the interaction between DNA and hydroxyl groups on the surface of TiO_2[5]. Similar principles apply for TiO_2 interactions with RNA (ref.[6]).

We tested two materials based on TiO_2 to determine their applicability for miRNA isolation – TiO_2 microspheres (GL Sciences, Japan) and TiO_2 nanotubes coated with Fe_3O_4 (CEMNAT, Czech Republic).

The authors gratefully acknowledge the financial support of the MEYS of the Czech Republic via project NANOBIO (Reg. No. CZ.02.1.01/0.0/0.0/17_048/0007421).

REFERENCES
1. Precazzini F., Detassis S., Imperatori A. S., Denti M. A., Campomenosi P.: Int. J. Mol. Sci. *22*, 1176 (2021).
2. Tomaselli S., Bonamassa B., Alisi A., Nobili V., Locatelli F., Gallo A.: Int. J. Mol. Sci. *14*, 22796 (2013).
3. Bartel D. P.: Cell *116*, 281 (2004).
4. Buckingham L. and Flaws M. L.: *Molecular diagnostics: fundamentals, methods, & clinical applications*, F.A. Davis, Philadelphia, 2007.
5. Amano T., Toyooka T., Ibuki Y.: Sci. Total Environ. *408*, 480 (2010).
6. Jimenez L. A., Gionet-Gonzales M. A., Sedano S., Carballo J. G., Mendez Y., Zhong W.: Anal. Bioanal. Chem. *410*, 1053 (2018).

CLINICAL-GRADE HUMAN EMBRYONIC STEM CELLS

TEREZA SOURALOVÁ[a,b], PAVEL VENTRUBA[c], MICHAL JEŠETA[c], DANIELA ŘEHÁKOVÁ[a,b], ALEŠ HAMPL[a,b], IRENA KOUTNÁ[a,b]*

[a]Masaryk University, Faculty of Medicine, Department of Histology and Embryology, Kamenice 3, 625 00 Brno; [b]St. Anne's University Hospital, International Clinical Research Center, Cell and Tissue Engineering Facility, Pekařská 664/53, 656 91 Brno; [c]Center of Assisted Reproduction, Department of Gynecology and Obstetrics, Faculty of Medicine, Masaryk University Brno and University Hospital Brno
irena.koutna@med.muni.cz

Human embryonic stem cells (hESC) have a unique ability to differentiate into every cell type of the human body. This ability makes them a tremendous cell source for regenerative medicine[1]. hESC lines are usually derived (established) with the use of mouse and bovine components. This approach is, unfortunately, highly variable and undefined. Furthermore, animal components can be an immunological risk for the patient. Luckily, feeder-free, xeno-free defined substrates with a combination of defined xeno-free media are replacing this old approach and are suitable for the derivation of hESC lines for cell therapies[2].

Here we present the derivation of clinical-grade hESC lines. We established these lines in cooperation with the Centre of assisted reproduction - University Hospital Brno which provided 6 to 7 days old blastocysts. Laminin 521 in

combination with Nutristem, Human serum albumin, and E-Cadherin was used for the mechanical derivation. hESC were cultured on laminin 521 in Nutristem medium.

The derivation itself and subsequent culture are defined, xeno-free and feeder-free and fulfill standards of current good manufacturing practices. In-depth quality control provides important information about the safety and differentiation potential of lines. We established clinical-grade hESC lines according to state-of-the-art technology in clean rooms that makes them excellent cell source for stem cell-based therapies.

Supported by Ministry of Health of the Czech Republic, grant nr. NV19-08-00147. *All rights reserved.* *Supported by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868). Funds by the Faculty of Medicine MU - MUNI/A/1398/2021. Tereza Souralová is Brno Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality.*

REFERENCES

1. Thomson J. A., Itskovitz-Eldor J., Shapiro S. S., Waknitz M. A., Swiergiel J. J., Marshall V. S., Jones J. M.: Science *282*, 5391 (1998).
2. Souralová T., Holubcová Z., Kyjovská D., Hampl A., Koutná I.: Stem Cell Res. *57*, 102574 (2021).

COMPARATIVE STUDY OF DIFFERENT HPLC COLUMNS FOR THE ANALYSIS OF POLAR COMPOUNDS IN METABOLOMICS

<u>TOMMASO STEFANI</u>[a,*], ANIČKA JELINKOVA[b], ZDENĚK KAMENÍK[a], JIŘÍ JANATA[a]

[a]Institute of Microbiology of the CAS, v. v. i., Vídeňská 1083, 142 20 Prague 4; [b]Department of Immunology and Gnotobiology, Institute of Microbiology ASCR, Novy Hradek
tommaso.stefani@biomed.cas.cz

Metabolomics involves large-scale analyses of diverse molecules with vast degrees of complexity[1]. Liquid Chromatography-Mass Spectrometry (LC-MS) has the advantage of tuneable chromatographic separation and high sensitivity in mass detection. Currently, there is no separation system, which allows the simultaneous analysis of all the constituents of a biological sample. However, by changing the stationary phase used in the LC separation is possible to select the class of desired analytes based on their physicochemical properties. If Reverse Phase (RP) is used, compounds with medium to low polarity are separated. Among these is C18, which for its broad range of application and efficiency represents currently the most used HPLC column. Meanwhile, with Hydrophilic Interaction Liquid Chromatography (HILIC), highly polar molecules can be retained. Nevertheless, HILIC analysis are usually more difficult to optimize due to their more complex mechanisms of analyte retention and are usually tuned just for a specific class of metabolites[2–4].

Because of the high quantity of polar compounds contained in biological samples and hence their importance in metabolomics, HILIC has found application as complementary technique to the already used RP systems[5]. Studies focused in the use of HILIC columns for metabolomics are raising, even if still not matching the use of RP columns reported in the literature[2]. However, only few studies are present that aim at comparing the performance of HILIC stationary phases with samples of different origin[6,7] and a general standardized method for the analysis of polar compounds is still lacking.

Therefore, in this study the performance of HILIC, multimode and C18 stationary phases, and different mobile phases was compared. 258 standards and 198 biological isolates (blood, stool, urine, skin and culture media) from humans, mice and microorganisms were analysed. From this data, a general method was developed selecting the best conditions for the simultaneous analysis of different classes of polar compounds (in different matrices) not handled by C18 column.

REFERENCES

1. Liu X., Zhou L., Shi X., Xu G.: TrAC Trends Anal. Chem. *121*, 115665 (2019).
2. Harrieder E.-M., Kretschmer F., Böcker S., Witting M.: J. Chromatogr. B *1188*, 123069 (2022).
3. Buszewski B., Noga S.: Anal. Bioanal. Chem. *402*, 231 (2012).
4. Hanai T.: Curr. Chromatogr. *5*, 43 (2018).
5. Cubbon S., Antonio C., Wilson J., Thomas-Oates J.: Mass Spectrom. Rev. 29(5), 671–684 (2010).
6. Elmsjö A., Haglöf J., Engskog M. K. R., Erngren I., Nestor M., Arvidsson T., Pettersson C.: J. Chromatogr. A 1568, 49 (2018).
7. Contrepois K., Jiang L., Snyder M.: Mol. Cell. Proteomics *14*, 1684 (2015).

CYANINES SUBSTITUTED ON HEPTAMETHINE CHAIN AND THEIR CONJUGATES AS FLUORESCENT LABELS

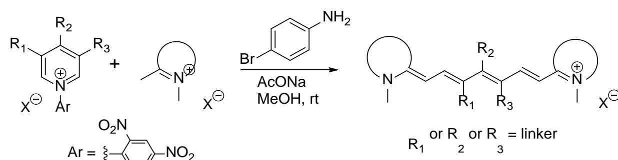
<u>REBECCA STRADA</u>[a], ZDENĚK FARKA[b], PETER ŠEBEJ[a]

[a]RECETOX, [b]Dept. of Biochemistry, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno
rebecca.strada@recetox.muni.cz

Cyanines belong to a family of polymethine dyes. Typical cyanine has a polymethine chromophore with an odd number of carbon atoms with two nitrogen-containing heterocycles as end-groups. Heptamethine cyanines (Cy-7) emit at around 800 nm (ref.[1]).

There is a strong demand for dyes emitting in the red part of the visible spectrum and the near-infrared region, especially in the tissue transparent window (650–950 nm), where tissues absorption is minimal, and light has its maximum depth of penetration[2]. Only one such dye is currently approved for use in human medicine – indocyanine green.

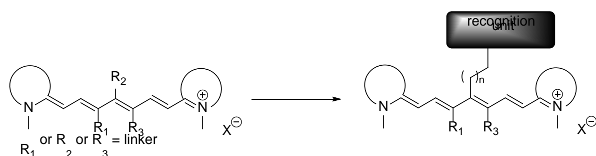
We prepared a small library of cyanines with a linker attached to the heptamethine fluorophore using a newly established synthetic route based on a condensation between pyridinium Zincke salt with suitable end-groups (Scheme 1)[3].



Scheme 1. **Synthesis of cyanines with linker attached to the heptamethine chain of the fluorophore**

The terminal aryls possess different substituents to address their electronic and steric effects on the dyes' properties.

We studied the properties of the dyes in the library with emphasis on the photophysical properties, conjugated them with antibodies, and evaluated the conjugates in cell cultures (Scheme 2).



Scheme 2. **Cyanines bioconjugation with antibodies**

This work was supported by the Czech Science Foundation (GJ20-30004Y), CETOCOEN EXCELLENCE Teaming 2 (CZ.02.1.01/0.0/0.0/17_043/0009632), and RECETOX RI (LM2018121).

REFERENCES

1. Li B., Zhao M., Zhang F.: ACS Mat. Lett. *2*, 905 (2020).
2. Weissleder R.: Nat. Biotechnol. *19*, 316 (2001).
3. Štackova L., Štacko P., Klan P.: J. Am. Chem. Soc. *141*, 7155 (2019).

ULTRASTRUCTURAL ANALYSIS OF CYTOPLASMIC ABNORMALITIES IN HUMAN OOCYTES

MARTINA TATÍČKOVÁ[a], ZUZANA TREBICHALSKÁ[a], DRAHOMÍRA KYJOVSKÁ[b], PAVEL OTEVŘEL[b], SOŇA KLOUDOVÁ[b], ZUZANA HOLUBCOVÁ[a,b]

[a] Department of Histology and Embryology, Faculty of Medicine, Masaryk University; [b]Reprofit International, Brno, Czech Republic
martina.tatickova@med.muni.cz

Egg quality is a limiting factor of female fertility. Human oocytes with the highest developmental potential are identified by spherical shape and uniformly clear cytoplasm. However, a large proportion of female gametes retrieved for fertility treatment exhibit cytoplasmic irregularities such as various sized vacuoles, prominent granularity, diverse inclusions, or round flat plaques termed the smooth endoplasmic reticulum disks. While current studies mainly focus on linking affected oocyte morphology with the postfertilization outcome, structural bases of these abnormalities and mechanisms behind their development remain largely unknown[1-3].

In this work, a total of 121 human oocytes donated for research were used to study the structural basis of the process by which the egg acquires its post-fertilization competence. Firstly, 39 morphologically normal human oocytes were used to examine ultrastructural changes associated with *in vitro* maturation. Conventional transmission electron microscopy allowed high-resolution analysis of peculiar ooplasmic features, and large volume electron microscopy revealed how individual microstructures interact in 3D. Next, 10 donated eggs with prominent dysmorphic features were examined. Ultrastructural analysis showed that the underlying cause of observed cytoplasmic abnormalities is the altered distribution of intracellular components. To address the mechanism of maturation-induced organelle relocation, cytoskeleton dynamics was experimentally perturbated in 72 oocytes maturing *in vitro*. Microscopic analysis showed that disruption of actin (but not microtubule) network in human oocytes led to inordinate organelle clustering resembling morphological pattern seen in *in vivo* matured egg with the abnormal granular ooplasm. This finding indicates that the actin network plays a pivotal role in ensuring homogenous organelle distribution during human oocyte maturation. In conclusion, this work extends knowledge of human oocyte morphology, cytoplasmic maturation, and egg quality markers.

The work was funded by the Grant Agency of the Czech Republic (GJ19-14990Y). The authors acknowledge the Tescan Orsay Holding for expert support and access to FIB-SEM systems. We also thank the staff of Reprofit International for the recruitment of egg donors and the administration of informed consents.

REFERENCES

1. Rienzi L., Balaban B., Ebner T., Mandelbaum J.: Hum. Reprod. *27* (suppl 1), i2 (2012).
2. Otsuki J., Iwasaki T., Katada Y., Tsutsimi Y., Tsuji Y., Furuhashi K., Kokeguchi S., Shiotani M.: J. Assisted Reprod. Genetics *35*, 899 (2018).
3. Ebner T., Moser M., Tews G.: Reprod. BioMed. *12*, 507 (2006).

HYBRID ORGANOSILICA MATERIALS: SYNTHESIS, CHARACTERISATION AND APPLICATIONS

DAVID TETOUR[a], VERONIKA MÁKOVÁ[b], BARBORA NIKENDEY HOLUBOVÁ[b], TOMÁŠ PAŠKA[b], MARIKA NOVOTNÁ[a], MICHAL ŘEZANKA[b], JANA HODAČOVÁ[a]

[a]Department of Organic Chemistry, UCT Prague, Technická 5, 166 28, Prague 6; [b]Institute for Nanomaterials, Advanced Technologies and Innovation, TU Liberec, Studentská 1402/2, 461 17, Liberec
david.tetour@vscht.cz

Hybrid organic-inorganic silica materials combine a functionality of incorporated organic units with unique physical and chemical stability of the inorganic silica network[1]. Bridged organosilicas could be prepared by direct self-condensation of organo-bridged bis(triethoxysilyl) precursors. Compared to other types of organosilicas (prepared by co-condensation or grafting methods) bridged silsesquioxanes allow the highest possible loading of organic fragments uniformly distributed over the whole material (Fig. 1).

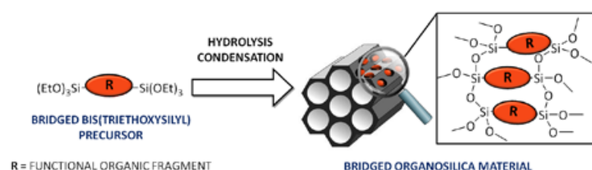


Fig. 1. **Preparation of bridged organosilica materials from bis(triethoxysilyl) precursors**

Herein, we report syntheses of various chiral bis(triethoxysilanes) based on *Cinchona* alkaloids and their transformation into hybrid materials. Next, characterisation of the materials by ^{13}C and ^{29}Si CP/MAS NMR, FTIR, SEM, TEM, TGA and gas sorption analyses will be discussed.

Subsequently, we used the materials as heterogenized ligands in catalysis of enantioselective Sharpless dihydroxylations (Fig. 2). Desired diols were obtained in yield up to 96% with *er* up to 99:1. Further experiments revealed that the materials were recyclable and no leaching of the organic fragments occurred[2].

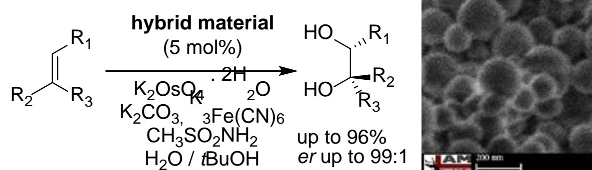


Fig. 2. **Sharpless dihydroxylation of alkenes catalyzed by *Cinchona* alkaloid-based hybrid material. SEM image of the material on the right**

REFERENCES

1. Ferré M., Pleixats R., Wong Chi Man M., Cattoën X.: Green Chem. *18*, 881 (2016).
2. Tetour D., Paška T., Máková V., Nikendey Holubová, B., Karpíšková J., Řezanka M., Brus J., Hodačová J.: J. Catal. *404*, 493 (2021).

RELEASE OF BISPHENOL A FROM DENTAL POLYCARBONATE MATERIALS

ANTONÍN TICHÝ[a]*, MARKÉTA ŠIMKOVÁ[b,c], JOSEF SCHWEIGER[d], JAN-FREDERIK GÜTH[e], PAVEL BRADNA[a]

[a]Institute of Dental Medicine, First Faculty of Medicine of the Charles University and General University Hospital in Prague, Karlovo Namesti 32, 121 11 Prague; [b]Institute of Endocrinology, Narodni 8, 116 94 Prague; [c]University of Chemistry and Technology, Technicka 6, 160 00 Prague; [d]University Hospital, Ludwig Maximilian University Munich, Goethestraße 70, 80336 Munich, Germany; [e]Center for Dentistry and Oral Medicine, Goethe University Frankfurt, Theodor-Stern-Kai 7, 60596 Frankfurt, Germany
antonin.tichy@lf1.cuni.cz

Polycarbonates are polymers of bisphenol A (BPA), a well-known endocrine disruptor. The use of polycarbonates in prosthetic dentistry includes denture bases, prefabricated temporary crowns and CAD/CAM-fabricated occlusal splints. This study evaluated the release of BPA from polycarbonate crowns that were (1) milled from Temp Premium Flexible (ZPF, Zirkonzahn, Italy) or Tizian Blank Polycarbonate (TBP, Schütz Dental, Germany), or (2) 3D-printed (Makrolon 2805, Covestro, Germany). Commercial prefabricated polycarbonate crowns (3M, USA) and milled poly(methyl methacrylate) (PMMA) crowns (Temp Basic, Zirkonzahn, Italy) were used for comparison. The crowns were stored at 37 °C in artificial saliva (AS) or methanol, which represented the worst-case scenario of BPA release. Extracts were collected after 1 day, 1 week, 1 month and 3 months. BPA concentrations were measured using a validated LC-MS/MS method with dansyl chloride derivatization. The amounts of released BPA were expressed in micrograms per gram of material (μg/g). After 1 day, the highest amounts of BPA were released from milled polycarbonates, TBP (methanol: 32.2 ± 3.8 μg/g, AS: 7.1 ± 0.9 μg/g) and ZPF (methanol 22.8 ± 7.7 μg/g, AS: 0.3 ± 0.03 μg/g), followed by 3D-printed crowns (methanol: 11.1 ± 2.3 μg/g, AS: 0.1 ± 0.1 μg/g) and prefabricated crowns (methanol: 8.0 ± 1.6 μg/g, AS: 0.07 ± 0.02 μg/g). Between 1 week and 3 months, the average daily release of BPA in methanol and AS decreased below 2 μg/g and 0.6 μg/g, respectively. No BPA was released from PMMA in AS, and the cumulative amount of BPA released in methanol was 0.2 ± 0.06 μg/g. In conclusion, polycarbonates could be a relevant source of BPA, but the current tolerable daily intake of BPA (4 μg/kg body weight/day) should not be exceeded. Nevertheless, clinicians should remain vigilant, because the effects of BPA on human health have not been fully clarified to date.

This study was supported by Charles University (GAUK 379421 and PROGRES Q29/1LF), and by the Ministry of Health of the Czech Republic (RVO 00023761-IE).

DESIGN AND DEVELOPMENT OF BIOLOGICALLY ACTIVE IMIDAZOPYRIDINES

DAVID VANDA[a], **PAWEŁ ZAJDEL**[b], **MIROSLAV SOURAL**[a*]

[a]Faculty of Science, Department of Organic Chemistry, Palacký University, 17. listopadu 12, 771 46 Olomouc; [b]Faculty of Pharmacy, Department of Organic Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, Kraków 30-688, Poland
david.vanda@upol.cz

Imidazopyridines represent interesting group of heterocyclic compounds with the large applicability in the field of medicinal chemistry and chemical biology[1]. For this reason, their synthesis and biological evaluation has been a subject of a long-term research in our group for almost a decade. We managed to develop an effective synthetic strategies leading to polysubstituted imidazo[4,5-*b*]pyridines and imidazo[4,5-*c*] pyridines using either traditional solution-phase synthesis or high-throughput synthesis concept[2]. Eventually, we were able to discover the diverse potential of specific imidazopyridines (Figure 1) to act as ligands of various biological targets – from serotonin receptor ligands[3,4] across phosphodiesterases[5] to selected kinase inhibitors[6]. In this short contribution, a key points and outcomes from individual projects will be summarized.

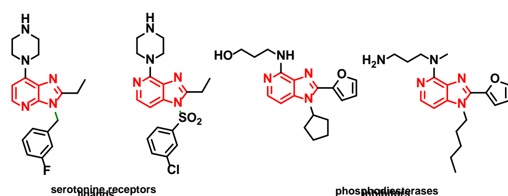


Fig. 1. **Examples of developed inhibitors/modulators**

REFERENCES

1. Vanda D., Zajdel P., Soural M.: Eur. J. Med. Chem. *181*, 111569 (2019).
2. Lemrová B., Smyslová P., Popa I., Oždian T., Zajdel P., Soural M.: ACS Comb. Sci. 16, 558 (2014).
3. Vanda D., Soural M., Canale V., Chaumont-Dubel S., Satała G., Kos T., Funk P., Fülöpová V., Lemrová B., Koczurkiewicz P., Pękala E., Bojarski A. J., Popik P., Marin P., Zajdel P.: Eur. J. Med. Chem. *144*, 716 (2018).
4. Vanda D., Canale V., Chaumont-Dubel S., Kurczab R., Satała G., Koczurkiewicz-Adamczyk P., Krawczyk M., Pietruś W., Blicharz K., Pękala E., Bojarski A. J., Popik P., Marin P., Soural M., Zajdel P.: J. Med. Chem. *64*, 1180 (2021).
5. Ručilová V., Świerczek A., Vanda D., Funk P., Lemrová B., Gawalska A., Bucki A., Nowak B., Zadrożna M., Pociecha K., Soural M., Wyska E., Pawłowski M., Chłoń-Rzepa G., Zajdel P.: Eur. J. Med. Chem. *209*, 112854 (2021).
6. Krajčovičová S., Jorda R., Vanda D., Soural M., Kryštof V.: Eur. J. Med. Chem. *211*, 113094 (2021).

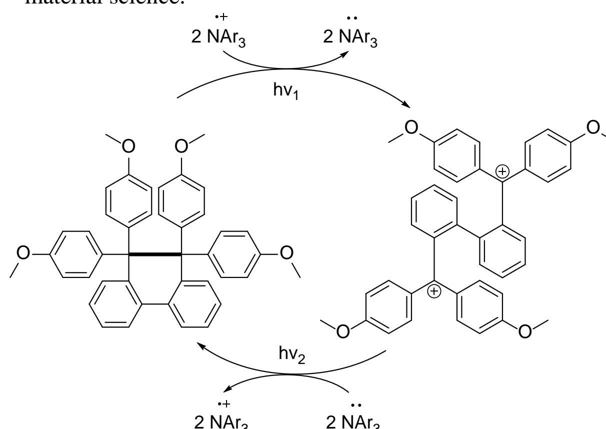
DEVELOPMENT OF STABLE PHOTOCONTROLLED CHARGE MANIPULATION SYSTEM

ANNA VASILEVSKA[a,b], **TOMÁŠ SLANINA**[a]

[a]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nám. 2, 16610, Prague 6
[b]Charles University, Ovocný trh 5, 116 36, Prague 1
anna.vasilevska@uochb.cas.cz

The formation of charge-separated systems via photoinduced electron transfer (PeT) is one of the basic processes in nature. However, as charge recombination is thermodynamically favourable, such states are rather short-lived. Theoretically, the charge recombination could be slowed or blocked completely by changing the HOMO-LUMO energies of the compounds just after PeT occurred.

Here, we propose a photocontrolled charge manipulation system based on hexaphenylethane redox switches that change their properties upon forming or breaking a C-C bond[2-4]. Triarylamines and their respective radical cations were found to be suitable for the light-mediated reduction and oxidation of the switches. The photophysical properties of the switches and amines, performance and stability of the system has been studied. As the system is reasonably stable and reversible, it can be further utilised in the field of solar energy storage and material science.



Scheme 1. **Reduction and oxidation cycle of the charge manipulation system**

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

REFERENCES

1. Hou Y., Zhang X., Chen K., Liu D., Wang Z., Liu Q., Zhao J., Barbon A.: J. Mater. Chem. C *7*, 12048 (2019).
2. Suzuki T., Nishida J., Tsuji T.: Angew. Chem. Int. Ed. Engl. *36*, 1329 (1997).
3. Higuchi H., Ohta E., Kawai H., Fujiwara K., Tsuji T., Suzuki T.: J. Org. Chem. *68*, 6605 (2003).
4. Ishigaki Y., Hayashi Y., Sugawara K., Shimajiri T., Nojo W., Katoono R., Suzuki, T.: Molecules *22*, 1900 (2017).

INTERACTION OF P53 PROTEIN WITH A HEME MOLECULE AND ITS POTENTIAL USE FOR BIOLOGICAL DOSIMETRY PURPOSES

JAKUB VÁVRA[a]*, ARTUR SERGUNIN[a], PETR POMPACH[a], DARIIA SAVCHENKO[b], JAKUB HRANÍČEK[c], MARKÉTA MARTÍNKOVÁ[a]

[a]Charles University, Faculty of Science, Department of Biochemistry, Hlavova 2030/8, 128 00 Praha 2; [b]Institute of Physics CAS, Na Slovance 1999/2, 182 21 Praha 8
[c] Charles University, Faculty of Science, Department of Analytical Chemistry, Hlavova 2030/8, 128 00 Praha 2
jakub.vavra@natur.cuni.cz

Biological dosimetry is an emerging scientific area important for radiation protection. Considering a possible future increase in the utilization of nuclear technologies, it will be necessary to develop new methods to evaluate the biological effects of ionizing radiation. Specifically, the indirect effect of ionizing radiation interaction with living matter generates free radicals leading to oxidative stress processes in cells. As a result of the natural radiation background, there has been a necessity for organisms to evolve adapting mechanisms to these processes[1]. Heme sensor proteins serve as an example of such adaptation. They can detect changes in either heme or gas molecules concentration in cells to regulate their specific functions and consequently many cellular actions[2].

Recently, there has been observed that p53 protein interacts specifically with a heme molecule[3]. Also, p53 protein is an essential regulator of many cell processes, including apoptosis and cell cycle regulation making it a model target for radiobiology[3]. Therefore, we decided to focus on the thorough biophysical description of the interaction of p53 with a heme molecule. Following that, we determined the exact number of heme molecules that interact with p53 and provided an accurate description of the ligand field of its iron center. In addition to that, structural changes of p53 protein upon heme binding were analyzed. Furthermore, we tested the effect of heme binding on the function of p53 as a transcription factor.

The possibility of p53 protein being also heme sensor protein will be discussed together with its potential applicability for oxidative stress processes detection. The results will contribute to the further development of new biological dosimetry methods.

The study was supported by the Charles University, project GA UK 158120.

REFERENCES

1. International Commission on Radiation Units and Measurements: J. ICRU *19*, 124 (2019).
2. Shimizu T., Lengálová A., Martínek V., Martínková M.: Chem. Soc. Rev. *48*, 5624 (2019).
3. Shen J., Sheng X., Chang Z., Wu Q., Wang S., Xuan Z., Li D., Wu Y., Shang Y., Kong X., Yu L., Li L., Ruan K., Hu H., Huang Y., Hui L., Xie D., Wang F., Hu R.: Cell Rep. *7*, 180 (2014).

CONSERVED PROLINE 246 IS AN IMPORTANT RESIDUE DETERMINING SUBSTRATE SPECIFICITY AND TRANSPORT ACTIVITY OF HOMO SAPIENS NA^+/H^+ ANTIPORTER NHA2

DIEGO VELÁZQUEZ[a], GAL MASRATI[b], NIR BEN-TAL[b], HANA SYCHROVÁ[a], OLGA ZIMMERMANNOVÁ[a]

[a]Laboratory of Membrane Transport, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic
[b] Department of Biochemistry and Molecular Biology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat-Aviv, Tel-Aviv, Israel
Diego.Velazquez@fgu.cas.cz

Human Na^+/H^+ antiporter NHA2 (*SLC9B2*) transports Na^+ or Li^+ across the plasma membrane in exchange for protons. In mammals, it is predominantly expressed in the kidney (distal tubule) and has been found to be implicated e.g. in essential hypertension or in insulin secretion. Thus, *Hs*NHA2 seems to be an important clinical marker. Nevertheless, information about its structure and mechanism of transport remains limited. Topology prediction algorithms suggest that this 537 amino acids long protein may consists of 14 transmembrane helices. In this work, we functionally expressed *Hs*NHA2 in a salt sensitive Saccharomyces cerevisiae strain and conducted rationally designed mutagenesis analysis to: (i) identify new structural and functional elements involved in ion selectivity, and (ii) validate the current 3D model of *Hs*NHA2. Mutated *Hs*NHA2 versions were characterized in terms of their localization, transport activity and substrate specificity in yeast cells.

Our data show that a highly conserved P246, localized in the core of the protein, plays a crucial role in ion selectivity. The substitution of this residue with polar amino acids (serine, threonine) altered the substrate specificity of the antiporter and changed its pH profile. The mutants became highly active not only at acidic pH 4.0 (as the native antiporter), but also at pH 7.0. Our experiments also suggest that the two titratable residues (E215 and R432) may be spatially close to each other and engaged in a salt bridge, predicted in silico by the model structure, since mutual exchange of both residues resulted in an active antiporter that transports Li+, while the respective single mutants, i.e., E215R and R432E, were inactive. Overall, our data bring new knowledge into the structure of human Na+/H+ antiporter NHA2 and confirm S. cerevisiae as an ideal organism for the structural analysis of mammalian membrane transporters.

This work was supported by a GAČR grant 21-08985S.

BIODEGRADABLE POLYHYDROXYALKANOATES AS CANDIDATE MATERIALS FOR WIDE RANGE OF APPLICATIONS

JURAJ VODICKA, MONIKA TRUDICOVA, ANETA POSPISILOVA, MONIKA WIKARSKA, MICHAL KALINA, ZUZANA JUGLOVA, STANISLAV OBRUCA, PETR SEDLACEK*

Faculty of Chemistry, Brno University of Technology, Purkynova 464, 612 00 Brno
sedlacek-p@fch.vut.cz

Polyhydroxyalkanoates (PHA), bacterial biopolymers represent a green island in the petrochemical flooding. The most common member of PHA family – poly(3-hydroxybutyrate) (P3HB) is already well explored. Alternatively, various PHA copolymers promise widespread applicability due to its favourable mechanical properties[1]. Recently, an extensive hunt for novel bacterial producents of such materials is in process. Thermophilic bacterium *Aneurinibacillus* sp. H1 appears to be a unique producent of PHA copolymers, since able to accumulate various 3HB to 4HB ratio copolymer corresponding to selected cultivation conditions[2].

In this study, we have applied basic biophysical characteristics of manufactured P(3HB-co-4HB) copolymer films to establish broad background for further applicability assessments. To ensure sufficient material for this purpose, cultivations of *Aneurinibacillus* sp. H1 were held. As a reference material, homopolymer P3HB was employed, while produced by mesophilic bacterium *Cupriavidus necator* H16. Thin films were prepared by solution casting. Tensile tests were performed in order to demonstrate mechanical differences as a motivation to focus on the monomer content. Simple dye release kinetics experiments were carried out. Rhodamine 6G and Nile red were used as eluates. Some barrier properties were tested. Further, tests of biodegradability in simulated body fluids (SBFs) were performed to evaluate degradation kinetics (weight and molar mass changes) and mechanism (changes in surface morphology by scanning electron microscopy – see Figure 1).

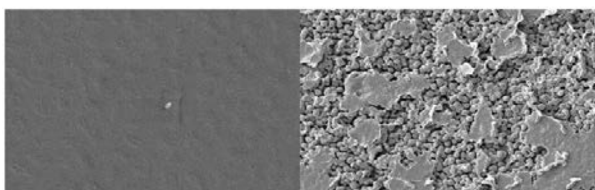


Fig. 1. **P(3HB-co-4HB) before (left) and after (right) 12 weeks incubation in simulated colonic fluid**

REFERENCES

1. Utsunomia C., Ren Q., Zinn M.: Front. Bioeng. Biotechnol. *8*, 257 (2020).
2. Pernicova I., Novackova I., Sedlacek P., Kourilova X., Kalina M., Kovalcik A., Koller M., Nebesarova J., Krzyzanek V., Hrubanova K., Slaninova E., Obruca S.: Polymers *12*, 1235 (2020).

SYNTHESIS OF STEROIDS WITH UNNATURAL CONFIGURATION

MARIIA VODOLAZHENKO, EVA KUDOVA

Institute of Organic Chemistry and Biochemistry CAS, Flemingovo namesti 2, 166 10 Prague 6
mariia.vodolazhenko@uochb.cas.cz

Steroids are an important class of biologically active compounds, participating in various processes in the human organism. Besides, they can show cardioactivity[1], be anticancer, anti-angiogenic, anti-inflammatory, antimicrobial, neuromuscular blocking agents[2]. Neurosteroids can be implicated in synaptic plasticity, age-related neurodegenerative diseases, learning and memory function, or disturbances associated with certain neuropsychiatric disorders[3-5].

Despite much attention being given to the production of highly functionalized synthetic steroids with varying substitutions and stereochemistry, their preparation remains challenging. Most of them have steroid skeletons with the natural configuration at stereocenters C-8, C-9, C-10, C-13, and C-14 to simplify their synthesis. From this point of view, an intriguing task is the synthesis of novel steroids that can display a similar *in vitro* effect as their natural counter partners. For example, ent-17β-estradiol shows similar or higher activity *in vitro* and *in vivo* then 17β-estradiol against different disorders, but doesn’t show hormonal activity in contrast to its natural enantiomer[6].

In our study, we have prepared a series of novel steroids with modified relevant stereocenters and functionalities. Starting from dehydroepiandrosterone, we developed a simple methodology for the synthesis of steroids with the modified configuration of C-13 and C-14 stereocenters and made variations of their main functional groups.

This work was supported by European Regional Development Fund–ERDF/ESF Project “PharmaBrain,” No. CZ.02.1.01/-0.0/0.0/16_025/0007444 and Research Project of the Academy of Sciences of the Czech Republic: RVO grant 61388963.

REFERENCES

1. Drouin M., Ruel R., Michel A.G.: Acta Cryst. *C47*, 1689 (1991).
2. Solum E. J., Mohamed Ya. M. A.: Synth. Commun. *49*, 1159 (2019).
3. Mellon S. H., Griffin L. D.: Trends Endocrin. Met. *13*, 35 (2002).
4. Reddy D. S.: Prog. Brain Res. *186*, 113 (2010).
5. Ratner M. H., Kumaresan V., Farb D. H.: Front. Endocrinol. *10*, 169 (2019).
6. Green P. S., Yang S.-H., Nilsson K. R., Kumar A. S., Covey D. F., Simpkins J. W.: Endocrin. *142*, 400 (2000).

ADVANCES IN STUDY OF STRUCTURE-PROPERTIES RELATIONSHIP OF CROCONAINE DYES

MICHAL VORBA[a], ZDENĚK FARKA[b], PETER ŠEBEJ[c]

[a]Dept. of Chemistry, [b]Dept. of Biochemistry and [c]RECETOX, Masaryk University, Kamenice 5, 625 00 Brno
m.vorba@seznam.cz

Croconaine dyes are less-known members of the large family of polymethine fluorescent dyes[1,2]. Structurally they possess a characteristic five-membered central carbocycle integrated into their polymethine chain and both semi-croconaines **1** and croconaines **2**, both possessing distinctive properties, belongs to this group.

While they show relatively small Stokes shifts, **1** absorbs in the visible region and **2** absorbs in NIR region. In the case of **2,** the fluorescence emission maxima lie in the tissue-transparent window (650–950 nm) which makes it a good candidate for bioimaging.

We synthesized a small library of croconaine and semi-croconaine dyes (Figure 1) by condensation of croconic acid with different derivatives of 2,3,3-trimethyl-3*H*-indole through the exocyclic methyl group[3]. With time-resolved spectroscopy and steady-state irradiation experiments in polar protic solvents, we studied their photophysical and photochemical properties to determine the structure-properties relationship. This would serve as a foundation for future design of croconaine dyes.

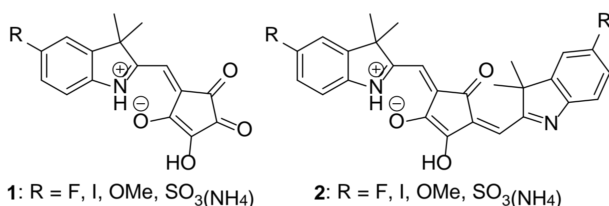


Fig. 1. **Semi-croconaine (1) and croconaine (2) dyes**

This work was supported by the Czech Science Foundation (GJ20-30004Y), CETOCOEN EXCELLENCE Teaming 2 (CZ.02.1.01/0.0/0.0/17_043/0009632), and RECETOX RI (LM2018121).

REFERENCES

1. Encinas C., Otazo E., Rivera L., Miltsov S., Alonso J.: Tetrahedron Lett. *43*, 8391 (2002).
2. Lynch D .E., Hamilton D. G.: Eur. J. Org. Chem. *27*, 3897 (2017).
3. Capozzi M. A. M., Punzi A., Babudri F., Musio R., Farinola G. M.: J. Org. Chem. *83*, 14396 (2018).

CHALCOGEN-BASED BODIPY PROBES FOR REVERSIBLE REDOX SENSING

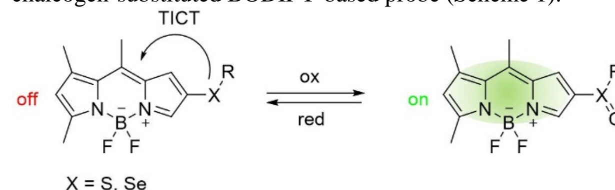
LUCIE WOHLRÁBOVÁ[a], MICHAL POLJAK[b], PETR KLÁN,[b] TOMÁŠ SLANINA[a]

[a]Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 542/2, 160 00 Prague 6
[b]Department of Chemistry and RECETOX, Masaryk University, Kamenice 5, 625 00 Brno-Bohunice
lucie.wohlrabova@uochb.cas.cz

Reactive oxygen species (ROSs) are a series of oxygen-based compounds commonly present in cells at constant levels, where they play a crucial role in the protection against pathogens[1]. However, when a specific level of ROSs is exceeded, they can cause degenerative damage to proteins, DNA, or fatty acids. Such behaviour can cause irreversible damage leading to cell death. This phenomenon is so-called oxidative stress, often connected with severe diseases such as cancer[2], pulmonary hypertension[3], or retinopathy[4]. The oxidative stress mechanism has not yet been described in detail, so it is vital to develop new probes for monitoring this complex process.

The damage caused by oxidative stress is not always irreversible. For example, the enzyme MsrA/MsrB can reduce oxidized sulfoxides back to the original sulfides and restore the original function of the damaged molecules. Therefore, monitoring the activity of these repairing enzymes is of great importance.

In this work, we developed an approach that allows monitoring redox changes and MsrA activity using a unique chalcogen-substituted BODIPY-based probe (Scheme 1).



Scheme 1. **Chalcogen-based BODIPY probe switching on and off upon oxidation and reduction**

This work was supported by the Czech Science Foundation (19-20467Y) and by the Ministry of Education, Youth and Sports (LTC20076).

REFERENCES

1. Dada L. A., Chandel N. S., Ridge K. M., Pedemonte C., Bertorello A. M., Sznajder J. I.: Clin. Invest. *111*, 1057 (2003).
2. Szatrowski T. P., Nathan C. F.: Cancer Res. *51*, 794 (1991).
3. Jernigan N. L., Naik J. S., Weise-Cross L., Detweiler N. D., Herbert L. M., Yellowhair T. R., Resta T. C.: PLoS ONE *12*, e0180455 (2017).
4. Volpe C. M. O., Villar-Delfino P. H., dos Anjos P. M. F., Nogueira-Machado J. A.: Cell Death Dis. *9*, 119 (2018).

AUTHOR INDEX

Ament, Anita 3
Aubrecht, Petr 30
Bacakova, Lucie 37
Baďurová, Lucia 3, 37
Balouch Martin 31
Baráth, Peter 21
Bárta, Tomáš 18
Bartošík, Martin 26
Bavlovič Piskáčková, Hana 4
Bednář David 28
Bednar, David 20
Bednář, David 28
Beier, Petr 17
Beneš, Petr 33, 36
Ben-Tal, Nir 47
Bergmann, Olaf 22
Bičíková, Marie 18
Bilavčíková, Kristýna 13
Bilkova, Zuzana 20, 42
Bilyeu, Kristin D. 4
Biová, Jana 4
Blšáková, Anna 5
Bobáľ Pavel 28
Bober, Patrycja 40
Borko, Simeon 20
Bouř, Petr 39
Bradna, Pavel 45
Brandmeier, Julian C. 39
Brázdová, Lada 5
Břenková, Barbora 6
Brhelová, Eliška 22
Brunoni, Federica 3
Bryja, Vitezslav 29
Buchtíková, Jana 25
Buchtová, Marcela 21
Burýšková, Barbora 6
Cahová, Hana 24
Capáková, Zdenka 40
Čelárová, Diana 18
Celec, Peter 17
Celušáková, Hana 18
Černá, Eva 7
Černá, Tereza 7
Chmela, Václav 8
Chrenko, Daniel 8
Chvíla Stanislav 31
Cibulka, Radek 28
Cigánková, Hana 9
Cimermanová, Michaela 10
Copko, Jakub 10
Coufalík, Pavel 13
Culenova, Martina 11
Damborsky, Jiri 20, 32
Daněk, Ondřej 11
Danisovic, Lubos 11
Debreova, Michaela 11
Derks, Wouter 22
Dobias, Adam 20
Dorazilová, Jana 7
Dostál, Zdeněk 25
Drašar, Pavel 38
Ďuriš, Marta 12
Dvořák, Pavel 6
Džubák, Petr 34
Farka, Zdeněk 39, 43, 49
Fili, Klevinda 12
Foret, František 16, 25
Friedlová, Nela 13
Fuksová, Markéta 13
Gabrielová, Viktorie 26
Gahurová, Lenka 24
Garaj, Vladimír 14
Gorris, Hansheiner 39
Güth, Jan-Frederik 45
Guzman, Juan David 5
Hampl, Aleš 18, 26, 31, 42
Havel, Josef 31
Havlica, Jaromír 30
Havránková, Eva 14
Hejátko, Jan 3, 37
Hernychová, Lenka 13
Hidasová, Denisa 15
Hill, Martin 18
Hlaváč, Jan 34
Hložková, Jana 22
Hodačová, Jana 44
Hodoň, Jiří 34
Holubcová, Zuzana 44
Horka, Vladimír 15
Hradski, Jasna 12
Hraníček, Jakub 47
Hrstka, Roman 26
Hrušková, Helena 16
Hudeček, Martin 16
Humpolíček, Petr 40
Hupp, Ted 13
Jahn, Ullrich 8, 35
Jampílek, Josef 14
Janata, Jiří 5, 32, 43
Janecký, Lukáš 17
Janega, Pavol 21
Janko, Jakub 17
Jankůjová Marie 28
Janovicova, Lubica 17
Janšáková, Katarína 18
Jaroš, Josef 18
Jelinkova, Anička 43
Ješeta, Michal 42
Jiráček, Jiří 29
Jongen, Vincent A. 18
Joshi, Rohit 19
Juglova, Zuzana 48
Kadlecová, Zuzana 19
Kalina, Michal 48
Kameník, Zdeněk 5, 32, 43
Kaminský, Jakub 39
Kandra, Mário 18
Kašpárková, Věra 40
Kastrati, Gylxhane 20
Katrlík, Jaroslav 21
Kauerová Tereza 28
Kianičková, Kristína 21
Killinger, Michael 21
Kittová, Patrícia 22
Klán, Petr 49
Klepárník, Karel 21
Kloudová, Soňa 44
Knopfová, Lucia 33, 36
Kocholatá, Michaela 36
Kohout, Michal 7, 41
Kohoutková, Eliška 22
Kolar, Frantisek 37
Kolátorová, Lucie 38
Kollár Peter 28
Kollár, Peter 37
Korecka, Lucie 20
Koutná, Irena 42
Krejčí, Lenka 26
Kren, Vladimir 37
Kristek, Jozef 23
Kubala, Lukáš 22
Kučera, Radim 4
Kudova, Eva 48
Kundalia, Paras 21
Květoň, Filip 5, 20
Kyjovská, Drahomíra 44
Lehocký, Marián 40
Leonhardt, Tereza 12
Lesayova, Alexandra 17
Lhoták, Pavel 23
Liegertová, Michaela 30
Lochmanová, Gabriela 3
Lorencová, Lenka 5
Máčová, Ludmila 18
Magdaléna, Onuščáková
Makhneva, Ekaterina 39
Máková, Veronika 44
Malina, Jakub Dávid 7
Malý, Jan 30, 36
Mamleev, Kamil 23
Mancini, Flaminia 24
Mandrla, Jan 25
Marchetti-Deschmann, Martina 31
Marek, Martin 32
Markos, Athanasios 17
Martínková, Markéta 47
Masák, Jan 5, 32
Masár, Marián 12
Masrati, Gal 47
Melicherová, Natália 25
Ménová, Petra 11
Micka, Mirek 29
Mikulík, Robert 22
Mikuška, Pavel 9
Mitrová, Katarína 29
Modrianský, Martin 25
Moráň, Lukáš 26, 31
Moráňová, Ludmila 26
Musilkova, Jana 37
Neděla, Vilém 7
Nejedlá, Zuzana 30
Nekulová, Marta 13
Nemčovič, Marek 21
Nicodemou, Andreas 11
Nikendey Holubová, Barbora 44
Nosáľová, Lea 27
Novák, Ondřej 3, 16
Novotná, Marika 44
Novotná, Vladimíra 41
Obertík, Róbert 28
Obruca, Stanislav 48
Obsil, Tomas 19, 33
Obsilova, Veronika 19, 33
Onuščáková Magdaléna 28
Ostatníková, Daniela 18
Otevřel, Pavel 44
Pacherník, Jiří 40
Paclikova, Petra 29

Pakanová, Zuzana 21
Pančík, Filip 21
Páníková, Terezie 29
Panuška, Petr 30, 35
Paška, Tomáš 44
Pastorek, Michal 17
Pažitná, Lucia 21
Pečinka, Lukáš 26, 31
Pešková, Michaela 22
Petrová Eliška 31
Piknová, Mária 10, 27
Pinto, Gaspar 20
Pížová Hana 28
Plechatá, Michaela 32
Plíhal, Ondřej 16
Pluskal, Daniel 32
Pohl, Pavel 33
Pokludová, Jana 33
Pokorna, Katerina 37
Pokorna, Petra 20
Poláček, Petr 7
Poljak, Michal 49
Pompach, Petr 47
Porokh, Volodymyr 26, 31
Porubský, Martin 34
Poryvai, Anna 35
Pospíšil, Jiří 8, 13, 15, 23
Pospisilova, Aneta 48
Poustka, David 35
Prener, Ladislav 35
Pristaš, Peter 10, 27
Prokop, Zbyněk 32
Průšová, Michaela 36
Radaszkiewicz, Katarzyna Anna 40
Řeháková, Daniela 42
Řemínek, Roman 16
Repiská, Gabriela 18
Řezanka, Michal 44
Říhová, Kamila 33, 36
Rudolf, Jiří 3, 37
Růžičková, Lucie 39
Savchenko, Dariia 47
Scheer, Peter 22
Schihada, Hannes 29
Schulte, Gunnar 29
Schweiger, Josef 45
Šebej, Peter 43, 49
Šebestová Janoušková, Olga 36
Sedlacek, Petr 48
Sedláková, Veronika 26
Sedlar, Antonin 37
Sergunin, Artur 67
Šimková, Markéta 38, 45
Skalák, Jan 3, 37
Skalický, Vladimír 16
Sklenář, Adam 39
Sklenárová, Dorota 39
Skopalová, Kateřina 40
Škrabišová, Mária 4
Slaby, Ondrej 20
Slanina, Tomáš 10, 15, 35, 41, 46, 49
Šmahel, Michal 41
Smejkal, Jiří 30
Smělá, Denisa 42
Smrčková, Anna 32
Soural, Miroslav 46
Souralová, Tereza 42
Staník, Michal 26
Stanková, Jarmila 34
Stefani, Tommaso 43
Stejskal, Jaroslav 40
Sterba, Jaroslav 20
Štěrbová-Kovaříková, Petra 4
Štofik, Marcel 30, 36
Stourac, Jan 20
Strada, Rebecca 43
Supuran, Claudiu T. 14
Sychrová, Hana 47
Szotkowská, Tereza 21
Szucs, Roman 12
Tariq Khan, Rayyan 20
Tatíčková, Martina 44
Tetour, David 44
Thalerová, Sandra 22
Tichý, Antonín 45
Tichý, Vlastimil 13
Tihlaříková, Eva 7
Tkáč, Ján 5
Tolar, Nikolas 24
Trčka, Filip 36
Trebichalská, Zuzana 44
Trudicova, Monika 48
Tůma, Jiří 7
Václavek, Tomáš 21
Vanda, David 46
Vaňhara, Petr 26, 31
Varchulova Novakova, Zuzana 11
Vasilevska, Anna 46
Vašinová Galiová, Michaela 13
Vávra, Jakub 47
Velázquez, Diego 47
Ventruba, Pavel 42
Vesselá, Tereza 26
Víteček, Jan 22
Vítků, Jana 38
Vodicka, Juraj 48
Vodolazhenko, Mariia 48
Vojtěšek, Bořivoj 13
Vojtová, Lucy 7, 19
Vopálenský, Pavel 24
Vorba, Michal 49
Wikarska, Monika 48
Wohlrábová, Lucie 49
Zajdel, Paweł 46
Žáková, Lenka 29
Zbytovská Jarmila 31
Zdráhal, Zbyněk 3
Zimmermannová, Olga 47

REMARKS AND NOTES

CZECH CHEMICAL SOCIETY SYMPOSIUM SERIES • ročník/volume 20 (2022), čís./no. 1 • ISSN 2336-7202 (Print), ISSN 2336-7210 (On-line) • ISSN 2336-7229 (CD-ROM) • evidenční číslo MK ČR E 21999 • Vydává Česká společnost chemická jako časopis Asociace českých chemických společností ve spolupráci s VŠCHT Praha, s ČSPCH a ÚOCHB AV ČR za finanční podpory Rady vědeckých společností ČR, Akademie věd ČR, Nadace Český literární fond a kolektivních členů ČSCH • IČO 444715 • Published by the Czech Chemical Society • VEDOUCÍ REDAKTOR/EDITOR-IN-CHIEF: V. Vyskočil • REDAKTOŘI/ EDITORS: J. Barek, E. Benešová, P. Drašar, P. Holý, P. Chuchvalec, M. Jurášek, Z. Kolská, B. Kratochvíl, J. Masák, J. Podešva, P. Šmejkal, V. Vyskočil; Webové stránky: P. Drašar • TECHNICKÁ REDAKTORKA/EDITORIAL ASSISTANT: R. Řápková • Redakce čísla (ISSUE EDITOR) P. Drašar, M. Fusek • ADRESA PRO ZASÍLÁNÍ PŘÍSPĚVKŮ/ MANUSCRIPTS IN CZECH, SLOVAK OR ENGLISH CAN BE SENT TO: Chemické listy, Novotného lávka 5, 116 68 Praha 1; tel./phone +420 221 082 370, e-mail: chem.listy@csvts.cz • PLNÁ VERZE NA INTERNETU/FULL VERSION ON URL: http://www.ccsss.cz • TISK: Garamon s.r.o., Wonkova 432, 500 02 Hradec Králové • SAZBA, ZLOM: ČSCH, Chemické listy • Copyright © 2022 Czech Chemical Society Symposium Series/Česká společnost chemická • Cena výtisku / Sigle issue price 180 Kč • This journal has been registered with the Copyright Clearance Center, 2322 Rosewood Drive, Danvers, MA 01923, USA, where the consent and conditions can be obtained for copying the articles for personal or internal use • Pokyny pro autory najdete na http://www.ccsss.cz , zkratky časopisů podle Chemical Abstract Service Source Index (viz http://cassi.cas.org/search.jsp) • Molekulární námět na obálce: Vladimír Palivec • Dáno do tisku 20.4.2022.