

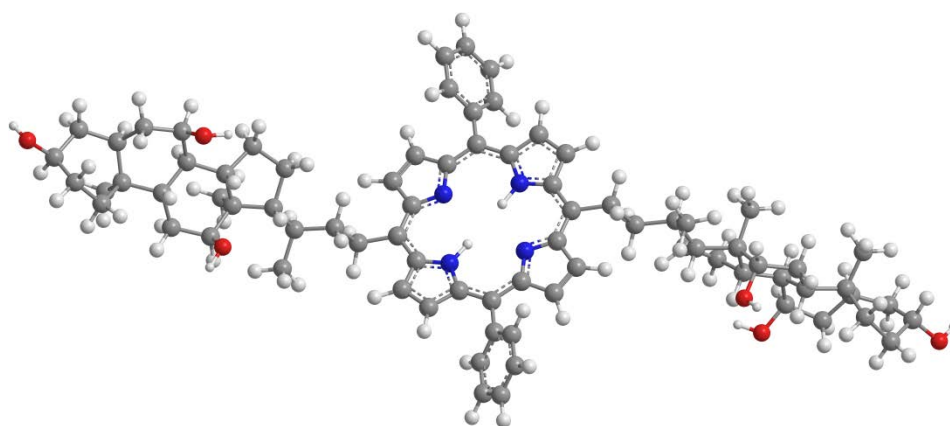


**MERCK**



**IOCB Tech**

**Chemické listy**



**XX<sup>th</sup> INTERDISCIPLINARY MEETING  
OF YOUNG RESEARCHERS AND STUDENTS IN THE FIELD  
OF CHEMISTRY, BIOCHEMISTRY, MOLECULAR BIOLOGY,  
AND BIOMATERIALS**

May 11 – May 14, 2020

Devět Skal Milovy hotel

Edited by  
Radmila Řápková, Martin Fusek, Pavel Drašar



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.



**SELECTION OF A NANOBODY AGAINST NKp30 FOR GENERATION OF FUSION IMMUNOLIGANDS AIMED AT ACTIVATION OF NK CELL CYTOTOXICITY AGAINST TUMOUR CELLS**

**CELESTE ABREU<sup>a,\*</sup>, SANDRA OLOKETUYI<sup>b</sup>, ARIODE MARCO<sup>b</sup>, ONDREJ VANĚK<sup>a</sup>**

<sup>a</sup>Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030, 12840 Prague, Czech Republic,  
<sup>b</sup>Laboratory of Environmental and Life Sciences, University of Nova Gorica, Rožna Dolina, Slovenia  
desousac@natur.cuni.cz

Natural killer (NK) cells play a pivotal role in innate immunity due to their ability to eliminate infected, stressed, autoreactive or tumour cells without prior antigen sensitization. Directed cytotoxicity by production of proinflammatory cytokines and cytotoxic machinery occurs through binding of specific ligands exposed on the surface of harmed cells to activating receptors on NK cells<sup>1</sup>. Coevolutionary arms race between tumour cells striving to escape immune surveillance and anti-tumour mechanisms often result in the effective escape of malignant cells to all protective mechanisms in place. Thus, the manipulation of cytotoxic recognition and activity via the development of NK cell-based immunotherapies coupled with nanobody delivery systems could provide an innovative approach towards directed tumour immunotherapy.

The preparation and characterization of bivalent fusion proteins able to simultaneously recognize specific tumour markers and activate NK cells has been recently achieved. The fusion proteins consist of VHH nanobody targeting NKp30 tumour cell marker and of the extracellular domain of NK cell activating ligand B7-H6<sup>2,3</sup>. The selection of a nanobody against NKp30 was achieved via panning the available naïve phage-displayed nanobody library against the soluble ligand binding domain of the activating NK cell receptor, NKp30 followed by selection in a nonspecific and in a competitive manner, thus broadening the screening population.

Expression and characterization of the fusion immunoligands will be performed and compared with the known information of the free ligand interactions. Further studies involving microscopy-based approaches as well as biological assays will be equally employed for a better understanding of the mechanisms associated with NK cell receptor:ligand interaction with the long-term aim of using bivalent immunoligands in NK cell-based immunotherapeutics for cancer therapy or suppression of autoimmune disorders.

*This work was supported by Czech Science Foundation (18-10687S) and Ministry of Education, Youth and Sports of the Czech Republic (LTC17065). This work benefited from STSM funding by COST Action CA15126 ARBRE-MOBIEU.*

**REFERENCES**

1. Vivier E., Raulet D.H., Moretta A., Caligiuri M.A., Zitvogel L., Lanier L.L., Yokoyama W.M., Ugolini S.: *Science*. 331, 6013 (2011).

2. Kellner C., Günther A., Humpe A., Repp R., Klausz K., Derer S., Valerius T., Ritgen M., Brüggemann M., van de Winkel J.G., Parren P.W., Kneba M., Gramatzki M., Peipp M.: *Oncoimmunology*, 5, 1 (2016).
3. Kellner C., Maurer T., Hallack D., Repp R., van de Winkel J.G.J., Parren P.W.H.I., Valerius T., Humpe A., Gramatzki M., Peipp M.: *J. Immunology* 189, 10 (2012).

**SEEDING MECHANISMS OF FIBRILLAR TAU REPEAT PEPTIDE AGGREGATES**

**NARENDRAN ANNADURAI<sup>a</sup>, LUKÁŠ MALINA<sup>a</sup>, MARIAN HAJDÚCH<sup>a,b</sup>, VISWANATH DAS<sup>a,b,\*</sup>**

<sup>a</sup>Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc;  
<sup>b</sup>Cancer Research Czech Republic, Hněvotínská 5, 779 00 Olomouc, Czech Republic  
viswanath.das@upol.cz

Tau aggregation and its progression are seminal events in tauopathies such as Alzheimer's disease, Progressive supranuclear palsy, etc.<sup>1</sup> Tau is a microtubule-associated protein that regulates the stability of axonal microtubules. Under pathological conditions, mutation and hyperphosphorylation of tau mainly at tau repeat (R1, R2, R3 and R4) regions in the microtubule-binding domains (MBD) induce tau misfolding and aggregation.<sup>2</sup> The presence of extracellular pre-formed tau aggregates induces the aggregation of tau *in vitro* or native tau in neighboring cells by a process termed as 'tau seeding'.<sup>3</sup> This seeding potency of aggregates is defined by the ability of tau to form  $\beta$ -sheet-rich fibrils, to enter the cells and escape the protein quality control machinery, such as ubiquitin-proteasome, endosomal-lysosomal, and autophagic protein degradation pathways.<sup>4</sup> Therefore, identifying tau repeats in MBD that defines the seeding ability of tau will be beneficial for pharmacological interventions for tauopathies.

With this goal in mind, we established an *in vitro* Thioflavin T (ThT)-binding assay to identify the aggregating tau repeat regions. We used synthetic peptides of R1, R2, R3 and R4 regions for aggregation assay. The formation of fibrils by each repeat was determined using atomic fluorescence microscopy (AFM). To find out the cross-seeding potential of fibrils, we used the lipofectamine-based transfection of peptide aggregates in HEK293-Tau-RD-P301S biosensor cells, expressing P301S mutant tau. The extracellular fibril-induced aggregation of intracellular P301S tau was monitored using an Operetta™ High-Content Screening System. The internalization of peptide aggregates in cells was visualized by live-cell imaging. The endosomal-lysosomal escape of peptide aggregates was examined in the presence of inhibitors of endocytosis pathways, such as chloroquine, dynasore, and genistein.

We found that tau R2 and R3 peptides were able to form fibrillar  $\beta$ -sheet-rich aggregates *in vitro* as indicated by the increase in the fluorescence of ThT. Only the repeat regions that formed fibrils, as identified by AFM, were able to induce the intracellular tau aggregation in biosensor cells. Dynasore,

an inhibitor of GTPase dynamin reduced the entry of extracellular peptide aggregates and their co-localization into endosomes in cells. Dose-dependent dynasore treatment also decreased the aggregation of Tau-RD in biosensor cells by peptide aggregates. There was no co-localization of peptide aggregates with lysosomes. Our results show that peptide aggregates enter cells through endocytosis and endosomal rupturing resulted in the escape of peptide aggregates from lysosomal degradation and its cytoplasmic release. In the cytoplasm, the escaped peptide aggregates seeded the endogenous tau aggregates. Understanding these escape mechanisms of protein aggregates will provide a foundation for therapeutic approaches in protein misfolding diseases.

*This work was supported by the European Regional Development Fund -Project ENOCH (No. CZ.02.1.01/0.0/0.0/16\_019/0000868).*

## REFERENCES

- Gao Y.L., Wang N., Sun F.R., Cao X.P., Zhang W., Yu J.T.: *Ann. Transl. Med.* **6**, 175 (2018).
- Annadurai N., Agrawal K., Džubák P., Hajdúch M., Das V.: *Cell. Mol. Life Sci.* **74**, 4159 (2017).
- Holmes B.B., Furman J.L., Mahan T.E., Yamasaki T.R., Mirbaha H., Eades W.C., Belaygorod L., Cairns N.J., Holtzman D.M., Diamond M.I.: *Proc. Natl Acad. Sci. USA* **111**, 4376 (2014).
- Piras A., Collin L., Grüniger F., Graff C., Rönnbäck A.: *Acta Neuropathol. Commun.* **4**, 22 (2016).

## DETECTION OF NEW METABOLITES WITH UNUSUAL ALKYLPROLINE MOIETIES BY ISOTOPICALLY LABELLED L-DOPA

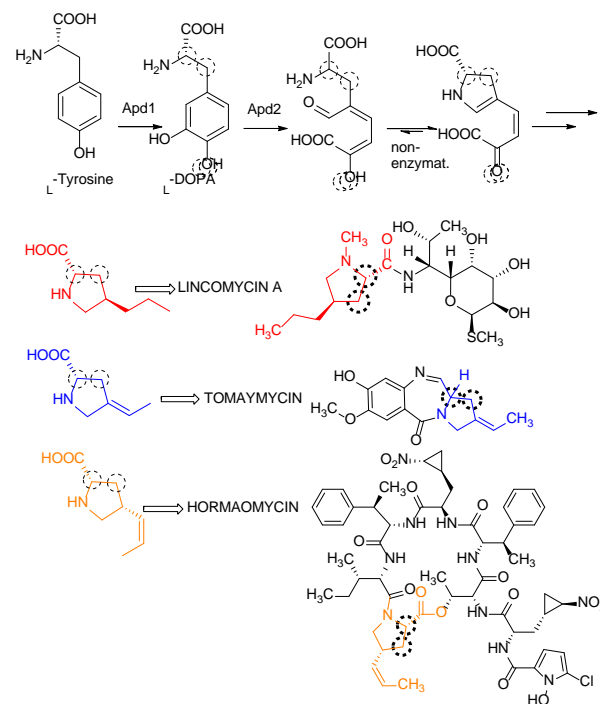
**LADA BRÁZDOVÁ<sup>a,b,\*</sup>, NATALIA CÉSPEDES<sup>a,c</sup>, ZDENĚK KAMENÍK<sup>a</sup>, STANISLAV KADLČÍK<sup>a</sup>, JIŘÍ JANATA<sup>a</sup>, JAN MASÁK<sup>b</sup>**

<sup>a</sup>Institute of Microbiology of the CAS, v. v. i., Vídeňská 1083, 142 20 Prague 4; <sup>b</sup>Department of Biotechnology, UCT Prague, Technická 5, 166 28 Prague 6; <sup>c</sup>Pontificia Universidad Javeriana, Bogota, Colombia  
lada.brazdova@biomed.cas.cz

Actinobacteria produce important bioactive metabolites with e.g. antimicrobial and antitumor properties. These compounds are used in human and veterinary medicine. Our laboratory focuses on specialized metabolites with unusual alkyl-L-proline derivatives (APD) incorporated into their structure. APD is similar to proteinogenic L-proline; however, it is biosynthesized in a special biosynthetic pathway from L-tyrosine, L-leucine or L-isoleucine. APD is incorporated into structurally and functionally diverse groups of natural compounds<sup>1</sup>. One of them is a small group of clinically used antibiotics, lincosamides (e.g. lincomycin)<sup>2</sup>. Further APD compounds include pyrrolbenzodiazepines, a large group of antitumor agents (e.g. tomaymycin)<sup>3</sup>, and hormaomycin, a signaling molecule<sup>4</sup>. APDs of these compounds are

biosynthesized L-tyrosine via L-DOPA (Fig. 1).

We tested a mass spectrometry-based method to facilitate the detection of new compounds with APD moieties. Specifically, we used to supplement production media of Actinobacteria strains, which bear a cryptic biosynthetic gene cluster for the biosynthesis of a new APD compound. Then, we compared the MS spectra of the produced metabolites in the medium with and without the isotopically labelled L-DOPA feeding. Incorporation of <sup>13</sup>C<sub>2</sub>, <sup>18</sup>O-L-DOPA into an APD metabolite would lead to the increase of the corresponding pseudomolecular ion by two in the supplemented culture compared to the control culture without L-DOPA feeding. In this way, we detected a new metabolite with an APD moiety, hascofovomycin, which is distinct from lincosamides, pyrrolbenzodiazepines, and hormaomycin.



**Fig. 1. Biosynthesis pathway of APD precursor from the amino acid L-tyrosine. The dotted ring is showed the fate of labeled atoms when fed with <sup>13</sup>C<sub>2</sub>, <sup>18</sup>O – L-DOPA**

*The work was supported by the project 17-13436Y from the Czech Science Foundation.*

## REFERENCES

- Janata J., Kamenik Z., Gazak R., Kadlcik S., Najmanova L.: *Nat. Prod. Rep.* **257**, 35 (2018).
- Mason D., Dietz A., DeBoer C.: *Antimicrob. Agents Chemother.*, **553**, 2 (1962).
- Gerratana B.: *Med. Res. Rev.* **254**, 32 (2012).
- Omura S., Mamada H., Wang N. J., Imamura N., Oiwa R., Iwai Y., Muto N.: *Jour. Antibiot.*, **700**, 37 (1984).

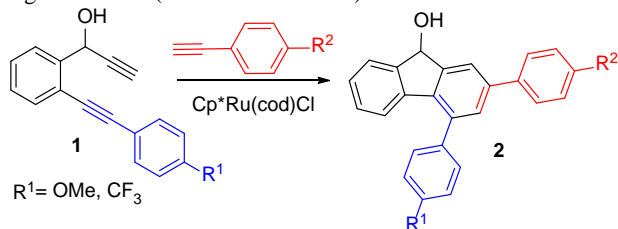
## CATALYTIC [2+2+2] CYCLOTRIMERIZATION FOR THE SYNTHESIS OF 2,4-DISUBSTITUTED 9,9'-SPIROFLUORENES AND FLUORINATED DISPIROINDENO[2,1-c]FLUORENES

**ILARIA CAIVANO<sup>a</sup>, REINHARD KAISER<sup>a</sup>, FLORIAN SCHNURRER<sup>a</sup>, JIRÍ MOSINGER<sup>b</sup>, IVANA ČISAŘOVÁ<sup>b</sup>, DAVID NEČAS<sup>a</sup>, MARTIN KOTORA<sup>a\*</sup>**

<sup>a</sup>Department of Organic Chemistry, Charles University, Albertov 6, 12843 Prague; <sup>b</sup>Department of Inorganic Chemistry, Charles University, Albertov 6, 12843 Prague  
caivano@natur.cuni.cz

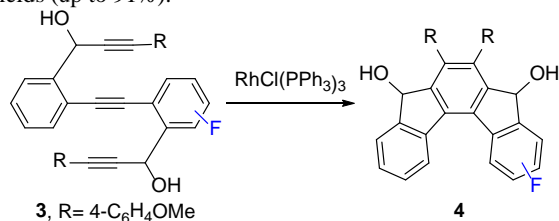
The catalytic [2+2+2] cyclotrimerization is a straightforward reaction for the preparation of decorated benzene rings. In this field, we have recently shown that Rh-catalyzed [2+2+2] cyclotrimerization is a convenient method for the synthesis of substituted fluorenes<sup>1</sup>. Herein, we present how this method can be extended for the preparation of 2,4-disubstituted fluorenes and fluorinated indeno[2,1-c]fluorenes.

The use of a [Ru] catalyst, instead of a [Rh] one promotes a regioselective cyclotrimerization of **1** to the meta-regioisomers **2** (meta/ortho = 3-10:1)<sup>2</sup>.



Scheme 1. Ru-catalyzed [2+2+2] cyclotrimerization

Moreover, the intramolecular Rh-catalyzed [2+2+2] cyclotrimerization of fluorinated triynols **3** afforded fluorinated indeno[2,1-c]fluorene-5,8-diols **4** in high isolated yields (up to 91%).



Scheme 2. [2+2+2] intramolecular cyclotrimerization

In addition, fluorenols **2** and **4** were converted to the corresponding bispirofluorenes, whose photophysical properties and single crystal X-ray structure analysis were also studied.

### REFERENCES

- Kaiser R. P., Nečas D., Cadart T., Gyepes R., Čisařová I., Mosinger J., Pospíšil L., Kotora M.: *Angew. Chem. Int.* 58, 7 (2019).
- Caivano I., Kaiser R. P., Schnurrer F., Mosinger J., Čisařová I., Nečas D., Kotora M.: *Catalysts* 9, 942 (2019).

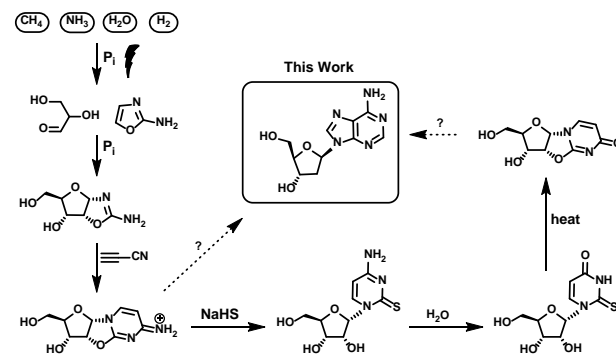
## PREBIOTIC SYNTHESIS OF 2'-DEOXYADENOSINE

**VÁCLAV CHMELA<sup>\*</sup>, JIANFENG XU, JOHN SUTHERLAND**

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK  
chmela@seznam.cz

We have previously discovered a short, highly efficient route to activated ribonucleotides from plausible prebiotic feedstock molecules such as cyanamide, cyanoacetylene, glycoaldehyde, glyceraldehyde and inorganic phosphate<sup>1</sup>. We can accomplish the synthesis of all precursors of ribonucleotides, amino acids and lipids by the reductive homologation of hydrogen cyanide and some of its derivatives. The key steps are driven by UV using hydrogen sulphide as a reductant and can be accelerated by Cu<sup>I</sup>-Cu<sup>II</sup> photoredox cycling<sup>2</sup>. Anomerization of  $\alpha$ -ribonucleosides to  $\beta$ -anomers is extremely inefficient. However, an extraordinarily efficient anomerization can be accomplished using  $\alpha$ -2-thionucleosides<sup>3</sup>.

Here we describe a high-yielding, completely stereo-, regio-, and furanosyl-selective prebiotic synthesis of 2'-deoxyadenosine. Our results lend considerable credence to the notion that purine deoxyribonucleosides and pyrimidine ribonucleosides may have coexisted before the emergence of life.



Scheme 1. Prebiotic synthesis of 2'-deoxyadenosine

This work was supported by Erasmus+ funding.

### REFERENCES

- Powner M. W., Gerland B., Sutherland J. D.: *Nature* 459, 239 (2009).
- Patel B. H., Percivalle C., Ritson D. J., Duffy C. D., Sutherland J. D.: *Nature Chemistry* 7, 301 (2015).
- Xu J., Tsanakopoulou M., Magnani C. J., Szabla F., Šponer J. E., Šponer J., Góra R. W., Sutherland J. D.: *Nature Chemistry* 9, 303 (2017).

## CATHEPSIN D PROTEASE OF PARASITIC BLOOD FLUKE *SCHISTOSOMA MANSONI* AS A TARGET FOR INHIBITORY DRUGS

**NIKOLA CHMÚRČIAKOVÁ, RADKA HOUŠTECKÁ, MARTIN HORN, MICHAEL MAREŠ\***

*Institute of Organic Chemistry and Biochemistry CAS CR, Flemingovo náměstí 542, 160 00 Prague michael.mares@uochb.cas.cz*

Schistosomiasis, a parasitic disease caused by blood flukes of the genus *Schistosoma*, is a global health problem with over 240 million people infected. Treatment relies on just one drug, and new therapies are needed<sup>2</sup>. Adult schistosomes live in the cardiovascular system, and host blood proteins are a primary source of nutrients<sup>1</sup>. In the schistosome gut, a network of proteases performs the digestion of host proteins and represents a potential intervention target. *Schistosoma mansoni* cathepsin D (SmCD) is a pepsin-family aspartic protease that initiates host hemoglobin digestion in schistosomes<sup>3</sup>. Recombinant SmCD was produced in the *Leishmania tarantolae* protozoan expression system. Screening of a library of more than 30 macrocyclic statin-based peptidomimetics against SmCD selected potent inhibitors with low nanomolar activity. These inhibitors displayed anti-schistosomal properties and thus represent a new lead scaffold for developing potential drugs for schistosomiasis treatment. Furthermore, we solved the crystal structure of the SmCD zymogen, and currently we are working on the structural analysis of SmCD complexes with macrocyclic inhibitors.

*This work was supported by the grant number 6061/23.*

### REFERENCES

1. Wilson R. A., Coulson P. S.: *Parasitology* 92 (Pt 1), 83 (1986).
2. Greenberg R. M.: *Parasitology* 140, 1534 (2013).
3. Caffrey C. R., McKerrow J. H., Salter J. P., Sajid M.: *Trends Parasitol.* 20, 241 (2004).

## MASS SPECTROMETRY OF TRIACYLGLYCEROL ESTOLIDES – FRAGMENTATION STUDY

**LUKÁŠ CUDLMAN<sup>a,b</sup>, VLADIMÍR VRKOSLAV<sup>a</sup>, ALEŠ MACHARA<sup>a</sup>, JOSEF CVAČKA<sup>a,b</sup>**

*<sup>a</sup>Institute of Organic Chemistry and Biochemistry AS CR, 166 10 Prague, Czech Republic; <sup>b</sup>Faculty of Science, Charles University, 128 00 Prague, Czech Republic lukas.cudlman@uochb.cas.cz; vladimir.vrkoslav@uochb.cas.cz*

Mass spectrometry is one of the most used techniques for analysis of lipids. For accurate structural identification of these compounds are widely used fragmentation techniques as collision-induced dissociation (CID), higher-energy collisional dissociation (HCD)<sup>1</sup> or ultraviolet photodissociation (UVPD)<sup>2</sup>.

Estolide is the common name for a linear oligomeric polyester of hydroxyfatty acid. It was discovered that estolides play role for treating diabetes and other diseases. Their metabolism is widely related to triacylglycerol estolides (TGest)<sup>3</sup>, which have one (or more) of three acyl chains of triacylglycerol containing a hydroxyl group esterified by another ester acyl chain.

In the present project, we studied a mass spectrometric high-resolution (Orbitrap) fragmentation study by CID, HCD and UVPD. We used a chip-based nano-electrospray system (Nanomate, Advion) for ionization of synthetic standards. We studied three types of TG-est isomers: fatty acid ester of hydroxyfatty acid is in position - $\omega$  (TG- $\omega$ -est), - $\alpha$  (TG- $\alpha$ -est) and -10 (TG-10-est) for description of its structure. Ammonium formate was added to solvent as an additive and fragmentation of  $[M+NH_4]^+$  molecular adduct was studied.

Using all fragmentation techniques we identified the ions related to loss of fatty acid and esterified hydroxyfatty acid from glycerol moiety. Nevertheless, we observed significant differences in MS/MS spectra between TG-est isomers in fragmentation of hydroxyfatty acids esterified by another ester acyl chain. We identified specific HCD MS/MS fragments correspond to the loss of fatty acid from hydroxyfatty acid of TG-est. TG- $\alpha$ -est obtain fragments of acylium ion from hydroxyfatty acid, while for TG-10-est we identified the specific fragment ions of neutral loss of esterified hydroxyfatty acid. Fragmentation CID provides the same results as HCD, except the above described specific fragments of TG- $\alpha$ -est and TG-10-est. UVPD provided more complex spectra with low abundant fragments. Their interpretation is difficult.

The best fragmentation technique of  $[M+NH_4]^+$  was HCD. HCD MS/MS spectra distinguished positional isomers of TG- $\omega$ -est, TG- $\alpha$ -est and TG-10-est.

### REFERENCES

1. Sleno L., Volmer D.A.: *J. Mass Spectrom.* 39, 1091 (2004).
2. Madsen J.A., Cullen T.W., Trent M.S., Brodbelt J.S.: *Anal Chem.* 83, 5107 (2011).
3. Tan D., Ertunc M.E., Konduri S., Zhang J., Pinto A.M., Chu Q., Kahn B.B., Siegel D., Saghatelyan A.: *J. Am. Chem. Soc.* 141, 8978 (2019)

## DEVELOPMENT OF BIOPOLYMERIC MATRIX TO VISUALISE CELL-TO-MATRIX INTERACTIONS USING LABEL-FREE COHERENCE-CONTROLLED HOLOGRAPHIC MICROSCOPY

**JANA DORAZILOVÁ\*, LENKA ŠTRBKOVÁ, MIROSLAV ĎURIŠ, LUCY VOJTOVÁ**

*CEITEC BUT – Central European Institute of Technology of Brno University of Technology, Research Groups of Advanced Biomaterials and Experimental Biophotonics, Purkyňova 123, 612 00 Brno. jana.dorazilova@ceitec.vutbr.cz*

Coherence-controlled holographic microscopy (CCHM)

is an emerging imaging technique used for fast processes visualisation<sup>1</sup>. Unlike fluorescence microscopy, which is currently one of the most used bioimaging technique, CCHM is a non-invasive, label-free technique with the capability to visualise cells in real-time. A challenging aspect of this technique is light scattering as the imaging in CCHM is based on the interference of the object and the reference light beams, which enables to detect the phase delay of light transmitted through the specimen<sup>2</sup>. Cells are overall weakly scattering absorbing specimens and highly scattering environment, such as polymeric substrates, can distort final images. Therefore, till this day, CCHM has been successfully used mostly in visualising only cell-to-cell interaction in cellular media. Within the context, the presented work aimed to establish a microstructured material resembling closely extracellular matrix by its composition for visualisation of cell-to-matrix interaction using CCHM.

Biopolymeric scaffolds were prepared inside chambered microscopic slides using the process of freeze-drying. For the visualisation, by CCHM the cell line of normal human dermal fibroblasts nHDF was used. The optimal concentration of biopolymeric substances was set to low concentrations of 0,2 % (w/w). At these concentrations, microstructured materials were still formed inside chamber slides and material exhibited negligible light scattering effect causing no disruption during image formation. Biopolymeric nature of the scaffolds does not negatively interfere with the viability of cells. Cells were able to attach and align to biopolymeric fibres within the microstructure of the scaffolds without forming a cluster or unaccustomed morphology. After addition of model presumably toxic substance, there were visible changes in cellular behaviour and morphology.

*This work was supported by the CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II, by Special Research project of Brno University of Technology under no. CEITEC VUT-J-19-6086 and by the Ministry of Health under the project no. 17-31276A.*

#### REFERENCES

1. Slabý T., Kolman P., Dostál Z., Antoš M., Lošťák M., Chmelík R.: *Opt. Express* 21, 14747 (2013).
2. Popescu G.: *Quantitative Phase Imaging of Cells and Tissues (McGraw-Hill Biophotonics)*, New York: McGraw-Hill, 2011.

#### RESORBABLE POLYMER-PHOSPHATE BONE CEMENT: THE EFFECT OF MICROFIBERS MODIFICATION

**MATEJ DZUROV<sup>a,\*</sup>, EDGAR B. MONTUFAR<sup>a</sup>, KRISTÝNA VALOVÁ<sup>a</sup>, KLÁRA LYSÁKOVÁ<sup>a</sup>, LADISLAV ČELKO<sup>a</sup>, JOSEF KAISER<sup>a</sup>, MATĚJ BUZGO<sup>b</sup>, MICHALA RAMPICHOVÁ<sup>b</sup>, LUCY VOJTOVÁ<sup>a</sup>**

*<sup>a</sup>Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 621 00 Brno, Czech Republic, <sup>b</sup>Institute of Experimental Medicine, Videňská 1083, 142 20 Prague 4, Czech Republic  
matej.dzurov@ceitec.vutbr.cz*

Complications such as fractures with various severity, osteoporosis – loss of bone density and different joint replacement surgeries are just a few of the most common bone-related complications. Bone cements have been developed and used in practice to fix these complications in a surgical manner. In the current state of the art, synthetic poly(methyl methacrylate) (PMMA) bone cements are favored due to their suitable mechanical parameters. Sadly, PMMA is non-degradable, causing complications due to polymers questionable cytotoxic behavior<sup>1</sup>. As an alternative to the polymer-based bone cements, resorbable calcium phosphate cements (CPCs) are emerging as a better alternative. Their inorganic structure, strikingly resembling bone minerals like hydroxyapatite, setting ability at physiological conditions and the possibility of injectability make them a perspective candidate for minimum invasive surgery and bioimplantology. However, mechanical parameters of CPCs are quite inferior compared to PMMA counterpart. A proposed solution to the low mechanical parameters is the introduction of biodegradable polymer microfibers to cement as a reinforcing agent.

In this work, hydrophobic and hydrophilic bioresorbable electrospun microfibers were used to reinforce the cement. Addition of these fibers increased mechanical properties in terms of compressive strength. The fiber amount was optimized in terms of final cement injectability. X-ray diffraction analysis proved full transformation of calcium phosphate to hydroxyapatite after fiber addition. Scanning electron microscopy was used to study microstructure and failure modes at crack interferences. For better resorption rates, microporosity was modulated. Experiments proved utilization of microfibers as bone cement reinforcement and when loaded with bioactive molecules, they can be used in cements as drug delivery systems.

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

#### REFERENCES

1. Poitout D. G.: *Biomechanics and Biomaterials in Orthopedics*, pp 40-89, Springer-Verlag London, 2004, DOI: 10.1007/978-1-4471-3774-0.
2. Vojtová L., Michlovská L., Valová K., et. al.: *Int. J. Mol. Sci.* 20, 391 (2019), DOI: 10.3390/ijms20020391.

**PHOTON-UPCONVERSION NANOPARTICLES FOR SINGLE-MOLECULE SENSING AND BIOIMAGING****ZDENĚK FARKA<sup>a</sup>, MATTHIAS J. MICKERT<sup>b</sup>, HANS H. GORRIS<sup>b</sup>, PETR SKLÁDAL<sup>a</sup>**<sup>a</sup>CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic<sup>b</sup>Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, 93053 Regensburg, Germany  
farka@mail.muni.cz

Fluorescence-based detection is a very popular readout method in immunoassays and cell imaging. However, it is limited by autofluorescence and light scattering of the surrounding matrix. This optical background can be elegantly avoided by using photon-upconversion nanoparticles (UCNPs), which emit shorter-wavelength light under near-infrared excitation (anti-Stokes emission). These unique properties enabled us to image single UCNPs by wide-field epilluminescence microscopy and allowed the development of single-molecule (digital) immunoassays<sup>1</sup>.

We have synthesized conjugates of polyethylene glycol-coated UCNPs with streptavidin and applied them as a label for the detection of cancer biomarker prostate-specific antigen (PSA). The digital detection based on the counting of individual sandwich immunocomplexes provided a limit of detection (LOD) of 23 fg·ml<sup>-1</sup> (800 aM) in 25% human serum, which is 20× more sensitive than the analog readout based on the measurement of output light intensity<sup>2</sup>.

The conjugates of UCNPs with streptavidin are also useful as a label in the diagnosis of bacterial diseases. We have developed an immunoassay for the detection of bacterium *Melissococcus plutonius*, the causative agent of European foulbrood. The assay provided an LOD of 340 CFU·ml<sup>-1</sup>, and its practical applicability was verified by the analysis of real samples of bees, larvae, and bottom hive debris<sup>3</sup>.

Furthermore, the unique optical properties make UCNPs suitable for cell imaging. We have employed PEG-based conjugates for the staining of HER2 biomarker on breast cancer cells. Due to the low optical background, as well as low non-specific binding, the UCNPs provided superior signal-to-background ratio ( $S/B = 319$ ) compared to the fluorescence-based experiment with the identical immunoreagents ( $S/B = 6$ ).

## REFERENCES

1. Farka Z., Mickert M. J., Pastucha M., Mikušová Z., Skládal P., Gorris H. H.: *Angew. Chem. Int. Ed. Accepted*, DOI: 10.1002/anie.201913924.
2. Mickert M. J., Farka Z., Kostiv U., Hlaváček A., Horák D., Skládal P., Gorris H. H.: *Anal. Chem.* 91, 9435 (2019).
3. Poláchová V., Pastucha M., Mikušová Z., Mickert M. J., Hlaváček A., Gorris H. H., Skládal P., Farka Z.: *Nanoscale.* 11, 8343 (2019).

**SEX-DETERMINATION IN GYNODIOECIOUS SPECIES IN GENUS *SILENE*****ROMAN GOGELA, JITKA ZLUVOVA, BORIS VYSKOT, ROMAN HOBZA, HELENA STORCHOVA, BOHUSLAV JANOUSEK**Department of Plant Developmental Genetics, Institute of Biophysics of the Czech Academy of Sciences, Kralovopolska 135, 61265, Brno, Czech Republic  
roman.gogela@gmail.com

Dioecious species represents only about 6 % (15 000 in total) of all angiosperm, but they can be found in more than 43 % angiosperm families. This is one piece of the evidence showing that dioecy has evolved multiple times during the evolution of angiosperms.

Our previous studies have focused on the evolution of sex-determining systems in genus *Silene*, section *Otites*. This section is specific by presence of dioecious species with both ZW-sex determining system and XY sex-determining system.

Identification of W-linked and Y-linked genes helped us to discover that the ZW sex-determining system (female heterogamety) is ancestral for section *Otites* and a switch from female heterogamety to male heterogamety occurred during the evolution of section *Otites*. There are also several pieces of evidence about introgression of sex determining system via interspecific hybridization.

To better understand basic steps in the evolution of sex-determination in section *Otites*, we have focused our research on the study of the *Silene sibirica*. *Silene sibirica* is a gynodioecious species closely related to dioecious species in section *Otites*. To study sex-determination in *Silene sibirica*, we have used RNA-seq analysis. The data that we obtained were used for genetic mapping of chromosome carrying male fertility restorer. We were also able to identify putative male fertility restorer as well as a rearrangement of proto-Y and proto-X chromosome.

The same analysis runs with other gynodioecious species from genus *Silene*, *Silene vulgaris*.

Our results about evolution of sex-determining system in *Silene* interestingly correspond with results of the research done in *Fragaria*. It suggests similarities in the pathways of the evolution of sex-determining systems in *Fragaria* and *Silene* in spite of their large phylogenetic distance.

## REFERENCES

1. Hobza R., Hudzieczek V., Kubat Z., Cegan R., Vyskot B., Kejnovsky E., Janousek R.: *Ann. Bot.* 122, 1085 (2018).
2. Slancarova V., Zdanska J., Janousek B., Talianova M., Zschach C., Zluzova J., Siroky J., Kovacova V., Blavet H., Danihelka J., Oxelman B., Widmer A., Vyskot B.: *Evolution.* 67, 3669 (2013).
3. Balounova V., Gogela R., Cegan R., Cangren P., Zluzova J., Safar J., Kovacova V., Bergero R., Hobza R., Vyskot B., Oxelman B., Charlesworth D., Janousek B.: *Sci Rep.* 9, 1045 (2019).
4. Tennessen J.A., Wei N., Straub S.C.K., Govindarajulu R., Liston A., Ashman T.-L. *PLoS Biol.* 16, e2006062 (2018).



**LIPOPOLYSACCHARIDE FROM *MICROCYSTIS AERUGINOSA*-DOMINATED WATER BLOOM ACTIVATES DIFFERENT CELL TYPES *IN VITRO* AND *EX VIVO***

**ZITA GOLIÁŠOVÁ<sup>a</sup>, ZDENA MOOSOVÁ<sup>a,b</sup>, GABRIELA AMBROŽOVÁ<sup>a</sup>, ONDŘEJ VAŠÍČEK<sup>a</sup>, VANDA HOŠEKOVÁ<sup>a</sup>, LUKÁŠ KUBALA<sup>a</sup>, PAVEL BABICA<sup>b,c</sup>, LENKA ŠINDLEROVÁ<sup>a</sup>**

<sup>a</sup>Dept of Biophysics of Immune System, Institute of Biophysics, AS CR, Královopolská 135, 612 00 Brno; <sup>b</sup>RECETOX, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno; <sup>c</sup>Institute of Botany AS CR, Dept of Experimental Phycology and Ecotoxicology, Lidická 25/27, 602 00, Brno  
z.goliasova@gmail.com

Cyanobacterial water blooms (HAB) are a global problem of freshwater reservoirs posing a significant risk to human health. Apart from highly toxic cyanotoxins, cyanobacterial and bacterial lipopolysaccharides (LPS) are toxins with not well described effects on human cells. LPS activates inflammatory responses of the cell via Toll-like receptor 4 (TLR 4). Exposure to environmental cyano-LPS happens primarily via oral intake targeting intestinal epithelium. Therefore, differentiated Caco-2 and confluent HT-29 human intestinal cell lines were used to study effects of LPS isolated from *Microcystis aeruginosa* (MA)-dominated HAB (WB). Exposure to the WB LPS induced increased production of pro-inflammatory cytokines interleukin-12 (IL-12), IL-8 and IL-1 $\beta$ . Moreover, the LPS showed endotoxin activity two times higher than *E. coli* (EC) LPS. Taking into account the ability of LPS to pass into the bloodstream via activated intestinal epithelium, the major phagocytes – polymorphonuclear leukocytes (PMNL) and monocytes from human whole blood - were tested and shown to be activated. Expression of CD11b and CD66b surface markers and activation of NF- $\kappa$ B and p38-MAPK was observed.

For deeper insight into the mechanism, macrophage cell line RAW 264.7 was employed. Contrary to the EC LPS, only tumor necrosis factor- $\alpha$ , but not IL-6 and nitric oxide (NO) production was induced by WB LPS. Furthermore, inhibition of TLR 4 did not decrease WB LPS effect.

In conclusion, LPS from MA-dominated water bloom exerts high endotoxin activity and pro-inflammatory effects in intestinal epithelial cells as well as in immune cells *in vitro* and *ex vivo* but the mechanism differs from model EC LPS.

*This study was supported by the Czech Science Foundation (19-09980S).*

**A SUSTAINABLE SYNTHETIC APPROACH TOWARD GRAPHENE QUANTUM DOTS FOR BIOIMAGING APPLICATIONS**

**I. JÉNNIFER GÓMEZ<sup>a,\*</sup>, JIŘINA MEDALOVÁ<sup>a</sup>, MANUEL VÁZQUEZ SULLEIRO<sup>b</sup>, NADĚŽDA PIZÚROVÁ<sup>c</sup>, LENKA ZAJÍČKOVÁ<sup>a,d,\*</sup>**

<sup>a</sup>CEITEC Masaryk University, Kamenice 5, 62500 Brno, Czech Republic; <sup>b</sup>IMDEA Nanociencia, Faraday 9, 28049 Madrid, Spain; <sup>c</sup>Institute of Physics of Materials AS CR, Žitkova 22, 61662 Brno, Czech Republic; <sup>d</sup>Faculty of Science Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic  
gomez.perez@ceitec.muni.cz

In the last few decades, significant advances were achieved in the research of carbon nanomaterials. Nowadays, the nanocarbon family spans from fullerene, the first member, to graphene quantum dots (GQDs), the last to join. GQDs are a fascinating class of nanocarbons that comprise quasi-spherical nanoparticles with sizes below 10 nm<sup>1</sup>. They typically display excitation wavelength dependence, excellent photostability and chemical stability, good biocompatibility, high-water solubility and low toxicity<sup>2</sup>. Furthermore, they can be easily functionalized with biomolecules. Due to these unique properties, GQDs have attracted tremendous interests for their potential in a lot of applications, especially in the biomedical field<sup>3</sup>.

In this work, the main objective was to obtain GQDs through a bottom-up approach: microwave-assisted hydrothermal method using glucose and ethylenediamine as molecular precursors. The as-prepared nanoparticles showed excitation-dependent visible and near-ultraviolet emission. Moreover, they have shown near-infrared emission, one of the research millstones of fluorescent carbon nanoparticles. The synthesized GQDs were nitrogen-doped and presented amine groups on their surface. Therefore, a high-water solubility due to the high nitrogen content. The morphology and size were studied by atomic force microscopy (AFM) and transmission electron microscopy (TEM), and the quasi-spherical crystalline nanoparticles of 1.5-7 nm were found. Finally, based on the *in vitro* analysis, the synthesized GQDs showed excellent biocompatibility and tuneable bioimaging applications.

*This work was supported by CEITEC Nano Research Infrastructure supported by MEYS CR (LM2018110).*

REFERENCES

1. Zheng X. T., Ananthanarayanan A., Luo K. Q., Chen P.: *Small*, *11*, 1620 (2015).
2. Reddy K. R., Haque E., Hossain M. S. A., Ward A. C., You J., Malgras V., Bando Y., Yamauchi Y., Kim J.: *Small Methods*, *2*, 1800050 (2018).
3. Tian P., Tang L., Teng K. S., Lau S. P.: *Mater. Today Chem.*, *10*, 221 (2018).

## POLYMER-PHOSPHATE BONE CEMENT MODIFIED BY ANTIBIOTICS FOR JOINT REPLACEMENT

**VERONIKA GRÉZLOVÁ<sup>a\*</sup>, KLÁRA LYSÁKOVÁ<sup>a</sup>, KRISTÝNA VALOVÁ<sup>a</sup>, KRISTÝNA ŠMERKOVÁ<sup>b</sup>, PAVEL KOPEL<sup>b</sup>, VOJTĚCH ADAM<sup>b</sup>, SILVIA KOČIOVÁ<sup>b</sup>, JAN VOJTÍŠEK<sup>c</sup>, EDGAR BENJAMIN MONTUFAR<sup>d</sup>, LADISLAV ČELKO<sup>d</sup>, JOSEF KAISER<sup>d</sup>, LUCY VOJTOVÁ<sup>a</sup>**

<sup>a</sup>Brno University of Technology, CEITEC, Adv. Biomat., Purkyňova 656/123, 612 00 Brno; <sup>b</sup>Mendel University in Brno, Fac. AgriSci., Dept Chem. Biochem., Zemědělská 1, 613 00 Brno; <sup>c</sup>Brno University of Technology, Fac. chem., Materials Research Centre, Purkyňova 118, 612 00 Brno; <sup>d</sup>Brno University of Technology, CEITEC, Mat. Character. Adv. Coatings, Purkyňova 656/123, 612 00 Brno  
Veronika.Grezlova@ceitec.vutbr.cz

Joint replacement surgery is quite common nowadays. However, these replacements are not life-long and often have to be reoperated. Surgery is an external intervention in the body that can cause an inflammatory reaction. Bacteria strains are becoming more and more resistant. This is the main reason, why we should find the solution and discover a novel way how to defend it. One way could be to add some antibacterial element into the bone cement and treat inflammation directly at the point of its origin.

In this work, tricalcium phosphate powder (TCP) was mixed with thermosensitive biodegradable copolymer PLGA-PEG-PLGA. This mixture created bone cement, which was modified by antibiotic Vancomycin. We tested antibacterial properties by disk dilution method, release of Vancomycin, mechanical strength, injectability, flowability, and setting reaction time. Transformation of TCP to crystals of calcium-deficient hydroxyapatite was verified by X-ray diffraction. This conversion was also figured by scanning electron microscopy.

The addition of Vancomycin to bone cement influence injectability and thixotropic properties. Prepared cement was effective on a gram-positive bacteria *Staphylococcus aureus* (SA) and its methicillin resistant form (MRSA), even in small amount. Moreover, Vancomycin accelerated self-hardening process of the bone cement.

Due to the positive effect of Vancomycin on antibacterial properties, the bone cement can be useful in action against inflammatory reaction.

This work was supported by the CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II, by the Ministry of Health under the project no. NV18-05-00379 and CEITEC VUT/FCH-J-19-5927.

## STOCHASTIC MODIFICATION OF BIOACTIVE SMALL MOLECULES FOR PROBE DEVELOPMENT AND PROTEIN TARGET IDENTIFICATION

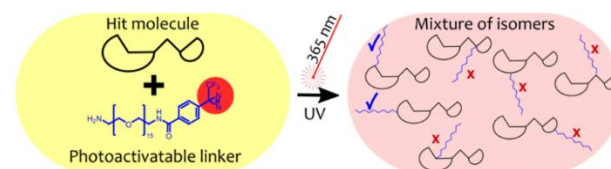
**MARTIN HADZIMA<sup>a,b</sup>, TEREZA ORMSBY<sup>a</sup>, PETR ŠIMON<sup>a</sup>, KRISTÝNA BLAŽKOVÁ<sup>a</sup>, PAVEL MAJER<sup>a</sup>, PAVEL ŠÁCHA<sup>a</sup>, LIBOR KOSTKA<sup>c</sup>, VLADIMÍR ŠUBR<sup>c</sup>, JAN KONVALINKA<sup>a,b</sup>**

<sup>a</sup>Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo 2, 166 10 Prague, Czechia; <sup>b</sup>Dept of Organic Chemistry, Faculty of Science, Charles University, 128 43 Prague, Czechia; <sup>c</sup>Institute of Macromolecular Chemistry AS CR, Heyrovského 2, 162 06 Prague, Czechia  
martin.hadzima@uochb.cas.cz

Phenotypic screening is a powerful tool for drug discovery used both in academia and industry. Identification of a protein target of the hit compound is complex, yet inevitable step in drug development. Most approaches rely on the preparation of an affinity probe which is a laborious and time-demanding process. Generally, a probe is derived from the hit compound, with a reporter tag attached via linker at certain position. Selection of the appropriate position is crucial but unpredictable without information on the protein-ligand structure.

We developed a method for stochastic modification of small molecules using a photoactivatable diazirine linker<sup>1</sup>. This procedure leads to an isomeric mixture of the compound of interest modified at multiple positions. Theoretically, at least a portion of the stochastic derivatives retains the affinity towards target and enables its visualization, isolation and identification. In addition, separation of the mixture into crude fractions leads to higher concentration of the active isomer(s) in one or multiple fractions.

Furthermore, the affinity of the active isomers can be improved by conjugation to iBodies, macromolecular antibody mimetics. iBodies exhibit increase of binding thanks to avidity caused by several ligand molecules bound to a single carrier.



Scheme 1. Stochastic modification of bioactive small molecules

The work is supported from European Regional Development Fund; OP RDE; Project: "ChemBioDrug" (No. CZ.02.1.01/0.0/0.0/16\_019/0000729).

## REFERENCE

- Šimon P., Knedlík T., Blažková K., Dvořáková P., Březinová A., Kostka L., Šubr V., Konvalinka J., Šácha P.: ACS Chem. Biol. 13, 3333 (2018).

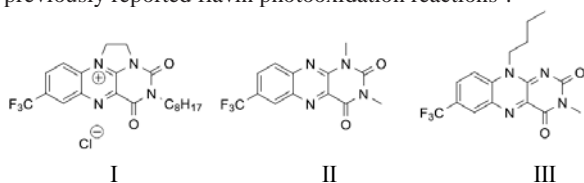
## TUNING FLAVIN-BASED PHOTOCATALYTIC SYSTEMS FOR APPLICATION IN THE MILD CHEMOSELECTIVE AEROBIC OXIDATION OF BENZYLIC SUBSTRATES

AMAL HASSAN\*, RADEK CIBULKA

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

Direct oxidation of C-H bonds and non-activated alcohols to the corresponding carbonyl compounds is one of the reaction pathways which has much attention because of the great potential in commercial applications such as in the fragrance or pharmaceutical industries<sup>1</sup>. Several attempts have been carried out for these transformations, nevertheless, excited ethylene-bridged alloxazinium salt **I** has been reported as the strongest biomimetic oxidant to date<sup>2</sup>. Actually, the excited form **I** has displayed its efficiency in the oxidation of strongly electron-deficient benzylic substrates to benzoic acids in a good yield, which was not accessible by the previously reported photooxidation catalysts<sup>2</sup>. Unfortunately, it was troublesome to cease the oxidation at aldehyde stage. In addition, flavinium salt starts to decompose after about 1 h of irradiation and converts to the neutral form. In spite of that, it still has a great catalytic activity with different types of substrates.

To overcome such limitations, new flavin-based photocatalytic systems used for chemoselective aerobic visible-light oxidations have been developed by tuning the flavin structure and reaction conditions. 1,3-Dimethyl-7-trifluoromethylalloxazine **II** and 10-butyl-3methyl-7-trifluoromethylalloxazine **III** were shown to mediate the selective oxidation of benzyl alcohols to form aldehydes in the presence of Cs<sub>2</sub>CO<sub>3</sub>. Flavin **III** was outstanding in the selective oxidation of toluene derivatives into aldehydes in the presence of trifluoroacetic acid. The usefulness of the developed catalytic systems using **I-III** was also demonstrated in the oxidation of secondary benzylic and aliphatic alcohols, and benzylic methylene groups to form the corresponding ketones. The systems have the advantage of a broad substrate scope and metal-free conditions, which distinguish them from the previously reported flavin photooxidation reactions<sup>3</sup>.



### REFERENCES

1. Nechab M., Einhorn C., Einhorn J.: Chem. Comm. 2004, 1500.
2. Zelenka J., Svobodová E., Tarábek J. N., Hoskovcová I., Boguschová V., Bailly S., Sikorski M., Roithová J., Cibulka R.: Org. Lett., 21, 114 (2018).

3. Tolba A. H., Vávra F., Chudoba J., Cibulka R.: Eur. J. Org. Chem. 2020, 1579; DOI: 10.1002/ejoc.201901628.

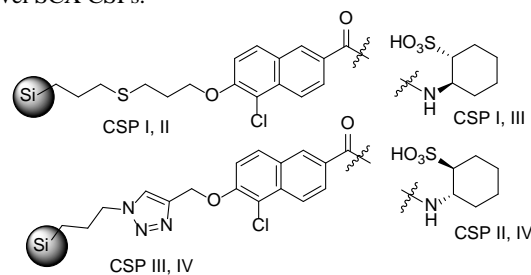
## SYNTHESIS AND EVALUATION OF NAPHTALENE-BASED CHIRAL STRONG CATION EXCHANGERS FOR CHIRAL SEPARATION OF BASIC DRUGS

JANA HERCIKOVÁ, MICHAL KOHOUT

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

Chiral separation of racemic mixtures represents an important tool in the production of enantiomerically pure substances. While researches can choose from a broad variety of commercially available chiral stationary phases (CSPs), the demand for novel, highly specific CSPs remains strong<sup>1</sup>. Specific CSPs are usually required for the chiral separation of charged or chargeable compounds. To address this need, chiral ion exchangers, which can separate a broad variety of such substances, have been developed<sup>2</sup>.

In this work, we have focused on novel type of strong cation-exchange (SCX) CSPs, which are well suited for chiral separation of basic analytes that act as cationic species upon protonation<sup>3</sup>. We have prepared four different SCX selectors and immobilized them by two different ways onto 3-mercaptopropyl- and 3-azidopropyl-modified silica using a radical reaction and copper(I)-catalyzed click-reaction, respectively (Scheme 1). The prepared CSPs were slurry packed and tested in a polar organic mode for the chiral separation of a set of basic drugs. The best results have been obtained for a mobile phase composed of methanol and acetonitrile with formic acid and diethylamine as a buffer. We show that, similarly to previously described *Cinchona* alkaloids-based chiral anion exchangers<sup>4</sup>, the structure of the linker to the silica support has an important effect on the chromatographic properties of the novel SCX CSPs.



Scheme 1. General structures of studied SCX CSPs differing in immobilization and absolute configuration of the sulfonic acid unit.

The authors thank P. Friihauf (University of Vienna) for assistance with column packing.

### REFERENCES

1. Lämmerhofer M.: J. Chromatogr. A 1217, 814 (2010).

- Lämmerhofer M.: Anal. Bioanal. Chem. 406, 6095 (2014).
- Wolrab D., P Frühauf., Kohout M., Lindner W.: J. Sep. Sci. 36, 2826 (2013).
- Kohout M., Wernisch S., Tůma J., Hettegger H., Pícha J., Lindner W.: J. Sep. Sci. 41, 1355 (2018).

### THE INHIBITORY EFFECT OF ANTIDEPRESSANT DRUGS ON PLACENTAL SEROTONIN HOMEOSTASIS

**HANA HORACKOVA, RONA KARAHODA, VERONIKA VACHALOVA, LUKAS CERVENY, CILIA ABAD, FRANTISEK STAUD\***

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

Depression affects up to 25% of pregnant women and approximately 10% are under pharmacotherapy. However, safety of this treatment is still controversial, since poor pregnancy outcomes such as organ malformations, neurological disorders and preeclampsia have been reported in pregnant woman using antidepressants (ADs). Selective serotonin reuptake inhibitors are the most frequently prescribed ADs in pregnancy, but there is lack of information about their interaction with placental serotonin homeostasis.

For accurate fetal development/programming, proper serotonin levels are of crucial importance and, therefore, concentrations of this monoamine must be tightly regulated in the fetoplacental unit. Two transporters have been described in the maternal-fetal interface, mediating serotonin uptake by the placenta. Serotonin transporter (SERT) takes up serotonin from the maternal circulation across the apical membrane to trophoblast. In addition, we have recently characterized that organic cation transporter 3 (OCT3) takes up serotonin from fetal circulation across basal membrane. In the current study, we hypothesize that ADs can influence serotonin homeostasis in placenta via inhibition of SERT and/or OCT3.

Experiments were performed using *in situ* dually perfused rat term placenta and *ex vivo* membrane vesicles isolated from human term placenta. In detail we tested the following ADs: paroxetine, citalopram, fluoxetine, fluvoxamine, sertraline and venlafaxine. In both human and rat placenta, we observed significant inhibitory effect of all tested drugs on placental handling of serotonin. Moreover, our data indicate the role of fetal gender in inhibition of OCT3 in rat placenta. We propose that use of ADs in pregnancy may affect placental homeostasis of serotonin and potentially alter placental and/or fetal development.

This project was funded by GAUK 1464119/C/2019, SVV 2019/260414, GACR 17-16169S.

### NOVEL OBETICHOLIC ACID DERIVATIVES AND ISOMERS AS ACTIVATORS OF THE FARNESOID X RECEPTOR (FXR) AND G PROTEIN-COUPLED BILE ACID RECEPTOR 1 (GPR1)

**ALZBETA HORVATOVA<sup>a</sup>, MIROSLAV KASPAR<sup>b</sup>, EVA KUDOVA<sup>b</sup>, PETR PAVEK<sup>a\*</sup>**

<sup>a</sup>Department of Pharmacology and Toxicology, Charles University, Heyrovského 1203, 500 05 Hradec Kralove, Czech Republic; <sup>b</sup>Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo 2, 166 10 Prague, Czech Republic  
pavek@faf.cuni.cz

Metabolic diseases with altered cholesterol and triglyceride levels are serious healthcare problem, emerging in western population, and are tightly linked to inflammation. Bile acids are important signalling regulating metabolism and inflammation via activation of several receptors, mainly farnesoid X receptor (FXR) and TGR5 receptor<sup>1,2</sup>. They are metabolized in hepatocyte and colon into their epimers and dehydrogenated forms. Recently, obeticholic acid (OCA), a potent (FXR) agonist, has been proposed as a promising treatment against inflammatory hepatic disorders<sup>3</sup>. Supposing that OCA undergoes the same metabolic modifications, we aimed to examine OCA epimers and oxidized derivatives for possible interactions with FXR, TGR5 and other nuclear receptors known to be activated by bile acid including vitamin D (VDR) and pregnane X (PXR) receptors.

A set of OCA derivatives was synthesized and analysed for their activity on nuclear receptor *in vitro* and *in vivo*. Gene reporter assays were performed to determine their capacity to activate nuclear receptors of interest. The results were confirmed *in silico* by TR-FRET assay and docking experiments. Changes on expression of target genes were evaluated by real time qPCR in human hepatic cells and C57BL/6 mice. Moreover, the conversion of the 3 hydroxy epimers and their pharmacokinetics in mice were studied employing LC-MS.

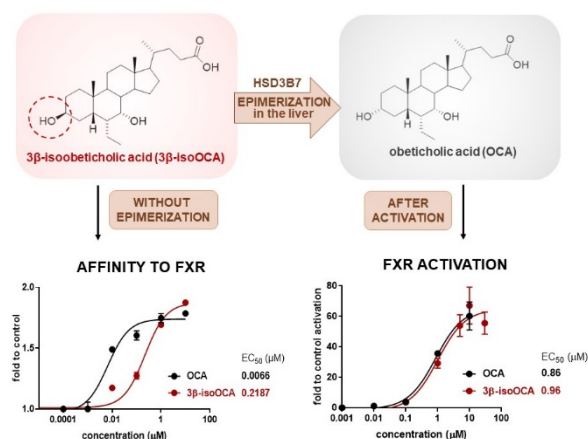


Fig. 1. 3β-hydroxy epimer of OCA acts as hepatocyte-activated prodrug of OCA

We found that 3 $\beta$ -hydroxy epimer of OCA has lower affinity to FXR than OCA; however, it readily converts to OCA, displaying the same capacity to activate FXR in human hepatocytes as well as in mice (Fig.1). This conversion and subsequent activation of FXR were inhibited by the hydroxy- $\Delta$ -5-steroid dehydrogenase inhibitor trilostane. In addition, we found that 3,7-dehydrobeticholic acid is a potent TGR5 agonist with marginal activity towards FXR.

We conclude that 3 $\beta$ -hydroxy-OCA is a prodrug of OCA activated by in liver and that 3,7-dehydro-OCA represents a novel selective TGR5 activator. These findings warrant further pharmacological examination of these compounds.

Study was supported by GAUK 170/50/85006 and SVV 260 414.

## REFERENCES

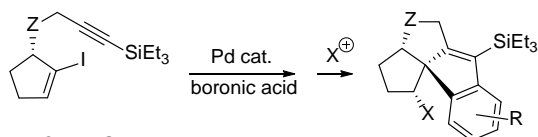
1. Molinaro A., Wahlstrom A., Marschall H.U.: Trends Endocrinol. Metab. 29, 31 (2018).
2. Pathak P., Liu H., Boehme S., Xie C., Krausz K., Gonzalez F., Chiang J.: J. Biol. Chem. 292, 11055 (2017).
3. Fiorucci S., Distrutti E., Ricci P., Giuliano V., Donini A., Baldelli F.: Expert Opin. Ther. Targets 18, 1449 (2014).

## SYNTHESIS OF ALKALOID-LIKE COMPOUNDS WITH ALL-CARBON QUATERNARY CENTRES

**PETR JANSA<sup>a</sup>, ELIŠKA MATOUŠOVÁ\***

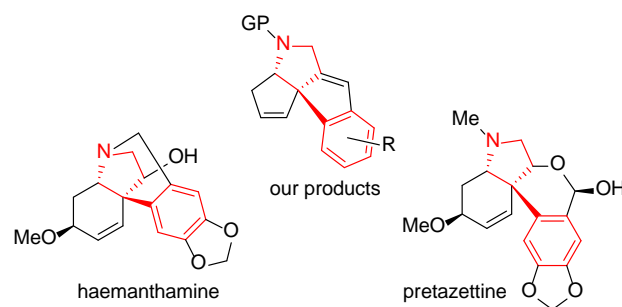
Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 8, 128 43 Prague, Czech Republic  
petr.jansa@natur.cuni.cz

Recently, we have developed a method for the enantioselective synthesis of compounds containing all-carbon quaternary centres<sup>1</sup>. The key transformations of the reaction sequence are tandem cyclisation/Suzuki cross-coupling and halocarbocyclisation (Scheme 1).



Scheme 1. The key transformations

Herein we present the application of this method in the synthesis of compounds with a similar structural pattern as is present in the alkaloids of the *Amaryllidaceae* plant family. Docking-based molecular dynamic simulation of these compounds was also performed, suggesting their acetylcholinesterase-inhibitory activity.



Scheme 2. Structural resemblance of our products to the selected *Amaryllidaceae* alkaloids

Moreover, further transformations of our products to advanced intermediates in the synthesis of haemanthamine or pretazettine analogues are discussed. These *Amaryllidaceae* alkaloids (Scheme 2) exhibit significant antiproliferative activity<sup>2,3</sup>.

This work was supported by Charles University (Projects PRIMUS/18/SCI/14 and SVV 260429).

## REFERENCES

1. Mikušek J., Jansa P., Jagtap P. R., Vašíček T., Císařová I., Matoušová E.: Chem. – Eur. J. 24, 10069 (2018).
2. McNulty J., Nair J. J., Codina C., Bastida J., Pandey S., Griffin C. Phytochemistry 68, 1068 (2007).
3. Weniger B., Italiano L., Beck J. P., Bastida J., Bergoñon S., Codina C., Anton R. Planta Med. 61, 77 (1995).

## CAN X-RAY POWDER DIFFRACTION BE A SUITABLE ANALYTICAL METHOD FOR ILLICIT DRUG IDENTIFICATION?

**BRONISLAV JURÁSEK<sup>a</sup>, VILÉM BARTŮŇEK<sup>b</sup>, ŠTĚPÁN HUBER<sup>b</sup>, PATRIK FAGAN<sup>c</sup>, VLADIMÍR SETNÍČKA<sup>c</sup>, FRANTIŠEK KRÁLÍK<sup>c</sup>, WIM DEHAEN<sup>d</sup>, MARTIN KUCHAR<sup>a\*</sup>**

<sup>a</sup>Forensic Laboratory of Biologically Active Substances and Dept Chemistry of Natural Compounds, <sup>b</sup>Dept Inorganic Chemistry, <sup>c</sup>Dept Analytical Chemistry, <sup>d</sup>Dept of Informatics and Chemistry, University of Chemistry and Technology Prague, Technická 5, Prague, 166 28, Czech Republic  
kuchara@vscht.

When the chemical structure of an illicit substance is carefully changed, the result can be a substance with similar psychoactive effects that subverts drug control legislation. These new psychoactive substances (NPS) exert considerable pressure on medicinal and law enforcement centres, as they are associated with an enormous number of fatalities and a great amount of chemically novel structures. As a result, there is significant pressure for the development of fast, cheap and reliable analytical methods capable of identifying these substances directly on a crime scene.

Raman and infrared (IR) spectroscopies are suitable for such detection, but they have limitations; for example,

fluorescence in Raman can overlay the signal and when the sample is a mixture sometimes neither Raman nor IR can identify the compounds. Therefore, we investigated the potential of X-ray powder diffraction (XRPD) for the identification of drugs.

First, a series of 8 NPS standards was used to verify whether XRPD can distinguish substances with similar chemical structures<sup>1</sup>. Subsequently, a series of seized samples obtained from the law enforcement agency was analysed by XRPD to verify the ability to identify substances in real samples. The series of seized samples was also measured by IR and Raman, and the resulting capabilities of individual instrumentations to identify street samples were compared.

XRPD was able to distinguish all of the NPS with similar molecular structure and was able to identify each of the seized substances. Both Raman and IR spectroscopies were not able to detect the substances in all of the samples. These results show that XRPD could be a valuable addition to the range of analytical tools used to detect these compounds in illicit drug samples.

*This study was funded by a specific university research (project MSMT No. 21-SVV/2019), by the Ministry of Interior of the Czech Republic (project VI20172020056).*

#### REFERENCE

1. Jurásek B., Bartůněk V., Huber S., Kuchař M.: *Talanta* 195, 1 (2019).

#### THE ROLE OF miR-183 FAMILY IN THE DIFFERENTIATION OF THE RETINAL ORGANIDS

**DENISA JURČÍKOVÁ, LUCIE PEŠKOVÁ, JAN KRIVÁNEK, MICHAELA CAPANDOVÁ, MAGDALÉNA KOLOUŠKOVÁ, HANA KOTASOVÁ, TOMÁŠ BARTA**

*Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 3, Brno 62500, Czech Republic  
Denisa.jurcikova@seznam.cz*

MicroRNAs (miRNAs), a class of small, non-coding RNA molecules represents important regulators of gene expression. Recent reports have implicated their role in cell specification process acting as "fine tuners" of gene expression to ensure the precise gene expression at specific stage of cell differentiation.

Evolutionary conserved miR-183 family contains three individual miRNA members - miR-183, miR-96, and miR-182. It has been reported that miR-183 family is specifically expressed in retina as well as in pluripotent stem cells. Given its tissue-specific expression pattern, miR-183 family represents likely an important player in differentiation of pluripotent stem cells into sensory organs [1].

Here we used human retinal organoids differentiated from human pluripotent stem cells (hPSCs) as a model to closely investigate the role of the miR-183 family. Using

miRNA tough decoy approach, we inhibited miR-183 family in hPSCs. Inhibition of miR-183 family resulted in an increased expansion of neuro-epithelium leading to abnormal "bulged" neural retina in organoids associated with up-regulation of neural-specific and retinal-specific genes.

Importantly, we identified PAX6 – a well-known essential gene in neuroectoderm specification, as a target of miR-183 family members [2]. Taken together, miR-183 family not only represents an important regulator of PAX6 expression in the formation of neuroectoderm, but it also plays a crucial role in retinal tissue morphogenesis presumably acting via regulation of PAX6 expression.

*This work has been supported by the projects: Grant Agency of Masaryk University (GAMU) – MUNI/G/1391/2018, Brno Ph.D. Talent, the Faculty of Medicine MU to junior researchers T.B. and L.P. We acknowledge the core facility CELLIM of CEITEC supported by the Czech-BioImaging large RI project (LM2018129 funded by MEYS CR) for their support with obtaining scientific data presented in this work.*

#### REFERENCES

1. Pierce M.L., Weston M.D., Fritzsche B., Gabel H.W., Ruvkun G., Soukup G.A.: *Evol. Dev.* 10, 106 (2008).
2. Dahl E, Koseki H, Balling R.: *Bioessays* 19, 755 (1997).

#### ROLE OF PROLINE/GLYCINE KINK IN PORE FORMATION BY ANTIMICROBIAL PEPTIDES

**ALZBETA TUERKOVA<sup>a,b</sup>, IVO KABELKA<sup>b</sup>, TEREZA KRÁLOVÁ<sup>b</sup>, LUKÁŠ SUKENÍK<sup>b</sup>, ŠÁRKA POKORNÁ<sup>c</sup>, MARTIN HOF<sup>c</sup>, ROBERT VÁCHA<sup>b\*</sup>**

*<sup>a</sup>Department of Pharmaceutical Chemistry, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; <sup>b</sup>CEITEC, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic; <sup>c</sup>J. Heyrovský Institute of Physical Chemistry, Dolejškova 3, 182 23 Prague, Czech Republic  
robert.vacha@mail.muni.cz*

Antimicrobial peptides (AMPs) can selectively disrupt bacterial membranes by the formation of leaky pores (see Fig. 1)<sup>1</sup>. Their selectivity and potency make them an appealing subject for drug development. Unfortunately, matching the peptide properties with their activity remains elusive. For instance, the role of proline/glycine kink in  $\alpha$ -helical peptides was reported to both enhance and reduce the antimicrobial activity<sup>2,3</sup>.

In this work, we combined molecular dynamics simulations and fluorescence leakage assays to demonstrate that a helical kink stabilizes toroidal pores but disrupts barrel-stave pores. In addition, the exact position of the proline/glycine kink in the peptide sequence further controls the structure of toroidal pores. The provided molecular-level insight could be utilized for the design and modification of pore-forming antibacterial peptides.

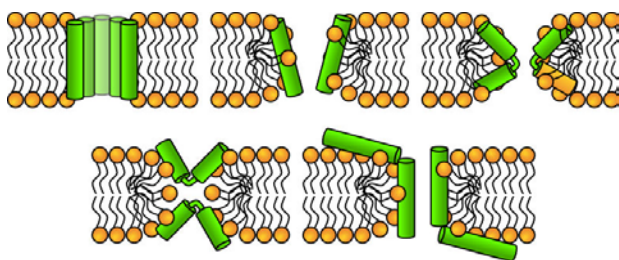


Fig. 1. Various types of membrane pores formed by antimicrobial peptides

This research was supported by GAČR (17-11571S) and the CEITEC 2020 (LQ1601) project. Computational resources were provided by the CESNET (LM2015042) and the CERIT Scientific Cloud (LM2015085).

#### REFERENCES

1. Peters B. M., Shirtliff M. E., Jabra-Rizk M. A.: *PLoS Pathog.* 6, e1001067 (2010).
2. Park C. B., Yi K. S., Matsuzaki K., Kim M. S., Kim S. C.: *Proc. Natl. Acad. Sci. U.S.A.* 8245, 97 (2000).
3. Liu L., Fang Y., Huang Q., Wu J.: *PLoS One* 6, e16441 (2011).

#### BIOMECHANICAL, STRUCTURAL AND *IN VITRO* EVALUATION OF A RESORABLE BIOPOLYMER-BASED SCAFFOLDS FOR SKIN TISSUE ENGINEERING APPLICATIONS

**KATARÍNA KACVINSKÁ<sup>a\*</sup>, VERONIKA PAVLIŇÁKOVÁ<sup>a</sup>, PETR POLÁČEK<sup>a</sup>, NIKOLA PTÁČKOVÁ<sup>b</sup>, VERONIKA ŠTĚPÁNKOVÁ<sup>b</sup>, VERONIKA BLAHNOVÁ<sup>c</sup>, EVA FILOVÁ<sup>c</sup>, LUCY VOJTOVÁ<sup>a</sup>**

<sup>a</sup>CEITEC –Brno University Technology, Purkyňova 656/123, 612 00 Brno, Czech Republic; <sup>b</sup>Enantis s.r.o., Kamenice 34, 625 00 Brno, Czech Republic; <sup>c</sup>Institute of Experimental Medicine, AS CR, Vídeňská 1083, 142 20 Prague 4, Czech Republic

katarina.kacvinska@ceitec.vutbr.cz

The addition of polysaccharides and blood derivatives in protein-based scaffolds has shown some perspective future views for skin wound applications affecting both antibacterial and healing properties<sup>1</sup>.

In the presented study, the effect of polysaccharides and bioactive coating on the biomechanical, structural and *in-vitro* properties of protein-based scaffolds was evaluated. Due to the ionic interactions with proteins, polysaccharides influence the swelling capacity, porosity and biomechanical properties of protein scaffolding material considering it as desirable and beneficial for restoring the function of the skin. In general, the addition of polysaccharides had increased strain without any detectable deformation during biomechanical test, which is referred to the improved elasticity of microfibrils. *In-vitro* biocompatibility assay on mouse fibroblasts 3T3 reported that

polysaccharide addition as well as bioactive coating of protein-based porous scaffolds had enhanced cell adhesion and proliferation, together with a high proportion of viable cells maintained for a significantly longer time throughout the whole culture period of four weeks.

Grafts used to treat skin defects requires a right balance between elasticity and stiffness, together with soft porous architecture allowing cells to infiltrate, adhere and proliferate. The polysaccharide-protein skin grafts are ideal to meet these requirements, therefore serve as good skin engineered substitutes. Moreover, we found that by the amount and type of polysaccharide and bio-coating, the enzymatic degradation of protein-based scaffolds (its "life-time") can be tailored in order to follow the rate of skin regeneration.

This work was supported by the Czech Health Research Council under the project no. 17-29874A.

#### REFERENCE

1. Babrnáková J., Pavliňáková V., Brtníková J., Sedláček P., Prosecká E., Rampichová M., Filova E., Hearnden V., Vojtová L.: *Mater. Sci. Eng., C* 100, 236 (2019).

#### METABOLIC PROFILING OF NEUROACTIVE STEROIDS IN PATIENTS WITH MULTIPLE SCLEROSIS

**MICHAL KALETA, JANA OKLEŠŤKOVÁ, ONDŘEJ NOVÁK**

Laboratory of Growth Regulators, Institute of Experimental Botany, The Czech Academy of Sciences & Faculty of Science, Palacký University, Šlechtitelů 27, 78371 Olomouc, Czech Republic  
michal.kaleta@upol.cz

Neuroactive steroids are all steroid substances, of natural, as well as synthetic origin, that can modulate the nervous system functions<sup>1</sup>. They are involved in shaping the structure and function of the nervous system throughout the lifespan<sup>2</sup>. The important role of these steroids in the pathology and therapy of a variety of neurological and psychiatric disorders, such as depression, anxiety, epilepsy or multiple sclerosis, cannot be overlooked<sup>3</sup>. To support this claim changes in neuroactive steroid levels have been observed in some neurodegenerative diseases<sup>4</sup>. Such changes can be used as biomarkers for pathological processes in the nervous system. Neurodegeneration is typical for multiple sclerosis – an inflammatory demyelinating disease of the CNS<sup>5</sup>.

The aim of this study was to determine the levels of selected neuroactive steroids in the blood serum of men suspected of multiple sclerosis and to compare the results with the control group. The processing of blood serum samples started with their extraction. Serum proteins were precipitated with methanol and subsequently removed. Ultra-high-performance liquid chromatography combined with tandem mass spectrometry was then used to profile progesterone,

pregnenolone, allopregnanolone, testosterone, dehydroepiandrosterone and pregnanolone conjugates. Hormonal differences were observed between patients with sclerosis and control group in this study. For both groups, dehydroepiandrosterone, testosterone, pregnenolone and progesterone were quantified. Surprisingly, allopregnanolone and pregnanolone conjugates were not detected. Next research dealing with changes in levels of steroid hormones in people with multiple sclerosis in comparison to healthy population might help to expand knowledge of sclerosis pathophysiology, discover new diagnostic options or even find a new therapeutic approach.

#### REFERENCES

- Melcangi R. C., Garcia-Segura L. M., Mensah-Nyagan A. G.: *Cell. Mol. Life Sci.* **65**, 777 (2008).
- Belvederi Murri M., Fanelli F., Pagotto U., Bonora E., Triolo F., Chiri L., Allegri F., Mezzullo M., Menchetti M., Mondelli V., Pariante C., Berardi D., Tarricone I.: *Schizophr. Res. Treat.* **2016** (2016).
- Zheng P.: *Prog. Neurobiol.* **89**, 134 (2009).
- Caruso D., Melis M., Fenu G., Giatti S., Romano S., Grimoldi M., Crippa D., Marrosu M. G., Cavaletti G., Melcangi R. C.: *J. Neurochem.* **130**, 591 (2014).
- Inglese M., Petracca M.: *Schizophr. Res.* **161**, 94 (2015).

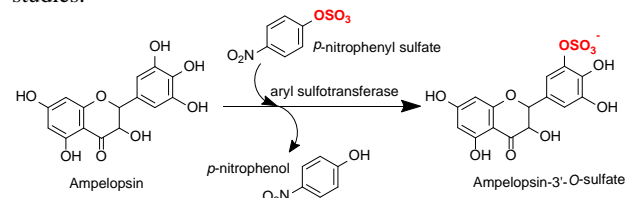
#### SULFATED METABOLITES OF QUERCETIN DERIVATIVES: PREPARATION AND BIOPHYSICAL PROPERTIES

**KRISTÝNA KÁŇOVÁ<sup>a,b</sup>, KATEŘINA VALENTOVÁ<sup>a</sup>, LUCIE PETRÁSKOVÁ<sup>a</sup>, VLADIMÍR KŘEN<sup>a</sup>**

<sup>a</sup>Institute of Microbiology of the Czech Academy of Sciences, Videňská 1083, 14220 Prague; <sup>b</sup>Department of Biochemistry and Microbiology, University of Chemistry and Technology, Technická 5, 16628 Prague  
k.kanova93@gmail.com

Flavonoids are well known for their significant biological activity. Together with their authentic human metabolites they are important standards for metabolic studies. Therefore, the preparation of such metabolites under laboratory conditions is essential to better understand the metabolism and pharmacological effects of the parent compounds. The aim of this study was preparation and purification of sulfated metabolites of luteolin, myricetin and ampelopsin. Using aryl sulfotransferase from *Desulfitobacterium hafniense* and *p*-nitrophenyl sulfate (Scheme 1), fractions of mono-, di- and trisulfates with a purity of >95% were obtained and subsequently characterized by HPLC, MS and NMR. After characterization, six *in vitro* methods were used to test the compounds for their ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)) and DMPD<sup>+</sup> (*N,N*-dimethyl-*p*-phenylenediamine) radicals, to reduce ferric ions and the Folin-Ciocalteu reagent, and to inhibit the rat liver microsomal membrane lipid peroxidation

induced by *tert*-butyl hydroperoxide. We prepared and identified 12 new sulfated metabolites of luteolin, myricetin and ampelopsin, and tested their antiradical, reducing and anti-liperoxidant activities. The activity of the prepared compounds differed considerably; surprisingly even between the monosulfate isomers. Together with myricetin-3'-*O*-sulfate, the parent compounds were the most active while the other compounds displayed significantly less activity, particularly the luteolin sulfates. Our findings show that sulfation considerably affects the biological activity of flavonoids. The prepared sulfated metabolites can now be used as authentic standards in future *in vitro* and *in vivo* metabolic studies.



Scheme 1. Example of sulfation reaction

This project was funded by GA 19-00043S, MEYS LTC18003 and LTC19039 (COST Actions CA16112 NutRedOx and CA16205 UNGAP).

#### SEROTONIN HOMEOSTASIS IN THE MATERNO-FETAL INTERFACE AT TERM

**RONA KARAHODA, HANA HORACKOVA, PETR KASTNER, ANDREAS MATTHIOS, LUKAS CERVENY, RADIM KUCERA, JURJEN DUINTJER TEBBENS, CILIA ABAD, FRANTISEK STAUD\***

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

Serotonin (5-HT) is an important trophic factor and tight regulation of its levels are required for proper placental function and fetal development. Fetal 5-HT supply switches from external (placental and/or maternal) sources early in pregnancy, towards own synthesis later in gestation. Here we reinvestigate the role of placenta in 5-HT handling at term and show that the Organic Cation Transporter 3 (OCT3/*SLC22A3*) is a key molecule controlling fetal 5-HT levels at term.

Using dually perfused rat term placenta and placental membrane vesicles isolated from human term placenta we observed high concentration-dependent uptake of 5-HT from the basal, fetus-facing membrane of the placenta. We characterized this uptake to be mediated by OCT3, inhibitable by endogenous compounds (glucocorticoids) and pharmacological agents (antidepressants, antidiabetics). Population analyses in rat placenta revealed that this phenomenon is significantly influenced by the fetal sex. Furthermore, we report negligible 5-HT levels in the fetal



circulation during maternal-to-fetal 5-HT transport studies and *de novo* synthetic studies at term.

Altogether, we provide novel evidence of 5-HT handling by term placenta. While no longer a 5-HT ‘donor’, placenta at term actively takes up 5-HT from both maternal and fetal circulations for degradation by monoamine oxidase-A within the trophoblast. We hypothesize that this interplay of uptake transporters and degradation enzyme offers a protective mechanism against the local vasoconstrictive effects of 5-HT in the placenta on both maternal and fetal sides.

Our results open new windows for so far unforeseen complications during pregnancy, including prenatal glucocorticoid excess and/or pharmacotherapy of pregnant women with OCT3 inhibitors.

*This project was funded by SVV 2020/260414 and EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16\_019/0000841)*

### FULLY AUTOMATED PIPELINE FOR ANCESTRAL SEQUENCE RECONSTRUCTION

**RAYYAN TARIQ KHAN<sup>a,b</sup>, MILOS MUSIL<sup>a,c</sup>, JAN STOURAC<sup>a,c</sup>, HANNES KONEGGER<sup>a,b</sup>, DAVID BEDNAR<sup>a,b</sup>, JIRI DAMBORSKY<sup>a,b</sup>**

*<sup>a</sup>Loschmidt Laboratories, Dept Exp. Biol. and RECETOX, Masaryk University, Kamenice 5/A13, 625 00 Brno, Czech Republic; <sup>b</sup>Intl Clin. Res. Ctr., St. Anne’s Univ. Hospital Brno, Pekarska 53, 656 91 Brno, Czech Republic; <sup>c</sup>Dept Information Systems, Faculty of Information Technology, Brno University of Technology, 612 66 Brno, Czech Republic  
Rayyan.Tariq.Khan@gmail.com*

During the course of evolution, nature produced a large number of diverse solutions to environmental problems by evolving proteins. This rich functional diversity is still encoded in DNA sequences spread over several branches of the tree of life<sup>1</sup>. Phylogenetic sequence reconstruction methods allow us to access, and process the exponentially growing DNA and protein sequence databases<sup>2</sup>, in order to reveal patterns which are representative for stable protein folds. Insights gathered from such analyses may help to facilitate the process of engineering thermostability in a multitude of proteins<sup>3</sup>. These methods are generally not accessible to scientists outside communities of evolutionary biologists. The purpose of this research is to construct a fully automated and robust pipeline. This pipeline allows anyone, including those who lack specialist knowledge, to reconstruct phylogenetic trees and derive ancestral sequences, thus reducing the academic gap and barrier to entry. The pipeline was extended with novel gap reduction algorithms and a method for handling prokaryotic datasets without the need for an out-group. This will allow its use even in cases where enough data is not available to determine a suitable out-group. The automation friendly nature will allow users to perform calculations remotely. The pipeline was verified against work that was previously done in the lab; on the ancestral sequence reconstruction of haloalkane dehalogenase family<sup>4</sup>. The newly

developed pipeline will be integrated to the web tool FireProt for protein stabilization<sup>5</sup>. A tentative link to the tool can be accessed at: <https://loschmidt.chemi.muni.cz/fireprotasr/>.

*The author would like to express his gratitude for the amazing support provided by the entire team of Loschmidt Laboratories.*

### REFERENCES

1. Pauling L., Zuckerkandl E.: *Acta Chem. Scand. suppl. 17*, S9 (1963).
2. Liberles D. A., ed. *Ancestral sequence reconstruction*. Oxford University Press on Demand, (2007).
3. Gaucher E., Sridhar G., Omjoy K.: *Nature* **451**, 704 (2008).
4. Babkova P., Sebestova E., Brezovsky J., Chaloupkova R., Damborsky J.: *ChemBioChem* **18**, 1448 (2017).
5. Bednar D., Beerens K., Sebestova E., Bendl J., Khare S., Chaloupkova R., Prokop Z., Brezovsky J., Baker D., Damborsky J.: *PLoS Comp. Biol.* **11**, e1004556 (2015).

### ENZYBIOTICS: HELP IN TREATMENT OF STAPHYLOCOCCAL INFECTION

**ŠÁRKA KOBZOVÁ, LUBOMÍR JANDA\***

*Department of Immunology, Veterinary Research Institute, Hudcova 296/70 Brno, Czech Republic  
kobzova@vri.cz*

Increasing resistance of bacteria to antibiotics is a huge problem today. The enzyme therapy presents some hope in the treatment of bacterial infections. Staphylococci, especially *Staphylococcus aureus*, are the most common cause of skin and soft-tissue infection<sup>1</sup>. Potential enzyme antibiotics (enzybiotics) include lysostaphin and endolysin, peptidoglycan hydrolases isolated from bacteria or bacteriophages, which effectively cleave a peptidoglycan in the staphylococcal cell wall leading to rupture due to osmotic pressure<sup>2</sup>.

For using these enzymes as a drug, it is not only necessary to improve its properties (solubility, production, stability), but also to minimize the possibility of developing resistance<sup>3</sup>. Our goal was to prepare thermostable enzybiotics against *Staphylococcus aureus* and incorporated these enzymes to collagen/carboxymethylcellulose dressing potentially with the impact for patients with acute injuries (burns) or with early chronic wounds.

We have already prepared lysostaphin and endolysin recombinantly in *Escherichia coli* and tested their activity in vitro with two detection method: plate lysis assay and turbidity assay. We have tested 15 sequenced types of *Staphylococcus aureus* for susceptibility to lysostaphin and endolysin  $\phi$ 812 in solution and to collagen/carboxymethylcellulose dressing enriched with lysostaphin. We have analyzed some staphylococcal strains with mass spectrometry to identify differences responsible for higher resistance to enzymes.

Using a bacterial expression system, we can produce the protein in satisfactory quantity. Both detection methods showed that lysostaphin is a highly potent antimicrobial agent, inhibiting the growth of all tested strains at low concentrations. However, the results of the plate lysis assay also show reduced susceptibility to lysostaphin during three months in refrigerator, especially for strains ST30, ST398 and ST15. The turbidity assay revealed that the ST 30 strain also exhibited reduced susceptibility to lysostaphin. Mass spectrometer analysis of *S. aureus* pointed out the differences in proteome of strain ST30.

#### REFERENCES

1. Eckmann C., Lawson W., Nathwani D., Solem C. T., Stephens J. M., Macahilig C., Simoneau D., Hajek P., Charbonneau C., Chambers R., Li J. Z., Haider S.: *Int. J. Antimicrob. Agents* 44, 56 (2014).
2. Hojckova K., Stano M., Klucar L.: *BMC Microbiol.* 13, 53 (2013).
3. Grishin A. V., Lavrova N. V., Lyashchuk A. M., Strukova N. V., Generalova M. S., Ryazanova A. V., Shestak N. V., Boksha I. S., Polyakov N. B., Galushkina Z. M., Soboleva L. A., Vetchinin S. S., Pavlov V. M., Karyagina A. S., Lunin V. G.: *Molecules* 24, 1879 (2019).

#### ORTHOLOG OF TELOMERE REPEAT BINDING PROTEIN 1 (TRB1) IN ONION (*ALLIUM CEPA*)

**KAROLÍNA KOLÁŘOVÁ<sup>a,b</sup>, PAVEL VEVERKA<sup>b,d</sup>, JAN PALEČEK<sup>c</sup>, PETR FAJKUS<sup>a,c</sup>, IVONA NEČASOVÁ<sup>a,d</sup>, JIRÍ FAJKUS<sup>a,b,c,\*</sup>, VRATISLAV PEŠKA<sup>a,\*</sup>**

<sup>a</sup>*Inst. Biophys. AS CR, 612 65 Brno, Czech Republic; <sup>b</sup>Lab. Funct. Genom. Proteom., Natl Ctr Biomol. Res., Fac. Sci., Masaryk University, 611 37 Brno, Czech Republic; <sup>c</sup>Mendel Ctr Plant Genom. Proteom., CEITEC, Masaryk University, 625 00 Brno, Czech Republic, <sup>d</sup>LifeB, Chromatin Mol. Comp., CEITEC Funct. Genom. Proteom., Natl Ctr Biomol. Res., Fac. Sci., Masaryk University, 625 00 Brno, Czech Republic*  
vpeska@ibp.cz; fajkus@sci.muni.cz

The very ends of linear eukaryotic chromosomes are formed by telomeres, which usually consist of tandemly repeated short DNA motifs and associated proteins. Across the plant kingdom, the Arabidopsis-type telomeric sequence with the motif (TTTAGGG)<sub>n</sub> is dominant. However, numerous exceptions were found, for example, in plants from the orders Solanales and Asparagales, which reflect evolutionary transitions in telomere sequence. Most recently, the unusual telomeric sequence (CTCGGTTATGGG)<sub>n</sub> was characterized in *Allium* species (Asparagales) and its synthesis by telomerase was demonstrated <sup>1</sup>. In mammals, the regulation and protection of telomeres is mediated by telomere specific protein complex termed as shelterin. One of the most essential component of shelterin complex is named Telomeric Repeat binding Factor (TRF). TRFs directly bind the double-stranded telomeric DNA in a sequence specific way. Numerous putative

shelterin-like proteins were found also in plants, whereas the best functional homologues of TRFs were found in a family of telobox proteins called SMH (Single Myb histone)<sup>2</sup>. SMH family has several plant specific features e.g., structural three domain organization. Telomere localisation and functions of SMH members e.g., telomere repeat binding proteins from *Arabidopsis thaliana* (AtTRBs) have been successfully demonstrated <sup>3</sup>. Interestingly, we found five homologues coding for TRB candidates in *A. cepa*, where AcTRB1 is the best candidate component for *Allium* shelterin-like complex, with similar characteristics to AtTRB1 protein from *A. thaliana*. AcTRB1 localizes in the nucleus and nucleolus, forms homomeric complexes, and also binds telomeric repeats of *A. cepa*. Importantly, it also interacts with telomerase reverse transcriptase of *A. cepa* (AcTERT) subunit of telomerase, indicating its possible role in telomerase recruitment to telomeres. Our results obtained by a broad panel of methods from bioinformatics, genomics, proteomics, and microscopy (RACE cloning, yeast two hybrid system, bimolecular fluorescence complementation, fluorescence anisotropy, expression in bacteria and transiently in plants) suggest that despite the significant difference between *Arabidopsis* and *Allium* telomere target sequence, the function of AcTRB1 has been conserved.

*This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project SYMBIT – Reg. No. CZ.02.1.01/0.0/0.0/15\_003/0000477 financed by the ERDF and by the Czech Science Foundation project 20-01331X. Computational resources were provided by the ELIXIR-CZ project (LM2015047), part of the international ELIXIR infrastructure and by the CESNET LM2015042 and the CERIT Scientific Cloud LM2015085, provided under the programme "Projects of Large Research, Development, and Innovations Infrastructures".*

#### REFERENCES

1. Fajkus P.: *Plant J.* 85, 337 (2016).
2. Marian C. O.: *Plant Physiol.* 133, 1336 (2003).
3. Procházková Schruppfová P.: *Plant J.* 77, 770 (2014).

#### UNCONVENTIONAL CHIRAL SUPERCRITICAL FLUID CHROMATOGRAPHY OF CATHINONES USING ION EXCHANGER AND GRADIENT ELUTION

**NATALIE KOLDEROVÁ<sup>a,\*</sup>, MARTIN KUČAŘ<sup>a</sup>, MICHAL KOHOUT<sup>b</sup>**

<sup>a</sup>*Department of Chemistry of Natural Compounds, UCT Prague, Technická 5, 166 28, Prague 6, Czech Republic; <sup>b</sup>Department of Organic Chemistry, UCT Prague, Technická 5, 166 28, Prague 6, Czech Republic*  
kolderon@vscht.cz

Synthetic cathinones are derivatives of (S)-cathinone, the major psychostimulant found in leaves of plant *Catha edulis*. Nowadays, cathinone analogues represent a large group of new

psychoactive substances, which are broadly abused as an alternative to classic drugs (such as cocaine, ecstasy or methamphetamine). Cathinones are usually available as racemic mixtures<sup>1,2</sup>. Their chiral separation can not only further extend information about their production but also provide the ratio of individual enantiomers in metabolomic studies giving important insight into their preferential biotransformation.

The recent progress in technology of supercritical fluid chromatography (SFC) allow chiral analysis of drugs in hyphenation with mass spectrometry (MS). However, there is only paucity of methods for cathinones enantioseparation using SFC on various chiral stationary phases (CSP)<sup>2-4</sup>.

Hereby, we introduce an unconventional application of zwitterion ion exchange CSP under SFC-MS conditions. As a mobile phase, we have tested three different alcohols as organic modifiers in supercritical CO<sub>2</sub> and screened various additives added into these modifiers. Finally, we have optimized the flow rate of a make-up solvent, which is typically added post-column to enhance ionization efficiency of analytes and stabilize the ion spray.

In conclusion we have developed a unique method combining the gradient elution of cathinones with gradient of the make-up solvent. To the best of our knowledge, this is the first gradient elution using ion-exchange-type of columns.

*This work is a part of the research project supported by the Ministry of Interior of the Czech Republic (grant No. MV0/VI20172020056).*

#### REFERENCES

1. European Monitoring Centre for Drugs and Drug Addiction. *European Drug Report 2019: Trends and Developments*, Publications Office of the European Union, Luxembourg (2019).
2. Carnes S., O'Brien S., Szweczek A., Treméau-Cayel L., Rowe W. F., McCord B., Lurie I. S.: *J. Sep. Sci.* **40**, 3545 (2017).
3. Albals D., Heyden Y. Vander, Schmid M. G., Chankvetadze B., Mangelings D.: *J. Pharm. Biomed. Anal.* **121**, 232 (2016).
4. Geryk R., Kalíková K., Schmid M. G., Tesařová E.: *Anal. Chim. Acta* **932**, 98 (2016).

#### IMPACT OF LIPIDIZED PROLACTIN-RELEASING PEPTIDE TREATMENT ON METABOLIC SYNDROME AND LEPTIN-RELATED SIGNALING PATHWAYS IN THREE MOUSE MODELS

**LUCIA KOŘÍNKOVÁ<sup>a</sup>, VERONIKA PRAŽIENKOVÁ<sup>a</sup>, BARBORA NEPRAŠOVÁ<sup>a,b</sup>, MARTINA HOLUBOVÁ<sup>a</sup>, LUCIE HRUBÁ<sup>a</sup>, BLANKA ŽELEZNÁ<sup>a</sup>, JAROSLAV KUNEŠ<sup>a,b</sup>, LENKA MALETÍNSKÁ<sup>a</sup>**

<sup>a</sup>*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic;*

<sup>b</sup>*Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*  
*lucia.korinkova@uochb.cas.cz*

Anorexigenic neuropeptides such as prolactin-releasing peptide (PrRP) are promising tools for the treatment of obesity. Lipidization of PrRP leads to increased stability of the peptide and half-life and allow its application to the periphery causing the central effect. The aim of this study was to characterize the effect of palmitoylated PrRP analog (palm<sup>11</sup>PrRP31) in db/db mice with non-functional leptin receptor. Besides, the effect of PrRP was studied alone or in combination with leptin in ob/ob mice lacking of endogenous leptin. Moreover, its anorexigenic impact was studied in model of diet-induced obesity (DIO) mice.

Db/db mice were treated with palm<sup>11</sup>PrRP31 subcutaneously (SC) twice a day for 14 days. Ob/ob mice were injected with palm<sup>11</sup>PrRP31, leptin, or their combination SC twice a day for 28 days. DIO mice were treated with palm<sup>11</sup>PrRP31 for 28 days SC twice a day. Food intake and body weight (BW) and metabolic parameters were monitored during the treatment. Oral glucose tolerance test was performed and hypothalamic signaling was determined.

The treatment with palm<sup>11</sup>PrRP31 decreased BW but did not improve glucose tolerance in db/db mice compared to saline treated db/db mice. The treatment of ob/ob mice with palm<sup>11</sup>PrRP31 alone and in combination with leptin significantly decreased BW. This combination also lowered fasting blood glucose more than single leptin or palm<sup>11</sup>PrRP31. In DIO mice the treatment with palm<sup>11</sup>PrRP31 significantly decreased BW and increased phosphorylation of leptin-related signaling pathways in hypothalamus.

Palm<sup>11</sup>PrRP31 did not affect BW and intolerance to glucose in db/db mice, probably because of the impaired leptin receptor signaling. Synergism of lipidized PrRP and leptin in ob/ob mice, namely decrease of BW and fasting glucose, was clearly demonstrated. Impact of palm<sup>11</sup>PrRP31 on decrease of BW was also confirmed in DIO mice together with improved leptin signaling. Those data indicate, that anti-obesity effect of PrRP is related to intact leptin signaling.

#### REFERENCES

1. Holubová M., Hrubá L., Neprašová B., Majerčíková Z., Lacinová Z., Kuneš J., Maletínská L., Železná B.: *J. Mol. Endocrinol.* **60**, 85 (2018).
2. Kořínková L., Holubová M., Neprašová B., Hrubá L., Pražienková V., Bencze M., Haluzík M., Kuneš J., Maletínská L., Železná B.: *J. Mol. Endocrinol.* **64**, 77 (2020).
3. Pražienková V., Holubová M., Pelantová H., Bugáňová M., Pírník Z., Mikulášková B., Popelová A., Blechová M., Haluzík M., Železná B., Kuzma M., Kuneš J., Maletínská L.: *PLoS One* **12**, e0183449 (2017).

#### REGULATION OF BCR SIGNALING DURING THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

**LENKA KOŠŤÁLOVÁ<sup>a,b</sup>, VÁCLAV ŠEDA<sup>a,b</sup>, LAURA ONDŘIŠOVÁ<sup>b</sup>, EVA VOJÁČKOVÁ<sup>a</sup>, DANIEL FILIP<sup>b</sup>, VERONIKA ŠANDOVÁ<sup>a,b</sup>, SONALI SHARMA<sup>a</sup>, MAREK MRÁZ<sup>a,b,\*</sup>**

<sup>a</sup>Molecular Medicine, CEITEC Masaryk University, Brno;

<sup>b</sup>Department of Internal Medicine, Hematology and Oncology, University Hospital Brno and Faculty of Medicine MU, Brno  
marek.mraz@email.cz

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults in western countries. It is characterized by an accumulation of malignant B lymphocytes in blood and lymphatic tissues. CLL cells are highly dependent on interactions with the tissue microenvironment for their survival and proliferation. These interactions lead to the activation of several pro-survival pathways, especially B cell receptor (BCR) signaling. Small molecule inhibitors including the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib targeting BCR signaling have been recently approved and show remarkable clinical activity in CLL<sup>1</sup>. We and others have shown that ibrutinib does not affect only the BCR signaling pathway, but also the cell-cell interactions and chemokine signaling in the microenvironment<sup>2</sup>. Notably, ibrutinib therapy only leads to a relatively slow decrease in CLL cell numbers with a lymphocytosis persisting for several months, and this could contribute to the development of a resistant disease.

To describe the mechanisms contributing to CLL survival in peripheral blood we have performed gene expression profiling (N=14 paired samples; RNAseq, Illumina) of primary CLL samples obtained from CLL patients before and during treatment with ibrutinib. Subsequently, we have selected candidate genes that could be potentially responsible for the early adaptation to ibrutinib treatment. We focused on the role of protein tyrosine phosphatases (PTPs) and adaptor molecules that in normal B cells are responsible for the negative regulation of BCR-signaling. We have shown that after ibrutinib treatment in vitro and also in vivo the expression of several PTPs (P<0.001) is downmodulated suggesting that CLL cells tend to maintain their BCR activity despite inhibition of BTK kinase. We demonstrated by siRNA-based silencing that repression of a particular PTP positively affects BCR signaling and maintains a high basal activity of down-stream pro-survival kinases irrespectively of antigen-binding.

Altogether, the downregulation of PTP could contribute to the survival of CLL cells in peripheral blood by decreasing the threshold for tonic or antigen-induced BCR activation and this might be of relevance for adaptation to BCR inhibitor therapy in CLL.

*This project was supported by ERC under the EU Horizon 2020 programme (grant agreement No 802644); by Ministry of Health of CR, grant nr. NV18-03-00054; by the Czech Science Foundation (20-02566S) and Ministry of Education, Youth and Sports of the Czech Republic, project CEITEC 2020 (LQ1601). Supported by MH CZ - DRO (FNBr, 65269705) and MUNI/A/1395/2019.*

#### REFERENCES

1. Seda V., Mraz M.: *Eur. J. Haematol.* 94 (2015).
2. Pavlasova G., Borsky M., Seda V., Cerna K., Osickova J., Doubek M., Mayer J., Calogero R., Trbusek M.,

Pospisilova S., Davids M. S., Kipps T. J., Brown J. R., Mraz M.: *Blood* 128 (2016).

#### SCREENING PLATFORM FOR IDENTIFICATION OF NOVEL AGONISTS AND ANTAGONISTS OF ADENOSINE RECEPTORS

**JANA KOTULOVA, PETR DZUBAK, MARIAN HAJDUCH**

*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, 779 00 Olomouc, Czech Republic  
jana.kotulova@upol.cz*

Having four subtypes (A1, A2A, A2B and A3), adenosine receptors (AdoRs) belong to the family of G protein-coupled receptors (GPCRs) and are involved in regulation of a plethora of biological processes, including pathophysiological conditions such as cardiovascular and neurodegenerative disorders, inflammation, diabetes, and cancer.

Currently, only few of AdoR ligands are among GPCR targeting FDA-approved drugs (used as analgesics, bronchodilators, CNS stimulants and anti-inflammatory agents). Given the ubiquitous expression of AdoRs and the lack of specificity of compounds targeting them, there is a prevailing need for highly selective and potent agonists and antagonists of AdoRs<sup>1</sup>.

Hence, we aimed to develop a GPCR screening platform for the identification and characterization of novel agonists and antagonists of AdoRs displaying high selectivity and/or high potency. Our pivotal method utilizes aequorin luminescence induced by activation of GPCR of interest in reporter cell lines<sup>2</sup>.

First, we demonstrate the overall suitability of our method as a reproducible and generic high throughput screening platform when we evaluate each AdoR reporter cell line performance in the screening. Afterwards, the small molecule proprietary chemical library of IMTM (over 5,000 compounds) was analysed in primary screen of potential AdoR agonists and antagonists. Compounds were retested in counter-screen for unspecific calcium release possibly interfering with the assay, followed by dose-response secondary screen. We identified several potent modulators of ARs. Most promising compounds were subjected to another series of verification. Data analysis includes assay robustness parameters and quantification of the biological response of compounds.

*This study was supported by the ERD Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16\_019/0000868), internal grant agency of Faculty of Medicine and Dentistry, Palacky University Olomouc IGA\_LF\_2019\_018, and EATRIS LM2015064.*

#### REFERENCES

1. Chen J. F., Eltzhig H. K., Fredholm B. B.: *Nat. Rev. Drug Discov.* 12, 265 (2013).

2. Sheu Y. A., Kricka L. J., Pritchett D. B.: *Anal. Biochem.* 209, 343 (1993).

### COMBINATION OF EPIGENETIC DRUG SCREEN AND CRISPR KNOCKOUT SCREEN AS AN UNBIASED APPROACH TO REVEAL POSSIBLE CD20 THERAPY IMPROVEMENT

**VERONIKA KOZLOVÁ<sup>a,b</sup>, ANETA LEDEREROVÁ<sup>a</sup>, VERONIKA MANČÍKOVÁ<sup>a,b</sup>, MICHAEL DOUBEK<sup>a,b</sup>, JIŘÍ MAYER<sup>a,b</sup>, ŠÁRKA POSPÍŠILOVÁ<sup>a,b</sup>, MICHAL ŠMÍDA<sup>a,b</sup>**

<sup>a</sup>Central European Institute of Technology (CEITEC), Masaryk University, 625 00 Brno; <sup>b</sup>Department of Internal Medicine - Hematology and Oncology, Medical Faculty of Masaryk University and University Hospital Brno 625 00, Czech Republic  
kozlovaveronica@gmail.com

Standard of care for B-lymphoid malignancies nowadays still relies on the administration of monoclonal antibodies, with CD20 antigen being the prime target. Although effective at first, repeated cycles of anti-CD20 treatment, e.g. Rituximab (RTX), often result in the loss of CD20 from the surface of malignant B cells and consequently in therapy resistance<sup>1,2</sup>.

In our first approach, we mimicked the situation in patients through chronic exposure of B-lymphoid cell line (Ramos) to gradually increasing doses of anti-CD20 antibody RTX. In this way, we have generated cell lines that are resistant to additional treatment with anti-CD20 antibodies and have low CD20 expression. Since epigenetic changes were predicted to play a role in CD20 regulation<sup>3,4</sup>, we aimed to uncover which epigenetic modifiers could enhance the expression levels of CD20 and recover its presence on the cell surface. Therefore, we screened our resistant CD20-low cells against a library consisting of 182 small-molecule compounds targeting various epigenetic modifying enzymes to determine surface CD20 expression changes by flow cytometry.

In our second approach, we used CRISPR/Cas9 system for functional knockout screen of WT Ramos cells. We infected them with the CRISPR screen library to generate a genome-scale collection of single gene knockouts. Infected cells were either treated with RTX or used as control without RTX treatment and cultured for 2 weeks. The abundance of single gene knockout at the beginning versus the end of the incubation period was compared using next-generation sequencing. This approach allowed us to reveal enriched and lost gene knockouts after RTX administration. These genes could play a role in RTX therapy response.

Unbiased combination of epigenetic drug screen and CRISPR knockout screen helped us to reveal possible treatment targets. Our results indicate the role of Aurora kinases in CD20 regulation and possible involvement of vesicle trafficking in RTX antibody therapy resistance. Further analysis of mechanisms regulating CD20 expression is ongoing in order to validate the effect of detected targets.

*This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601) and grant MUNI/A/1395/2019.*

### REFERENCES

- Davis T. A., Grillo-López A. J., White C. A., McLaughlin P., Czuczman M. S., Link B. K., Maloney D. G., Weaver R. L., Rosenberg J., Levy R.: *J Clin Oncol* 18, 3135 (2000).
- Doubek M., Šmída M.: *Expert review of hematology*: 8 (6), p. 743 – 764 (2015).
- Hiraga J., Tomita A., Sugimoto T., Shimada K., Ito M., Nakamura S., Kiyoi H., Kinoshita T., Naoe T.: *Blood* 113, 4885 (2009).
- Shimizu R., Kikuchi J., Wada T., Ozawa K., Kano Y., Furukawa Y.: *Leukemia* 24, 1760 (2010).

### NEW POST-TRANSLATIONAL MODIFICATION OF PROTEIN DISHEVELLED REGULATES ITS PHASE SEPARATION AND SIGNALOSOME FORMATION

**MAREK KRAVEC<sup>a</sup>, ONDŘEJ ŠEDO<sup>c</sup>, DAVID POTĚŠIL<sup>c</sup>, KRISTÍNA GÖMÖRYOVÁ<sup>a</sup>, CARSTEN JANKE<sup>b</sup>, ZBYNĚK ZDRÁHAL<sup>c</sup>, VÍTĚZSLAV BRYJA<sup>a</sup>**

<sup>a</sup>Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic; <sup>b</sup>Genotoxic stress and Cancer department, Institut Curie, Orsay Cedex, France; <sup>c</sup>Central European Institute of Technology (CEITEC), Brno, Czech Republic  
mar.kravec@gmail.com

Dishevelled protein is a key signal transducer of highly conserved Wnt signaling pathway. This pathway is crucial in regulation of embryonic development as well as in maintenance of homeostasis in adult organisms. Its deregulation is associated with developmental disorders and with many types of cancer. Protein dishevelled acts as a switch between various downstream signaling events. It consists of three structured domains and the major part of protein that is intrinsically disordered (IDP). As most of IDPs, it is target to numerous post-translational modifications that regulate its functions.

Post-translational modifications (PTMs) form different protein variants, what is crucial in regulation of complex biological processes such as signaling pathways, where crosstalk between numerous interacting partners results in a fine-tuned cellular response. PTMs are also known to regulate formation of biomolecular condensates which are membraneless cellular compartments that are formed through liquid-liquid phase separation driven by intermolecular multivalent interactions<sup>1</sup>. This compartment has spatiotemporally enriched concentration of molecules allowing less favourable interactions<sup>2</sup>. They are shown to be involved in various signaling pathways<sup>1</sup>, but their regulation is poorly understood.

We have shown that polyglutamylase TTL11 interacts with all three human homologues of dishevelled protein and mediates their reversible PTM-polyglutamylation. We have biochemically analysed the modification process and we propose that this modification regulates conformational dynamics of protein dishevelled. We demonstrate, that this PTM modulates interaction of dishevelled protein with its key interacting partners and also affects formation of its biomolecular condensates.

*Brno Ph.D. Talent Scholarship holder – Funded by the Brno City Municipality. This work was supported by Czech Science Foundation, grant 18-17658S.*

#### REFERENCES

1. Sear R. P.: *Soft Matter* 3, 680 (2007).
2. Gammons M. V., Renko M., Johnson C. M., Rutherford T. J., Bienz M.: *Mol. Cell* 64, 92 (2016).

#### PHENOTYPIC CHANGES CAUSED BY NOVEL SNV OF RPS7 GENE

**AGATA KUBICKOVA<sup>a</sup>, PETR VOJTA<sup>a</sup>, MARTIN ONDRA<sup>a</sup>, JANA VOLEJNIKOVA<sup>a,b</sup>, ZUZANA MACECKOVA<sup>a</sup>, PAVLA KORALKOVA<sup>c</sup>, ALEXANDRA JUNGVA<sup>d</sup>, ZUZANA SAXOVA<sup>c</sup>, RENATA MOJZIKOVA<sup>c</sup>, IVANA HADACOVA<sup>f</sup>, JAROSLAV CERMAK<sup>g</sup>, MONIKA HORVATHOVA<sup>c</sup>, DAGMAR POSPISILOVA<sup>a,b,\*</sup>, MARIAN HAJDUCH<sup>a,\*</sup>**

<sup>a</sup>*Inst Mol. Transl. Med., Palacky University, 779 00 Olomouc;* <sup>b</sup>*Dept Paediatrics, Fac. Med. Dent., Palacky University and University Hospital, 779 00 Olomouc;* <sup>c</sup>*Dept Biol., Fac. Med. Dent., Palacky University, 775 15 Olomouc;* <sup>d</sup>*Dept Haematoolcol., Charles University, 304 60 Pilsen-Lochotin;* <sup>e</sup>*Dept Haemato-Oncol., Fac. Med. Dent., Palacky University, 779 00 Olomouc;* <sup>f</sup>*Dept Haematol., Charles University, 150 06 Prague;* <sup>g</sup>*Inst. Haematol. Blood Transfusion, 128 20 Prague, Czech Republic*  
agata.kubickova92@gmail.com

**Introduction:** *RPS7* gene encodes a ribosomal protein that is a component of the 40S ribosomal subunit<sup>1</sup>. Mutations in *RPS7* gene causes rare inherited erythrocyte aplasia called Diamond Blackfan anaemia. DBA is considered as Dameshek's riddle, because patients with this disease have increased incidence of cancer in adulthood. In our study, we focused on an SNV in *RPS7* which was found in a family suffering from DBA.

**Methods and materials:** For characterization of phenotypic changes caused by heterozygous SNV in *RPS7*, we designed and prepared a cellular model by CRISPR/Cas9 technology. This model was used for studying not only p53-mediated ribosomal stress, by measurement of protein levels and stability, by Western blotting. Alterations in the processing of ribosomal RNA were determined by Northern blotting and rRNA FISH, changes in nucleolar morphology by immunofluorescence, cell cycle progression and protein

synthesis were measured by flow cytometry, cell proliferation was determined by MTS viability assay after 72 hours.

**Results and conclusions:** Our results suggest that this SNV in the *RPS7* gene causes significantly downregulated global protein synthesis coupled with slower proliferation with small changes in cell cycle progression. p53-mediated ribosomal stress pathway is slightly activated whereas a significantly decreased ability of rRNA processing in its initial steps was found. Most probably these phenotypic changes are caused by higher extra-ribosomal accumulation of mutated *RPS7* protein.

*This project is supported by the European Regional Development Fund - Project ENOCH (No.CZ.02.1.01/0.0/0.0/16\_019/0000868) and by grants of the Czech Ministry of Health (AZV16-32105A), IGA\_LF\_2019\_018.*

#### REFERENCE

1. Zhu Y., Poyurovsky M. V., Li Y., Biderman L., Stahl J., Jacq X., Prives, C.: *Mol Cell.* 35, 316 (2009).

#### GUANYLATED NON-NATURAL OLIGOPEPTIDES FOR GLYCOLYX INTERACTIONS

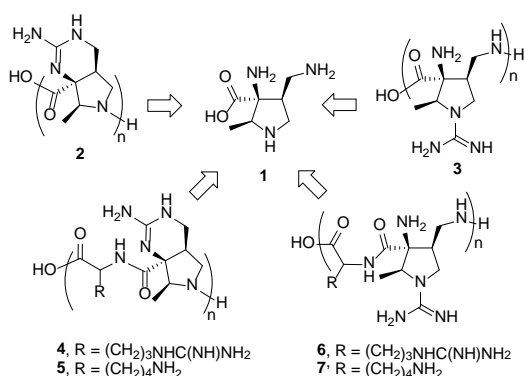
**RADKA KUCHERKOVÁ, ULLRICH JAHN**

*Institute of Organic Chemistry and Biochemistry AS CR  
Flemingovo nám. 542, 160 00 Praha 6  
radka.kucherkova@uochb.cas.cz*

Arginine containing proteins play an interesting role in processes occurring at the glycocalyx and cell membrane based on interaction of their positive charge with negatively charged glycosaminoglycans.

Therefore, peptides prepared from non-natural amino acids with guanidine units could play a similar role. Moreover, such derivatives might be more resistant to enzymatic cleavage because enzymes will not be able to recognize them. This led us to design polyfunctional peptides that have defined secondary structure and offer both covalent and supramolecular linkages to active drugs or fluorescent markers and could be used for studying the glycocalyx structures.

Our synthetic approach (Scheme 1) is based on a tandem process consisting of asymmetric conjugate addition reaction and 1,3-dipolar cycloaddition<sup>1</sup> which allows an easy access to functionalized cyclic non-natural  $\alpha,\beta,\gamma$ -triamino acid **1**. Subsequent introduction of guanidine units<sup>2</sup> to the nitrogen atoms followed by peptide coupling will be used for preparation of homooligomers **2**, **3** or heterooligomers containing arginine **4**, **6** or lysine **5**, **7** units.



Scheme 1. Strategy for preparation of homo/heterooligomers from non-natural amino acid 1

This project was supported from European Regional Development Fund; OP RDE; Project: "ChemBioDrug" (No. CZ.02.1.01/0.0/0.0/16\_019/0000729).

#### REFERENCES

1. Kapras V., Pohl R., Císařová I., Jahn U.: *Org. Lett.* 16, 1088 (2014).
2. Zhang W., Xu L., Xi Z.: *Chem. Commun.* 51, 254 (2015).

#### FLUORESCENT NANODIAMONDS MODIFIED WITH BIOCOMPATIBLE POLYMERS

**KLAUDIA KVAKOVÁ<sup>a,b</sup>, JIŘÍ SCHIMER<sup>a</sup>, MARTIN ONDRA<sup>c</sup>, MIROSLAV TOPPER<sup>c</sup>, MILOŠ PETŘÍK<sup>c</sup>, ZBYNĚK NOVÝ<sup>c</sup>, HELENA RAABOVÁ<sup>a,d</sup>, MARIÁN HAJDÚCH<sup>c</sup>, PETR CÍGLER<sup>a\*</sup>**

<sup>a</sup>Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 166 10 Prague 6; <sup>b</sup>Charles University, First Faculty of Medicine, 121 08 Prague 2; <sup>c</sup>Institute of Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, 772 00 Olomouc; <sup>d</sup>University of Chemistry and Technology, 166 28 Prague 6, CR [klaudia.kvakova@uochb.cas.cz](mailto:klaudia.kvakova@uochb.cas.cz)

In the past decade, sentinel node(s) mapping became standard procedure used in cancer diagnostics. However, agents commonly used in these applications, mostly molecular dyes and radiotracers, still have several disadvantages. Fluorescent nanoprobe currently show the most promising results as potential alternatives. Fluorescent nanodiamond (FND) is a biocompatible material which exhibit unique optical properties. The origin of the nanodiamond fluorescence is based on artificially created nitrogen-vacancy (NV) centers. Emission maximum of NV centres is in near-infrared region (approximately 700 nm) which belongs to the tissue imaging window. NV centers are extremely resistant towards photobleaching. These properties make FND a promising candidate for bioimaging applications.

This work is focused on preparation of FNDs coated with D-mannosylated polyglycerol for sentinel node(s) visualization. Polyglycerol coating overcomes limited colloidal stability of FNDs in the biological environment and enables surface modification. D-Mannose targets macrophages, which are abundantly present in the sentinel nodes. The functionalization with D-mannose was achieved using click chemistry (azide-alkyne cycloaddition). First, treatment with glycidyl propargyl ether provided alkyne-modified polyglycerol which was connected with azidated D-mannose via click reaction. The optimal polymerization and click reaction conditions was extensively studied. Resulting particles (both mannosylated and non-mannosylated) were highly stable in the high-salt condition (1 M NaCl) and non-specific protein binding in FBS was completely eliminated. The mannosylated particles interacted specifically with macrophages and showed enhanced retention in mice lymphatic nodes, providing a clear imaging contrast.

This work was supported by the Czech Science Foundation project no. 18-17071S.

#### CONTROLLED RELEASE OF PRO-HEALING PROTEINS FROM "SMART" HYDROGEL-BASED DRUG DELIVERY SYSTEM

**KLÁRA LYSÁKOVÁ<sup>a</sup>, NIKOLA PTÁČKOVÁ<sup>b</sup>, JANA BRTNÍKOVÁ<sup>a</sup>, MARKĚTA ZÁMEČNÍKOVÁ<sup>b</sup>, JIŘÍ DAMBORSKÝ<sup>c</sup>, LUCY VOJTOVÁ<sup>a</sup>**

<sup>a</sup>CEITEC, Brno University of Technology, Purkyňova 656/123, Brno, CZ, <sup>b</sup>Enantis L.t.d., Kamenice 771/34, Brno, CZ, <sup>c</sup>Loschmidt Laboratories of Protein Engineering, Masaryk University, Kamenice 753/5, Brno, CZ [Klara.Lysakova@ceitec.vutbr.cz](mailto:Klara.Lysakova@ceitec.vutbr.cz)

Controlled drug delivery systems have several advantages over traditional drug forms. A reduction in the frequency and accurate amount of administered drug can be achieved. In this work stable Fibroblast growth factor 2 (FGF2-stab) was used as a protein-based drug. FGF2-stab stimulate the growth and the development of new vessels (angiogenesis), leading to improved wound healing, tissue development and contributes to the pathogenesis of several diseases (cancer or atherosclerosis). Other members of the FGFs family have the potential for the use in tissue engineering, for example FGF18 is believed to be a product to restore cartilage tissues<sup>1</sup>.

Amphiphilic PLGA-PEG-PLGA triblock copolymer was used as the biodegradable water-soluble copolymer that undergoes to a gel at physiological temperature. Along with increasing temperature in an aqueous environment, the PLGA-PEG-PLGA copolymer forms micelles having the hydrophobic core (PLGA) and the hydrophilic shell surface (PEG)<sup>2</sup>. Nowadays, drug carriers based on PLGA-PEG-PLGA copolymers are already commercially available under the

names ReGel<sup>®</sup> and OncoGel<sup>®</sup> loaded with insulin or paclitaxel, respectively.

The aim of this work was to synthesize the thermosensitive PLGA-PEG-PLGA hydrogel with specific properties and ensure reproducible measurement of the FGF2-stab released amount from the hydrogel at 37 °C in PBS. Second goal was to determine the protein-hydrogel interactions in order to tune the protein release into a controlled way for medical applications. The released amount of proteins was measured by UV-VIS spectrophotometry in the presence of a Bradford reagent and by SDS-page electrophoresis. Controlled release exhibiting first order kinetics dependent only on the diffusion and protein concentration. Further work will be concerned in protein release from functionalized copolymer.

*This work was supported by the CEITEC 2020 (LQ1601) with financial support from the MEYS of the Czech Republic and by the Czech Health Research Council under the project no. 17-29874A.*

## REFERENCES

1. Nugent M., Renato I.: *Int. J. Biochem. Cell Biol.* 32, 115 (2000).
2. Michlovská L., Vojtová L., Mravcová L., Hermonová S., Kučeřík J., Jančář J.: *Macromol. Symp.* 295, 119 (2010).

## POLAR-TO-RADICAL SWITCH STRATEGY FOR THE SYNTHESIS OF DIVERSE NATURAL POLYPHENOLS

**TOMÁŠ MAŠEK<sup>a</sup>, GABRIELA PRESOVÁ<sup>a</sup>, IVANA ČISAŘOVÁ<sup>b</sup>, ULLRICH JAHN<sup>a\*</sup>**

<sup>a</sup>*Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo nám. 542/2, Prague, 166 10*

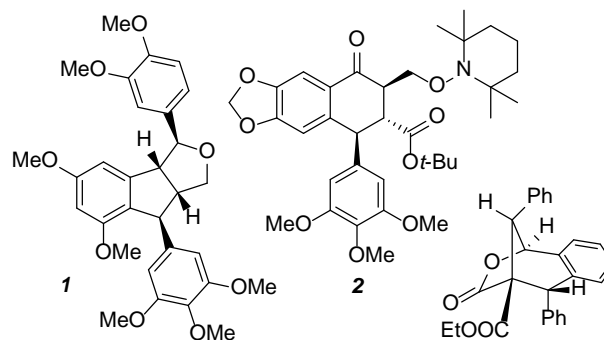
<sup>b</sup>*Department of Inorganic Chemistry, Faculty of Science, Charles University, Hlavova 8, Prague, 128 43*  
masek@uochb.cas.cz

Trivalent carbon-centred reactive intermediates, *i.e.*, carbanions, C-centred radicals and carbenium ions, differ greatly in their reactivity, but all have found wide application in synthesis. Carbanions, thanks to their manageable reaction kinetics, are well suited for fragment union and stereoselective C-C bond formation via conjugate addition to electron poor alkenes. Higher reactivity of radicals allows reaction with unactivated olefins to form small rings. Finally, electrophilic carbenium ions easily undergo C-heteroatom bond formation.

Cascading these processes using SET oxidants is a strategy extremely well suited for the synthesis of natural polyphenols, because the aromatic rings stabilize intermediate arylmetals and benzylic radicals and carbenium ions. This strategy is applicable to several classes of classical lignans as well as furoindane stilbenolignans and stilbene dimers, all examples of plant metabolites from the polyphenol family<sup>1</sup>.

Methodological background including stereochemical aspects and comparison to fully polar processes will be

discussed. Total synthesis of a derivative of Kompasinol A **1**, Thuriferic acid **2** as well as progress towards total synthesis of Lehmbackol D and Gnetifolin F will be presented.



Scheme 1. Examples of prepared structures

*This work was supported by The Czech Science Foundation (reg. No. 19-22085S) and the Gilead Sciences Research Centre at IOCB.*

## REFERENCE

1. Begum S. A., Sahai M., Ray A. B., in: *Prog. Chem. Org. Nat. Prod.* Kinghorn A., Falk H., Kobayashi J. Eds.; Springer: Wien, New York, 2010, Vol 93, p 1-70.

## INTACT-CELL MASS SPECTROMETRY AS A TOOL FOR MONITORING AND QUALITY CONTROL OF STEM CELL CULTURES AND DERIVATION OF LUNG CELL PROGENITORS

**LUKÁŠ MORÁŇ<sup>a,b</sup>, ALEŠ HAMPL<sup>a,b</sup>, JOSEF HAVEL<sup>c</sup>, HANA KOTASOVÁ<sup>a</sup>, VENDULA PELKOVÁ<sup>a</sup>, VOLODYMYR POROKH<sup>a</sup>, PETR VAŇHARA<sup>a,b\*</sup>**

<sup>a</sup>*International Clinical Research Center, St. Anne's University Hospital Brno, Czech Republic;* <sup>b</sup>*Faculty of Medicine, Masaryk University, Brno, Czech Republic;* <sup>c</sup>*Faculty of Science, Masaryk University, Brno, Czech Republic*  
408080@mail.muni.cz

Human embryonic stem cells (hESCs) represent a promising tool for pharmaceutical, bio-industrial or biomedical applications. However, these applications require strict quality control of cell cultures. In routine long-term cell line cultivation, cross-contamination with other cell types and gradual phenotypical changes, as well as substantial heterogeneity in differentiation processes are major risks. Accurate biotyping of cultured cells is thus essential to unambiguously characterize their condition.

Simple and commercially available technique for cell line authentication is an analysis of repetitive DNA sequences, such as short tandem repeats (STR). However, this technique provides only qualitative information whose interpretation is dependent on reference databases. Similarly, karyotyping or analysis of gene or protein markers (eg. pluripotency or



differentiation) that target only a particular type of instability, such as genome abnormalities or abnormal phenotype features, and do not provide sufficient sensitivity. For these reasons, there is a need for robust, easy-to-perform and sensitive methods for determining and confirming cell status and for revealing potential divergences from optimal cell status.

Here we modelled typical scenarios, that may occur in long-term cultures of human embryonic stem cells (hESC), involving the development of hESCs aberrations, cross-contamination with other cell types, and differentiation of hESCs to early lung progenitors (ELEPs). We analysed the cell cultures by intact cell mass spectrometry followed by advanced mass spectra analysis. Spectral fingerprints allowed the proper clustering of cell samples and the classification of "unknown", blind, spectra by artificial neural networks (ANN).

We demonstrated that intact cell mass spectrometry, when coupled to artificial intelligence, is a robust and routinely useful tool for detecting, and even quantifying, hidden heterogeneity in stem cell cultures.

*This study was supported by Ministry of Health of the Czech Republic, grant nr. NV18-08-00299. All rights reserved.*

#### REFERENCE

1. Vaňhara P, Kučera L, Prokeš L, Jurečková L, Peňa-Méndez EM, Havel J, Hampel A.: *Stem Cells Transl. Med.* 7, 109 (2018). doi: 10.1002/sctm.17-0107.

#### ELECTROCHEMICAL LAMP-BASED ASSAY FOR SCREENING OF HPV16/HPV18 INFECTION IN CERVICAL SAMPLES

**LUDMILA MORÁŇOVÁ<sup>a</sup>, ROMAN HRSTKA<sup>a</sup>, MILAN ANTON<sup>b</sup>, MARTIN BARTOŠÍK<sup>a</sup>**

<sup>a</sup>Masaryk Memorial Cancer Institute, RECAMO, Žlutý kopec 7, 656 53, Brno, Czech Republic; <sup>b</sup>Department of Obstetrics and Gynecology, University Hospital Brno and Medical Faculty Masaryk University, Obilní trh 11, 602 00 Brno, Czech Republic  
ludmila.moranova@mou.cz

Cervical cancer is caused by a persistent infection with high risk human papillomavirus (HPV) strains. Since untreated long-term infection can be present without morphological changes on cervix, HPV testing has become an important alternative to standard cytological screening. Standard methods are often laborious, expensive and time-consuming. Here, we propose new detection technique for HPV determination based on loop-mediated amplification (LAMP)<sup>1</sup> with hybridization on magnetic beads and electrochemical readout<sup>2</sup>. This method was validated on clinical samples containing HPV16 and HPV18, the two most widespread HPV subtypes.

To demonstrate usefulness of the method, we screened 61 cervical samples for both these subtypes and compared our results with standard PCR and also with two commercially

available techniques used in healthcare – COBAS (Roche) and INNO-LiPA (Fujirebio). The best concordance was obtained with PCR reaction; we reached high significance (p-value <0.0001) and positive and negative predictive values over 90%<sup>3</sup>.

These results demonstrate that our electrochemical assay may serve as an interesting tool for rapid and inexpensive screening of oncogenic HPVs.

*The work was supported by the projects MH CZ - DRO (MMCI, 00209805) and by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16\_019/0000868).*

#### REFERENCES

1. Notomi T., Okayama H., Masubuchi H., Yonekawa T., Watanabe K., Amino N., Hase T.: *Nucleic Acids Res.* 28, E63 (2000).
2. Bartosik M., Jirakova L., Anton M., Vojtesek B., Hrstka R.: *Anal. Chim. Acta* 1042, 37 (2018).
3. Anton M., Moranova L., Hrstka R., Bartosik M.: *Anal. Methods* 12, 822 (2020); DOI: 10.1039/C9AY02383F.

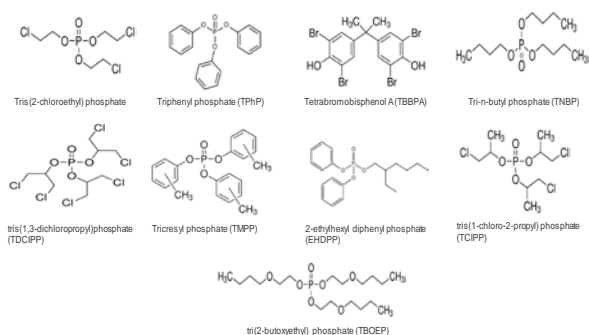
#### IN VITRO ASSESSMENT OF NOVEL FLAME RETARDANTS INDUCED HEPATIC STEATOSIS TO DEVELOP (QUANTITATIVE) ADVERSE OUTCOME PATHWAYS, (Q)AOPS

**CHANDER KANT NEGI, LOLA BAJARD, LUDĚK BLAHA**

Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Kamenice 753/5, pavilion A29, 625 00 Brno, Czech Republic  
chandernegi09@gmail.com

Following the ban of long-used brominated flame retardants, replacements used as a novel flame retardant (nFRs) are frequently detected in the environment and in human matrices. Some evidence indicates the toxic effects of individual nFRs in mammals, but generally, toxicity data is limited. Considering the pervasive presence of these compounds, this is a crucial knowledge gap, and more toxicological studies on nFRs, individually and as mixtures are critically needed<sup>1</sup>.

The aim of the present study is to understand the molecular mechanisms driving the liver toxicity (hepatic steatosis) of nine highest priority nFRs (fig. 1), utilizing OECD endorsed framework adverse outcome pathway (AOP).



**Fig. 1. Highest prioritized flame retardants**

For hepatic steatosis, AOPs and AOP network linking several molecular initiating events (MIEs) have been suggested and current evidence indicates that the prioritized nFRs affect some relevant MIEs. *In vitro* toxicological studies on HepG2 2D and 3D cell culture are being conducted to quantify the effects of these nFRs individually and mixtures on different Key Events of the AOPs proposed for hepatic steatosis.

Our first results indicate that several nFRs may induce lipid accumulation in the hepatocytes at sub-cytotoxic concentrations. Further *in vitro* assessment of nuclear receptor activation and expression of associated downstream proteins are currently being done to understand the molecular mechanism and to develop quantitative data for follow-up development of qAOP using Artificial Intelligence-based tools.

#### REFERENCES

1. Bajard L., Melymuk L., Blaha L.: *Environ. Sci. Eur.* **31**, 14 (2019).

#### TACKLING BRAF INHIBITORS RESISTANCE IN HUMAN MELANOMA

**MICHAELA NOSKOVÁ, TOMASZ WITOLD RADASZKIEWICZ, VÍTĚZSLAV BRYJA**

*Institute of Experimental Biology, Department of Animal Physiology and Immunology, Faculty of Science, Masaryk University, Kamenice 5, Bld. A36, 625 00 Brno, Czech Republic*  
 NOSKOVA.MICHAELA@email.cz

Malignant melanoma is one of the most aggressive cancers, characterized by high invasive and metastatic potential. It is responsible for the majority of skin cancer related deaths. Initially, drugs targeting constitutively active MAPK pathway caused by *BRAF* V600E mutation occurring in melanoma (e.g. Vemurafenib) had excellent results and increased patient's survival<sup>1,2</sup>. Unfortunately, patients receiving BRAF inhibitor (BRAFi) treatment relapsed after therapy, because of acquired resistance to this drug<sup>3</sup>. It is known that Wnt signaling plays a role in development of resistance to targeted therapy. We found that activity of the

canonical and non-canonical pathways is crucial for melanoma cells survival upon BRAF inhibition. We observed plasticity in the activation of Wnt pathway components in responses to the temporary and chronic Vemurafenib treatments.

Interestingly, we found out that RNF43 in human melanoma cells is a negative regulator of the Wnt signaling pathway. Moreover, RNF43 expression is significantly lower in the metastatic growth phase of melanoma and it is a negative survival factor for melanoma patients. Importantly, cancer cells overexpressing RNF43 lose their plasticity in the response to the vemurafenib treatment.

Furthermore, during our experiments we identified that for resistance development can be also important casein kinase 1 $\epsilon$  (Ck1 $\epsilon$ ), which can influence *BRAF* V600E inhibitor resistance in melanoma metastasis. Our results indicated its increased expression in the vemurafenib resistant cells. Strikingly, kinases are druggable targets by i.e. small molecule inhibitors, what makes Ck1 $\epsilon$  and possibly other Ck1 isoforms an attractive targets in melanoma.

#### REFERENCES

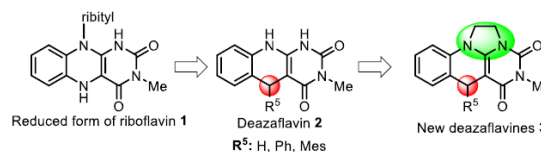
1. Chapman P.B., Hauschild A., Robert C., et al.: *N. Engl. J. Med.* **364**, 2507 (2011).
2. Flaherty K. T., Puzanov I., Kim K. B., et al.: *N. Engl. J. Med.* **363**, 809 (2010).
3. Johnson D. B., Menzies A. M., Zimmer L., et al.: *Eur. J. Cancer* **51**, 2792 (2015).

#### 5-DEAZA-1,10-BRIDGED FLAVINES AS NEW CATALYSTS FOR PHOTOREDOX REACTIONS

**RÓBERT OBERTÍK, TETIANA PAVLOVSKA, RADEK CIBULKA**

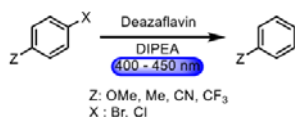
*Department of Organic Chemistry, University of Chemistry and Technology, Prague, Technická 5, Prague 6,166 28*  
 obertiko@vscht.cz

Flavines as photocatalysts have already been known for several decades but their application has still been limited only on photooxidations<sup>1</sup>. The reason, why flavines have not been used for photoreductions is low stability of reduced form of common riboflavine derivatives **1** (cit.<sup>2</sup>). It is oxidizes on air very rapidly in order of milliseconds.



**Scheme 1. Design of new ethylene bridged deazaflavines**

Deazaflavines are flavin derivatives with carbon on the position 5 instead of "classic" flavines (Scheme 1)<sup>2</sup>. Very recently, simple deazaflavins **2** has been proven as the strong reductive organic photocatalyst allowing dehalogenation of *p*-halogenanisole with very negative reduction potential (*p*-chloroanisole:  $E_{ox}^* = -2,8$  V) (Scheme 2)<sup>3</sup>.



Scheme 2. Photoreduction catalysed by deazaflavine

Despite the high reductive ability, simple deazaflavines exhibit some disadvantages, e.g. low yields of photoreductions of more difficult substrates. Therefore, within the work new ethylene-bridged flavines **3** have been designed as additional five-member ring is proposed to increase the reduced form stability. New deazaflavines **3** will be tested in photoreduction, namely in dehalogenation of *p*-halogenoanisoles and in Birch reductions<sup>3</sup>.

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

## REFERENCES

1. König B., Kümmel S., Svobodová E., Cibulka R., in *Physical Sciences Reviews*, Vol. 3, 2018.
2. Zilly F. E., Taglieber A., Schulz F., Hollmann F., Reetz M. T.: *Chem. Commun.* 2009, 7152.
3. König B., Chatterjee A.: *Angew. Chem., Int. Ed. Engl.* 2019, 14289.

## SPECTROSCOPIC PROPERTIES OF NAPHTHALENE ON ICE SURFACES

GABRIELA ONDRUŠKOVÁ<sup>a</sup>, DOMINIK HEGER<sup>b</sup>

<sup>a</sup>Faculty of Science, Masaryk University, RECETOX, Kamenice 5, 62500 Brno, Czech Republic; <sup>b</sup>Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5/A8, 625 00 Brno, Czech Republic, [ondruskovag1@gmail.com](mailto:ondruskovag1@gmail.com)

Ice and snow in nature are not composed only of pure water, but they also contain numerous other chemical compounds – inorganic and organic, of both natural (sea salt, dust) and anthropogenic (pollution) origin<sup>1</sup>. In general the contaminants on Earth are moving from warmer to colder regions (e.g. by global distillation or grasshopper effect). In these regions the chemicals from atmosphere are soaked up by snow and move slowly deeper into the glacier over the time and change the behavior of snow and ice<sup>1</sup>. These processes explain relatively high concentrations of some pollutants in polar regions<sup>2,3</sup>. During both freezing and deposition the compounds concentrate in between ice grains and on the grain boundaries, which is called freeze-concentration<sup>4</sup>. The ice surface is a dynamic and constantly changing system which is covered with the freeze concentrated solution (FCS)<sup>5</sup> under these conditions.

We used fluorescence emission spectroscopy to study behavior of naphthalene and 1-methylnaphthalene (model compounds of polyaromatic hydrocarbons, a common pollutant in the environment) on various ice surfaces and under

different conditions (e.g. temperature). Comparison with quantum chemical calculations allows us to suggest orientation of molecules in the aggregates (Figure 1)<sup>6</sup>.

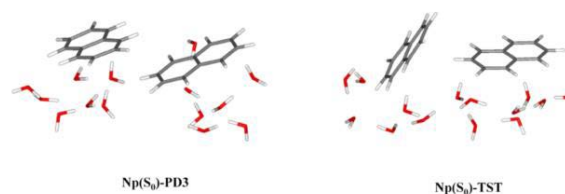


Fig. 1. Cluster models of naphthalene dimer associated on the ice surface

Authors would like to thank to Mgr. Peter Šebej, Ph.D.

## REFERENCES

1. Bartels-Rausch T.: *Nature*, 494, 27 (2013).
2. Wania F.: *Env. Sci. Technol.*, 37, 1344 (2003).
3. Gouin T., Mackay D., Jones K. C., Harner T., Meijer S. N.: *Environ. Pollution* 128, 139 (2004).
4. Takenaka N., Ueda A., Daimon T., Bandow H., Dohmaru T., Maeda Y.: *J. Phys. Chem.* 100, 13874 (1996).
5. Bogdan A., Molina M. J., Tenhu H., Bertel E., Bogdan N., Loerting T.: *Sci. Rep.* 4, 7414 (2014).
6. Krausko J., Malogwe J., Bičanová G., Klán P., Nachtigallová D., Heger D.: *J. Phys. Chem. A*, 119, 8565 (2015).

IMMUNOMODULATORY CATHEPSIN B FROM THE HOUSE DUST MITE *DERMATOPHAGOIDES FARINAE*: FUNCTIONAL AND STRUCTURAL CHARACTERIZATION

## KATARÍNA ORSÁGHOVÁ, JANA PYTELKOVÁ, ADÉLA JÍLKOVÁ, MICHAL BUŠA, MICHAEL MAREŠ\*

*Institute of Organic Chemistry and Biochemistry CAS, Flemingovo náměstí 2, 16000 Prague*  
[michael.mares@uochb.cas.cz](mailto:michael.mares@uochb.cas.cz)

Mites are a major source of allergens and contributor to the rising incidence of allergic diseases, including bronchial asthma, rhinitis, and atopic dermatitis. Digestive enzymes produced by mites and released into the environment are potent allergens and a target for the treatment of allergic hypersensitivity. We performed the first detailed profiling of digestive proteolytic enzymes in the house dust mite *Dermatophagoides farinae* using functional proteomics and identified cathepsins B (DfCB) as a new major component protease. Recombinant DfCB was produced in the yeast expression system and enzymologically characterized. Furthermore, the purified DfCB was crystallized, and its preliminary 3D structure was solved by X-ray crystallography.

This work was supported by the grant number 6061/23.

## WNT PATHWAY CRISPRING

**PETRA PAČLIKOVÁ<sup>a</sup>, TOMASZ WITOLD RADASZKIEWICZ<sup>a</sup>, ANNA KOTRBOVÁ<sup>a</sup>, VITEZSLAV BRYJA<sup>a,b\*</sup>**

<sup>a</sup>Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic; <sup>b</sup>Institute of Biophysics, The Czech Academy of Sciences, Brno, Czech Republic  
394465@mail.muni.cz

Communication between the cells about their current position and state is essential for proper developmental decisions of the tissues, organs and of the whole organism as a functional unit *per se*. This process is mediated *via* signalling molecules, receptors and downstream signalling components that function as a relay mechanism for their specific signalling pathways.

Wnt signalling pathways are one of the most important signalling cascades involved basically in all the developmental aspects during the embryogenesis and also during homeostasis of the adult organism. They have a key role during proliferation, differentiation, migration and establishment of polarity of the cells within a plane of a tissue. Therefore, deregulation of Wnt signalling is causative for development of several types of cancer and other diseases. Wnt/ $\beta$ -catenin pathway is the most studied of all Wnt pathways. One of the reasons is that it is a "classical" type of signalling cascade, where binding of the ligand to the receptor causes activation of intracellular transducers which relay the signal to the nucleus where a target genes transcription is commenced.

In our study we took advantage of CRISPR/Cas9 editing technique and created cell line that lack individual Wnt/ $\beta$ -catenin components and by testing these unique cell lines we are able to properly access roles of these molecules in Wnt/ $\beta$ -catenin pathway. Among the proteins of our interest are: a key molecule of all Wnt pathways- Dishevelled (DVL1, DVL2, DVL3 in human), correceptors LRP5/6, E3 ubiquitin-protein ligases that act as a negative regulators of Wnt/ $\beta$ -catenin pathways- RNF43 and ZRNF3, protein AXIN1 that is part of the destruction complex and AXIN2 and scaffold protein APC that is also part of the destruction complex.

We used these cell lines in series of experiments testing ability to mediate Wnt signals (reporter TopFLASH assay, DVL shift assay, rescue assays etc.) and ability to rescue observed phenotypes. These cell lines represent more physiological systems than studies using gain-of-function experiments with overexpression of different Wnt pathways components and are much better and precise in elucidating their real functions within Wnt/ $\beta$ -catenin pathway without any interference with endogenously expressed proteins. In addition, these cell lines are useful tools for testing mutant proteins by rescue assays established in our lab<sup>1</sup>.

Our work represents complex but easy and elegant way how to dissect roles of individual proteins involved in Wnt/ $\beta$ -catenin signalling pathway using CRISPR/Cas9 technique that can be applied generally in many other studies focusing on

different pathways and proteins whose precise role is not known.

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

## REFERENCE

1. Pačliková P., Bernatík O., Radaszkiewicz T. W., Bryja V.: Mol. Cell Biol. 37, e00145 (2017).

## EVALUATION OF ANTIMICROBIAL AND ECOTOXICOLOGICAL PROPERTIES OF NEW TYPES OF NANOFIBER MEMBRANES

**NINA PÁLEŠOVÁ<sup>a</sup>, EVA TRÁVNÍČKOVÁ<sup>a</sup>, MICHAL BITTNER<sup>a</sup>, PŘEMYSL MIKULA<sup>a,b</sup>**

<sup>a</sup>RECETOX Centre, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czechia; <sup>b</sup>Department of Veterinary Public Health and Forensic Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1946/1, 612 42 Brno, Czechia.  
nina.palesova@centrum.sk

Membrane biofouling, undesirable formation of the biofilm on the filtration membrane surface, renders membrane filtration technologies for water purification expensive and ineffective. There are several ways for its mitigation and all of them have their pros and cons (e.g. formation of disinfection by-products for chlorine disinfection or expensive pre-treatment of filtered samples). One of the solutions is modification of filtration membrane with antimicrobial agents resulting in decreased formation of biofilm on the surface<sup>1</sup>. Evaluation of the modification efficacy encompasses antimicrobial and ecotoxicological assays. There are plenty of possible approaches for testing of these two aspects, probably due to the lack of standardized methodology.

In the experimental part of this work, a battery of bioassays was designed for the antimicrobial and ecotoxicological evaluation of the 18 membrane materials varying in support layers and antimicrobial modification agents (different forms of silver nanoparticles and quaternary ammonium salts). These membranes were manufactured by Synpo Inc, COC Ltd. and SPUR Inc. for South-Moravian water treatment company ASIO Inc.

Resazurin test with model bacteria *Escherichia coli* and the sample of natural microbial community from wastewater treatment effluent; acute inhibition test with water crustacea *Daphnia magna* and growth inhibition test with microalgae *Raphidocelis subcapitata* were employed to assess both antimicrobial efficacy (A) and ecotoxicological effects on non-target organisms. Some materials did not exhibit antimicrobial efficacy, some exhibited strong antimicrobial, but also strong ecotoxicological effects at the same time, which is undesirable.

The results indicate the membranes for particulate filtration modified with silver nanoparticles are the best ones, due to their high A and low ecotoxicity. The combination of relatively easy and quick biotests developed in this study provides the first valuable information about the modified membranes and potential ecotoxicity, and can recommend the manufacturers which materials have a real-life application potential.

*This research was supported by RECETOX Research Infrastructure (LM2015051 and CZ.02.1.01/0.0/0.0/16\_013/0001761).*

#### REFERENCE

1. Al-Juboori R.A., Yusaf T.: *Desalination* 302, 1 (2012).

### METHOD OPTIMIZATION AND DEVELOPMENT IN CF GENOMICS

#### FILIP PARDY

CEITEC MU Genomics Core Facility, Kamenice 5,  
616 00 Brno  
f.pardy@gmail.com

As there is an explosion of possible sequencing applications, we see an ever-increasing demand for genomic services, namely Next Gen Sequencing, project counselling and application development. Here we present a selection of the workflows that have been developed in CEITEC CFG for diagnostic and research purposes for Illumina and Oxford Nanopore platforms.

Genomic breakpoint detection using (ligation dependent inverse) LDI-Seq<sup>1</sup> and Crispr-Cas9 enrichment<sup>2</sup> are the methods of choice for pinpointing a breakpoint location across a large breakpoint region that cannot be reliably analysed using a short read technology. LDI-seq utilizes restriction endonucleases to cut the genomic DNA, inverse PCR then amplifies region of interest neighboring with the breakpoint. Up to 10 kb can be analysed with one set of primers. Crispr-Cas9 enrichment is a complementary method that makes use of the fact that Cas9 nuclease leaves a phosphorylated nucleobase in the restriction site that is then used for adapter ligation.

Four-primer PCR enrichment is a preferred method for large taxonomical studies where processing of hundreds of samples is necessary. Library generation is divided in two rounds of PCR. In the first round, composite primers containing target specific (TS) part and a short 5' overhang amplify targeted sequences. Most common targets are 16S/18S, ITS, COI genes. In second round, Illumina-specific primers are used to barcode and finalize the libraries. Final pools are then prepared using densitometry-based gel analysis and Pippin prep size selection. The optimized workflow can be finished in under 3 days for up to 384 samples.

Third featured method is currently under development in our facility to complete the single-cell analysis portfolio. To perform "Single cell targeted DNA resequencing", we

repurpose a MSND instruments to dispense cells into a metal chip patterned with ~5000 hundred-nanoliter wells. Single cell-per-well is obtained by using an optimal dilution of the cell suspension, according to poisson distribution probability. Next, random primer extension followed by single strand ligation and indexing PCR enables us to multiplex thousands of individual samples. Sequencing libraries are pooled afterwards and subjected to target capture protocol.

#### REFERENCES

1. Schneider B., Drexler H. G., MacLeod R. A. F.: *Atlas Gen. Cytogen. Oncol. Haematol.* 2010, 20087; <http://atlasgeneticsoncology.org/Deep/LDI-PCRinCancerID20087.html> (staženo 20.3.2020).
2. Stangl C., de Blank S., Renkens I., et al.: *bioRxiv* 807545; doi: <https://doi.org/10.1101/807545> (staženo 20.3.2020).

### NEW SYNTHETIC CATHINONES: DESIGN, SYNTHESIS AND CHARACTERIZATION

**MARTIN PAŠKAN<sup>a</sup>, ŽANTEA JAVORSKÁ<sup>a</sup>, DITA SPÁLOVSKÁ<sup>b</sup>, VÁCLAV KOZMÍK<sup>a</sup>, MICHAL KOHOUT<sup>a</sup>**

<sup>a</sup>Department of Organic chemistry; <sup>b</sup>Department of Analytical chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague  
paskanm@vscht.cz

New psychoactive substances (NPS), a heterogenous array of psychoactive compounds, are designed as legal alternatives to established illicit drugs<sup>1</sup>. The second most abundant class of NPS represent synthetic cathinones<sup>2</sup>, which are derived from the natural substance (*S*)-cathinone (present in leaves of *Catha edulis*). Cathinones are popular among the public, particularly young people, because of their psychostimulative effect; however, they can also induce serious adverse effects, such as tachycardia and hypertension. The effects of synthetic cathinones may significantly vary depending on their structure<sup>3,4</sup> leading potentially to pharmaceutically interesting substances. We have focused on the introduction of different substituents in the *para* position of the aromatic ring (Fig. 1). Since cathinones are chiral substances, we have also elaborated methods for the chiral separation of the synthesized compounds. Using a combined approach of *ab initio* calculations and chiroptical methods we have determined the absolute configuration of individual enantiomers. We have also prepared optically pure formal metabolites and studied their cytotoxicity *in vitro*.

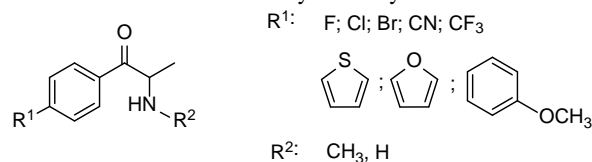


Fig. 1. General structure of synthetic cathinones

This work was supported by the Ministry of Interior of the Czech Republic (grant No. MV0/V120172020056).

## REFERENCES:

1. Katz D., Bhattacharya D.: *Toxicol. Lett.* 229, 349 (2014).
2. European Monitoring Centre for Drugs and Drug Addiction: *European drug report 2018: trends and developments*. Publications Office of the European Union, Luxembourg 2018.
3. Kelly J.: *Drug Test. Anal.* 3, 439 (2011).
4. Prosser J. M., Nelson L. S.: *J. Med. Toxicol.* 8, 33 (2012).

## NEAR-INFRARED POLYMETHINE DYES FOR TARGETED BIOMARKER DETECTION

MATĚJ PASTUCHA<sup>a,b</sup>, MARTIN POTRUSIL<sup>c</sup>, ZDENĚK FARKA<sup>a</sup>, PETER ŠEBEJ<sup>b</sup>

<sup>a</sup>CEITEC MU and <sup>b</sup>RECETOX, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno; <sup>c</sup>Department of Surgery, University Hospital Brno, Faculty of Medicine, Masaryk University, Jihlavská 20, 625 00 Brno  
mpastucha@mail.muni.cz

Fluorescent dyes have a wide scope of use in life-sciences and medicine, most commonly as imaging agents, either alone, or in connection with antibodies in immunofluorescence experiments. Biological tissues absorb visible light and a tissue-transparent window (TTW) in the near-infrared (NIR) region is preferred for imaging purposes. This is particularly true in the fluorescence image-guided surgery.

Polymethine dyes (Fig. 1a) are good candidates for applications in the TTW and their properties can be tuned by changes in the polymethine chain length and substituents<sup>1</sup>. We chose the indocyanine green (ICG, Fig. 2b) as an initial model as it is the only NIR dye certified by the FDA for use in clinical medicine. It is usually used as a dye with very limited selectivity, but when conjugated to an antibody, it can also be used for specific visualization of a biomarker of interest.

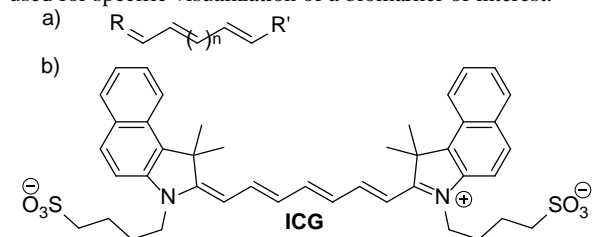


Fig. 1. Polymethine dyes: a) general formula; b) indocyanine green (ICG)

We chose the carbohydrate antigen (CA) 19-9 as a promising target because it is already successfully used in the diagnosis and monitoring of treatment outcome in patients with pancreatic cancer<sup>2</sup>. First, we labeled the mouse anti CA 19-9 antibody with the ICG NHS ester. The conjugate was

successfully tested in a microtiter plate-based fluorescence immunoassay. Testing of the conjugate for detection of CA 19-9 in cell lines and tissue sections follows. We aim to develop a platform for assessing the performance of the newly synthesized polymethine fluorescent labels in complex biological systems.

Financial support: GAČR GJ20-30004Y, MZČR-RVO (FNBr, 65269705), MEYS CR CEITEC 2020 (LQ1601).

## REFERENCES

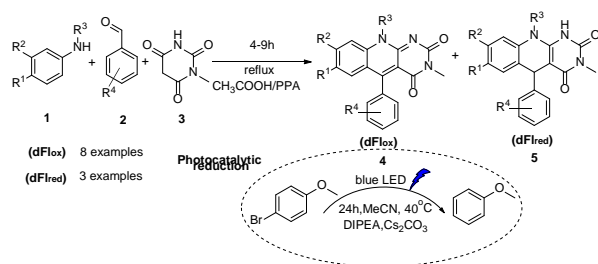
1. Rastede E. E., Tanha M., Yaron D., Watkins S. C., Waggoner A. S., Armitage B. A.: *Photochem. Photobiol. Sci.* 14, 1703 (2015).
2. Goonetilleke K. S., Siriwardena A. K.: *Eur. J. Surg. Oncol.* 33, 266 (2007).

## THE ONE-POT SYNTHESIS OF 5-DEAZAFLAVINS AND THEIR USE IN ORGANIC PHOTOREDOX CATALYSIS

TETIANA PAVLOVSKA, RADEK CIBULKA

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

Modern synthetic chemistry tends to finding efficient low energy protocols by developing novel catalytic systems. Discovering of the coenzyme factor F420, which possesses a 5-deazaflavin nucleus, has opened new horizons for organic catalysis<sup>1</sup>. The 5-deazaflavin skeleton is structurally similar to flavins with a position N-5 of flavin (isoalloxazine) ring replaced by methine group. They have previously shown oxidation-reduction properties similar to those in flavins as well as high potency as antitumor agents<sup>2</sup>. It should be noted, that reduced form of 5-deazaflavins (1,5-dihydro-5-deazaflavins) is much more stable comparing to flavin analogues, that makes them perspective for reductive photochemistry. Despite the number of publications on synthesis of 5-deazaflavin core, most methods implement disadvantageous or several steps procedures. We have firstly applied the multicomponent (MCRs) approach by the simple and efficient three-component condensation of N-substituted anilines (1), aromatic aldehydes (2) and N-methyl barbituric acid (3) in AcOH/PPA media that leads to formation of 5-deazaflavins (4) and 1,5-dihydro-5-deazaflavins (5) in moderate yields. With the deazaflavins derivatives prepared, photocatalytic dehalogenation was studied as model reaction using 4-bromoanisole as substrate. With a one-electron reduction potential of  $-2.75 \text{ V}^3$ , 4-bromoanisole is beyond the limits of still known organic photoredox catalysts. With all deazaflavin derivatives, we observed about 90% conversion to the reduced substrate, which shows high potential of deazaflavins as photoredox catalysts in reduction processes.



Author thanks Czech Science Foundation (project No 19-09064S) for financial support.

## REFERENCES

1. Eirich L. D., Vogels G. D., Wolfe R. S.: *Biochemistry* 17, 4583 (1978).
2. Ali H.I., Tomita K., Akaho E., Kambara H., Miura S., Hayakawa H., Ashida N., Kawashima Y., Yamagishi T., Ikeya H., Yoneda F., Nagamatsu T.: *Biorg. Med. Chem.* 15, 242 (2007).
3. Neumeier M., Sampedro D., Májek M., O'Shea V., Wangelin A.J., Pérez-Ruiz R.: *Chem. Eur. J.* 24, 105 (2018).

## CRISPR/CAS9 TECHNOLOGY: A USEFUL TOOL IN THE STUDY OF CHRONIC LYMPHOCYTIC LEUKEMIA

**HELENA PESCHELOVÁ<sup>a</sup>, VERONIKA KOZLOVÁ<sup>a,b</sup>,  
VERONIKA MANČÍKOVÁ<sup>a,b</sup>, ADRIANA  
LADUNGOVÁ<sup>a</sup>, VÁCLAV HEJRET<sup>a</sup>, MICHAL  
ŠMÍDA<sup>a,b</sup>**

<sup>a</sup>Central European Institute of Technology (CEITEC), Masaryk University, 625 00 Brno; <sup>b</sup>Department of Internal Medicine - Hematology and Oncology, Medical Faculty of Masaryk University and University Hospital Brno 625 00, Czech Republic  
helenapeschelova@gmail.com

Chronic lymphocytic leukemia (CLL) patients carry a variety of somatic mutations<sup>1</sup>, whose exploration could shed light on the disease etiology, or even lead to the discovery of potential novel drug targets. To establish suitable models mimicking the genetic heterogeneity observed in patients, we generated isogenic cell lines harboring some of the most frequent CLL mutations. We aim to investigate unique vulnerabilities specific to these mutations.

We made use of HG3 and MEC1 cells, two well-established CLL cell lines<sup>2,3</sup>, and using CRISPR/Cas9 technology we generated monoclonal isogenic knockout cell lines in *ATM* and *TP53* genes and a knock-in HG3 cell line in *MYD88* gene. In addition to these monoclonal cell lines, we also established a polyclonal CD20 knockout HG3 cell line.

The *ATM* knockout cell line was used for CRISPR/Cas9 functional screening to reveal genes synthetic lethal to *ATM* by comparing the abundance of gene knockouts in the beginning of the experiment vs. after 3-week incubation. Gene knockouts

that were depleted in the *ATM* knockout cells but not in wildtype control should have a synthetic lethal relationship with the *ATM* gene. Altogether we obtained 11 candidate *ATM*-synthetic lethal genes, which are now being validated. After thorough validation, some of the most interesting genes might serve as potential therapeutic targets for patients with *ATM* mutations.

Apart from the functional screening, the established knockout and knock-in cell lines serve as suitable models for CLL and have a wide range of uses to develop novel personalized therapies. We have utilized these cells to generate CLL mouse models for CAR T-cell study, to investigate CD20 function and also for a drug screening with an FDA-approved drug library.

This research is financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

## REFERENCES

1. Landau D., Carter S., Stojanov P., et al.: *Cell* 152, 714 (2013).
2. Rosén A., Bergh A., Gogok P., Evaldsson C., Myhrinder A., Hellqvist E., Rasul A., Björkholm M., Jansson M., Mansouri L., Liu A., Teh B., Rosenquist R., Klein E.: *OncoImmunology* 1, 18 (2012).
3. Stacchini A., Aragno M., Vallario A., Alfarano A., Circosta P., Gottardi D., Faldella A., Rege-Cambrin G., Thunberg U., Nilsson K. and Caligaris-Cappio F.: *Leukemia Res.* 23, 127 (1999).

## ON THE EDGE OF THE LIMITS – SUPERCRITICAL FLUID CHROMATOGRAPHY OF CYTOKININS

**IVAN PETŘÍK<sup>a,b</sup>, ALEŠ PĚNČÍK<sup>a,b</sup>, PETRA  
AMAKOROVÁ<sup>a</sup>, MIROSLAV STRNAD<sup>a</sup>, ONDŘEJ  
NOVÁK<sup>a\*</sup>**

<sup>a</sup>Laboratory of Growth Regulators, AS CR, Inst. Exp. Botany & Palacký University, Fac. Sci., 78371 Olomouc, Czech Republic; <sup>b</sup>Dept Chem. Biol. Genet., Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, 78371 Olomouc, Czech Republic  
ivan.petrik@upol.cz

Cytokinins are naturally occurring compounds and one of the most studied plant growth regulators. They control important growth and developmental processes in plants, such as inhibition of leaf senescence, promoting of lateral shoot formation, leaf expansion or inhibition of apical dominance<sup>1</sup>. Similarly, to the other phytohormones cytokinins act in living organisms in very low concentrations (up to 10<sup>-15</sup> mol per gram of fresh weight). Consequently, cytokinin analysis includes salt removal and isolation of analytes from complex plant matrix. This process is crucial to determine quantity of cytokinins in real samples<sup>2</sup>. Furthermore, the identification and quantification of cytokinins requires very sensitive instrumental approaches. Ultra-high performance liquid

chromatography coupled with tandem mass spectrometry detection (UHPLC-MS/MS) represents one of the well-established techniques for this purpose<sup>3</sup>. However, rapid expansion of supercritical fluid chromatography (SFC) has emerged in the last decade. This method utilizes CO<sub>2</sub> in supercritical state as the mobile phase. Therefore, SFC combines the advantages of gas chromatography and liquid chromatography resulting in high separation performance in relatively short time<sup>4</sup>. Moreover, in the combination with the mass spectrometry detection SFC promises powerful tool for analysis of low abundant small molecules such as cytokinins. We used SFC-MS/MS instrumentation in order to determine full cytokinin profile in *Arabidopsis thaliana*.

This work was supported by Ministry of Education, Youth and Sports of the Czech Republic (European Regional Development Fund-Project 'Centre for Experimental Plant Biology' no. CZ.02.1.01/0.0/0.0/16\_019/0000738) and Internal Grant Agency of Palacký University (IGA\_PrF\_2019\_020)

#### REFERENCES

1. Mok M. C.: CRC Press Boca Raton 1994, 155.
2. Dobrev P., Kamínek M.: J. Chromatogr. A 950, 21 (2002).
3. Novák O., Napier R., Ljung K.: Ann. Rev. Plant Biol. 68, 323 (2017).
4. Lesellier E.: J. Chromatogr. A, 1614, 460582 (2020); DOI: 10.1016/j.chroma.2019.460582.

#### INVESTIGATION OF PLGA NANOPARTICLES FOR TOFACITINIB DELIVERY INTO HAIR FOLLICLES

**ELIŠKA PETROVÁ<sup>a</sup>, REBEKKA CHRISTMANN<sup>b</sup>, BRIGITTA LORETZ<sup>b</sup>, CLAUS-MICHAEL LEHR<sup>b</sup>, JARMILA ZBYTOVSKÁ<sup>a\*</sup>**

<sup>a</sup>University of Chemistry and Technology Prague, Technická 6, 16628 Prague, Czech Republic; <sup>b</sup>Helmholtz Institute of Pharmaceutical Research Saarland, Campus E8.1, 66123 Saarbrücken, Germany  
Jarmila.Zbytovska@vscht.cz

Tofacitinib is a selective inhibitor of Janus Kinase enzyme and a promising drug for treatment of alopecia areata (reversible, fast and completely circumscribed hair loss). However, a lot of severe side effects hinder tofacitinib to expand into clinical praxis<sup>1</sup>.

One of the potential options to decrease its side effects is topical application of tofacitinib direct into hair follicles. As tofacitinib is hardly soluble in the usually used solvents, the choice of an optimum drug formulation is crucial. Our idea is to encapsulate tofacitinib into PLGA (lactic acid:glycolic acid 50:50) nanoparticles using nanoprecipitation method and to target them directly in hair follicles.

The amount of incorporated tofacitinib was increased gradually in PLGA nanoparticles. The optimum amount of tofacitinib in formulation was determined concerning the

acceptable nanoparticle size and polydispersity. The most suitable PLGA nanoparticles were tested on porcine skin by the differential stripping method (see Fig. 1)<sup>2</sup>. The amount of tofacitinib in the skin and hair follicles was determined by relative fluorescent intensity of nanoparticles. Based on these results, the efficiency of tofacitinib delivery to the hair follicles by PLGA nanoparticles was confirmed.

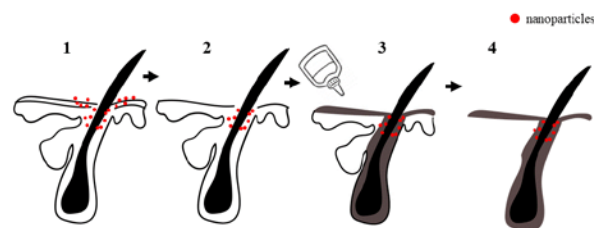


Fig. 1. Differential stripping: 1 - nanoparticles application, 2 - after tape stripping, 3 - glue application, 4 - biopsy

Authors thank the Czech Science Foundation (GACR19-09600S) for financial support.

#### REFERENCES

1. Xing L., Dai Z., Jabbari A., Cerise J. E., Higgins C. A., Gong W., De Jong A.; Harel S., Destefano G. M., Rothman L., Siengh P., Petukhova L., MacKay-Wiggan J., Christiano A. M., Clynes R.: Nat. Med. 20, 1043 (2014).
2. Raber A. S., Mittal A., Schafer J., Bakowky U., Reichrath J., Vogt T., Schaefer U. F., Hansen S., Lehr C. M.: J. Control. Release 179, 25 (2014).

#### THE POTENTIAL OF SUPERCRITICAL FLUID CHROMATOGRAPHY IN PHARMACEUTICAL ANALYSIS

**KATEŘINA PLACHKÁ, MARIA KHALIKOVA, BARBORA BABIČOVÁ, ZDEŇKA NĚMCOVÁ, LUCIE ROUBÍČKOVÁ, LUCIE NOVÁKOVÁ**

Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové  
plachkka@faf.cuni.cz

Ultra-high-performance supercritical fluid chromatography (UHPSFC) is an advanced version of supercritical fluid chromatography using CO<sub>2</sub>-based mobile phase, sub-2-micron particles, and dedicated instrumentation. The interest in ultra-high performance supercritical fluid chromatography (UHPSFC) separations is constantly growing due to inherent benefits such as high speed, high efficiency and versatility in method development. However, the usability in strictly controlled fields such as pharmaceutical analysis still needs to be proven.

The first part of this study is focusing on searching for screening approach and generic UHPSFC conditions which could be used as starting point for method development. Ten



various quality control mixtures including overall 70 compounds were selected and analysed in 4 min by UHPSFC method with UV and mass spectrometry detection using eight stationary phases, three organic modifiers, and five additives in methanol. Resulting 640 chromatograms were compared in six selected parameters including number of eluted peaks, number of separated peaks, resolution between active pharmaceutical ingredient and following impurity, peak symmetry, peak width at 50 % of peak high, and separation selectivity. The generic combination of diol stationary phase and 0.1 % ammonium hydroxide in methanol as modifier was proposed as a starting point for UHPSFC method development based on the obtained results.

Several challenges were described during following optimization of the final UHPSFC methods and their solution was proposed. To prove applicability of resulting methods in pharmaceutical quality control, validation was carried out using parameters based on ICH guidelines Q2 and Q3 including limit of detection and quantification, linearity, range, accuracy, and precision. Besides that, validation approach using total error approach was applied on several selected methods.

The interlaboratory precision has to be proven before application of UHPSFC method in pharmaceutical laboratories. Therefore, a study using UHPSFC method for the determination of salbutamol impurities was carried out at 19 laboratories in 9 countries. Statistic evaluation confirmed the method reproducibility with results comparable or even better than for LC method described in European Pharmacopeia.

All obtained results proved the potential of UHPSFC in pharmaceutical analysis. Not only that UHPSFC offers fast and environmentally friendly methods, but these methods are able to pass strict validations required by international guidelines.

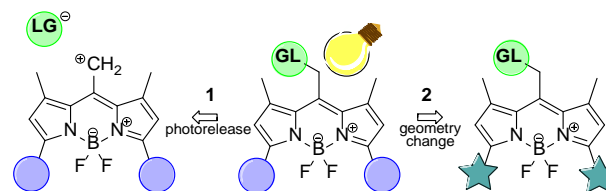
*The study was supported by the Project of Specific Research, SVV No. 260412/2017, and by the STARSS project (Reg. No. CZ.02.1.01/0.0/0.0/15\_003/0000465) co-funded by ERDF.*

## NOVEL BODIPY-BASED PHOTOCAGES BEARING LIPIDS

**ANNA PORÝVAJ, TOMÁŠ SLANINA\***

*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 542/2, 160 00 Prague 6  
anna.poryvai@uochb.cas.cz*

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



Scheme 1. Targeted modifications of the structure

In this study we focused on two modifications of a BODIPY scaffold (Scheme 1). Firstly, designed PhCs were used to protect several lipids (represented as a green circle) to further study their photorelease in cells. Second modification lies in the connection of the BODIPY scaffold to moieties with controllable geometry (blue circles and stars). Such combination enables induction of reversible changes of properties of the BODIPY scaffold.

*This research was supported by the program INTER-EXCELLENCE, subprogram INTER-COST of the Ministry of Education, Youth and Sports CR, grant No. LTAI19166.*

## REFERENCES

1. Klán P., Šolomek T., Bochet Ch. G., Blanc A., Givens R., Rubina M., Popik V., Kostikov A., Wirz J.: *Chem. Rev.* **113**, 119 (2013).
2. 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).
3. Peterson J. A., Wijesooriya Ch., Gehrman 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).

## ROLE OF RAD51 IN METABOLISM OF G4 STRUCTURES

**MICHAELA POSPÍŠILOVÁ<sup>a</sup>, MÁRIO ŠPÍREK<sup>a,b</sup>, FEDOR NIKULENKOV<sup>a,b</sup>, MARK SKEHEL<sup>c</sup>, LUMÍR KREJČÍ<sup>a,b</sup>**

*<sup>a</sup>Laboratory of Recombination and DNA Repair, NCBR & Dept Biol., Masaryk University, Kamenice 5/A7, 625 00 Brno; <sup>b</sup>Intl Clinical Research Ctr, St Anne's University Hospital, Brno, Czech Republic; <sup>c</sup>Biological Mass Spectrom. Proteom., MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, UK  
436900@mail.muni.cz*

RAD51 is DNA binding protein with a key role in the maintenance of genomic stability including double strand break repair<sup>1</sup> and protection, reversal and restart of stalled replication fork<sup>2</sup>. Replication fork progression can be blocked by formation of stable quadruplexes (G4s)<sup>3</sup>, four-stranded DNA secondary structures<sup>4</sup>. Although G4s were long considered an *in vitro* artefact, high-throughput sequencing revealed more than 700,000 potential G4 structures in the human genome, which are proposed to play regulatory roles in

multiple biological processes<sup>5</sup>. Subsequently several evidences pointed out the role of RAD51 in G4 processing, including reduced viability of human RAD51 or BRCA2-depleted cells upon G4 stabilization<sup>7</sup>. Moreover, adversely effect of persistent G4 on progression of replication fork in cells lacking RAD51 protein suggested relevance of RAD51 for fluent replication through regions prone to form quadruplex structure<sup>6</sup>.

Nevertheless, all previous studies proposed role of RAD51 in repair of generated DNA damage as a result of G4 stabilization. Surprisingly, our results uncovered direct role of RAD51 in G4 metabolism due to its tight G4 binding with higher affinity in comparison to single-stranded DNA (ssDNA). Moreover, RAD51 selectively recognize parallel compared to anti-parallel G4 and also the kinetics of binding shows unexpected differences in comparison to ssDNA binding. We will provide further characterization of this interaction and tested effect of G4 binding on other known RAD51 activities both *in vitro* and *ex vivo*.

Obtained data provide new specific role of RAD51 protein in processing G4 containing DNA sequences and its biological consequences. These results may also contribute to understand of cancer development, since RAD51 is associated with different types of cancer and G4s have become a target of anti-cancer therapy.

#### REFERENCES

1. Arnaudeau C., Lundin C., Helleday T.: *J. Mol. Biol.* **307**, 1235 (2001).
2. Kolinjivadi AM., Sannino V., de Antoni A., Técher H., Baldi G., Costanzo V.: *FEBS Lett.* **591**, 1083 (2017).
3. Kruisselbrink E., Guryev V., Brouwer K., Pontier DB., Cuppen E., Tijsterman M.: *Curr. Biol.* **12**, 900 (2008).
4. Rhodes D., Lipps HJ.: *Nucleic Acids Res.* **43**, 8627 (2015).
5. Chambers VS., Marsico G., Boutell JM., Di Antonio M., Smith GP., Balasubramanian S.: *Nat. Biotechnol.* **33**, 877 (2015).
6. Zimmer J., Tacconi EMC., Folio C., Badie S., Porru M., Klare K., Tumiati M., Markkanen E., Halder S., Ryan A., Jackson SP., Ramadan K., Kuznetsov SG., Biroccio A., Sale JE., Tarsounas M.: *Mol Cell.* **61**, 449 (2016).

#### 12-O-TETRADECANOYLPHORBOL-13-ACETATE INCREASES CARDIOMYOGENESIS IN EMBRYONIC STEM CELLS THROUGH PKC/ERK SIGNALLING

**KATARZYNA RADASZKIEWICZ<sup>a</sup>, DEBORAH BECKEROVÁ<sup>a</sup>, LUCIE WOŁOSZCZUKOVÁ<sup>a</sup>, TOMASZ WITOLD RADASZKIEWICZ<sup>a</sup>, LUKÁŠ KUBALA<sup>a,b</sup>, PETR HUMPOLÍČEK<sup>c</sup>, JIŘÍ PACHERNIK<sup>a,\*</sup>**

<sup>a</sup>Dept Exper. Biol., Faculty of Science, Masaryk University, Brno; <sup>b</sup>Dept Free Radical Pathophysiol., Institute of Biophysics, AS CR, Brno; <sup>c</sup>Centre of Polymer Systems and

Faculty of Technology, T. Bata University, 760 01 Zlin, Czech Republic  
jipa@sci.muni.cz

12-O-Tetradecanoylphorbol-13-acetate (TPA) is the most widely used diacylglycerol (DAG) mimetic agent and inducer of protein kinase C (PKC)-mediated cellular response in biomedical studies. TPA has been proposed as a pluripotent cell differentiation factor, but results obtained have been inconsistent.

In the present study we show that TPA can be applied as a cardiomyogenesis promoting factor for the differentiation of mouse embryonic stem (mES) cells *in vitro*. The mechanism of TPA action is mediated by the induction of extracellular signal-regulated kinase (ERK) activity and the subsequent phosphorylation of GATA4 transcription factor. Interestingly, general mitogens (FGF, EGF, VEGF and serum) or canonical Wnt signalling did not mimic the effect of TPA. Moreover, on the basis of our results, we postulate that a TPA sensitive population of cardiac progenitor cells exists at a certain time point (after days 6-8 of the differentiation protocol) and that the proposed treatment can be used to increase the multiplication of ES cell-derived cardiomyocytes.

#### RNF43 SUPPRESSES MELANOMA METASTASIS BY NON-CANONICAL Wnt SIGNALLING INHIBITION

**TOMASZ RADASZKIEWICZ<sup>a</sup>, MICHAELA NOSKOVÁ<sup>a</sup>, KRISTÍNA GÖMÖRYOVÁ<sup>a</sup>, KATARZYNA RADASZKIEWICZ<sup>a</sup>, MARKETKA PICKOVÁ<sup>d</sup>, TOMÁŠ GYBEL<sup>a</sup>, ANNA KOTRBOVÁ<sup>a</sup>, LUCIA BINO<sup>d</sup>, JAN VERNER<sup>a</sup>, RADEK FEDR<sup>d</sup>, KAROL KAISER<sup>a</sup>, LUCIA DEMKOVA<sup>b</sup>, LUCIA KUČEROVÁ<sup>b</sup>, DAVID POTĚŠIL<sup>c</sup>, ZBYNĚK ZDRÁHAL<sup>c</sup>, KAREL SOUČEK<sup>b</sup>, VÍTĚZSLAV BRYJA<sup>a,b,\*</sup>**

<sup>a</sup>Dept Exp. Biol., Fac. Sci., Masaryk University, 625 00 Brno, Czech Republic; <sup>b</sup>Lab. Mol. Oncol., Cancer Res. Inst., Biomed. Res. Ctr AS SR, Bratislava, Slovakia; <sup>c</sup>CEITEC, Masaryk University, 625 00 Brno, Czech Republic; <sup>d</sup>Dept Cytokinetics, Inst. Biophys. CAS, 612 65 Brno, Czech Republic  
bryja@sci.muni.cz

Melanoma is the deadliest human skin cancer with worldwide rising incidence. Non-canonical Wnt signalling pathway controls various aspects of oncogenesis and cancer progression, i.e. proliferation and invasion. We show here that high expression of the key Wnt planar cell polarity (PCP) pathway players is a negative survival factor for melanoma patients. Next, we focused on the E3 ubiquitin ligase called RNF43, which expression dramatically decreases with melanoma progression. RNF43 and related ZNRF3 are transmembrane ligases with an ability to inhibit canonical Wnt signalling. Their activity leads to the internalization and degradation of Frizzled proteins, which are Wnt ligands receptors. Existing data suggest that PCP pathway can also be negatively regulated by RNF43. Thus, we were intrigued to

ask if RNF43 can modulate also the non-canonical Wnt signalling in human cells by distinct mechanism. To address this question, we utilized proximity-dependent Biotin Identification (BioID). We showed that RNF43 interacts with the non-canonical Wnt signalling receptor complexes components, which are also RNF43 substrates. Briefly, RNF43 efficiently suppress the Wnt5a-driven non-canonical signalling cascade.

These phenomena have direct consequences also for melanoma. Apart from *in vitro* studies, our key findings were verified also by the *in vivo* approach. Human melanoma cell line was labelled with luciferase and modified to express RNF43 in the doxycycline presence. Intradermally injected cells in the immunodeficient NRG mouse xenograft formed tumors and metastasized. Interestingly, doxycycline forced expression of RNF43 significantly decreased number of invading melanoma cells. Moreover, number of circulating tumor cells was also reduced in comparison to the control cohort. We observed decreased expression epithelial to mesenchymal transition (EMT) markers.

Taken together, we propose that RNF43 downregulation allows efficient Wnt5a signalling and is beneficial for melanoma progression. Additionally, we provided new *in vivo* model of metastatic melanoma, which can be used in the following studies.

#### CYANOBACTERIAL LIPOPOLYSACCHARIDE CAN ACTIVATE HUMAN KERATINOCYTES PRO-INFLAMMATORY WAY

**PETRA RAPTOVÁ<sup>a</sup>, VERONIKA SKOČKOVÁ<sup>a,d</sup>, PAVEL BABICA<sup>b,c</sup>, LENKA ŠINDLEROVÁ<sup>a</sup>**

<sup>a</sup>Institute of Biophysics AS CR, The Department of Biophysics of Immune System, 612 00 Brno; <sup>b</sup>Masaryk University, Fac. Sci., RECETOX, 625 00 Brno; <sup>c</sup>Inst. Botany AS CR, Dept Exp. Phycol. Ecotoxicol., 602 00 Brno; <sup>d</sup>Masaryk University, Fac. Sci., Dept Exp. Biol., 625 00 Brno  
zwoey323@gmail.com

Cyanobacteria-dominated harmful algae blooms (HAB) are an important source of various toxins in the aquatic environment. Not well studied one is a lipopolysaccharide (LPS) which can originate not only from Gram-negative heterotrophic bacteria associated with HAB but also from cyanobacteria. Although bacterial LPS is known to be a potent pro-inflammatory agent, the cyanobacterial LPS is not studied well. The most probable exposure to HAB-LPS is via dermal contact with contaminated water during recreational activities like swimming, canoeing, windsurfing etc. Taking into account the pro-inflammatory properties of LPS and dermal irritations observed after exposure to HAB, contribution of LPS to the overall skin-irritating activity of the HAB. Human immortalized keratinocytes HaCaT were chosen as an *in vitro* model to study LPS effects. Four different water bloom samples from the Czech Republic dominated by different cyanobacterial species were chosen and LPS was isolated using hot-phenol extraction. Moreover, axenic cultures of

respective cyanobacterial species (*Microcystis aeruginosa*, *Dolichospermum flos-aquae*, *Planktothrix aghardii*, and *Aphanizomenon flos-aquae*) were used to obtain pure cyanobacterial LPS. As a positive control, LPS from *Escherichia coli* was used. Toxicity of all LPS samples was tested by LDH and BCA assays and non-toxic concentrations were used for further experiments. HAB dominated by *D. flos-aquae* and *M. aeruginosa* activated the keratinocytes to produce pro-inflammatory cytokines interleukin (IL) 6 and IL8 and the activation was comparable or even higher than the activation by positive control. Interestingly, exposure to LPS from pure culture of *A. flos-aquae* also induced production of both cytokines, though not as much as the positive control. Nevertheless, it is the first time when pro-inflammatory effect of pure cyanobacterial LPS on keratinocytes was proven. Based on our results we can say that environmental mixtures of LPS from HAB as well as at least some LPS from pure cyanobacteria are able to activate human keratinocytes and therefore they could contribute to skin irritations observed after dermal contact with HAB-contaminated water.

*This project was supported by the Czech Science Foundation project No. GA19-09980S.*

#### ALKALOIDS OF *GEISSOSPERMUM VELLOSI* ALEMÃO AND THEIR BIOLOGICAL ACTIVITY

**MARCELA ŠAFRATOVÁ<sup>a</sup>, DANIELA HULCOVÁ<sup>a</sup>, JAKUB CHLEBEK<sup>b</sup>, LUBOMÍR OPLETAL<sup>b</sup>**

<sup>a</sup>Department of Pharmacognosy, FAF UK, Akademia Heyrovského 1203/8, 500 05 Hradec Králové<sup>a</sup>

<sup>b</sup>Department of Pharmaceutical Botany, FAF UK, Akademia Heyrovského 1203/8, 500 05 Hradec Králové<sup>b</sup>  
safratom@faf.cuni.cz

Plants produce a wide range of secondary metabolites and are classified according to their chemical structure. One of the main classes are alkaloids. Alkaloids are basic molecules with nitrogen atom in their structure. These chemical structures show interesting biological activities eg. antimalarial (quinine), anticancer (lycorine), analgesic (morphine) and cholinomimetic activity (galanthamine).<sup>1</sup>

Plants of Apocynaceae family are well known source of indole alkaloids. One of them is *Geissospermum vellosii* Alemão (Pao pereira), it is a rainforest tree with small white flowers. The inner bark from the tree has many uses in folk medicine eg. sexual stimulant, against malaria, inflammation, and tinctures are used in anticancer therapy. Pao pereira is a source of indole and beta-carboline alkaloids that are also known as antiparasitic agents.<sup>2</sup>

Our study was based on a previous screening study where many other plant drugs were tested for inhibition activity against human acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Alkaloidal extract from *G. vellosii* exhibited the best results (IC<sub>50,AChE</sub> = 10,51 ± 3,03 µg/ml, IC<sub>50,BuChE</sub> = 0,39 ± 0,057 µg/ml) and has been chosen for further phytochemical study. All isolated compounds

undergo biological test that are connected with Alzheimer's disease (AD) (inhibition of AChE, BuChE and glycogen synthase kinase 3 $\beta$ (GSK3)). The most interesting isolated alkaloids were quebrachamine (IC<sub>50, BuChE</sub> = 3,72  $\pm$  0,05  $\mu$ M, IC<sub>50, GSK3</sub> = 87,81  $\pm$  11,03 %) and vellosimine (IC<sub>50, BuChE</sub> = 3,72  $\pm$  0,05  $\mu$ M, IC<sub>50, GSK3</sub> = 7,18  $\pm$  1,12  $\mu$ M). The main pathological changes of AD take place in brain, for this reason active compounds were tested for their ability to cross the blood-brain barrier.

*The study was supported by SVV 260 412.*

#### REFERENCES

1. Cahlíková, L., Macáková, K., Benešová N., Chlebek J., Hošťálková A., Opletal L.: in *Studies in Natural Products Chemistry*, kap. 6, s. 153, Elsevier Amsterdam 2014.
2. Lima J. A., Costa R., Epifânio R.A., Castro N. G., Rocha M. S., Pinto A. C.: *Pharmacology, Biochemistry and Behavior* 508, 92 (2009).

#### ROLE OF GALECTIN-3 IN CELL-MATRIX ADHESION

ANTONIN SEDLAR<sup>a</sup>, JANA MUSILKOVA<sup>a</sup>, MARTINA TRAVNICKOVA<sup>a</sup>, VLADIMIR KREN<sup>b</sup>, LUCIE BACA KOVA<sup>a</sup>

<sup>a</sup>Institute of Physiology AS CR, Videnska 1083, 142 20 Praha 4 – Krc; <sup>b</sup>Institute of Microbiology AS CR, Videnska 1083, 142 20 Praha 4 – Krc  
antonin.sedlar@fgu.cas.cz

Galectins are mammalian lectin proteins. Their characteristic properties are high affinity to beta-galactosides and highly conserved carbohydrate-recognition domain (CRD).

So far, 15 different galectins were found and characterized. These proteins can be involved in processes including cell-cell interaction, cell-matrix adhesion, endocytosis or intracellular signalling. Biological activity of galectins is mediated mainly by their ability to bind beta-galactosides naturally present on the surface of various glycoproteins<sup>1</sup>.

Galectin-3 is a 28 kDa protein. In comparison with other galectin proteins, galectin-3 has rather unique structure. It is composed of C-terminal carbohydrate recognition domain and N-terminal domain containing collagen-like short tandem repeats of amino acid segments (P-G-A-Y-P-G)<sup>2</sup>. The N-terminal domain enables the protein to form pentamers. Galectin-3 can be localized in cell nucleus, cytoplasm, plasma membrane or extracellular space. It can mediate cell adhesion by interacting with beta-galactoside-capped glycoproteins in extracellular matrix (e.g. fibronectin, laminin, tenascin) or at cell surface (e.g. integrins, Mac-2 binding protein)<sup>3</sup>.

Our results show that recombinant galectin-3 binds to the plasma membrane of endothelial cells in a carbohydrate-inhibitable manner. Surprisingly, when adsorbed to plastic surface, galectin-3 mediates cell adhesion independently on

the presence of beta-galactoside ligand LacDiNAc. These results suggest that two possible modes of interaction of galectin-3 with cell plasma membrane exist. The first one is typical for galectin protein family. It is mediated by interaction of galectin-3 CRD with carbohydrates. The second one is probably mediated by protein-protein interaction and needs to be further elucidated. Further experiments will be focused on the integrins and other cell adhesion molecules as possible binding partners in cell adhesion to galectin-3 coated surface.

*This work is supported by the Grant Agency of the Czech Republic (Grant no.18-01163S).*

#### REFERENCES

1. Yang R.-Y., Rabinovich G. A., Liu F.-T.: *Expert. Rev. Mol. Med.* 10, e17 (2008).
2. Gao X., Liu J., Liu X., Li L., Zheng J.: *Cancer Metastasis Rev.* 36, 367 (2017).
3. Hughes R. C.: *Biochimie* 83, 667 (2001).

#### NOVEL DISEASE-CAUSING MUTATIONS DISCOVERED IN HUMAN ADAR2

JIRÍ SEDMÍK<sup>a</sup>, TIONG Y. TAN<sup>b,c,d</sup>, MARK P. FITZGERALD<sup>e,f</sup>, R. SUKENIK-HALEVY<sup>g,h</sup>, REZA MAROOFIAN<sup>i</sup>, MARY A. O'CONNELL<sup>a\*</sup>

<sup>a</sup>CEITEC, Masaryk University, 625 00 Brno, Czechia; <sup>b</sup>Murdoch Children's Res. Inst., Melbourne, Australia; <sup>c</sup>Victorian Clin. Genet. Services, Melbourne, Australia; <sup>d</sup>Dept Paediatr., Univ. Melbourne, Australia; <sup>e</sup>Depts Neurology & Pediatrics, Children's Hosp., Philadelphia, USA; <sup>f</sup>Perelman School Med., Univ. Pennsylvania, Philadelphia, USA; <sup>g</sup>Sackler Fac. Med., Tel Aviv Univ., Israel; <sup>h</sup>R. Recanati Genet. Inst., Rabin Med. Ctr - Beilinson Hosp., Petah Tikva, Israel; <sup>i</sup>Dept Neuromusc. Disord., UCL Queen Sq. Inst. Neurol., London, UK  
jiri.sedmik@ceitec.muni.cz

Double-stranded RNA-specific adenosine deaminases (ADARs) are a family of enzymes that catalyse the hydrolytic deamination of adenosine to inosine in dsRNA. The editing and RNA-binding activities of ADARs affect RNA processing, stability, and can even lead to RNA recoding<sup>1</sup>. The enzyme ADAR2 is essential for recoding of brain transcripts. Impaired ADAR2 editing causes early-onset epilepsy and premature death in mouse models<sup>2</sup>.

ADAR2 variants were found in five unrelated individuals with microcephaly, intellectual disability, and epilepsy. One patient carried a homozygous mutation in one of the double-stranded RNA-binding domains, whereas, in the remaining four patients, homozygous or biallelic mutations located in or around the deaminase domain were identified. To evaluate the effects of these variants on ADAR2 activity, *in vitro* assays with recombinant proteins expressed in HEK293T cells and *ex vivo* assays with fibroblasts derived from one of the individuals were performed.

We demonstrate that these ADAR2 variants lead to reduced editing activity on a known ADAR2 substrate. We also

detected changes in ADAR2 mRNA splicing induced by one of the mutations. Lastly, we observed altered ADAR2 mRNA and protein levels in patient fibroblasts. Together, these results provide evidence that the mutations have a small but significant effect on ADAR2 activity that may lead to the observed phenotype. Proper neuronal model is needed to characterise the effects of these mutations on brain development.



Funded under the FP7 project 'The ERA Chair Culture as a Catalyst to Maximize the Potential of CEITEC' (ID 621368) of EU.

#### REFERENCES

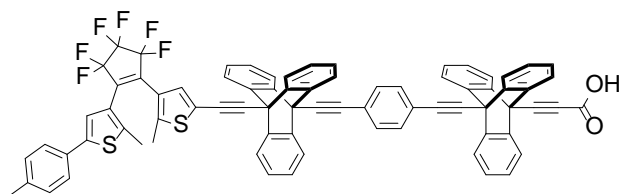
1. Nishikura K.: *Ann. Rev. Biochem.* 79, 321 (2010).
2. Brusa R., Zimmermann F., Koh D. S., Feldmeyer D., Gass P., Seeburg P. H., Sprengel R.: *Science* 270, 1677 (1995).

#### SYNTHESIS OF MOLECULAR SWITCHES FOR SELF-ASSEMBLY ON SURFACES

**LUKÁŠ SEVERA<sup>a</sup>, CARINA SANTOS HURTADO<sup>a</sup>, EVA KALETOVÁ<sup>a</sup>, MILAN MAŠÁT<sup>a</sup>, JAKUB ŠTOČEK<sup>a</sup>, IVANA ČISAŘOVÁ<sup>b</sup>, JIŘÍ KALETA<sup>a\*</sup>**

<sup>a</sup>Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo 2, 166 10 Prague 6, Czech Republic, <sup>b</sup>Department of Inorganic Chemistry, Faculty of Science, Charles University, Hlavova 2030, 128 40 Prague 2, Czech Republic  
severa@uochb.cas.cz, kaleta@uochb.cas.cz

Organizing of individual molecules into regular arrays is a promising route towards new generation of smart materials. Once equipped with properly designed photoactive units (various molecular switches), such systems could be of potential interest on the field of molecular electronics (memory devices, etc.). Work presented herein describes a new class of rod-shaped amphiphilic triptycene-based structures that should form regular 2-D films using Langmuir-Blodgett technique (LB) on the water-air interface (Scheme 1). All structures share several characteristic motives: (i) carboxylic group on one terminus that facilitate strong interaction with aqueous subphase, (ii) triptycene units that should stabilize 2-D structures due to intermolecular  $\pi$ - $\pi$  interactions, and (iii) light-driven molecular switches. Such LB films could be transferred to various solid substrates (quartz, mica, gold, etc.) and subsequently analysed using standard techniques (UV-vis, ellipsometry, PM-IRRAS, X-Ray Photoelectron Spectroscopy, STM or AFM).



Scheme 1. Amphiphilic molecular switch designed to form LB films.

This work was supported by the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences (RVO: 61388963), and Czech Science Foundation (grant number: 20-13745S).

#### REFERENCES

1. Kaleta J., Kaletová E., Čísařová I., Teat S. J., Michl J.: *J. Org. Chem.* 80, 10134 (2015).
2. Kaleta J., Wen J., Magnera T. F., Dron P. I., Zhu C., Michl J.: *PNAS* 115, 9373 (2018).

#### THE STRUCTURE-FUNCTION RELATIONSHIPS AND ENGINEERING OF *RENILLA*-TYPE LUCIFERASES

**ANDREA SCHENKMAYEROVA<sup>a,b</sup>, GASPAR P. PINTO<sup>a,b</sup>, MARTIN TOUL<sup>b</sup>, MARTIN MAREK<sup>b</sup>, LENKA HERNYCHOVA<sup>c</sup>, VERONIKA LISKOVA<sup>b</sup>, DANIEL PLUSKAL<sup>b</sup>, STEPHANE EMOND<sup>d</sup>, MARK DÖRR<sup>e</sup>, JOAN PLANAS<sup>b</sup>, RADKA CHALOUPOKVA<sup>b</sup>, DAVID BEDNAR<sup>a,b</sup>, ZBYNEK PROKOP<sup>a,b</sup>, UWE T. BORNSCHEUER<sup>e</sup>, FLORIAN HOLLFELDER<sup>d</sup>, JIRI DAMBORSKY<sup>a,b</sup>**

<sup>a</sup>Intl Clin. Res. Ctr, St. Anne's Univ. Hospital, 656 91 Brno, Czech Republic; <sup>b</sup>Loschmidt Labs, Dept Exp. Biol. RECETOX, Fac. Sci., Masaryk Univ., 625 00 Brno, Czech Republic; <sup>c</sup>Regional Ctr Appl. Mol. Oncol., Masaryk Memorial Cancer Inst., 656 53 Brno, Czech Republic; <sup>d</sup>Dept Biochem., Univ. Cambridge, Cambridge CB2 1GA, UK; <sup>e</sup>Dept Biotechnol Enzyme Catal., Inst. Biochem., Univ. Greifswald, 17487 Greifswald, Germany  
andrea.schenkmayerova@fnusa.cz

We have resurrected a bifunctional ancestral enzyme<sup>1</sup> that putatively existed prior to the functional diversification into modern-day haloalkane dehalogenases (EC 3.8.1.5) and coelenterazine-converting *Renilla* luciferase (EC 1.13.12.5). This ancestor, which exhibited markedly enhanced thermal stability, was subjected to an insertion-deletion mutagenesis to uncover molecular determinants important for the evolution of luciferase activity. Generated libraries were screened and the best hits carrying alterations in three hot-spot regions were comprehensively characterized. The most potent hit was crystallized and its structure was solved by protein crystallography. There are two monomers present in the asymmetric unit. Although the overall structure of both monomers is very similar, they markedly differ in the positioning of the cap-domain-forming  $\alpha 4$  helix. Moreover, electron density maps for the  $\alpha 4$  helix and its flanking loops are not perfectly resolved, which illustrates a conformational flexibility in this region. Complementary protein simulations, transient kinetic and HDX-MS experiments revealed that the gained conformational flexibility in the cap domain favours the accommodation of bulkier coelenterazine substrate. Finally, by transplantation of flexible fragment from modern *Renilla* luciferase into robust ancestor we created a mutant with enhanced affinity towards coelenterazine.

Crystallographic analysis of this light-emitting enzyme variant provided for the first time the biologically relevant molecular view of an enzyme-product complex.

#### REFERENCE

1. Chaloupkova R., Liskova V., Toul M., Markova K., Sebestova E., Herychova L., Marek M., Pinto G.P., Pluskal D., Waterman J., Prokop Z., Damborsky J.: ACS Catal. 9, 4810 (2019).

#### LONG-TERM RELEASE OF BISPHENOLS FROM DENTAL COMPOSITE MATERIALS

**MARKÉTA ŠIMKOVÁ<sup>a,b</sup>, ANTONÍN TICHÝ<sup>c</sup>, PAVEL DRAŠAR<sup>a</sup>, PAVEL BRADNA<sup>c</sup>**

<sup>a</sup>University of Chemistry and Technology, Technická 5, 166 28 Prague 6; <sup>b</sup>Institute of Endocrinology, Národní 8, 116 94 Prague, <sup>c</sup>Institute of Dental Medicine, First Faculty of Medicine of the Charles University, Kateřinská 32, 121 08 Prague 2  
simkovam@vscht.cz

The use of dental amalgam has been gradually restricted and since 2018, it has been banned in children under 15 years of age, pregnant and nursing women. Therefore, resin-based composite materials have become most used in restorative dentistry. However, these materials often contain bisphenol A glycidyl methacrylate (Bis-GMA) or derived monomers from which bisphenol A (BPA), known for its endocrine disrupting effects, may be released either as a residue or due to the degradation of the polymer matrix, and represent a long-term source of BPA for the human body. To overcome the problem of BPA presence, its structural analogues such as BPS, BPF, and BPAF are often used in industry. The aim of this work was to determine the maximal amounts of BPA released from 'BPA-free' and Bis-GMA-containing restorative composites, and to describe the kinetics of BPA release to methanol using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Bis-GMA-containing composites Charisma Classic (CC) and Filtek Ultimate (FU), and 'BPA-free' Charisma Diamond (CD) and Admira Fusion (AF) were used in this study. Specimens (diameter 6 mm, height 2 mm, n=5) were light-cured from one side (20 s, 1000 mW/cm<sup>2</sup>), and stored at 37 °C in 2 mL of methanol which was exchanged after 1, 4, 9, 16, 35, 65, and 130 days. Bisphenol concentrations were measured using LC-MS/MS. Dansyl chloride derivatization was carried out in order to gain high sensitivity<sup>1</sup>.

The total amounts of BPA released from 'BPA-free' materials CD (8.31±1.47 ng/g) and AF (5.18±1.30 ng/g) after 130 days were significantly lower compared with Bis-GMA-containing composites CC (146.17±6.99 ng/g) and FU (182.61±5.94 ng/g). The highest release of BPA was observed within the first day of incubation and followed by a gradual decrease. Since the 35-day values, the release was still ongoing at a similar daily rate until 130 days of incubation. Alternative bisphenols (BPS, BPF, BPAF) were not detected.

As the total amounts of BPA released within 130 days were several orders of magnitude lower than the tolerable daily intake 4 µg/kg bw/day, these materials should not pose a serious health risk according to the current criteria.

Supported by Charles University, project PROGRES Q29/ILF and MH CZ – DRO (Institute of Endocrinology, 00023761).

#### REFERENCE

1. Kolatorova Sosvorova L., Chlupacova T., Vitku J., Vlk M., Heracek J., Starka L., Saman D., Simkova M., Hampl R.: Talanta 174, 21 (2017).

#### METHODOLOGY FOR THE OPTIMIZATION OF LIBS ANALYSIS OF SOFT TISSUES

**ANNA ŠINDELÁŘOVÁ<sup>a</sup>, PAVEL POŘÍZKA<sup>a</sup>, PAVLÍNA MODLITBOVÁ<sup>a</sup>, LUCIE VRLÍKOVÁ<sup>b</sup>, KATEŘINA KUBÍČKOVÁ<sup>c</sup>, DAVID PROCHAZKA<sup>a</sup>, JAKUB VRÁBEL<sup>a</sup>, MARCELA BUCHTOVÁ<sup>b</sup>, JOZEF KAISER<sup>a</sup>**

<sup>a</sup>Central European Institute of Technology (CEITEC), University of Technology, Brno; <sup>b</sup>Institute of Analytical Chemistry as cr, Brno; <sup>c</sup>Charles University, Faculty of Medicine, Hradec Králové, Czech Republic  
anna.sindelarova@ceitec.vutbr.cz

The topic of soft tissues ablation has recently become very popular in the LIBS community due to the advancement in instrumentation and analytical performance. Elemental layout within the tissue is investigated and provides further understanding in biological research. The laser ablation of soft tissues is a complex issue and demands previous sample treatment which is not straightforward compared to typical LIBS experiment. In this article, we focus on the optimization of laser-tissue interaction and namely on the optimization of LIBS experimental setting prior the analysis of soft tissue. Considering soft heterogeneous nature of biological tissues, we aim to remain close to histological routine preparation, so LIBS could extend clinical research. Therefore, we propose a unique system of sample preparation and subsequent data treatment, which enables comparison of signal response from heterogeneous matrix for different parameters setting. On the example of zinc in murine kidneys we illustrate the influence of selected experimental parameters: defocus, gate delay, laser energy, gas used in purge and atmosphere, on the laser-tissue interaction presented as the zinc signal response. Moreover, we provide an extensive methodology for determination of the optimum LIBS experimental setting in the analysis of soft tissues.

This research has been financially supported by the Ministry of Education, Youth and Sports of CR, project CEITEC 2020 (LQ1601), project Action (no. 82p5), and GACR Junior (no. 20-19526Y). We are also very grateful to Ing. Pípalová from Masaryk University in Brno for the help during sample preparation.

## CROSS TALK OF EPIDERMAL CELLS MEDIATING PROTECTION AGAINST UV IRRADIATION

**HELENA SKÁLOVÁ, JIŘINA MEDALOVÁ**

*Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 629 00 Brno  
460968@mail.muni.cz*

Human skin is constantly exposed to many environmental impacts, like UV irradiation, that can cause many severe defects, for example extremely dangerous melanoma tumour. However, there are several protective mechanisms developed in the skin, which include intensive communication and many signalling pathways between epidermal cells.

The most well-known way of protection is the melanogenesis. Melanin is synthesized in melanocytes in specialised organelles called melanosomes<sup>1</sup>. There are several proteins participating this process or regulating it, the most important are PMEL17, p38, TRP1 and the central transcription factor MITF<sup>2</sup>. Once the melanin is produced and melanosomes are mature, they are transported along the cytoskeleton to the periphery of melanocyte and subsequently transferred to keratinocytes. This process is still quite unclear. However, some theories have been established<sup>3</sup>. The most promoted mechanism is exocytosis-endocytosis model. The process is regulated by several proteins, PAR2<sup>4</sup>, SCF<sup>5</sup> and melanoregulin<sup>6</sup> in particular.

We aimed to study pathways mentioned above in the co-cultivation systems simulating conditions in the epidermis and analyse the mechanism of melanin transport and transfer in melanotic and amelanotic cell lines (IPC298 and A375). The preliminary results show the early response to UV involves activation of p38 MAPK, which results in MITF activity. The best marker of melanosomes is increased expression of PMEL17, which migration we can observe via live imaging.

*This project was supported by scientific research grant of ministry of education MUNI/A11380/2018. We would like to thank to Dr. Stjepan Uldrian for giving us the studied cell lines.*

### REFERENCES

1. Raghunath A., Sambarey A., Sharma N., Mahadevan U. Chandra N.: *BMC Res. Notes* 8, 170 (2015).
2. Serre C., Busuttill V., Botto J. M.: *Int. J. Cosmetic Sci.*, 40, 328 (2018).
3. Tadokoro R., Takanashi Y.: *Curr. Opin. Genet. Dev.* 45, 132 (2017).
4. Seiberg M., Paine C., Sharlow E., Andrade-Gordon P., Constanzo M., Eisinger M., Shapiro S. S.: *Exp. Cell Res.*, 254, 25 (2000).
5. Kim J. Y., Kim D. S., Sohn H., Lee E. J., Oh S. H.: *Exp. Dermatol.* 25, 487 (2016).

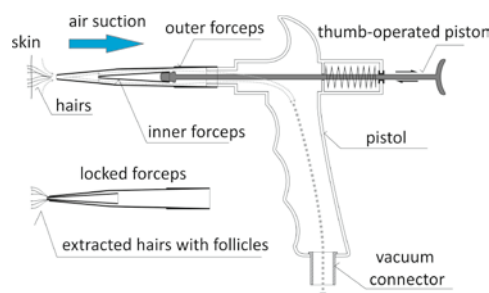
6. Wu X. S., Masedunskas A., Weigert R., Copeland N. G., Jenkins N. A., Hammer J. H.: *P. Natl. Acad. Sci. USA*, 109, e2101 (2012).

## AN EFFICIENT, NON-INVASIVE APPROACH TO IN-VIVO SAMPLING OF HAIR FOLLICLES: DESIGN AND APPLICATIONS IN MONITORING DNA DAMAGE AND AGING

**HANUS SLAVIK<sup>a</sup>, NATALIE KUDLOVA<sup>a</sup>, PAVLINA DUSKOVA<sup>a</sup>, TOMAS FURST<sup>b</sup>, JOSEF SROVNAL<sup>a</sup>, MARIAN HAJDUCH<sup>a</sup>, MARTIN MISTRIK<sup>a</sup>**

*<sup>a</sup>Institute of Molecular and Translational Medicine; <sup>b</sup>Faculty of Science, Palacky University and University Hospital in Olomouc, 17. listopadu 1192, 779 00, Czech Republic  
hanusslavik@seznam.cz*

Emphasizing the importance of the 3Rs-principle (Replacement, Reduction and Refinement) in current biomedical research involving animal models, here we have developed and applied a new approach for sampling and analyses of hair follicles in a variety of experimental settings. We designed and present a dedicated, simple-to-use device for non-invasive collection of hair follicles (construction of the device is summarized on Scheme 1) and the ensuing processing methods providing sufficient amounts of biological material to replace stressful and painful biopsies and tail and/or ear punches. Moreover, the major component of hair follicles provides live cells of epithelial origin, highly relevant for the bulk of cancer types which are derived from epithelia, thereby providing opportunities for research of aging-related pathologies including cancer. Here, we successfully employed mouse hair follicular cells for genotyping, quantitative PCR<sup>1</sup> and quantitative immunofluorescence. As a proof of concept, we report the applicability usefulness of this new method for routine genotyping and monitoring changes in quality and expression levels of selected proteins after gamma irradiation and during natural or experimentally induced aging of mice<sup>2</sup>. Our results highlight hair follicles as a valuable biological material for the innovative in-vivo sampling and processing of which, reported here, will facilitate both research and routine applications with a broad range of ethical and logistic advantages over the currently used biopsy-based approaches<sup>3</sup>.



Scheme 1. Design of vacuum collector for hair follicle sampling

*This work was financially supported by grants*

IGA\_LF\_2019\_003, TACR TE02000058 and VG20102014001.

## REFERENCES

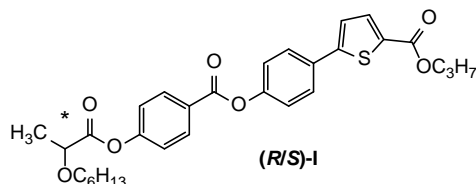
1. Kabacik S., Ortega-Molina A., Efeyan A., Finnon P., Bouffler S., Serrano M., Badie C.: *Cell Cycle*. 10, 7 (2011).
2. Li M., You L., Xue J., Lu Y.: *Front. Pharmacol.* 9, 522 (2018).
3. Albrecht H., Durbin-Johnson B., Yunis R., Kalanetra K., Wu S., Chen R., Stevenson T., Rocke D.: *Radiation Res.* 177, 1 (2012).

### SELECTIVE REFLECTION AND SELF-ASSEMBLING PROPERTIES OF PHENYLTHIOPHENE-BASED MATERIALS

**MICHAL ŠMAHEL<sup>a</sup>, ALEXEJ BUBNOV<sup>b</sup>, JIŘÍ SVOBODA<sup>a</sup>, MICHAL KOHOUT<sup>a</sup>**

<sup>a</sup>Department of Organic Chemistry, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic; <sup>b</sup>Institute of Physics, AS CR, Na Slovance 1999/2, 182 21 Prague 8, Czech Republic  
smahelm@vscht.cz

Chiral liquid crystals have attracted considerable attention due to their unique self-assembling properties, which can be driven by an applied external electric field. In our previous study<sup>1</sup>, we prepared photosensitive lactic acid-based materials and described the effect of UV light on their mesomorphic behaviour in bulk mesophases. In this follow up study, we have focused on phenylthiophene-based chiral materials **I** (Figure 1). We have synthesized both enantiomers of materials **I** with the emphasis on their optical purity and found that they selectively reflect light in the visible region. Subsequently, we have prepared mixtures with different enantiomeric excess (*ee*) of one enantiomer in the mixture, using chiral liquid chromatography to determine the exact composition of the mixtures<sup>2</sup>. Mixing both the enantiomerically pure materials allowed us to tune the wavelength of the selectively reflected light. Mesomorphic properties of the materials and the mixtures have been studied by means of differential scanning calorimetry and optical polarising microscopy. In conclusion, we have described the effect of *ee* on mesomorphic properties of non-racemic mixtures and on the wavelength of the selectively reflected light.



Scheme 1. Structure of final materials.

The financial support by Czech Science Foundation (projects

19-03564S and 18-14497S) is gratefully acknowledged.

## REFERENCES

1. Poryvai A., Bubnov A., Pocięcha D., Svoboda J., Kohout M.: *J. Mol. Liq.* 275, 829 (2019).
2. Poryvai A., Vojtylová-Jurkovičová T., Šmahel M., Kolderová N., Tomášková P., Sýkora D., Kohout M.: *Molecules* 24, 1099 (2019).

### RUTHENIUM-CAPPED CUCURBIT[N]URIL-BASED ROTAXANES WITH ANTIMETASTATIC PROPERTIES

**MARTIN SOJKA<sup>a,b</sup>, MICHAELA FOJTU<sup>b,c</sup>, MICHAL MASARIK<sup>b,c</sup>, MAREK NECAS<sup>a,b</sup>, RADEK MAREK<sup>a,b,\*</sup>**

<sup>a</sup>Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, CZ-62500 Brno, Czechia; <sup>b</sup>CEITEC, Masaryk University, Kamenice 5, CZ-62500 Brno, Czechia; <sup>c</sup>Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, CZ-62500 Brno, Czechia  
rmarek@chemi.muni.cz

Recent report highlights the dinuclear Ru complexes for the specific mode of apoptosis induction.<sup>1</sup> Cucurbituril inclusion complexes of Pt<sup>2+</sup> and Ru<sup>3+</sup> complexes show enhanced pharmacological effect caused by encapsulation of the drug. Thus, the combination of dinuclear Ru prodrugs with supramolecular carriers could be beneficial. Following our previous research,<sup>4,5</sup> we report the coupling of Ru(II) units with cucurbit[6/7]uril-based pseudorotaxanes for bioapplication.

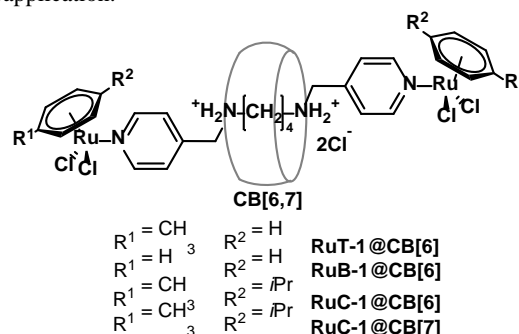


Fig. 1. Schematic representation of Ru molecular rotaxane.

Structure of one Ru-capped rotaxane was determined by X-ray diffraction. The effect of salt concentration on the hydrolysis of Cl was monitored by <sup>1</sup>H NMR spectroscopy. Evaluation of bioactivity was performed for HBL-100, MCF-7, and MDA-MB-231 cell lines. The antimetastatic activity of the Ru(II)-rotaxanes evaluated against MCF-7 and MDA-MB-231 cell lines is notably enhanced compared to the reference compound. The indicated synergistic effect opens a new direction in searching for anticancer metallodrugs.



## REFERENCES

1. Davey, G. E.; Adhikarsan, Z.; Ma, Z.; Riedel, T.; Sharma, D.; Padavattan, S.; Rhodes, D.; Ludwig, A.; Sandin, S.; Murray, B. S.; Dyson, P. J.; Davey, C. A. *Nat. Comm.*, 2017, 8, 1575.
2. Cao, L.; Hettiarachchi, G.; Briken, V.; Isaacs, L. *Angew. Chem. Int. Ed.*, 2013, 52, 12033.
3. Li, F.; Gorle, A. K.; Ranson, M.; Vine, K. L.; Kinobe, R.; Feterl, M.; Warner, J. M.; Keene, F. R.; Collins, J. G.; Day, A. I. *Org. Biomol. Chem.*, 2017, 15, 4172.
4. Novotný, J.; Sojka, M.; Komorovsky, S.; Nečas, M.; Marek, R. *J. Am. Chem. Soc.* 2016, 138 (27), 8445.
5. Novotný, J.; Přichystal, D.; Sojka, M.; Komorovsky, S.; Nečas, M.; Marek, R. *Inorg. Chem.* 2018, 57 (2), 652.

**ENZYMES PRODUCED BY PROBIOTIC BACTERIA MIGHT HELP TO STOP INCREASING PREVALENCE OF CELIAC DISEASE**

**MICHAELA ŠTASTNÁ, LUBOMÍR JANDA, ADAM NOREK, HELENA JUŘICOVÁ, IVAN RYCHLÍK**

*Department of Immunology, Veterinary Research Institute, Hudcova 296/70 Brno, Czech Republic  
stastna@vri.cz*

Celiac disease (CD) is defined as a chronic immune-mediated enteropathy caused by gluten uptake among genetically predisposed individuals. The manifestations of the disease have a wide range of symptoms, which can be both intestinal and digestive problems, making it difficult to recognize CDs. In the first half of the last century, the CD was considered rare, occurring mainly in childhood. Today, the CD has a worldwide prevalence of 0.7% to 1.4%, making it one of the most common autoimmune disorders that is steadily increasing. The only way to handle CDs is through a strict lifelong gluten-free diet. Untreated CD is associated with increased morbidity and mortality.

More than forty probiotic strains are used worldwide to improve human health. In the Czech Republic, 27 strains are available in various probiotic products. Many probiotic bacteria have an effect on strengthening the intestinal barrier, and other probiotic bacteria can be helpful in the cleavage of gluten peptides - prolamines. We have identified *Lactobacillus* sp., which contains the gene for X-prolyl dipeptidylaminopeptidase, having capacity to utilize proline and glutamine rich peptides - prolamines. Testing of enzymatic activity revealed large differences between strains of the same species *Lactobacillus* sp. and this was confirmed by bioinformatic genome analysis. The diversity is not only at the level of gene organization, but also at the amino acid level of the XPDAD gene sequence. These results may help to reduce the increase in the prevalence of celiac disease.

The intellectual property of results were protected by a functional sample 5791/2019 "The probiotic lysate reducing the immunotoxic prolamines" and by utility model PUV 2019-36811, "A set for identifying a probiotic lactobacillus strain cleaving immunotoxic gluten peptides".

## REFERENCES

1. Hoffmanová I.: *Celiakie, Edice postgraduální medicíny.*: Mladá fronta, Praha 2019. ISBN 978-80-204-5414-0.
2. Frič P., Keil R.: *Med. praxi.* 8, 354 (2011).
3. Altobelli E., Paduano R., Petrocelli R., di Orio F.: *Ann. Ig.* 26, 485 (2014).
4. Bron P., Kleerebezen M., Brummer R.J., et al.: *Br. J. Nutr.* 117, 93 (2017).

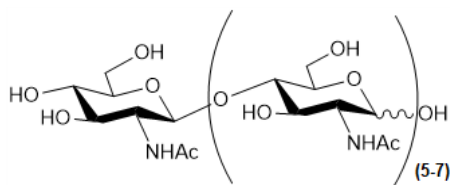
**FUNGAL TRANSGLYCOSIDASES FOR PREPARATION OF ANTIMICROBIAL CHITOLIGOSACHARARIDES**

**ZUZANA STRAKOVÁ<sup>a,b</sup>, KRISTÝNA SLÁMOVÁ<sup>a</sup>, LUCIE PETRÁSKOVÁ<sup>a</sup>, VLADIMÍR KŘEN<sup>a</sup>**

*<sup>a</sup>Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czech Republic; <sup>b</sup>University of Chemistry and Technology, Technická 5, 166 28 Prague, Czech Republic  
zuzana.strakova@biomed.cas.cz*

Chitooligosaccharides (COS) are  $\beta$ -(1 $\rightarrow$ 4)-linked oligomers of *N*-acetylglucosamine, which are increasingly being recognized as bioactive molecules featuring beneficial biological activities, such as antiangiogenic<sup>1</sup>, antioxidant<sup>2</sup>, and antifungal as they act as elicitors of the defence response in plants<sup>3</sup>. Plant diseases are a large issue and they account for losses up to 40% of the annual crop production worldwide. In this work, the route to the effective enzymatic synthesis of chitooligomers with degree of polymerization more than 5 employing a combined action of chitinase and mutant  $\beta$ -*N*-acetylhexosaminidase<sup>4</sup> is investigated. We discovered a fungal chitinase from *Talaromyces flavus* (*Tf*Chit), whose sequence is deposited in GenBank (ADV02754.1). In the sequence alignment with known fungal chitinases, the conserved sequence motifs in *Tf*Chit were confirmed. The genes of the respective enzyme were inserted into the yeast expression vector pPICZ $\alpha$ A containing the yeast  $\alpha$ -factor sequence for extracellular targeting of the produced enzymes. Robust and high-yielding method of protein expression using methylotrophic yeast *Pichia pastoris* followed by one-step purification was used.

The first step is using chitinase for the hydrolytic cleavage of chitin as residual sources with zero utilization to prepare shorter chitooligomers. The second step is using mutant  $\beta$ -*N*-Acetylhexosaminidase from *Aspergillus oryzae*, where tyrosine was exchanged for asparagine in the active site to reduce hydrolytic activity and increase transglycosylation activity with substrates for the synthesis of new derivatized chitooligomers (Scheme 1.).



Scheme 1. Antimicrobial chitooligosaccharides.

This work was supported by the Ministry of Education, Youth, and Sports of the Czech Republic mobility project no. LTC19035 (COST Action CA17118) and by the Czech Science Foundation grant 20-00477S.

## REFERENCES

1. Wang Z., Zheng L., Yang S., Niu R., Chu E., Lin X.: *Biochem. Biophys. Res. Commun.* 357, 26 (2007).
2. Ngo D.-N., Lee S.-H., Kim M.-M., Kim S.-K.: *J. Funct. Foods* 1, 188 (2009).
3. Kaku H., Nishizawa Y., Ishii-Minami N., Akimoto-Tomiya C., Dohmae N., Takio K., Minami E., Shibuya N.: *Proc. Natl. Acad. Sci.* 103, 11086 (2006).
4. Slámová K., Bojarová P., Petrásková L., Křen V.: *Biotechnol. Adv.* 28, 682 (2010).

### LIBS AS A USEFUL TOOL IN SPATIAL ELEMENTAL ANALYSIS OF PLANTS: QUANTUM DOTS DISTRIBUTION IN WHITE MUSTARD

SÁRA STRÍTEŽSKÁ<sup>a</sup>, PAVLÍNA MODLITBOVÁ<sup>a</sup>, DAVID PROCHAZKA<sup>a</sup>, ŠTĚPÁN ZEZULKA<sup>b</sup>, MARIE KUMMEROVÁ<sup>b</sup>, KAREL NOVOTNÝ<sup>c</sup>, PAVEL POŘÍZKA<sup>a</sup>, JOZEF KAISER<sup>a</sup>

<sup>a</sup>CEITEC, Brno University of Technology, Technická 3058/10, 616 00 Brno, Czech Republic; <sup>b</sup>Department of Experimental Biology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; <sup>c</sup>Department of Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic  
sara.stritezska@ceitec.vutbr.cz

Laser-Induced Breakdown Spectroscopy (LIBS) is an optical analytical method that is used to determine the elemental composition of samples. Its tremendous advantages lie mainly in its simplicity, ability of *in situ* analysis with minute sample preparation, multielemental analysis, low cost and short measurement times. Together with the mentioned benefits, its capability of mapping sample surface and thus revealing the spatial distribution of elements is what opens the doors to various applications<sup>1</sup>. While there are many fields where mapping of samples can be extremely useful, this work focuses entirely on the use of LIBS in analysing spatial distribution of chosen elements in plants.

Every year many new nano- and micro-materials, such as various nanoparticles (NPs), are created and leak into the environment. Thus, there is emerging need for evaluation of their stability and effect on various organisms, including

plants. LIBS is widely used for providing elemental distribution in plant tissues. In the previous years, the research focused mostly on essential elements (both macro- and micro-nutrients) as well as on non-essential ones. However, a very limited amount of publications deals with the toxicity of different NPs so far. This is likely caused by the major outbreak of nanomaterials in the last few years<sup>2</sup>.

Quantum dots (QDs) are fluorescent semiconductor spherical nanoparticles. Their fluorescence is highly stable as well as easily tuneable which makes them a promising tool for applications such as bioimaging. QDs structure usually includes two parts: core and shell. The core most commonly consists of CdTe, CdS or CdSe and the ZnS is most frequent material for encapsulation since it enhances chemical stability and fluorescence efficiency. Nowadays their use in medical applications is severely limited due to the risk of highly toxic cadmium ions (Cd<sup>2+</sup>) leaking into their surrounding environment, including tissues<sup>3</sup>.

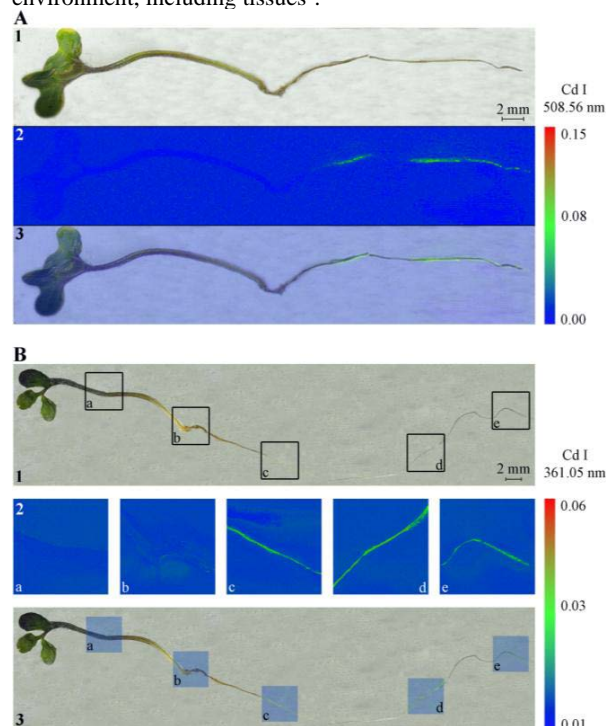


Fig. 1. *S. Alba* plants exposed to CdTe QDs in nominal concentration 20  $\mu$ M Cd. A) (1) Photograph of exposed plant before LIBS measurement. (2) LIBS map of Cd I 508.56 nm. (3) Overlap of photograph with LIBS map. B) (1) Photograph of exposed plant before micro LIBS measurement. (2) Micro LIBS maps of Cd I 361.05 nm. (3) Overlap of photograph with micro LIBS maps.

This study focuses on the comparison of toxicity and bioaccumulation of two distinct types of cadmium-based QDs: core QDs (CdTe) and core/shell QDs (CdTe/SiO<sub>2</sub>) in two concentrations dispersed in aqueous environment in white mustard (*Sinapis Alba*) with CdCl<sub>2</sub> used as a positive control. The exposition to QDs took place for 72 hours. After the exposure the overall content of cadmium in tested plants was assessed using Inductively Coupled Plasma Optical Emission

Spectrometry and bioaccumulation factors were calculated separately for the root, that was exposed directly to the dispersion of QDs, and stem with leaves. As a toxicological end-point the length of the root was observed. LIBS was applied to determine spatial distribution of Cd in tested samples. The achieved resolution for the mapping of whole plants analysis was 100  $\mu\text{m}$ . Specific areas that showed potentially interesting Cd distribution were measured with micro LIBS with a resolution of 25  $\mu\text{m}$ .

LIBS results have successfully shown that both Cd distribution and bioaccumulation patterns differed in QDs and positive control.

#### REFERENCES

1. Kaiser J., Novotný K., Martin M.Z., Hrdlička A., Malina R., Hartl M., Adam V., Kizek R.: *Surfaces Sci. Rep.* 67, 233 (2012); DOI: 10.1016/j.surfrep.2012.09.001.
2. Modlitbová P., Pořízka P., Kaiser J.: *TrAC Trend Anal. Chem.* 122, 115729 (2020); DOI: 10.1016/j.trac.2019.115729.
3. Modlitbová P., Pořízka P., Novotný K., Drbohlavová J., Chamradová I., Farka Z., Zlámalová-Gargošová H., Romih T., Kaiser J.: *Ecotoxicol. Environ. Saf.* 153, 23 (2018); DOI: 10.1016/j.ecoenv.2018.01.044.

#### STRUCTURAL ANALYSIS OF UNSATURATED TRIACYLGLYCEROLS IN *D. MELANOGASTER* USING ALDRITHIOL-2 DERIVATIZATION

**TIMOTEJ STRMEŇ<sup>a,b</sup>, JOSEF CVAČKA<sup>a,b,\*</sup>**

*Charles University in Prague, Hlavova 8 Prague 2<sup>a</sup>, Institute of Organic Chemistry and Biochemistry, Academy of Science Czech Republic, Flemingovo náměstí 542 Prague 6<sup>b</sup> Josef.cvacka@uochb.cas.cz*

Aldrithiol-2 reacts with unsaturated molecules to give permanently charged derivatives<sup>1</sup>. We have developed aldrithiol-2 derivatization of unsaturated lipids, which turned out to be a useful tool in their structural analysis (most notably double bond localization). The permanent charge of derivatized lipids dramatically enhanced their detectability in electrospray ionization mass spectrometry. This derivatization was applied to a natural sample – a chloroform extract of cuticular lipids from *D. melanogaster*. In addition to hydrocarbons, triacylglycerols were abundantly present in the extract. An example of the fragmentation mass spectrum of derivatized triacylglycerols at  $m/z$  858.8 and localization of double bond on one of their fatty acyl chains by MS<sup>3</sup> is depicted in Fig. 1. With aldrithiol-2 derivatization method, we were able to characterize all fatty acyls (from fragments in Fig 1a) including double bond positions (demonstrated on the fragmentation of fragment  $m/z$  364 in Fig 1b).

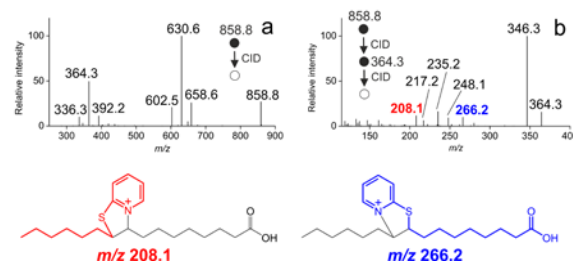


Fig. 1. Fragmentation mass spectrum (MS<sup>2</sup>) of triacylglycerol derivatives with  $m/z$  858.8 (a) and a subsequent fragmentation (MS<sup>3</sup>) of derivatized fatty acid with  $m/z$  364.3 (b). The diagnostic fragments that correspond to the localized double bond are illustrated on the derivatized fatty acid molecules.

*Project SVV and OP RDE; Project: "Chemical biology for drugging undruggable targets (ChemBioDrug)" (No. CZ.02.1.01/0.0/0.0/16\_019/0000729) are hereby acknowledged with appreciation.*

#### REFERENCE

1. Potapov V., Ishigeev R., Amosova S., Borodina T.: *Tetrahedron Lett.* 60, 475 (2019).

#### NOVEL LIPIDIZED ANALOG OF PROLACTIN-RELEASING PEPTIDE REDUCES PATHOLOGICAL LIPID ACCUMULATION IN A MOUSE MODEL OF ALZHEIMER'S-LIKE PATHOLOGY

**ŠTĚPÁN ŠTRNAD<sup>a,b</sup>, VERONIKA PRAŽIENKOVÁ<sup>a</sup>, DAVID SÝKORA<sup>b</sup>, JOSEF CVAČKA<sup>a</sup>, LENKA MALETÍNSKÁ<sup>a</sup>, VLADIMÍR VRKOSLAV<sup>a\*</sup>**

*<sup>a</sup>Institute of Organic Chemistry and Biochemistry AS CR, 160 00 Prague 6; <sup>b</sup>University of Chemistry and Technology, 166 28 Prague 6 stepan.strnad@uochb.cas.cz, vladimir.vrkoslav@uochb.cas.cz*

Alzheimer's disease (AD) is a progressive and neurodegenerative disorder, which results in dementia. Currently, there is no drug available to cure neurodegenerative diseases. Instead, several compounds managing symptoms or slowing the progression of the disease are available only. However, some peptides have shown enhanced potential for the treatment, specifically, palm11-PrRP31 (an anorexigenic and glucose-lowering analog of prolactin-releasing peptide) and liraglutide (a type 2 diabetes drug). In recent years, MALDI mass spectrometry imaging (MSI) has been repeatedly used for studying lipids in tissue sections and for clarification of their role in diseases such as AD. MSI is an analytical technique capable of direct distribution analysis of compounds of interest within the tissues.

In the present work, we examined the neuroprotective properties of palm11-PrRP31 and liraglutide, using the lipid

biomarkers found by the MSI method in the APP/PS1 mouse model of neurodegeneration.

For tracking of lipid changes in treated versus non-treated APP/PS1 model, we used visualization of GM2 36:1 because of its low concentration in the control model and the area surrounding plaques in APP/PS1 model. Two months of the treatment with palm11-PrRP31 or liraglutide reduced significantly area of lipids linked with senile plaques compared to controls treated with saline. The results obtained indicate that these drugs might be potentially useful in the treatment of neurodegenerative diseases.

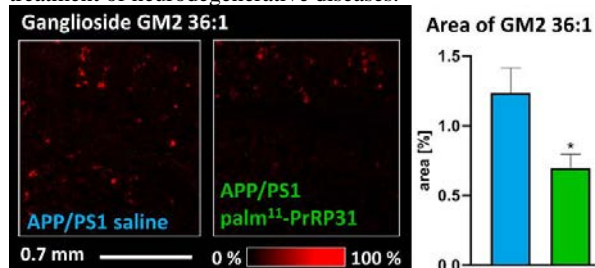


Fig. 1. MALDI MSI analysis of 8 month old APP/PS1 mice treated with saline or palm<sup>11</sup>-PrRP31. Ion images of ganglioside (GM2 36:1) were obtained in negative ion mode at a spatial resolution 15  $\mu$ m. Data are mean  $\pm$  SEM (4 mice in one group). Statistical analysis was performed as a Student t-test (\* $p < 0.05$ ).

## PRIMARY MAMMARY ORGANOID MODEL OF LACTATION AND INVOLUTION

**JAKUB SUMBAL<sup>a,b</sup>, AURÉLIE CHICHE<sup>b,\*</sup>, ELSA CHARIFOU<sup>b</sup>, ZUZANA KOLEDOVÁ<sup>a,\*</sup>, HAN LI<sup>b,\*</sup>**

<sup>a</sup>Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, 625 00, Czechia;

<sup>b</sup>Department of Developmental and Stem Cell Biology, Institut Pasteur, Paris, 75015, France  
aurelie.chiche@pasteur.fr; koledova@med.muni.cz; han.li@pasteur.fr

Mammary gland is a unique invention of mammals, providing evolutionary advantage for the survival of the progeny. During lifetime, mammary gland undergoes hormonally driven remodelling, coupled with each cycle of pregnancy, lactation and involution, but this plastic environment could provide a niche for breast cancer development. To achieve deep understanding of mammary gland biology, organoid cultures have been widely employed, however organoid models recapitulating key aspects of lactation and involution have been missing.

We aimed to develop a robust and straightforward mouse mammary organoid system modelling pregnancy associated branching lactation and involution.

Using time-lapse microscopy, qPCR, histology, immune staining and confocal imaging, we tracked the fate of organoids during fibroblast growth factor 2-induced branching and alveologenesis, prolactin-induced lactogenic differentiation and finally involution induced upon prolactin withdrawal.

We found that stimulated organoids produced milk proteins as well as lipid droplets, they retained functional myoepithelial coverage and contracted upon oxytocin stimulation. Furthermore, we proved the involution stage to be reversible by inducing second round of alveologenesis and lactation.

Taken together, we present a robust 3D organoid model of lactation and involution that could be applied to study various mechanisms of mammary gland, pharmacological disturbances of the milk production process as well as pregnancy-associated breast cancer.

Funding: MUNI/G/1446/2018, MUNI/A/1382/2019

## MOLECULAR MECHANISMS DRIVING BIOLOGICAL PROPERTIES OF HUMAN PLURIPOTENT STEM CELLS

**BARBORA VAVRUŠÁKOVÁ<sup>a</sup>, VOLODYMYR POROKH<sup>b</sup>, LIBOR ŠTREIT<sup>b</sup>, JAN KOCANDA<sup>c</sup>, ALEŠ HAMPL<sup>a,\*</sup>**

<sup>a</sup> Department of Histology and Embryology, Faculty of Medicine, Masaryk University, 625 00 Brno, <sup>b</sup> Department of Plastic and Aesthetic Surgery, St. Anne's University Hospital, 61200 Brno, <sup>c</sup> Orthopedic Clinic, University Hospital Brno, 625 00 Brno  
ahampl@med.muni.cz

Human pluripotent stem cells (hPSC) represent a cell type with unique properties: self-renewal and pluripotency. These properties predestine hPSC to use in tissue engineering and regenerative medicine to treat diseased or damaged tissue through cell-replacement therapies.

Before any clinical use of hPSC in regenerative medicine is possible, extensive and detailed study of their yet unexplained biological properties on the molecular level is necessary.

During continuous *in vitro* culture, hPSC are prone to changes in number and structure of chromosomes, many errors occur during mitosis. Loss or gain of chromosome is typically a result of a mitotic nondisjunction<sup>1</sup>. Errors in cell division and chromosome segregation are usually linked to errors in centrosome function. It was shown that high percentage of hPSC suffer from centrosomal amplifications. Their presence in cells during mitosis leads to the formation of a multipolar spindle, resulting in incorrect chromosome segregation to daughter cells<sup>2</sup>. The exact molecular mechanism driving the generation of multipolar spindle remains unknown.

In this study we focused on induced pluripotent stem cells (iPSC) obtained by genetic manipulation with adult somatic cells as the most clinically relevant source of human iPSC. Human primary fibroblasts were isolated from 15 healthy donors (6 – 71 years old) undergoing surgical procedures and reprogrammed by introducing transcription factors Oct4, Sox2, Klf4, MYC, and Lin28 with episomal vectors. To characterize the genetic stability of iPSC lines we analysed the cells for the presence of supernumerary

centrosomes during mitosis by indirect immunofluorescence and microscopy. The data show that in iPSC lines derived from young donors (less than 15 years old) the occurrence of multipolar cell division is on average 9,3 %. In iPSC lines derived from elderly donors (over 60 years old) the occurrence of multipolar mitoses is on average 4,1 % ( $p < 0,001$ ).

Here we indicate that the age of a donor is an important factor contributing to genetic stability and hence quality of iPSC. Further investigation of molecular link between donor's age and regulation of cell division is needed to tackle this medically important aspect of iPSC biology.

*Supported by funds from Faculty of Medicine of Masaryk University (MUNI/A/1382/2019).*

#### REFERENCES

1. Romito A., Cobellis G.: *Stem Cells Int.* 2016, 9451492 (2016).
2. Holubcová Z, Matula P., Sedláčková M., Vinarský V., Doležalová D., Bárta T., Dvořák P., Hampl A.: *Stem Cells* 29, 46 (2011).
3. Porokh V., Vaňhara P., Bárta T., Jurečková L., Bohačiaková D., Pospíšilová V., Mináriková D., Holubcová Z., Pelková V., Souček K., Hampl A.: *Stem Cells Dev.* 27, 1077 (2018).
4. Mayshar Y., Ben-David U., Lavon N., Biancotti J.C., Yakir B., Clark A.T., Plath K., Lowry W.E., Benvenisty N.: *Cell Stem Cell.* 74, 521 (2010).

#### MITOCHONDRIAL FUNCTIONAL AND METABOLIC ANALYSIS OF HUMAN PLATELETS: COMPARISON OF DIFFERENTIAL CENTRIFUGATION AND CONTINUOUS FLOW APHERESIS

**ANDREA VERNEROVÁ<sup>a,b</sup>, L. F. GARCIA-SOUZA<sup>c</sup>, E. GNAIGER<sup>c,d</sup>, L. KUJOVSKÁ KRČMOVÁ<sup>a,b</sup>, O. SOBOTKA<sup>e,f</sup>**

<sup>a</sup>Dept Anal. Chem., Faculty of Pharmacy Hradec Králové, Charles Univ., Czech Republic; <sup>b</sup>Dept Clin. Biochem. Diagnost., Univ. Hospital Hradec Králové, Czech Republic; <sup>c</sup>Oroboros Instruments, Innsbruck, Austria; <sup>d</sup>Medical Univ. Innsbruck, Innsbruck, Austria; <sup>e</sup>3<sup>rd</sup> Dept Int. Med., Univ. Hospital Hradec Králové, Czech Republic; <sup>f</sup>Dept Physiol., Faculty of Medicine in Hradec Králové, Charles Univ., Czech Republic  
verneran@faf.cuni.cz

Multiple non-aggregatory functions of human platelets (PLT) are widely acknowledged, yet their functional examination is limited mainly due to lack of suitable analytic methods. Optical aggregometry is easily accessible, but specificity is low entailing difficulties of interpretation<sup>1</sup>. Simply reporting PLT count can create a false impression of bleeding diathesis. Using modern high-resolution respirometry (HRR) the analysis of mitochondrial functions and the metabolic profile of PLT from a small sample of human blood

was made accessible<sup>2</sup>. A standardized isolation procedure for human PLT was developed in the frame of international COST Action MitoEAGLE and PLT prepared by this differential centrifugation (DC), securing a high inter-laboratory reproducibility of respirometry results<sup>2</sup>. We can investigate multiple metabolic pathways of PLT within a single experimental run. Combined with high sensitivity, this creates an ideal opportunity for diagnosis of functional disorders of human platelets. Continuous flow apheresis (CFA) is a clinical method for PLT isolation aiming at treatment of bleeding diathesis in severe thrombocytopenia based on many years of good clinical outcomes.

The aim of this project was to compare mitochondrial respiration of PLT obtained by two different methods: differential centrifugation and continuous flow apheresis. 20 healthy plasma donors were included in our study. CFA was performed using Haemonetics MCS and TRIMA blood separator. DC was performed using an established isolation protocol<sup>2</sup>. Isolated PLT respiration was measured by HRR (O2k-FluoRespirometer, Oroboros Instruments, Austria) at 37 °C in 0.5-mL glass chambers containing MiRO5-Kit.

According to our preliminary results, the ROUTINE respiration was about 20 % higher in PLT isolated by CFA versus DC. The isolation method did not affect respiratory capacity of PLT evaluated after uncoupler titration. PLT isolated by CFA resulted in a 30 % decrease of succinate oxidation in comparison to DC. Moreover, CFA affected PLT viability. These differences were eliminated after washing the PLT by phosphate buffer saline, suggesting the primary importance of applying an appropriate isolation medium.

We conclude that HRR is a highly sensitive method to describe mitochondrial function of human PLT. Our results support the concept that functional mitochondrial PLT diagnosis meets an acute clinical demand of biomedical research of patients suffering from thrombocytopenia, sepsis and metabolic diseases.

*The study was supported by Project SVV 260 412, the University Hospital in Hradec Králové MH CZ - DRO (UHHK, 00179906), COST Action CA15203 MitoEAGLE. The O2k respirometers were provided by Oroboros Instruments.*

#### REFERENCES

1. Hvas A. M., Favalaro E.: *J. Methods Mol. Biol.* 1646, 321 (2017).
2. Doerrier C., Garcia-Souza L. F., Krumschnabel G., Wohlfarter Y., Meszaros A. T., Gnaiger E.: *Methods Mol. Biol.* 1782, 31 (2018).

## BIOPHYSICAL CHARACTERIZATION OF ISOFORMS I AND II OF HUMAN AMELOBLASTIN PROTEIN

**VERONIKA VETYSKOVA<sup>a</sup>, MONIKA ZOUHAROVA<sup>a</sup>,  
LUCIE BEDNAROVA<sup>a</sup>, ROMANA HADRAVOVA<sup>a</sup>  
ONDREJ VANEK<sup>b</sup>, KRISTYNA BOUSOVA<sup>a</sup>, JIRI  
VONDRASEK<sup>a\*</sup>**

<sup>a</sup>Institute of Organic Chemistry and Biochemistry, CAS, Flemingovo 542, 160 00 Prague; <sup>b</sup>Faculty of Science, Charles University, Albertov 6, 128 00 Prague  
veronika.vetyiskova@uochb.cas.cz; jiri.vondrasek@uochb.cas.cz

Intrinsically disordered proteins (IDPs) play an important role in cellular processes such as biomineralization<sup>1</sup>. Ameloblastin (AMBN) is an IDP which is one of the major protein components of tooth enamel<sup>2</sup>. AMBN is an enamel matrix phosphoprotein<sup>3</sup> known for its oligomerization potential attributable to its specific interaction motif, which is encoded by its exon 5. Human AMBN is present in two isoforms: a full-length isoform I and isoform II, which is shorter by 15 amino acid residues. These isoforms are the result of RNA alternative splicing. The sequence encoded by the exon 5 occurs in both AMBN isoforms. One of the project aims is to characterize and compare biophysical, biochemical, and structural properties of both isoforms. AMBN proteins are expressed as recombinant proteins in an *E. coli* expression system and purified. Then they are characterized by circular dichroism spectroscopy (CD), dynamic light scattering (DLS), analytical ultracentrifugation (AUC), microscale thermophoresis and transmission electron microscopy (TEM). The CD characterization has confirmed the disordered character of both AMBN isoforms but has not identified any structural changes in AMBN in the presence or absence of calcium ions. Oligomerization properties have been revealed by DLS, AUC and TEM analyses. The AUC has shown differences in oligomerization properties of AMBN isoforms I and II in the presence and absence of calcium ions as well as TEM. So, there is a question how important is to have both of these isoforms and how much they differ in function?

*This work was supported by the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences (RVO: 61388963).*

### REFERENCES

1. Boskey A. L., Villarreal-Ramirez E.: *Matrix Biol.* 52, 43 (2016).
2. Wald T., Osickova A., Sulc M., Benada O., Semeradtova A., Rezabkova L., Veverka V., Bednarova L., Maly J., Macek P., Sebo P., Slaby I., Vondrasek J., Osicka R.: *J. Biol. Chem.* 288, 22333 (2013).
3. Stakkestad Ø., Lyngstadaas S. P., Thiede B., Vondrasek J., Skålhegg B. S., Reseland J. E.: *Front. Physiol.* 8, 531 (2017).

## ORGANOCHLORINE MIXTURE AND IT'S ENDOCRINE DISRUPTING EFFECTS ON MALE REPRODUCTIVE SYSTEM

**ISHITA VIRMANI, ELIŠKA SYCHROVÁ, JIŘÍ  
NOVÁK, IVA SOVADINOVÁ**

RECETOX, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Bohunice, Brno, Czech Republic  
ishita.virmani@recetox.muni.cz

We get exposed to the amalgamation of various chemicals on a regular basis. However, chemical toxicity and risk assessment is commonly conducted with isolated chemicals without considering the potential interaction effects of mixtures of chemicals. Even the regulations in EU are mostly based on a single chemical assessment and do not deal with a mixture of chemicals. Therefore, there is a lack of a proper toxicity assessment framework. Evaluating mixture toxicity and risk is a multistep process where *in vitro* models have nowadays a central role.

An exposure to a chemical cocktail is a highly discussed reason for male sub/fertility as it has become a global problem nowadays. Organochlorines are common environmental contaminants and humans get exposed to their mixtures on daily basis. There are studies confirming an association of organochlorines with adverse effects on male reproductive system (REF) however mechanistic-based studies dealing with organochlorine mixtures are scarce. To screen effects of plenty chemical mixtures *in vitro*, there is a need to develop high-throughput screening (HTS)/ high-content analysis (HCA) approaches to gain so many information we can.

In our study, we assessed a reproductive and testicular toxicity of an organochlorine mixture whose potential to disturb male reproductive health has been previously reported *in vivo*<sup>1</sup>. To address this issue, we developed a rapid and versatile battery of tests including transfected reporter gene assays to study interactions with a variety of nuclear receptors and assays based on testicular somatic cells in a single-cell-type culture models of immature Leydig or Sertoli cells as well as in their co-culture model targeting numerous different endpoints.

We observed strong antiandrogenic and weak estrogenic effects of the mixture as well as direct effects on both testicular cell types and their functions in a dose- and time-dependent manners. Finally, we did a hypothesis-driven mechanistic approach to study mechanisms involved. We identified that the mixture was able to rapidly dysregulate the gap junctional intercellular communication (GJIC) *in vitro* in both testicular cell types. Our results indicate that organochlorines and their mixtures could be a potential etiological agent contributing to reproductive dysfunctions in males through an impairment of testicular GJIC.

### REFERENCE

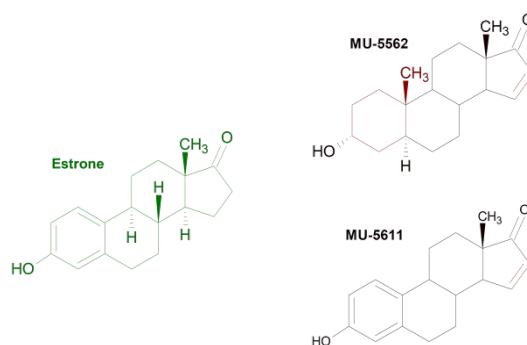
1. Campagna C., Guillemette C., Ayotte P., Bailey J.L.: *J. Androl.* 6, 317 (2009).

## MOLECULAR DOCKING AND STRUCTURE-FUNCTION RELATIONSHIP TO IDENTIFY NOVEL ANTI-OESTROGENS

PETR VOŇKA<sup>a,b</sup>, LUCIE RÁROVÁ<sup>b</sup>, VÁCLAV BAZGIER<sup>b,d</sup>, KAREL BERKA<sup>c,d</sup>, MIROSLAV KVASNICA<sup>b</sup>, JANA OKLEŠŤKOVÁ<sup>b</sup>, EVA KUDOVÁ<sup>e</sup>, MIROSLAV STRNAD<sup>b</sup>, ROMAN HRSTKA<sup>a,b,\*</sup>

<sup>a</sup>Res. Ctr Appl. Mol. Oncol., Masaryk Memorial Cancer Institute, 656 53 Brno; <sup>b</sup>Lab. Growth Regul., Inst. Exper. Bot. AS CR & Palacký Univ., 783 71 Olomouc; <sup>c</sup>Dept Phys. Chem., Fac. Sci., Palacký University, 771 46 Olomouc; <sup>d</sup>Region. Ctr Adv. Technol. Mater., Dept Phys. Chem., Palacký University, 779 00 Olomouc; <sup>e</sup>Institute of Organic Chemistry and Biochemistry AS CR, 166 10 Praha 6, Czech Republic  
hrstka@mou.cz

Oestrogen receptor alpha (hER $\alpha$ ) is a key biomarker for breast cancer, and presence or absence of hER $\alpha$  in breast and other hormone dependent cancers influences treatment regimens and patient prognosis. hER $\alpha$ s are activated after ligand binding - typically by oestradiol. Oestradiol belongs to steroids, which represent a group of chemical compounds with four rings skeleton. Approximately 3 000 steroid compounds were used for molecular docking studies to find novel potential ligands for hER $\alpha$ s to predict compounds that may show anticancer activity. Mainly two compounds, MU-5562 and MU-5611 (Scheme 1), showed hER $\alpha$  inhibitory activity comparable to the clinically used hER $\alpha$  inhibitors such as tamoxifen or fulvestrant (Fig. 1). The effect of tested compounds on hER $\alpha$  activity was determined by luciferase reporter test, which we developed for this purpose. The inhibitory effect of MU-5562 and MU-5611 on hER $\alpha$  is probably due to the presence of a double bond in their D ring, which protects activation of hER $\alpha$ s by decreasing the electron density of the keto group. This configuration blocks the development of hydrogen bonds, which represent an important prerequisite for conformational changes of the  $\alpha$  helix H12 associated with hER $\alpha$  activation. Moreover, immunochemical analysis revealed that MU-5611 decreases the level of hER $\alpha$  (similarly to fulvestrant), which is associated with reduced expression of AGR2 protein, well known downstream target of hER $\alpha$ . Further we found that these compounds influence cell cycle, which is associated with increased level of protein p21 as confirmed by immunochemical analysis. Interestingly, we also detected additional mechanism of action of these compounds. We found that they interact with mitochondrial structures, as shown by JC-1 staining. Accordingly, to our results, we can speculate that these compounds disrupt electron transport chain. In conclusion, the combination of computational and experimental methods represents rapid approach to determine the activity of compounds towards hER $\alpha$ . We believe that these data will be helpful not only in research of hER $\alpha$  activity and inhibition, but may also be useful for the development of novel drugs for clinical application.



Scheme 1. Structures of both potential anti-oestrogens, MU-5562 and MU-5611, are very similar to estrone (green), which is natural ligand of hER $\alpha$ . Structural differences between them are highlighted by red colour.

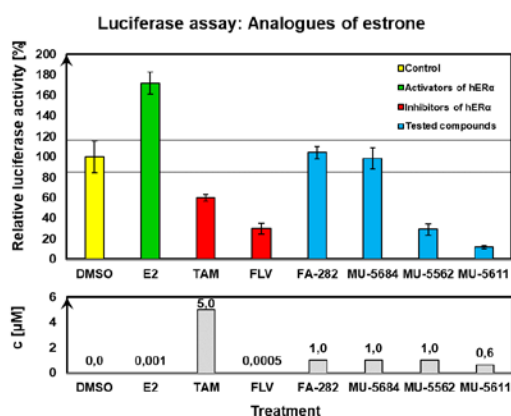


Fig. 1. Results of luciferase assay show that selected steroid compounds may influence hER $\alpha$  activity. Signal of natural activator (green) is higher than control (yellow). Signal of inhibitors (red) is lower than control. Tested steroid compounds selected from our library are in blue.

This work was supported by MH CZ DRO (MMCI, 00209805), by the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16\_019/0000868), by MEYS (project IT4Innovations National Supercomputing Center - LM2015070), by ASCR (RVO: 61388963) and by the Grant Agency of the Czech Republic GACR 19 01383S.

## ELUCIDATION OF ADAR1 FUNCTIONS IN THE IMMUNE RESPONSE BY USING INTERACTOME DATA

DRAGANA VUKIC<sup>a</sup>, LEENA YADAV<sup>b</sup>, SAMPATH KUMAR<sup>a</sup>, MARKKU VARJOSALO<sup>b</sup>, LIAM KEEGAN<sup>a</sup>, MARY O'CONNELL<sup>a</sup>

<sup>a</sup>CEITEC, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic; <sup>b</sup>Institute of Biotechnology, University of Helsinki, Biocenter 3, Viikinkaari 1, 00014 Helsinki, Finland  
dragana.vukic@ceitec.muni.cz

Adenosine deaminases acting on dsRNA (ADARs) are essential for a normal embryonic development and have a role in preventing innate immune response to endogenous dsRNA. ADARs deaminate adenosine to inosine by hydrolytic deamination, known as A-to-I editing. Our group was the first to demonstrate that this editing event in endogenous dsRNA prevents the interferon (IFN) signalling cascades from dsRNA sensors in the cytoplasm: RIG-I and MDA5. In accordance, mice lacking Adar1 die at embryonal stage with heightened levels of type-I IFN and widespread apoptosis. In humans, mutations in ADAR1 cause the autoimmune disorder Aicardi-Goutières syndrome (AGS). Most of the AGS mutations were shown to reduce editing activity of the protein, except ADAR1 D1113H. This mutation is in the deaminase domain and it can lead to the perturbation in protein-protein interactions. To answer this, we looked at ADAR1 interactome under different conditions. For this goal, we have prepared a tetracycline inducible HE239T cell line, expressing both isoforms of ADAR1. These proteins were tagged with Strep-tag or BirA at either N- or C-terminus of the protein. In addition, to further reflect biological function of ADAR1p150 cells were treated with type I IFN. Furthermore, we have induced immune response in HeLa cell line and performed co-IP of ADAR1 to look at the protein complexes formed at the endogenous level. Taken together, until now we have a comprehensive data set of ADAR1 protein complexes with or without induction of immune response; both with IFN and HMW Poly I:C. Our data are consistent between all sets and in agreement with all published interacting proteins of ADARs. In addition, not only that we have found differences in protein complexes upon immune response, but also, we have found that tags at the different terminus influences protein stability and interactions.

*Funded under FP7 project 'The ERA Chair Culture as a Catalyst to Maximize the Potential of CEITEC' (contract no. 621368).*

## CALCIUM-BINDING PROTEINS AND MODIFICATION OF THEIR PROPERTIES IN FUSION CONSTRUCTS

**MONIKA ZOUHAROVA<sup>a</sup>, VERONIKA VETYSKOVA<sup>a</sup>, LUCIE BEDNAROVA<sup>a</sup>, ONDREJ VANEK<sup>b</sup>, KRISTYNA BOUSOVA<sup>a</sup>, JIRI VONDRASEK<sup>a\*</sup>**

*<sup>a</sup>Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo 542, 160 00 Prague; <sup>b</sup>Faculty of Science, Charles University, Albertov 6, 128 00 Prague  
monika.vargova@uochb.cas.cz; jiri.vondrasek@uochb.cas.cz*

Functions of protein domains are strongly dependent on the allosteric effects of their domain context. By studying synthetic domain chimeras, information about possibilities of preparing proteins with improved properties can be obtained. Through fusions, the effect of the domain context on the functions of calcium-binding proteins, ameloblastin (AMBN) and calmodulin (CaM), was monitored. AMBN is poorly characterized calcium-binding intrinsically disordered protein involved in biomineralization of tooth enamel<sup>1,2</sup>. The affinity of AMBN to calcium ions was predicted to be ensured by calcium-binding regions at its C-terminal domain<sup>3</sup>. The fusion of AMBN C-terminal domain to structurally and functionally thoroughly characterized protein CaM allowed us to observe allosteric modulation of CaM by adjacent AMBN C-terminal domain. The results obtained in this study will extend the spectrum of information on AMBN and common possibilities of allosteric modulations in new synthetic fusion proteins.

*This work was supported by the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences (RV0: 61388963).*

## REFERENCES

1. Yamakoshi Y., Tanabe T., Oida S., Hu C. C., Simmer J. P., Fukae M.: Arch. Oral Biol. 46, 1005 (2001).
2. Wald T., Bednarova L., Osicka R., Pachel P., Sulc M., Lyngstadaas S. P., Slaby I., Vondrasek J.: Eur. J. Oral Sci. 119, 261 (2012).
3. Vymetal J., Slaby I., Spahr A., Vondrasek J., Lyngstadaas S. P.: Eur. J. Oral Sci. 116, 124 (2008).



## AUTHOR INDEX

Abad, Cilia	260, 264	Fajkus, Jiří	
Abreu, Celeste	251	Fajkus, Petr	266
Adam, Vojtěch	258	Farka, Zdeněk	256, 278
Amakorová, Petra	279	Fedr, Radek	282
Ambrožová, Gabriela	257	Filip, Daniel	267
Annadurai, Narendran	251	Filová, Eva	263
Anton, Milan	273	Fitzgerald, Mark P.	284
Babica, Pavel	257, 283	Fojtu, Michaela	288
Babičová, Barbora	280	Furst, Tomas	287
Bacaková, Lucie	284	Garcia-Souza, L. F.	293
Bajard, Lola	273	Gnaiger, E.	293
Bárta, Tomáš	262	Gogela, Roman	256
Bartošík, Martin	273	Goliášová, Zita	257
Bartůněk, Vilém	261	Gómez, I. Jénifer	257
Bazgier, Václav	295	Gömöryová, Kristína	269, 282
Beckerová, Deborah	282, 283	Gorris, Hans H.	256
Bednar, David	265, 285	Grézlová, Veronika	258
Bednarova, Lucie	294, 296	Gybel, Tomáš	282
Berka, Karel	295	Hadacova, Ivana	270, 294
Bino, Lucia	282	Hadravova, Romana	294
Bittner, Michal	276	Hadzima, Martin	258
Blaha, Luděk	273	Hajduch, Marian	268, 270, 287
Blahnová, Veronika	263	Hajdúch, Marián	251, 271
Blažková, Kristýna	258	Hampl, Aleš	272, 292
Bornscheuer, Uwe T.	285	Hassan, Amal	259
Bousova, Kristyna	294, 295	Havel, Josef	272
Bradna, Pavel	286	Heger, Dominik	275
Brázdová, Lada	252	Hejret, Václav	279
Brtníková, Jana	271	Herciková, Jana	259
Bryja, Vitezslav	276	Hernychova, Lenka	285
Bryja, Vítězslav	269, 274, 282	Hobza, Roman	256
Bubnov, Alexej	288	Hof, Martin	262
Buchtová, Marcela	286	Hollfelder, Florian	285
Buša, Michal	275	Holubová, Martina	267
Buzgo, Matěj	255	Horackova, Hana	260, 264
Caivano, Ilaria	253	Horn, Martin	254
Čapandová, Michaela	262	Horvathova, Monika	270
Čelko, Ladislav	255, 258	Horvatova, Alzbeta	260
Cermak, Jaroslav	270	Hošeková, Vanda	257
Cervený, Lukas	260, 264	Houštecká, Radka	254
Céspedes, Natalia	252	Hrstka, Roman	273, 295
Chaloupkova, Radka	285	Hrubá, Lucie	267
Charifou, Elsa	292	Huber, Štěpán	261
Chiche, Aurélie	292	Hulcová, Daniela	283
Chlebek, Jakub	283	Humpolíček, Petr	282
Chmela, Václav	253	Jahn, Ullrich	270, 272
Chmúřčiaková, Nikola	254	Janata, Jiří	252
Christmann, Rebekka	280	Janda, Lubomír	265, 289
Cibulka, Radek	259, 274, 278	Janke, Carsten	269
Cígler, Petr	271	Janousek, Bohuslav	256
Císařová, Ivana	253, 272, 285	Jansa, Petr	261
Cudlman, Lukáš	254	Javorská, Žantea	277
Cvačka, Josef	254, 291, 291	Jílková, Adéla	275
Damborsky, Jiri	265, 285	Jungova, Alexandra	270
Damborský, Jiří	271	Jurásek, Bronislav	261
Das, Viswanath	251	Jurčíková, Denisa	262
De Marco, Ario	251	Juřicová, Helena	289
Dehaen, Wim	261	Kabelka, Ivo	262
Demkova, Lucia	282	Kacvinská, Katarína	263
Dorazilová, Jana	254	Kadlčík, Stanislav	252
Dörr, Mark	285	Kaiser, Jozef	255, 258, 286, 290
Doubek, Michael	269	Kaiser, Karol	282
Drašar, Pavel	286	Kaiser, Reinhard	253
Đuriš, Miroslav	254	Kaleta, Jiří	285
Duskova, Pavlina	287	Kaleta, Michal	263
Dzubak, Petr	268	Kaletová, Eva	285
Dzurov, Matej	255	Kameník, Zdeněk	252
Emond, Stephane	285	Káňová, Kristýna	264
Fagan, Patrik	261	Karahoda, Rona	260, 264

Kaspar, Miroslav	260	Mayer, Jiří	269
Kastner, Petr	264	Medalová, Jiřina	257, 287
Keegan, Liam	295	Mickert, Matthias J.	256
Khalikova, Maria	280	Míkula, Přemysl	276
Khan, Rayyan Tariq	265	Mítrik, Martin	287
Kobzová, Šárka	265	Modlitbová, Pavlína	286, 290
Kocanda, Jan	292	Mojzikova, Renata	270
Kočiová, Silvia	258	Montufar, Edgar B.	255
Kohout, Michal	259, 266, 277, 288	Montufar, Edgar Benjamin	258
Kolářová, Karolína	266	Moosová, Zdena	257
Kolderová, Natalie	266	Moráň, Lukáš	272
Koledová, Zuzana	292	Moráňová, Ludmila	273
Koloušková, Magdaléna	262	Mosinger, Jiří	253
Konegger, Hannes	265	Mráz, Marek	267
Konvalinka, Jan	258	Musil, Milos	265
Kopel, Pavel	258	Musilkova, Jana	284
Koralkova, Pavla	270	Nečas, David	253
Kořínková, Lucia	267	Necas, Marek	288
Košálová, Lenka	267	Nečasová, Ivona	266
Kostka, Libor	258	Negi, Chander Kant	273
Kotasová, Hana	262, 272	Němcová, Zdeňka	280
Kotora, Martin	253	Neprašová, Barbora	267
Kotrbova, Anna	276	Nikulenkov, Fedor	281
Kotrbová, Anna	282	Norek, Adam	289
Kotulova, Jana	268	Nosková, Michaela	274, 282
Kozlová, Veronika	269, 279	Novák, Jiří	294
Kozmík, Václav	277	Novák, Ondřej	263, 279
Králík, František	261	Nováková, Lucie	280
Králová, Tereza	262	Novotný, Karel	290
Kravec, Marek	269	Nový, Zbyněk	271
Krejčí, Lumír	281	O'Connell, Mary	295
Kren, Vladimír	284	O'Connell, Mary A.	284
Křen, Vladimír	264, 289	Obertík, Róbert	274
Křivánek, Jan	262	Oklešťková, Jana	263, 295
Kubala, Lukáš	257, 282	Oloketuyi, Sandra	251
Kubickova, Agata	270	Ondra, Martin	270, 271
Kubičková, Kateřina	286	Ondřišová, Laura	267
Kucera, Radim	264	Ondrušková, Gabriela	275
Kučerová, Lucia	282	Opletal, Lubomír	283
Kuchař, Martin	261, 266	Ormsby, Tereza	258
Kucherková, Radka	270	Orságiová, Katarína	275
Kudlova, Natalie	287	Pachernik, Jiří	282
Kudová, Eva	260, 295	Paclíková, Petra	276
Kujovská Krčmová, L.	293	Paleček, Jan	266
Kumar, Sampath	295	Pálešová, Nina	276
Kummerová, Marie	290	Pardy, Filip	277
Kuneš, Jaroslav	267	Paškan, Martin	277
Kvaková, Klaudia	271	Pastucha, Matěj	278
Kvasnica, Miroslav	295	Pavek, Petr	260
Ladungová, Adriana	279	Pavliňáková, Veronika	263
Ledererová, Aneta	268	Pavlovská, Tetiana	274, 278
Lehr, Claus-Michael	280	Pelková, Vendula	272
Li, Han	292	Pěničik, Aleš	279
Liskova, Veronika	285	Peschelová, Helena	279
Loretz, Brigitta	280	Peška, Vratislav	266
Lysáková, Klára	255, 258, 271	Pešková, Lucie	262
Maceckova, Zuzana	270	Petrásková, Lucie	264, 289
Machara, Aleš	254	Petřík, Ivan	279
Majer, Pavel	258	Petřík, Miloš	271
Maletínská, Lenka	267, 291	Petrová, Eliška	280
Malina, Lukáš	251	Pickova, Marketa	282
Mančíková, Veronika	268, 279	Pinto, Gaspar P.	285
Marek, Martin	285	Pizúrová, Naděžda	257
Marek, Radek	288	Plachká, Kateřina	280
Mareš, Michael	254, 275	Planas, Joan	285
Maroofian, Reza	284	Pluskal, Daniel	285
Masák, Jan	252	Pokorná, Šárka	262
Masarik, Michal	288	Poláček, Petr	263
Mašát, Milan	285	Pořízka, Pavel	286, 290
Mašek, Tomáš	272	Porokh, Volodymyr	272, 292
Matoušová, Eliška	261	Poryvai, Anna	281
Matthios, Andreas	264	Pospisilova, Dagmar	270

Pospíšilová, Michaela	281	Sumbal, Jakub	292
Pospíšilová, Šárka	269	Sutherland, John	253
Potěšil, David	269, 282	Svoboda, Jiří	288
Potrusil, Martin	278	Sychrová, Eliška	294
Pražienková, Veronika	267, 291	Sýkora, David	291
Presová, Gabriela	272	Tan, Tiong Y.	284
Prochazka, David	286, 290	Tebbens, Jurjen Duintjer	264
Prokop, Zbynek	285	Tichý, Antonín	286
Ptáčková, Nikola	263, 271	Topper, Miroslav	271
Pytelková, Jana	275	Toul, Martin	285
Raabová, Helena	271	Trávníčková, Eva	276
Radaszkiewicz, Katarzyna	282	Travnickova, Martina	284
Radaszkiewicz, Tomasz	282	Tuerkova, Alzbeta	262
Radaszkiewicz, Tomasz Witold	274, 276, 282	Vácha, Robert	262
Rampichová, Michala	255	Vachalova, Veronika	260
Raptová, Petra	283	Valentová, Kateřina	264
Rárová, Lucie	295	Valová, Kristýna	255, 258
Roubíčková, Lucie	280	Vanek, Ondřej	294, 295
Rychlík, Ivan	289	Vaněk, Ondřej	251
Šácha, Pavel	258	Vaňhara, Petr	272
Šafratová, Marcela	283	Varjosalo, Markku	295
Šandová, Veronika	267	Vašíček, Ondřej	257
Santos Hurtado, Carina	285	Vavrušáková, Barbora	292
Saxova, Zuzana	270	Vázquez Sulleiro, Manuel	257
Schenkmayerova, Andrea	283	Verner, Jan	282
Schimer, Jiří	271	Vernerová, Andrea	293
Schnurrer, Florian	253	Vetyskova, Veronika	294, 296
Šebej, Peter	278	Veverka, Pavel	266
Šeda, Václav	267	Virmani, Ishita	294
Šedlar, Antonin	284	Vojáčková, Eva	267
Sedmík, Jiří	284	Vojta, Petr	270
Šedo, Ondřej	269	Vojtíšek, Jan	258
Setnička, Vladimír	261	Vojtová, Lucy	254, 255, 258, 263, 271
Severa, Lukáš	285	Volejnikova, Jana	270
Sharma, Sonali	267	Vondrasek, Jiri	294, 296
Šimková, Markéta	286	Voňka, Petr	295
Šimon, Petr	258	Vrábel, Jakub	286
Šindelářová, Anna	286	Vrkoslav, Vladimír	254, 291
Šindlerová, Lenka	257, 283	Vrlíková, Lucie	286
Skálová, Helena	287	Vukic, Dragana	295
Skehel, Mark	281	Vyskot, Boris	256
Skládal, Petr	256	Woloszczuková, Lucie	282, 283
Skočková, Veronika	283	Xu, Jianfeng	253
Slámová, Kristýna	289	Yadav, Leena	295
Slanina, Tomáš	281	Zajíčková, Lenka	257
Slavik, Hanus	287	Zámečnicková, Markéta	271
Šmahel, Michal	288	Zbytovská, Jarmila	280
Šmerková, Kristýna	258	Zdrahal, Zbyněk	269, 282
Šmída, Michal	269, 279	Železná, Blanka	267
Sobotka, O.	293	Zezulka, Štěpán	290
Sojka, Martin	288	Zluzova, Jitka	256
Souček, Karel	282	Zouharova, Monika	294, 296
Sovadinová, Iva	294		
Spálovská, Dita	277		
Špírek, Mário	281		
Srovnal, Josef	287		
Šťastná, Michaela	289		
Štaud, Frantisek	260, 264		
Štěpánková, Veronika	263		
Štoček, Jakub	285		
Storchova, Helena	256		
Stourac, Jan	265		
Straková, Zuzana	289		
Štrbková, Lenka	254		
Štreit, Libor	292		
Střítežská, Sára	290		
Strmeň, Timotej	291		
Strnad, Miroslav	279, 295		
Strnad, Štěpán	291		
Šubr, Vladimír	258		
Sukeník, Lukáš	262		
Sukenik-Halevy, R.	284		

**REMARKS AND NOTES**



**CZECH CHEMICAL SOCIETY SYMPOSIUM SERIES • ročník/volume 18 (2020), čís./no. 4 • 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: B. Kratochvíl • REDAKTOŘI/ EDITORS: J. Barek, Z. Bělohav, E. Benešová, P. Drašar, P. Holý, P. Chuchvalec, Z. Kolská, B. Kratochvíl, J. Podešva, V. Vyskočil; Webové stránky: V. Vyskočil • 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, +420 222 220 184, 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 © 2020 Czech Chemical Society Symposium Series/Česká společnost chemická • Cena výtisku / Single 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 20.10.2020.**