Chemistry towards Biology 10 Instruct

11 – 14 September, 2022

Bratislava, Slovakia

https://www.instruct.sav.sk/index.html

Conference Venue

Hotel Tatra

Námestie 1. mája 5, 811 06 Bratislava

Programme

Abstract Booklet

All the individual contributions were reviewed by members of the International Scietific Committee:

Miloš Hricovíni
Josef Jampílek
Roberta Pierattelli
Grazyna Stochel
Janez Plavec
Andras Perczel

ISBN 978-80-971665-3-3

Dear Colleagues,

The International Scientific Board of the „Chemistry towards Biology“ conference series and the Local Organizing Committee are pleased to announce the

Chemistry towards Biology 10 - Instruct conference.

This conference will be the 10th in the **Chemistry towards Biology** series of successful meetings aimed at the exchange of scientific results and ideas in the fields of chemistry and biology. The European Infrastructure **Instruct-ERIC** supports advances in structural biology research, particularly those linking atomic structure with molecular properties and cellular context. As these two meetings will be organized together, the theme of this year's Conference will be "Biomolecular structure” and will mainly cover the topics of structure and dynamics of biomolecules, intermolecular interactions, and experimental and theoretical methods in biomolecular research. The symposium will include plenary and invited lectures, oral presentations selected from submitted abstracts, and poster sessions.

The meeting venue is located in the central part of the city. The ancient heart of the old town is a few steps from the venue.

We welcome you and hope that you will have a great time in Bratislava at the Chemistry towards Biology 10 - Instruct meeting!

Miloš Hricovíni
Chairman of CTB10 - Instruct

Organizing committee

Miloš Hricovíni (Institute of Chemistry)
Josef Jampílek (Comenius University)
Olga Švančarová (Institute of Chemistry)
Juraj Kóňa (Institute of Chemistry)
Zuzana Hricovíniová (Institute of Chemistry)
Iveta Uhliariková (Institute of Chemistry)
Veronika Kotrasová (Institute of Molecular Biology)
Nina Kunová (Institute of Molecular Biology)

The CTB-Instruct gratefully acknowledges support from the following sponsors

RE• FEYN

WEIGHING MOLECULES WITH LIGHT

MERCK

Programme

Sunday
11 Sept.

Registration 13,00 – 17,15

17,15 – 17,30 Opening
17,30 – 18,00 H. Schwalbe
18,00 – 18,30 K. Djinovic Carugo

19,00 Welcome Party

	Lectures		Tea/Coffee	Lectures		Lunch	Lectures		Tea/Coffee	Lectures		Posters
Monday 12 Sept.	8,45 – 10,10		10,10 –10,35	10,35 – 12,15		12,15 – 13,30	13,30 – 15,20		15,20 – 15,45	15,45 – 17,15		17,15 – 18,15
	8,45 – 9,15	R. Piaratelli		10,35 – 11,00	J. Plavec		13,30 – 13,55	A. Perczel		15,45 – 16,05	F. Foret	
	9,15 – 9,45	W. Kozminski		11,00 – 11,25	J. Moncoľ		13,55 – 14,20	V. Bauerová		16,05 – 16,25	S. Samsonov	
				11,25 – 11,50	A. Slodek					16,25 – 16,45	V. Grolmusz	
	9,45 –10,10	O. Malkina		11,50 – 12,15	A. Bak		14,20 – 14,40	A. J. Kiss-Szemán		16,45 – 17,00	M. Domšicová (PhD)	
							14,40 – 15,00	J. Asher		17,00 – 17,15	M. Gadanecz (PhD)	
							15,00 – 15,20	H. Fernandes				
Tuesday 13 Sept.	8,30 – 10,15		10,15 –10,40	10,40 – 12,10		12,10 – 13,15	13,15 – 15,05		15,10 – 15,30	15,30 – 17,00		17,00 – 18,00
	8,30 – 9,00	L. Banci		10,40 – 11,10	P. Řezáčová		13,15 –13,45	R. Owens		15,30 – 15,50	M. Oszajca	
										15,50 – 16,10	O. Mazuryk	
										16,10 – 16,30	V. Kelemen	
	9,00 – 9,25	R. Musiol		11,10 – 11,30	D. Lőrinczy		13,45 – 14,05	J. Bauer		16,30 – 16,45	M. Procházková (PhD)	
	9,25 – 9,50	E. Kutejová		11,30 – 11,50	M. Maszota-Zieleniak		14,05 – 14,25	M. Brindell		16,45 – 17,00	F. Pilhál (PhD)	Social Dinner
	9,50 – 10,15	T. Martinek		11,50 – 12,10	V. Sládek		14,25 – 14,45	Z. Tokárová				19.00 – 22.00
							14,45 – 15,10	V. Pevala				
Wednesday 14 Sept.	8,45 – 10,10		10,10 –10,35	10,35 – 12,20		12,15 – 13,30	Departure					
	8,45 – 9,15	M. Májeková		10,35 – 10,55	W. Musiał							
	9,15 – 9,45	G. Stochel		10,55 – 11,20	P. Štarha							
				11,20 – 11,45	L. Urbaníková							
				11,45 – 12,10	A. Sobczak-Kupiec							
	9,45 – 10,10	Z. Pakanová										
				12,10 – 12,15	Closing							

Plenary lectures | Invited lectures | Oral presentations | PhD students

Scientific Programme

Sunday

11 September

13,00 – 17,15 **Registration** **Hotel Tatra,** Námestie 1.mája 5, Bratislava

17,15 – 17,30 **Opening** *Miloš Hricovíni (Slovak Academy of Sciences, Bratislava, Slovakia)*

Session 1

Chair *Janez Plavec (Slovenian NMR center, University of Ljubljana, Slovenia)*

17,30 – 18,00 **PL-1** **European Integrated Structural Biology as a Motor for Biomedical Translation**
Harald Schwalbe ([1]Instruct-ERIC, Oxford, UK, [2]Center for Biomolecular Magnetic Resonance (BMRZ), Goethe-University Frankfurt/M., Germany)

18,00 – 18,30 **PL-2** **Order from Disorder: Towards molecular architecture of the muscle Z-disk assembly**
Kristina Djinovic Carugo (University of Vienna, Austria)

19,00 ***Welcome Party***

Monday

12 September

Session 2

Chair *Miloš Hricovíni (Slovak Academy of Sciences, Bratislava, Slovakia)*

8,45 – 9,15	**PL-3**	**Just flexible linkers? Un-structural biology by NMR spectroscopy** *Roberta Pierattelli (CERM, University of Florence, Italy)*
9,15 – 9,45	**PL-4**	**High dimensionality and high resolution NMR experiments for IDPs** *Wiktor Koźmiński (University of Warsaw, Poland)*
9,45 – 10,10	**IL-1**	**Beyond the Dirac vector model: why one-bond reduced NMR spin-spin coupling can be negative** *Olga L. Malkina (Slovak Academy of Sciences, Bratislava, Slovakia)*

10,10 – 10,35 **Tea/Coffee**

Session 3

Chair *András Perczel (MTA-ELTE, Eötvös Loránd University, Budapest, Hungary)*

10,35 – 11,00	**IL-2**	**NMR structure and dynamic features of quadruplex DNA topologies** *Janez Plavec (Slovenian NMR center, University of Ljubljana, Slovenia)*
11,00 – 11,25	**IL-3**	**Structure types of transition metal complexes as potential anticancer drugs** *Ján Moncoľ (Slovak University of Technology, Bratislava, Slovakia)*
11,25 – 11,50	**IL-4**	**Donor-acceptor (D-A) systems based on the phenothiazine unit as modern materials for application in optoelectronics and bioimaging** *Aneta Slodek (University of Silesia, Katowice, Poland)*
11,50 – 12,15	**IL-5**	**Similarity-mediated Property Profiling in Computer-assisted Drug Design** *Andrzej Bak (University of Silesia, Katowice, Poland)*

12,15 – 13,30 **Lunch**

Session 4

Chair *Kristina Djinovic Carugo (University of Vienna, Austria)*

13,30 – 13,55	**IL-6**	**The Rule of Amyloid Control: Proglucagon derived polymorphic amyloid 3D-structures and their formation kinetics** *András Perczel (MTA-ELTE, Eötvös Loránd University, Budapest, Hungary)*

13,35 – 14,20	**IL-7**	**The effect of the mutations on the dynamic motion of the N terminal domain of the human ryanodine receptor 2: towards deeper understanding of cardiac arrhythmias** *Vladena Bauerová-Hlinková (Slovak Academy of Sciences, Bratislava, Slovakia)*
14,20 – 14,40	**OP-1**	**Cryo-EM structure of acylpeptide hydrolase: substrate selection by multimerization and a multi-state serine-protease triad** *Anna J. Kiss-Szemán (Eötvös Loránd University, Budapest, Hungary)*
14,40 – 15,00	**OP-2**	**A study of the photochemical behaviour and relaxation mechanisms of *anti-syn* isomerisation around quinazolinone ---N-N= bonds** *James R. Asher (Slovak Academy of Sciences, Bratislava, Slovakia)*
15,00 – 15,20	**OP-3**	**Towards a new biomarker for Diabetic Retinopathy: exploring RBP3 structure and retinoids binding for eyes functional imaging in vivo** *Humberto Fernandes (Institute of Physical Chemistry, Warsaw, Poland)*

15,20 – 15,45 **Tea/Coffee**

Session 5

Chair *Wiktor Koźmiński (University of Warsaw, Poland)*

15,45 – 16,05	**OP-4**	**Epitachophoresis - new tool for nucleic acid purification/concentration** *František Foret (Czech Academy of Sciences, Brno, Czech Republic)*
16,05 – 16,25	**OP-5**	**Theoretical chemistry approaches to biologically relevant problems in the molecular systems containing glycosaminoglycans** *Sergey A. Samsonov (University of Gdańsk, Poland)*
16,25 – 16,45	**OP-6**	**New docking strategy to enhance protein interactions with an application to the RAS G12D mutations** *Vince Grolmusz (Eötvös Loránd University, Budapest, Hungary)*
16,45 – 17,00	**PhD-1**	**The use of K562 aptasenzor as detection method for chronic myeloid leukemia** *Michaela Domšicová (Slovak Academy of Sciences, Bratislava, Slovakia)*
17,00 – 17,15	**PhD-2**	**Identifying druggable sites of the Mg^{2+}-free intermediate state of the catalytic cycle of K-Ras^{G12C} oncogenic protein by NMR spectroscopy** *Márton Gadanecz (Eötvös Loránd University, Budapest, Hungary)*

17,15 – 18,15 ***Poster Session***

Tuesday

13 September

Session 6

Chair *Ray Owens (University of Oxford & Rosalind Franklin Institute, UK)*

8,30 – 9,00	**PL-5**	**Cellular Structural Biology: from structural characterization to functional processes in a cellular context** *Lucia Banci (CERM, University of Florence, Italy)*
9,00 – 9,25	**IL-8**	**Should we have complexes with terpyridines?** *Robert Musiol (University of Silesia in Katowice, Poland)*
9,25 – 9,50	**IL-9**	**Lon protease – the essential component of mitochondrial homeostasis** *Eva Kutejová (Slovak Academy of Sciences, Bratislava, Slovakia)*
9,50 – 10,15	**IL-10**	**Degradation-free intracellular delivery of nanomolar protein cargoes with ganglioside-specific recognition tags** *Tamás A. Martinek (University of Szeged, Hungary)*

10,15 – 10,40 **Tea/Coffee**

Session 7

Chair *Roberta Pierattelli (CERM, University of Florence, Italy)*

10,40 – 11,10	**PL-6**	**Structure-assisted design of enzyme inhibitors** *Pavlína Řezáčová (Czech Academy of Sciences, Prague, Czech Republic)*
11,10 – 11,30	**OP-7**	**Thermal analysis of tendon, cartilage and bone samples of patients underwent shoulder replacement with differential scanning calorimetry (DSC) and thermogravimetry (DTA/TG)** *Dénes Lőrincy (University of Pécs, Hungary)*
11,30 – 11,50	**OP-8**	**Computational and experimental analysis of the interactions between glycosaminoglycans and small molecules** *Martyna Maszota-Zieleniak (University of Gdańsk, Poland)*
11,50 – 12,10	**OP-9**	**Residue Networks – protein structure, function and computational analysis** *Vladimir Sladek (Slovak Academy of Sciences, Bratislava, Slovakia)*

12,10 – 13,15 **Lunch**

Session 8

Chair *Lucia Banci (CERM, University of Florence, Italy)*

13,15 – 13,45 **PL-7** **Structural and functional analysis of nanobodies to the Spike protein of SARS-CoV-2**
Ray Owens (University of Oxford & Rosalind Franklin Institute, UK)

13,45 – 14,05 **OP-10** **Interpretation of Single-Molecule Force Microscopy Experiments Using Normal Mode Analysis**
Jacob Bauer (Slovak Academy of Sciences, Bratislava, Slovakia)

14,05 – 14,25 **OP-11** **Design new fluorescent turn off-on probes for hypoxia imaging**
Małgorzata Brindell (Jagiellonian University, Krakow, Poland)

14,25 – 14,45 **OP-12** **Photodynamic therapy „pre-quided by" organic chemistry & synthesis**
Zita Tokárová (University of ss Cyril and Methodius in Trnava, Slovakia)

14,45 – 15,10 **IL-11** **A new Slovak center for research and teaching activities in structural biology – Interreg V-A Slovakia – Austria project StruBioMol**
Vladimír Pevala (Slovak Academy of Sciences, Bratislava, Slovakia)

15,10 – 15,30 **Tea/Coffee**

Session 9

Chair *Pavlína Řezáčová (Czech Academy of Sciences, Prague, Czech Republic)*

15,30 – 15,50 **OP-13** **Air-pollution-derived metal ions induced decomposition of S nitrosothiols**
Maria Oszajca (Jagiellonian University in Kraków, Poland)

15,50 – 16,10 **OP-14** **Antimetastatic activity of polypyridyl ruthenium(II) complexes – in vitro functional and molecular studies**
Olga Mazuryk (Jagiellonian University in Kraków, Poland)

16,10 – 16,30 **OP-15** **Stereoselective synthesis of 1,2-cis-α-thioglycosides by two Sequential photoinitiated thiol-ene additions**
Viktor Kelemen (University of Debrecen, Hungary)

16,30 – 16,45 **PhD-3** **The new quantum dot luminescent probe for caspase-3/7 imaging inside cells**
Markéta Procházková ([1]Czech Academy of Sciences, [2]Masaryk University, Brno, Czech Republic)

16,45 – 17,00 **PhD-4** **Separation and identification of the α- and β-anomers of sugar amino acids in chimera peptides**
Fruzsina Pilhál (ELTE Eötvös Loránd University, Budapest, Hungary)

17,00 – 18,00 ***Poster Session***

19,00 – 22,00 ***Social Dinner***

Wednesday

14 September

Session 10

Chair *Josef Jampílek (Comenius University, Bratislava, Slovakia)*

8,45 – 9,15 **PL-8** **Structural changes of SERCA protein after ligand binding**
Magdaléna Májeková (Centre of Experimental Medicine SAS, Bratislava, Slovakia)

9,15 – 9,45 **PL-9** **Light - controlled processes and materials from biomedical application perspectives. Mechanistic insight and development strategies**
Grażyna Stochel (Jagiellonian University, Kraków, Poland)

9,45 – 10,10 **IL-12** **Mass spectrometry in glycoconjugate analysis**
Zuzana Pakanová (Slovak Academy of Sciences, Bratislava, Slovakia)

10,10 – 10,35 **Tea/Coffee**

Session 11

Chair *Eva Kutejová (Slovak Academy of Sciences, Bratislava, Slovakia)*

10,35 – 10,55 **OP-16** **Selected applications of Langmuir balance in pharmaceutical sciences**
Witold Musiał (Wroclaw Medical University, Poland)

10,55 – 11,20 **IL-13** **Transition metal complexes for cancer therapy**
Pavel Štarha (Palacký University Olomouc, Czech Republic)

11,20 – 11,45 **IL-14** **Bioinformatics analysis of the family GH13 trehalose synthases with focus on their maltokinase-like domain**
Ľubica Urbániková (Slovak Academy of Sciences, Bratislava, Slovakia)

11,45 – 12,10 **IL-15** **Materials for medicine - bioactive composites and coatings**
Agnieszka Sobczak-Kupiec (Cracow University of Technology, Poland)

12,10 – 12,15 **Closing** *Miloš Hricovíni (Slovak Academy of Sciences, Bratislava, Slovakia)*

12,15 – 13,30 **Lunch**

13,30 **Departure**

POSTERS

P-01 Electrical pulse stimulation as an in vitro model of exercise – comparison of the two protocols in differentiated human skeletal muscle cells

Klára Gabrišová[1], Tímea Kurdiová[1], Jozef Ukropec[1], Barbara Ukropcová[1,2]
[1]*Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences*
[2]*Institute of Pathological Physiology, Faculty of Medicine, Comenius University; Bratislava, Slovakia*

P-02 Graphene Oxide derivatives as potential drug nanocarriers

Marlena Paździor, Aleksandra Świetlicka, Agata Hadryś, Andrzej Bąk, Violetta Kozik
Institute of Chemistry, University of Silesia in Katowice, Poland

P-03 The effect of acute aerobic exercise on extracellular vesicles/EVs in serum and cerebrospinal fluid in humans: the comparison of two methods of EVs isolation

Oksana Mytiai[1,2], Kristína Balčoková[1], Kristína Ukropcová[1], Nikoleta Alchus Laiferová[1,2], Martin Schön[1], Mária Tomková[1], Jozef Ukropec[1], Barbara Ukropcová[1,2]
[1]*Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences*
[2]*Institute of Pathological Physiology, Faculty of Medicine, Comenius University; Bratislava, Slovakia*

P-04 Zoom in the brain sphingomyelin

Petra Maleš[1], Jana Munivrana[2], Danijela Bakarić[1]
[1]*Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Zagreb, Croatia*
[2]*University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia*

P-05 Novel carbohydrate-based amphiphiles for medical and pharmaceutical applications: Structure-biological activity relationships

Zuzana Hricovíniová[1*], Wojciech Smułek[2]
[1]*Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Institute of Chemical Technology and Engineering, Poznan University of Technology, Poznan, Poland*

P-06 Synthesis and anticoagulant activity of an L-guluronic acid containing idraparinux analogue pentasaccharide

Mihály Herczeg[1], Fruzsina Demeter[1], István Timári[2], Katalin E. Kövér[3,4], Anikó Borbás[1]
[1]*Department of Pharmaceutical Chemistry, University of Debrecen, Debrecen, Hungary*
[2]*Department of Organic Chemistry, University of Debrecen, Debrecen, Hungary*
[3]*Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary*
[4]*MTA-DE Molecular Recognition and Interaction Research Group, University of Debrecen, Debrecen, Hungary*

P-07 From nano- to milimolar: dimerization dissociation constant determination

Alexandra Náplavová[1,3], Aneta Kozeleková[1,3], Jozef Hritz[1,2*]
[1]*Central European Institute of Technology, Masaryk University, Brno, Czechia*
[2]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czechia*
[3]*National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czechia*

P-08 Evaluation of the effect of hydrogel substrate components on the stability of tetracycline hydrochloride

Agnieszka Kostrzębska, Karolina Pączek, Angelika Weselak, Witold Musiał
Department of Physical Chemistry and Biophysics, Faculty of Pharmacy, Wroclaw Medical University, Poland

P-09 Co-precipitation of viral glycoprotein HCMV UL144 and human NK cell activating ligand CD160 has revealed their mutual engagement

Andrej Bitala[1], Simona Lenhartová[1], Mário Benko[1], Marek Nemčovič[2], Ivana Nemčovičová[1]
[1]Biomedical Research Center, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

P-10 Physicochemical and mechanical behavior analysis of composite coatings designed for bone regeneration

Dagmara Słota, Wioletta Florkiewicz, Karina Piętak, Mateusz Dyląg, Agnieszka Sobczak-Kupiec
Department of Materials Science, Faculty of Materials Engineering and Physics, Cracow University of Technology, Krakow, Poland

P-11 Polymorphic amyloid-like crystal structures of proglucagon derived hexapeptides demonstrate pH-dependent reversible amyloid formation

Dániel Horváth[1], Zsolt Dürvanger[2], Nóra Taricska[2], Máté Sulyok-Eiler[2], András Perczel[1*]
[1]ELKH-ELTE Protein Modelling Research Group, Budapest, Hungary
[2]Laboratory of Structural Chemistry and Biology, Budapest, Hungary

P-12 The interaction of sodium hyaluronate with lidocaine hydrochloride and sodium naproxen

Dorota Wójcik-Pastuszka, Karolina Stawicka, Witold Musiał
Wroclaw Medical University, Faculty of Pharmacy, Department of Physical Chemistry and Biophysics, Poland

P-13 Semisynthetic teicoplanin derivatives against SARS-CoV-2 and multiresistant bacteria

Eszter Boglárka Lőrincz[1,2,3], Erzsike Rőth[1], Herczegh Pál[1], Borbás Anikó[1,4], Bakai-Bereczki Ilona[1,4]
[1]Department of Pharmaceutical Chemistry, University of Debrecen, Debrecen, Hungary
[2]Institute of Healthcare Industry, University of Debrecen, Debrecen, Hungary
[3]Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary
[4]National Laboratory of Virology, Szentágothai Research Centre, Pécs, Hungary

P-14 Indirect Nuclear Spin-Spin & Hyperfine Coupling pathway visualisation in heavy metal complexes

Florian Lemken, Olga L. Malkina, Vladimir Malkin, Stanislav Komorovský
Institute of Inorg. Chem., Slovak Academy of Sciences, Bratislava, Slovakia

P-15 Understanding the intrinsic Asn-Gly isomerization reaction of polypeptides and proteins by NMR kinetics and *ab initio* calculations

Fruzsina Pilhál[1], Imre Jákli[1], Ernő Keszei[2], András Láng[1], András Perczel[1]
[1]MTA-ELTE Protein Modeling Res. Group and Laboratory of Structural Chemistry and Biology, Institute of Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary
[2]Department of Physical Chemistry and Chemical Kinetics Laboratory; Institute of Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary

P-16 Influence of the poly(ethyleneglycol) dimethacrylates on the selected physicochemical properties of thermally sensitive polymeric particles of NIPA derivatives

Agnieszka Gola, Borys Podżus, Witold Musiał
Department of Physical Chemistry and Biophysics, Pharmaceutical Faculty, Wroclaw Medical University, Wroclaw, Poland

P-17 Improvement of Total Phenanthrene Quantification by a Novel LC-DAD Analytical Method

Petra Chaľová[1,2*], František Csicsay[2], Jaroslav Galba[2], Michaela Matušková[1,3], Ivana Čižmárová[1,3], Juraj Piešťanský[1,3], Ľudovít Škultéty[2]

[1]Comenius University Bratislava, Faculty of Pharmacy, Department of Pharmaceutical Analysis and Nuclear Pharmacy, Bratislava, Slovakia

[2]Biomedical Research Center of the Slovak Academy of Sciences, Institute of Virology, Bratislava, Slovakia

[3]Toxicological and Antidoping Center, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia

P-18 Effect of structures of newly synthesized peptides on the stability of the formed monolayers

Iwona Golonka[1], Patyrycja P. Petrus[1], Jakub E. Pucułek[1], Katarzyna E. Greber[2], Witold Musiał[1]

[1]Department of Physical Chemistry and Biophysics, Faculty of Pharmacy, Wroclaw Medical University, Wrocław, Poland

[2]Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland

P-19 Glycoprofiling of oligosaccharides from urine samples of α-mannosidosis patients using MALDI-TOF/TOF and ESI-MS/MS analysis

Filip Pančik[1], Maroš Krchňák[1], Zuzana Pakanová[1], Marek Nemčovič[1], Anna Hlavatá[2], Anna Šalingová[3], Stanislav Kozmon[1], Peter Baráth[1]

[1]Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

[2]Commenius University in Bratislava, Faculty of Medicine, National Institute of Children's Diseases, Department of Paediatrics, Bratislava, Slovakia

[3]National Institute of Children's Diseases, Centre for Inherited Metabolic Disorders, Bratislava, Slovakia

P-20 Bioconjugation of a fluorescent turn off-on probe to holo-transferrin for the selective delivery and imaging of hypoxic cancer cells

Ewelina Janczy-Cempa[1], Olga Mazuryk[1], Franck Suzenet[2], Malgorzata Brindell[1]

[1]Faculty of Chemistry, Jagiellonian University, Krakow, Poland

[2]Institute of Organic and Analytical Chemistry, University of Orléans, UMR-CNRS 7311, Orléans Cedex 2, France

P-21 Effect of co-solvents on the solubility and dissolution rate of cryptotanshinone from alcohol gels

Justyna Kobryń, Patryk Demski, Witold Musiał

Department of Physical Chemistry and Biophysics, Faculty of Pharmacy, Wroclaw Medical University, Wroclaw, Poland

P-22 Preparation and characterization of pullulan-enriched polymer-ceramic composites

Karina Piętak, Dagmara Słota, Wioletta Florkiewicz, Agnieszka Sobczak-Kupiec

Department of Materials Science, Faculty of Materials Engineering and Physics, Cracow University of Technology, Krakow, Poland

P-23 Synthesis of an effective MRSA inhibitor based on trifluoromethyl diamide scaffold

Eliska Pilarova[1], Karel Pauk[1], Pratibha Magar[1], Ales Imramovsky[1], Dominika Pindjakova[2], Alois Cizek[3], Josef Jampilek[2]

[1]Faculty of Chemical Technology, Institute of Organic chemistry and Technology, University of Pardubice, Pardubice, Czech Republic

[2]Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

[3]Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Brno, Czech Republic

P-24 Advances and challenges of liposome-assisted drug release in the presence of serum albumin molecules. Influence of surrounding pH

Danuta Pentak[1]*, Anna Ploch-Jankowska[1], Andrzej Zięba[2], Violetta Kozik[3]*
[1]*Faculty of Chemistry, University of Opole, Opole, Poland*
[2]*Department of Organic Chemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Sosnowiec, Poland*
[3]*Institute of Chemistry, University of Silesia, Katowice, Poland*

P-25 Homology modelling of α4β1 integrin and its interactions with ligands

Stanislav Kozmon[1,2], Barbora Stratilová[1]
[1]*Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Medical Vision o.z., Bratislava, Slovakia*

P-26 Effect of carotid endarterectomy on induction of ischemic conditioning in stroke

Marek Furman[1], Miroslava Nemethova[1], Lubica Macakova [1], Vladimir Sihotsky[3], Ivan Kopolovets[2], Michal Virag[2], Rastislav Mucha[1]
[1]*Institute of Neurobiology, Biomedical Research Center, Slovak Academy of Sciences, Košice, Slovakia*
[2]*Eastern Slovak Institute of Cardiovascular Diseases and Faculty of Medicine, Pavol Jozef Safarik University, Kosice, Slovakia*
[3] *Faculty of Medicine, Pavol Jozef Safarik University, Kosice, Slovakia*

P-27 Natural protection against aggregation: the role of the gatekeeper amino acids

Nóra Taricska[1], Dániel Horváth[2], András Perczel[1,2]
[1]*Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary*
[2]*MTA-ELTE Protein Modelling Research Group, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary*

P-28 Expression, purification, partial characterization and *in-silico* modeling of hRyR2 tandem Repeat1-2

Alica Petrová[1,2], Terézia Hromádková[1,2], Konrad Beck[3], Monika Zámocká[1], Jacob A. Bauer[1], Vladena Bauerová-Hlinková[1]
[1]*Institute of Molecular Biology Slovak Academy of Sciences, Department of Biochemistry and Protein Structure, Bratislava, Slovak Republic*
[2]*Faculty of Natural Sciences Comenius University in Bratislava, Department of Genetics, Bratislava, Slovak Republic*
[3]*Cardiff University School of Dentistry, Heath Park, Cardiff CF14 4XY, Wales*

P-29 Characteristics of interactions of cobalt and iron complexes with selected glutathione and nitrosoglutathione in aqueous solutions

Dominika Porębska[1], Maria Oszajca[1], Dorota Rutkowska-Żbik[2], Grażyna Stochel[1], Łukasz Orzeł[1]
[1]*Faculty of Chemistry, Jagiellonian University, Krakow, Poland*
[2]*Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland*

P-30 Theoretical investigation of small molecular binding to 17β-HSD type 1,2 and AKR1C enzymes

Vivien Erzsébet Resch[1], Erzsébet Mernyák[2], Eva Hafner[3], Tea Lanisnik-Rizner[3], Gábor Paragi[1,4]
[1]*Department of Medical Chemistry, University of Szeged, Dóm-tér 8, H-6720 Szeged, Hungary*
[2]*Department of Organic Chemistry, University of Szeged, Dóm-tér 8, H-6720 Szeged, Hungary*
[3]*Institute of Biochemistry and Molecular Genetics, University of Ljubljana, Ljubljana, Slovenia*
[4]*Department of Physics, University of Pécs Ifjúság útja 6. H-7624 Pécs, Hungary*

P-31 Host Cell Interaction with *Coxiella burnetii*

Shreein Ahmed[1,2], Katarína Palkovičová[2], Ľudovít Škultéty[2,3]
[1]*Comenius University Faculty of Natural Sciences, Bratislava, Slovakia*
[2]*Department of Rickettsiology, Institue of Virology, Biomedical Research Center, Slovak Academy of Sciences, Slovakia*
[3]*Laboratory of Molecular Structure Characterization, Institute of Microbiology, Czech Academy of Sciences, Czech Republic*

P-32 Biological response of substituted quinazolinones: Structure-activity relationship study

Jana Hricovíniová[1,2], Zuzana Hricovíniová[3]
[1]*Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovak Republic*
[2]*Comenius University Bratislava, Faculty of Pharmacy, Bratislava, Slovakia*
[3] *Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*

P-33 Effects of fatty acids on pregnane X receptor

Lucia Sládeková, Petra Pečinková, Zdeněk Dvořák
Department of Cell Biology and Genetics, Faculty of Science, Palacký University, Olomouc, Czech Republic

P-34 Study of acceptor substrate specificity of Xyloglucan endotransglycosylase (XET) using computational methods

Barbora Stratilová, Eva Stratilová, Stanislav Kozmon
Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

P-35 Diamides and dithioamides as compounds with potential bioactivity

Aleksandra Świetlicka, Marlena Paździor, Agata Hadryś , Violetta Kozik , Andrzej Bąk
Institute of Chemistry, University of Silesia, Katowice, Poland

P-36 Flexibility and function in human ileal bile acid-binding protein

Tamara Teski[1], András Micsonai[2], József Kardos[2], and Orsolya Tőke[1]
[1]*Research Centre for Natural Sciences, Centre for Structural Science, NMR Research Laboratory, Budapest, Hungary*
[2]*Department of Biochemistry, MTA-ELTE NAP B Neuroimmunology Research Group, Institute of Biology, Eötvös Loránd University, Budapest, Hungary*

P-37 *Nostoc* cf. *linckia* exopolysaccharide - a structural study

Iveta Uhliariková[1], Mária Matulová[1], Jaromír Lukavský[2], Peter Capek[1]
[1]*Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Institute of Botany, National Centre of Competence, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic*

P-38 Order & disorder: Carbonic anhydrase IX

Vojtěch Vařečka[1,2], Krishnendu Bera[1], Jozef Hritz[1,2*]
[1]*Central European Institute of Technology, Masaryk University, Brno, Czechia*
[2]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czechia*

P-39 Towards a new biomarker for Diabetic Retinopathy: exploring RBP3 structure and retinoids binding for eyes functional imaging in vivo

Vineeta Kaushik, Luca Gessa, Nelam Kumar, Humberto Fernandes
Integrated Structural Biology Lab, International Centre for Translational Eye Research, Institute of Physical Chemistry, Warsaw, Poland

P-40 Composite materials as drug carriers for controlled release of clindamycin

Wioletta Florkiewicz, Dagmara Słota, Karina Piętak, Julia Sadlik, Agnieszka Sobczak-Kupiec
Department of Materials Science, Faculty of Materials Engineering and Physics, Cracow University of Technology, Krakow, Poland

Abstracts

PL-1

European Integrated Structural Biology as a Motor for Biomedical Translation

Harald Schwalbe[1, 2]

[1]Instruct-ERIC, Oxford, UK. harald.schwalbe@instruct-eric.org
[2]Center for Biomolecular Magnetic Resonance (BMRZ), Goethe-University Frankfurt/M., Germany. schwalbe@nmr.uni-frankfurt.de

In this contribution, an account of the role of Integrated Structural Biology (ISB) in combatting human disease will be given. Special focus is on ISB in the support of vaccination campaigns as well as in the development of anti-viral drugs against SARS-CoV-21-3, but also in the fight against cancer.
Instruct-ERIC is a pan-European infrastructure that provides access to all methodologies in ISB4. The transforming impact of European science coherence, integration and democratization will be highlighted.

ACKNOWLEDGEMENT
This work was financed by iNEXT-Discovery, European Union's Horizon 2020 No 871037, DFG-funded collaborative research center 902, BMBF-funded Sprin-D, VW foundation, Goethe-Corona fund.

REFERENCES

1. A. Wacker, J. Weigand et al., (2020) *Nucl. Acids Res.,* 48 (22),12415-12435.
2. S. Sreeramulu, C. Richter et al., (2021) *Angew. Chem. In.t Ed. Engl.,*60 (35),19191-19200.
3. N. Altinecekic, S. Korn, N. Qureshi, M. Dujardin, M. Ninot-Pedrosa et al., (2021) *Front. Mol. Biosci.,* 8, 653148. doi: 10.3389/fmolb.2021.653148.
4. H. Wienk, L. Banci, S. Daenke, S. Pereiro, H. Schwalbe, D.I. Stuart, M.S. Weiss, A. Perrakis, (2021) *J. Vis. Exp.,* 177. doi: 10.3791/63435.

PL-2

Order from Disorder: Towards molecular architecture of the muscle Z-disk assembly

Kristina Djinović-Carugo[1,2,3]

[1]*European Molecular Biology Laboratory Grenoble, Grenoble, France*
[2]*Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Campus Vienna Biocenter 5, A-1030 Vienna, Austria*
[3]*Department of Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia*

In sarcomeres, α-actinin crosslinks actin filaments and anchors them to the Z-disk. FATZ proteins interact with α-actinin and five other core Z-disk proteins, contributing to myofibril assembly and maintenance as a protein interaction hub.
We generated an integrative model of α-actinin-2 in complex with a Z-disk partner, FATZ-1, which is best described as a conformational ensemble. We show that FATZ-1 forms a tight fuzzy complex with α-actinin-2 and propose a molecular interaction mechanism via main molecular recognition elements and secondary binding sites. The obtained integrative model reveals a polar architecture of the complex which, in combination with FATZ-1 multivalent scaffold function, might organise interaction partners and stabilise α-actinin-2 preferential orientation in the Z-disk. We further uncover FATZ-1 ability to phase-separate and form biomolecular condensates with α-actinin-2, raising the intriguing question of whether FATZ proteins can create an interaction hub for Z-disk proteins through membrane-less compartmentalization during myofibrillogenesis.

PL-3

Just flexible linkers? Un-structural biology by NMR spectroscopy

Roberta Pierattelli

Magnetic Resonance Center (CERM) and Department of Chemistry "Ugo Schiff", University of Florence, Via L. Sacconi 6, Sesto Fiorentino (Florence), Italy

Invisible in X-ray studies of protein crystals, intrinsically disordered regions (IDRs) of complex proteins have been for a long time considered just passive linkers connecting functional globular domains and thus often ignored in structural biology studies. However, in many cases they comprise a significant fraction of the primary sequence of a protein and likely have a role in protein function.

The characterization of highly flexible regions of large proteins as well as entire proteins characterized by the lack of a 3D structure, now generally referred to as intrinsically disordered proteins (IDPs), lies well behind that of their folded counterparts and is nowadays pursued by an increasingly large number of studies to fill this knowledge gap.

Nuclear magnetic resonance (NMR) spectroscopy plays a crucial role in IDPs and IDRs investigation, being the only method that allows a high-resolution description of their structural and dynamic features in solution. The high flexibility has several consequences on the NMR spectroscopic parameters that, if properly handled, can give precious information.

We will present recent results suggesting that more complex functions than expected can be ascribed to the long disordered chains connecting well-structured protein domains.

ACKNOWLEDGEMENT

The support of the CERM/CIRMMP center of Instruct-ERIC is gratefully acknowledged. This work has been funded in part by a grant of the Fondazione CR Firenze and by the Italian Ministry of University and Research.

PL-4

High dimensionality and high resolution NMR experiments for IDPs

Wiktor Koźmiński

Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Żwirki i Wigury 101, 02-089 Warsaw, Poland

Studies of biomolecular structure and dynamics by NMR spectroscopy at atomic resolution require acquisition of multidimensional spectra. However, the recording time of sufficiently resolved multidimensional spectra is often very long due to the sampling limitations. A variety of different methods, mostly based on non-uniform sampling, were proposed to overcome this limitation in multidimensional NMR spectroscopy. They could be utilized in two different ways, either to shorten the experiment duration without loss of resolution, or to perform experiments that are not obtainable conventionally, i.e. with significantly improved resolution and/or of high dimensionality. Most often first of these two, so called “Fast NMR” approach, is shown as the example of the utility of these methods, as it saves expensive spectrometer time. However, in many cases spectra which are not possible to record conventionally, featuring extraordinary resolution and high number of dimensions may be more interesting from scientific point of view as they reveal effects that are hidden, when spectral lines are broad, or enable resolving spectral ambiguities when peaks are overlapped. This second approach we refer to as “Accurate NMR”. Its full potential is manifested when the overall experiment time is less important than a new information available from spectra of high dimensionality (4-6D) or of high resolution approaching natural line-width. The new methods were applied for NMR studies of intrinsically disordered proteins, where the structural disorder in combination with highly repetitive amino-acid sequences causes severe peak overlap in the spectra. Several novel 4-7D pulse sequences are proposed. The new experiments employ non-uniform sampling that enables achieving high resolution in indirectly detected dimensions.

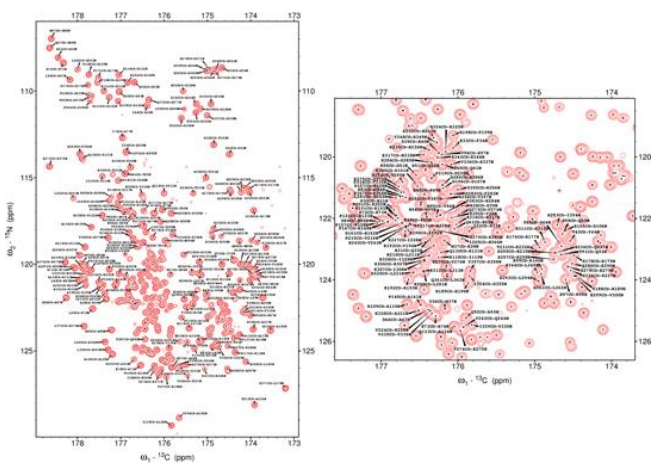


Figure 1 Resonance assignment of Tau3x (354 aa) shown on CON projection from 3D HNCO [1].

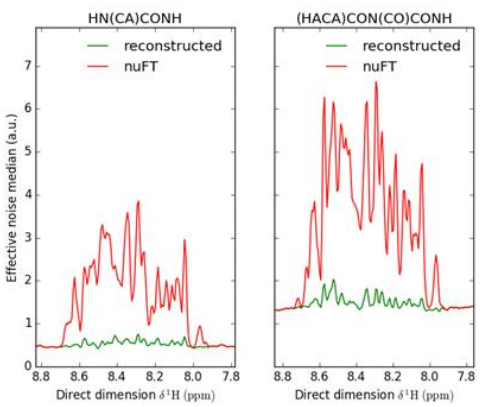


Figure 2 Noise median for 5D HN(CA)CONH (*left*) and 5D (HACA)CON(CO)CONH (*right*) SSA-reconstructed and nuFT spectra with respect to direct dimension δ^1H chemical shift [2].

ACKNOWLEDGEMENT
Polish National Science Centre MAESTRO grant 2015/18/A/ST4/00270 is gratefully acknowledged.

REFERENCES
1. S. Żerko, P. Byrski, P. Włodarczyk-Pruszyński, M. Górka, K. Ledolter, E. Masliah, R. Konrat, W. Koźmiński, (2016) *J. Biomol. NMR.,* 65.
2. K. Kosiński, J. Stanek, M.J. Górka, S. Żerko, W. Koźmiński, (2017) *J. Biomol. NMR.,* 68, 129-138.
3. K. Kazimierczuk, J. Stanek, A. Zawadzka-Kazimierczuk, W. Koźmiński, (2013) *Chem. Phys. Chem.,* 14, 3015-3025.
4. M. Nowakowski, S. Saxena, J. Stanek, S. Żerko, W. Koźmiński, (2015) *Prog. Nucl. Magn. Reson. Spectrosc.*, 90-91, 49-73.

PL-5

Cellular Structural Biology: from structural characterization to functional processes in a cellular context

Lucia Banci

CERM, University of Florence, Via L. Sacconi 6, Sesto Fiorentino, Italy, e-mail: banci@cerm.unifi.it

The characterization of functional processes and the understanding of molecular pathways require their description both at system (e.g. a cell) and at molecular level, (e.g. atomic-resolution characterization of biomolecules). This approach calls for the development of suitable methodologies, capable of addressing multiple, specific, and sometimes non-conventional, aspects and amenable to characterize functional processes in living cells. On this respect NMR spectroscopy is a unique tool not only for characterizing the structure and dynamics of biomolecules but also for describing transient interactions and functional events with atomic resolution, possibly in a cellular context. On this respect, in-cell NMR, i.e. high resolution NMR spectra of biomolecules in intact, living cells, can provide the description of these processes within living cells[4,5]. A further striking application is its use for drug screening in real time at cellular level, in human living cells. Finally, other spectroscopic techniques, such as EPR and Moessbauer, can be applied at cellular level, and their outcomes integrated with the NMR characterization.

Methodological aspects and innovations will be discussed and a few examples of the striking power of this approach for the characterization of functional processes, for the assessment of the protein redox state in the cellular environment, and for the study of effective drug screening will be presented. Particular focus will be on the cellular uptake and target binding in the cellular milieu of drugs and leads and on the meaningful differences observed between drug-target binding in living cells versus in vitro.

REFERENCES

1. E. Luchinat, M. Cremonini, L. Banci, (2022) *Chem. Rev.*, 122 (10), 9267-9306.
2. S. Matteucci, F. Camponeschi, M. Clémancey, S. Ciofi-Baffoni, G. Blondin, L. Banci, (2021) *Angew. Chem. Int. Ed.,* 60, 14841-1484.
3. E. Luchinat, L. Barbieri, M. Cremonini, A. Nocentini, C. Supuran, L. Banci, (2020) *Angew. Chem.*, 59, 6535-6539.
4. E. Luchinat, L. Barbieri, T.F. Campbell, L. Banci, (2020) *Anal. Chem.*, 92, 9997-10006.
5. L. Barbieri, E. Luchinat, L. Banci, (2016) *Nature Protocols,* 11, 1101.
6. E. Mercatelli, L. Barbieri, E. Luchinat, L. Banci, (2016) *BBA - Molecular Cell Research*, 1863, 198-204.
7. L. Barbieri, E. Luchinat, L. Banci, (2015) *Scientific Reports*, 5, 14456. DOI: 10.1038/srep14456.

PL-6

Structure-assisted design of enzyme inhibitors

Pavlina Rezacova

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nam. 2, 16610 Prague, Czech Republic, e-mail: rezacova@uochb.cas.cz

Structure-assisted ligand design, a method used as an alternative to screening random compounds, is the process of identifying new drugs through rational design of molecules based on knowledge of the structure of their biological target. Structural information is obtained through experimental methods such as X-ray crystallography and NMR spectroscopy, or through homology modeling. Enzymes involved in pathologies are good targets for rational design. Enzymes usually have a well-defined active site to bind substrates, and many have allosteric sites that bind regulators. These sites can be targeted by small molecules that mimic the structure of the substrate or regulator. Knowledge of the 3D structure of the enzyme, especially in complex with its natural ligand, is beneficial to lead the design. During the iterative process of rational design, the structure of the ligand is progressively altered to maximize the shape and charge complementarity to the enzyme binding site. Design also can be guided to ensure that the ligand has little to no affinity towards other off-target enzymes to prevent undesirable side effects.
Structure-assisted design of inhibitors targeting human carbonic anhydrase [1, 2] and human deoxynucleotidases [3, 4] will be presented.

REFERENCES

1. B. Gruner, J. Brynda, V. Das, V. Sicha, J. Stepankova, J. Nekvinda, J. Holub, K. Pospisilova, M. Fabry, P. Pachl, V. Kral, M. Kugler, V. Masek, M. Medvedikova, S. Matejkova, A. Nova, B. Liskova, S. Gurska, P. Dzubak, M. Hajduch, P. Rezacova, (2019) *J. Med. Chem.*, 62, 9560-9575.
2. J. Brynda, P. Mader, V. Sicha, M. Fabry, K. Poncova, M. Bakardiev, B. Gruner, P. Cigler, P. Rezacova, (2013) *Angew. Chem. Int. Edit.*, 52, 13760-13763.
3. P. Pachl, O. Simak, P. Rezacova, M. Fabry, M. Budesinsky, I. Rosenberg, J. Brynda, (2015) *MedChemComm*, 6, 1635-1638.
4. P. Pachl, O. Simak, M. Budesinsky, J. Brynda, I. Rosenberg, P. Rezacova, (2018) *Eur. J. Org. Chem.*, 5144-5153.

PL-7

Structural and functional analysis of nanobodies to the Spike protein of SARS-CoV-2

Ray Owens

University of Oxford & Rosalind Franklin Institute, UK

Single domain antibodies (nanobodies) have proved effective in neutralising SARS-CoV-2 viruses both in vitro and in animal models of COVID-19. Using a naive llama single-domain antibody library and PCR-based affinity maturation, we rapidly produced a first generation series of closely related nanobodies, that blocked the interaction between the receptor binding domain (RBD) of the virus spike protein and ACE2. Single-particle cryo-EM revealed that the nanobodies bind to all three RBDs in the spike trimer. Crystal structures of each nanobody–RBD complex in combination with biophysical analyses has shown how sequence changes through affinity maturation have led to an increase in binding. In parallel we produced a second generation series of nanobodies by immunisation with significantly increased affinity and have demonstrated their potential as therapeutics for the treatment of COVID-19.

REFERENCES

1. Huo et al., (2020) *Nat. Struct. Mol. Biol.,* 27, 846-854.
2. Huo et al., (2021) *Nat. Comms.*, 12, 5469.
3. Mikolajek et al., (2022) *Proc. Natl. Acad. Sci. USA,* 119, e2205412119.

PL-8

Structural changes of SERCA protein after ligand binding

M. Májeková[1], L. Kissová[1], V. Heger[1], J. Viskupičová[1], P. Žižková[1], Ľ. Horáková[1], Y. Rodríguez[3,4]

[1]Centre of Experimental Medicine SAS, Institute of Experimental Pharmacology and Toxicology,Dubravska 9, 841 04, Bratislava, Slovakia
[2]Department of Natural Sciences, CUNY's Hostos Community College, 475 Grand Concourse, Bronx, 10451, New York, United States of America
[3]Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, 1425 Madison Avenue, New York, NY 10029, USA

SERCA, a sarco/endoplasmic reticulum Ca^{2+}-ATPase, plays a key role in maintaining the calcium homeostasis in cells and represents an important target in treating various chronic diseases. During the catalytic process SERCA undergoes several structural changes connected with phosphorylation, cation binding and protonation. In our contribution we show the basic conformation changes of SERCA connected with the transition of E1 to E2 state and reversely as well as the possible influencing of individual states by external/internal ligands. We performed molecular docking and full optimization of SERCA bound with rutin derivatives (inhibitors) [1] and compound CDN1163 (activator) [2]. The complex of SERCA bound with rutin-arachidonate was used in molecular dynamics simulation in the dipalmitoyl-phosphatidylcholine bilayer membrane. According to our results, rutin arachidonate was bound in the transmembrane region of SERCA, affecting thus the transport of calcium ions and protons. Compound CDN1163 was bound in the cytoplasmic region, which may be the source of its allosteric activation of SERCA. Both results may be used in further studies of inhibition and activation mechanisms as well as in design of novel compounds with a therapeutic potential.

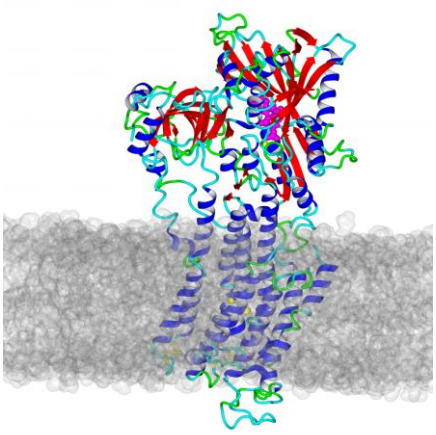


Fig. 1. Calcium pump SERCA immersed in the membrane.

ACKNOWLEDGEMENT
This work was supported by the VEGA 2/0103/22 and by the Slovak Research and Development Agency under the contract No. APVV-20-0543.

REFERENCES

1. Y. Rodríguez and M. Májeková, (2020) *Biomolecules*, 10, 214.
2. S. Kang S, Dahl R, et al., (2016) *J. Biol. Chem*., 291, 5185-5198.

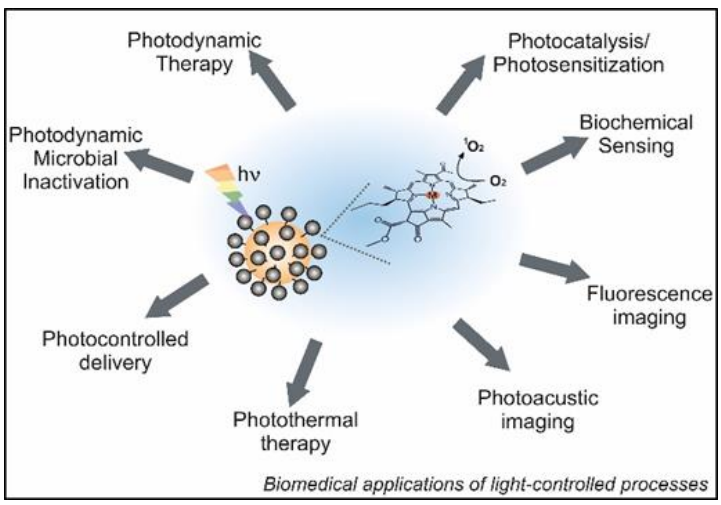
PL-9

Light - controlled processes and materials from biomedical application perspectives. Mechanistic insight and development strategies

Grażyna Stochel

Faculty of Chemistry Jagiellonian University, Kraków, Poland

A short overview of the current trends in the development of biomedical strategies based on photoresponsive materials will be presented. Both mechanistic insight into light-driven physical, chemical and biochemical processes applied in these strategies as well as challenges for molecular and nanostructured platforms designed for prophylactic, therapeutic, or diagnostics purposes will be illustrated by systems developed for reactive oxygen, nitrogen, and sulfur species photogeneration in cells and tissues.



ACKNOWLEDGEMENT

This work was financed by by the National Science Center in Poland for the OPUS Project no. 2019/35/B/ST4/04266.

REFERENCES

1. O. Mazuryk, E. Janczy-Cempa, J. Łagosz, D. Rutkowska-Zbik, A. Machnicka, A. Krasowska, P. Pietrzyk, G. Stochel, M. Brindell, (2022) *Dalton Transaction*, 51, 1888-1900.
2. Ch. Zhang, X. Wang, J. Du, Z. Gu, Y. Zhao(2021) *Advanced Science*, 8, 2002797.
3. M. S. Babtista, J. Cadet, A. Greer, A.H. Thomas (2021) *Photochemistry and Photobiology*, 97, 1456.
4. J. Kuncewicz, J.M. Dąbrowski, A. Kyzioł, M. Brindell, P. Łabuz, O. Mazuryk, W. Macyk, G. Stochel (2019) *Coordination Chemistry Revew*, 398, 113012.
5. A. Regiel-Futyra, J.M. Dąbrowski, O. Mazuryk, K. Śpiewak, A. Kyzioł, B. Pucelik, M. Brindell, G. Stochel (2017) *Coordination Chemistry Revew*, 351, 76-117.
6. L.G. Arnaut, M.M. Pereira, J.M. Dąbrowski, E.F.F. Silva, F.A. Schaberle, A.R. Abreu, L.B. Rocha, M.M. Barsan, K. Urbańska, G. Stochel, Ch.M.A. Brett (2014) *Chemistry A European Journal*, 20, 5346-5357.
7. G. Stochel, M. Brindell, W. Macyk, Z. Stasicka, K. Szaciłowski (2009) *Bioinorganic Photochemistry*, John Wiley@Sons.

IL-1

Beyond the Dirac vector model: why one-bond reduced NMR spin-spin coupling can be negative

Olga L. Malkina

Institute of Inorganic Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84536 Bratislava, Slovakia

A common explanation of the positive sign of the Fermi-contact contribution to the reduced indirect NMR spin-spin coupling through one bond and its sign alternation with increasing number of bonds is given by the so-called Dirac vector model. However, there are many examples, for which this simple model does not work and it does not predict the correct sign of spin-spin coupling. In the present work a deeper insight into the Fermi-contact contribution to one-bond spin-spin coupling is provided and the origin of the negative one-bond coupling is explained. The obtained results demonstrate that even when the Dirac vector model predicts the correct sign of one-bond coupling the overall picture can be more complicated than what is described by the model. We explain why the standard Dirac vector model may fail and we suggest how to predict the correct sign of the Fermi-contact contribution.

ACKNOWLEDGEMENT

This work received funding from the Slovak Research and Development Agency (grants APVV-19-0516) and VEGA (grant 2/0135/21).

IL-2

NMR structure and dynamic features of quadruplex DNA topologies

Janez Plavec[1,2,3]

[1]*Slovenian NMR center, National Institute of Chemistry, Ljubljana, Slovenia*
[2]*University of Ljubljana, Faculty of Chemistry and Chemical Technology, Slovenia*
[3]*EN-FIST Centre of Excellence, Trg OF 13, Ljubljana, Slovenia*
**e-mail: janez.plavec@ki.si*

Assessment of the impact of somatic mutations in human diseases can benefit from structural insights into DNA and can also facilitate biological interpretation of genome sequence data. A large portion of repetitive elements of DNA lies within the gene regulatory regions. DNA with its canonical Watson-Crick paired duplex plays a major role in inheritance of genetic material and gene expression. Alternative secondary structures including quadruplexes and i-motifs have been associated with many different biological functions of DNA [1-7]. The most well studied noncanonical DNA structures are G-quadruplexes. They are formed by G-rich sequences and consist of four-stranded columnar structures. I-motif formation relies on intercalation of hemi-protonated $C^{+}\cdots C$ base pairs that stabilize quadruplex structure. Our lab recently described a new family of tetrahelical structures that differ significantly from G-quadruplexes despite containing the G-quadruplex folding motif (*e.g.* d[$(GGGN_n)_3GGG$]). These sequences with N_n=AGCGA adopt topologies characterized by the tetrahelical core of AGCGA repeats, connected with edge-type loops of different lengths, stabilized by G-G base pairs in N1-carbonyl symmetric geometry. Notably, different DNA structures open diverse possibilities for targeting through their local and dynamic features.
Our laboratory has been using NMR spectroscopy in combination with complementary methods to uncover structural details of four-stranded DNA architectures in relation to sequence details, presence of cosolutes and inorganic salts, pH, interaction with (heterocyclic) ligands and folding pathways.

ACKNOWLEDGEMENT
The author is grateful to the colleagues named in the cited papers from his laboratory at Slovenian NMR centre. This work was supported partly by the Slovenian Research Agency (ARRS, grants P1-0242, J1-1704, J3-7245 and J7-9399).

REFERENCES
1. E. Ruggiero, I. Frasson, E. Tosoni, M. Scalabrin, R. Perrone, M. Marušič, J. Plavec, S. N. Richter (2022) *ACS Infect. Dis.*, *8*, 958-968.
2. D. Pavc, N. Sebastian, L. Spindler, I. Drevenšek-Olenik, G. K. Podboršek, J. Plavec, P. Šket (2022) *Nat. Commun.*, *13*, 1062.
3. M. L. Živković, M. Gajarský, K. Beková, P. Stadlbauer, L. Vicherek, M. Petrová, R. Fiala, I. Rosenberg, J. Šponer, J. Plavec, L. Trantírek (2021) *Nucleic Acids Res.*, *49*, 2317-2332.
4. S. Takahashi, A. Kotar, H. Tateishi-Karimata, S. Bhowmik, Z.-F. Wang, T.-C. Chang, S. Sato, S. Takenaka, J. Plavec, N. Sugimoto (2021) *J. Am. Chem. Soc.*, *143*, 16458-16469.
5. K. Peterková, I. Durník, R. Marek, J. Plavec, P. Podbevšek (2021) *Nucleic Acids Res.*, *49*, 8947-8960.
6. S. Lago, M. Nadai, E. Ruggiero, M. Tassinari, M. Marušič, B. Tosoni, I. Frasson, F. M. Cernilogar, V. Pirota, F. Doria, J. Plavec, G. Schotta, S. N. Richter (2021) *Nucleic Acids Res.*, *49*, 847-863.
7. S. Bielskutė, J. Plavec, P. Podbevšek (2021) *Nucleic Acids Res.*, *49*, 2346-2356.

IL-3

Structure types of transition metal complexes as potential anticancer drugs

Ján Moncoľ

Institute of Inorganic Chemistry, Technology and Materials, Faculta of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 82107 Bratislava, Slovak Republic

Cisplatin is one of the first metallodrugs introduced into clinical use, and is used to treat many types of cancer. The introduction of cisplanin was the first case of the successful introduction of a metallodrug into the cancer treatment process. Due to the side effects of cisplatin, the emergence of resistance and optimization, other platinum compounds were introduced into clinical practice. Further optimization of the use of metallodrugs for the treatment of cancer led to the preparation of other compounds also based on other metal elements. Two basic ways of effect of metallodrugs are via ligand exchange and/or redox processes.[1] Metallodrugs differ in their structure, which is influenced by the selection of a metal element as the central atom, thus it is possible to influence the effect of the complex to a certain extent.

For example platinum(II) complexes such as cisplatin, carboplatin and oxaliplatin have a conformationally planar structure and act through ligand exchange and chemically bind to DNA bases. Ruthenium(II) or ruthenium(III) complexes prefer an octahedral arrangement and can bind to DNA through intercalation. On the other hand, copper complexes can have different geometries around the central atom, whereby the effect depends on the ligands used. Complexes of copper(II), ruthenium(III), platinum(IV) and gold(III) can be formed through reduction to Cu(I), Ru(II), Pt(II) and Au(I).

This lecture summarizes an overview of metal complexes that are used or have the potential to be used as anti-cancer drugs, their structure and mode of therapeutic effect.

ACKNOWLEDGEMENT

This work was financed by the Scientific Grant Agency of the Slovak Republic (Project: 1/0482/20) and the Slovak Research and Development Agency under contact No. APVV-19-0087.

REFERENCES

1. U. Ndagi, N. Mhlongo,M.E. Soliman.(2017) *Drug Design, Development and Therapy*, 11, 599-616.

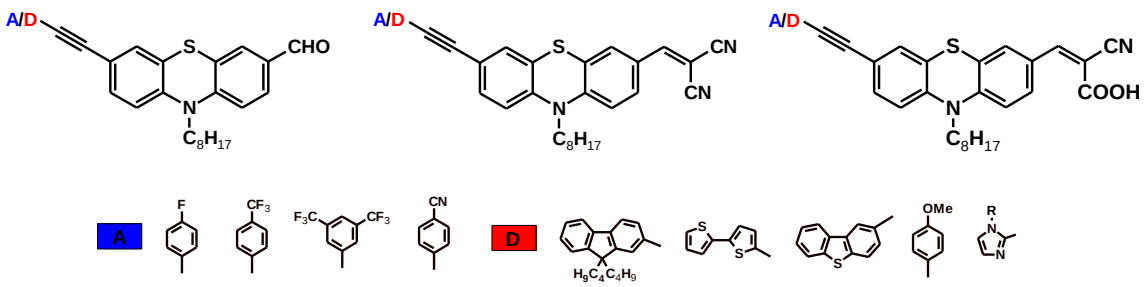
IL-4

Donor-acceptor (D-A) systems based on the phenothiazine unit as modern materials for application in optoelectronics and bioimaging

Aneta Slodek

Institute of Chemistry, Faculty of Science and Technology, University of Silesia, Szkolna 9, Katowice 40-007, Poland; a.slodek@wp.pl

Phenothiazine (PTZ) and its derivatives are interesting heterocycles that comprise electron-rich sulfur and a nitrogen atom. Phenothiazine, as a strong electron-donating group, is primarily used in donor-acceptor (D-A) systems, with its non-planar geometry providing exceptionally excellent photophysical properties. Phenothiazine and mainly its substituted derivatives are readily applied in optoelectronics as emissive layers in organic light-emitting diodes (OLEDs) and as photosensitizers in dye-sensitized solar cells (DSSCs), sensors, bio-imaging [1-3].



Scheme 1. Structures of phenothiazine derivatives.

We present the synthesis and investigation of D/A-π linker-D-A systems based on a phenothiazine framework connected via a π linker with various electron-donating (D) and electron-withdrawing substituents (A) (Scheme 1). The phenothiazine derivatives were designed and synthesized in order to determine the structure's relationship and photophysical properties. The phenothiazine derivatives were obtained via multistep reaction and were entirely characterized by spectroscopic methods (^{1}H NMR, ^{13}C NMR, HRMS). The photophysical properties of the presented compounds were systematically investigated and confronted with DFT and TD/DFT calculations. The phenothiazine derivatives were used as photosensitizer in DSSCs, showing power conversion efficiency (PCE) in the range of 4.22-6.22%, emissive layers in OLEDs, and bio-imaging as fluorescent dyes.
The presented compounds' photophysical properties indicate they are brilliant D-A materials for various applications.

ACKNOWLEDGEMENT
This work was financed by the National Science Centre (Poland) grant PW-2004-105.

REFERENCES

1. A. Slodek, D. Zych, S. Kotowicz, G. Szafraniec-Gorol, S. Zimosz, E. Schab-Balcerzak, M. Siwy, J. Grzelak, S. Maćkowski (2021) *Dyes Pigments*, 189, 109248.

2. A. Slodek, D. Zych, A. Maroń, R. Gawecki, A. Mrozek-Wilczkiewicz, K. Malarz, R. Musioł (2019) *J. Mol. Liq.*, 285, 515-525.
3. A. Slodek, D. Zych, S. Golba, S. Zimosz, P. Gnida, E. Schab-Balcerzak (2019) *J. Mat. Chem. C,* 7, 5830-5840.

IL-5

Similarity-mediated Property Profiling in Computer-assisted Drug Design

Andrzej Bak and Violetta Kozik

Department of Chemistry, University of Silesia, 40-006 Katowice, Szkolna 9, Poland

Finding the critical *in vitro* and/or *in silico* parameters on the hit→lead→seed→drug route is still a monumental challenge in the process of the 'rational production of properties' [1]. Thus, the quantitative mapping of the experimental/calculated properties/descriptors into the ADMET-driven molecular potency is fundamental in the multidimensional structure–activity (mD-QSAR) studies. In fact, molecular similarity is unarguably at the core of many SAR-based property profiling methods, assuming that small variations in structure lead to small changes in activity [2]. On the other hand, the validity of the 'similarity principle' is still questionable, since there is no absolute standard (or metric) of similarity. On the whole, similarity is a subjective concept directly related to aspects of human cognition; this phenomenon is sometimes called 'psychological proximity' [3].

Despite some drawbacks, the idea of specifying a numerical measure of the intermolecular similarity based on the degree of the resemblance between two objects, each described by a number of attributes, has been widely applied in chemical information systems. Obviously, a crucial question that confronts medicinal chemists is, which properties or features should be employed in the receptor-dependent (RD-QSAR) methods that rely on the principle of complementarity or in the receptor-independent (RI-QSAR) ones that are mainly based on the principle of similarity?

In practice, the similarity-driven methodologies have been engaged in the comparable molecular shape analysis (e.g., CoMSA), where machine learning 'fuzzy' techniques are coupled with descriptor elimination/selection algorithms in order to approximate roughly the complex biological reality. Moreover, finding the optimal balance between ADMET-related properties and the desired drug potency ('sweet spot') can be rationalized graphically by extension of the 2D similarity-based projection with the activity data in the form of a 'biological response surface' or SAR landscapes (SALI), where the smooth/flat (homogenous) areas are striated with sharp (heterogeneous) activity cliffs [4]. Additionally, the intermolecular similarity-based examination of the structure/property-related descriptors using the projection procedures (e.g., principal component analysis, self-organizing maps, hierarchical clustering) can be combined with the experimental data (e.g., biological activity, lipophilicity).

As a matter of fact, the clever management of the similarity-related information can provide hints, which might be incorporated at the synthetic stage in order to modulate pharmacological effects and/or demolish unwanted side effects.

REFERENCES

1. J. Kos, V. Kozik, D. Pindjakova, T. Jankech, A. Smolinski, S. Stepankova, J. Hosek, M. Oravec, J. Jampilek, A. Bak (2021) *Int. J. Mol. Sci.*, 22(7), 3444.
2. A. Bak, H. Pizova, V. Kozik, K. Vorcakova, J. Kos, J. Treml, K. Odehnalova, M. Oravec, A. Imramovsky, P. Bobal, A. Smolinski, Z. Trunek, J. Jampilek (2019), *Int. J. Mol. Sci*., 20, 5385.
3. A. Bak, J. Kos, H. Michnova, T. Gonec, S. Pospisilova, V. Kozik, A. Cizek, A. Smolinski, J. Jampilek (2020) *Int. J. Mol. Sci*., 21, 6583.
4. A. Empel, A. Bak, V. Kozik, M. Latocha, A. Cizek, J. Jampilek, K. Suwinska, A. Sochanik, A. Zieba (2021) *Int. J. Mol. Sci*., 22, 12826.

IL-6

The Rule of Amyloid Control: Proglucagon derived polymorphic amyloid 3D-structures and their formation kinetics

Dániel Horváth, Nóra Taricska, Fruzsina Bencs, Zsolt Dürvanger, Máté Sulyok-Eiler, Veronika Harmat Viktor Farkas, András Perczel

Laboratory of Structural Chemistry and Biology & MTA-ELTE Protein Modeling Research Group at the Institute of Chemistry, Eötvös Loránd University, 1117, Pázmány Péter st. 1/A, Budapest, Hungary

Before use, a significant part of hormone-acting oligo- and polypeptides (e.g. glucagon, β-endorphin) are stored (in acidic secretion vesicles, pH~5.2), as functional amyloid in our body. Prior entering the bloodstream, they adopt their native 3D- structure and exert their biological effect by binding to the appropriate GPCR. These key molecules of blood sugar regulation, the gastrointestinal peptides of the glucagon family, adopt two completely different 3D-shapes for their dual role. They are i) amyloids during storage, while ii) they adopt their unique native structures as hormones. We have determined the mechanism of amyloid formation of these polypeptides and identified truncated sections of elevated amyloidicity. We determined the aggregation nuclei of GLP-1, GLP-2, Exenatide and their derivatives by X-ray, and analyzed the polymorphic forms of the amyloid crystalline structures. we studied the molecular background of pH-dependent reversible amyloid formation, by characterizing the complex ECD, VCD and X-ray data driven parameter space: f(c(peptide), pH, T, c(ion), t). Thus, amyloid aggregation of bioactive polypeptides is a double-edged sword! We figured out how the protonation state of the Glu and/or Asp side chains, as molecular switches, controls amyloidicity. Furthermore, we studied how gatekeeper residues (Lys, Arg) regulate the size of the amyloidogenic particles, and why amino acids with aromatic side chains are Janus-faced. In the light of these new data, the aggregation potential of peptide drugs (e.g. Exenatide) is not only a threat while manufacturing, but also an opportunity to develop suitable subcutaneous depot analogues.

REFERENCES

1. J. Author1, R. Author2, ...(2018) *Biochemistry*, 200, 7-10.
2. D. Otzen, R. Riek (2019) *Cold Spring Harb. Perspect. Biol.,* 11 (12), a033860. https://doi.org/10.1101/cshperspect.a033860.
3. N. Taricska, D. Horváth, D. K. Menyhárd, H. Ákontz-Kiss, M. Noji, M. So, Y. Goto, T. Fujiwara, A. Perczel (2020) *Chemistry A European Journal, 2*6 (9), 1968-1978. doi.org/10.1002/chem.201903826.
4. D. Horváth, D.K. Menyhárd, A. Perczel (2019) *Current Protein & Peptide Science,* 20 (11), 1077-1088.

IL-7

The effect of the mutations on the dynamic motion of the N-terminal domain of the human ryanodine receptor 2: towards deeper understanding of cardiac arrhythmias

Jacob Bauer and Vladena Bauerová-Hlinková

Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 845 51 Bratislava, Slovakia

The human cardiac ryanodine receptor (hRyR2) is the ion channel responsible for the release of Ca^{2+} ions from the sarcoplasmic reticulum into the cytosol. It plays an important role in cardiac muscle contraction. Mutations of this channel are associated with inherited cardiac arrhythmias, including CPVT1 and ARVC/D2, LQTS, SUO, SCD and SIDS, which decrease quality of the life and in some cases cause death [1]. These mutations appear to cluster in distinct parts of the hRyR2 channel: the N-terminal, central and C-terminal. We used molecular dynamics simulation to examine the effects of three disease-associated mutations: R414L, I419F and R420W, which are located in the N-terminal region of hRyR2, on the dynamics of this domain [2]. We find that the R414L and I419F mutations diminish the overall amplitude of motion without greatly changing the direction of motion of the individual domains, whereas R420W both enhances the amplitude and changes the direction of motion. Based on these results, we hypothesize that R414L and I419F hinder channel closing, whereas R420W may enhance channel opening [2].

ACKNOWLEDGEMENT

Authors thank to Eva Kutejová for general support while performing this study. This work was financially supported by the Slovak Grant Agency VEGA, grants 2/0140/16 and 2/0131/20.

REFERENCES

1. V. Bauerova-Hlinkova, D. Hajdúchová (2020) *J.A. Bauer. Molecules,* 25, 4040.
2. J.A. Bauer, Ľ. Borko, J. Pavlović, E. Kutejová, V. Bauerová-Hlinková (2020) *J. Biomol. Struct. Dyn*., 38, 1054-1070.

IL-8

Should we have complexes with terpyridines?

Robert Musiol

Faculty of Science and technology, University of Silesia in Katowice, Poland

Polypyridine systems, are particularly known for their ability to form complexes with various metal cations. In this regard, the bipyridines and even more terpyridines were investigated in detail. The large fused aromatic scaffold that is available in terpyridines makes them typical non-innocent ligands [1]. Their contribution to the electronic energy of the central metal results in a less-defined oxidative state on this atom and permits the redox activity. This feature opens the possibility of creating a variety of applications in supramolecular chemistry [2], photovoltaic cells [3], pollutant degradation [4] or catalysis. The low redox barrier in terpyridine complexes also contributes to their biological activity including antiprotozoal [5], anti-tubercular [6] or antifungal [7] and the anticancer potency that have been probably the most explored filed. The terpyridines were investigated as complexes with transition metals among whose first row and selected metals for the next rows such as Pt, Pd and Au are predominant. Recently, an Ru-complex with a polypyridyl system (TLD1433) has entered clinical trials as photosensitizer that is used in the photodynamic therapy of bladder cancer [8]. One of the prerequisites that determines the success of metal-based drugs is a specific three-dimensional configuration and electronic potential, which are unavailable in more typical, purely organic molecules. A wide range of properties and biological activities can be obtained in combination with various organic ligands. Trivial to say, the complexes cannot be investigated in isolation from their ligands. Nevertheless in case of terpyridines the studies on biological activity have been conducted mostly on complexes and independently from ligands. This has resulted in a strong biased opinion about the biological potency of the terpyridines and their complexes, leading to situation when conclusions about the specific structure have been drawn without a proper scientific background. Indeed, today it is easier to find a statement that specific Tpy complexes are more active than their ligands than sound comparison of the data for either. On the other hand, the compounds with highly and independent from complexing phenomena activity have been described among terpyridines. Moreover the complexes themselves may be not as stable as expected in many approach and the effect that was really observed may be a result of different interactions. Finally the mechanism of action can be different for ligands and their complexes. Therefore a critical analysis of the literature data is necessary to reveal what really have been done so far.

ACKNOWLEDGEMENT

This work was financed by National Science Center (grant number: 2018/31/B/NZ7/02122).

REFERENCES

1. C. Wei, Y. He, X, Shi, et al. (2019) *Coord. Chem. Rev.,* 15, 1-19.
2. G. Schwarz, I. Hasslauer, D.G. Kurth (2014) *Adv. Colloid Interface Sci.,* 207, 107-20.
3. D. Saccone, C. Magistris, N. Barbero, et al. (2016) *Materials,* 27, 3.
4. Y.X. Ye, C. Wen, J.W. Wang, et al. (2020) *Chem. Commun.,* 21, 5476-5479.
5. A. Uneja, T.S. Macedo, D.R. Magalhaes Moreira, et al. (2014) *Eur. J. Med. Chem*., 21, 203-10.
6. T.B. Hadda, M. Akkurt, M.F. Baba, et al. (2009*) J. Enzyme Inhib. Med. Chem.,* 24, 457-63.
7. A. Anthonysamy, S. Balasubramanian, V. Shanmugaiah (2008) *Dalton Trans.*, 28, 2136-43.
8. S. Monro, K.L. Colon, H. Yin (2019) *Chem. Rev.,* 23, 797-828.

IL-9

Lon protease – the essential component of mitochondrial homeostasis.

Eva Kutejova[1], Nina Kunová[1], Gabriela Ondrovičová[1], Barbora Stojkovičová[1], Ján Frankovský[2], Sami Kereïche[3], Veronika Lukáčová[4], Veronika Krajčovičová[1], Henrieta Havalová[1], Jacob A. Bauer[1], Vladimír Pevala[1], Peter Baráth[4,5], Ľubomír Tomáška[2]

[1] *Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, Bratislava, Slovakia*
[2] *Department of Genetics, Comenius University in Bratislava, Faculty of Natural Sciences, Bratislava, Slovakia*
[3] *Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague, Czech Republic*
[4] *Medirex Group Academy, Nitra, Slovakia*
[5] *Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*

Mitochondria are essential semiautonomous organelles present in most eukaryotic cells. In humans, their malfunction often leads to severe pathologies, including myopathies, neurodegenerative diseases, and cancer. The majority of mitochondrial proteins is encoded by the nuclear DNA, synthetized in the cytosol and transported into the mitochondria, where they combine with subunits synthesized within the mitochondria themselves and form the essential protein complexes. For proper functioning, mitochondria possess their own protein quality control systems consisting mainly of ATP-dependent proteases and chaperones [1]. Lon is a unique mitochondrial ATP-dependent protease that forms specific hexameric structure where both, ATPase and protease domains, are located on the same subunit. The first full-length human Lon structures determined by cryo-EM have shown that ATP hydrolysis induces conformational changes to the Lon hexamer and the N-terminal domain plays a crucial role in stability and activity of the enzyme [2]. The rearrangement of Lon's structure is essential for degradation of its substrates, including the subunits of mitochondrial processing peptidase MPP, StAR protein, helicase Twinkle and ribosomal subunit MrpL32 in human cells, and mtDNA-packaging protein Abf2 and mtDNA-maintenance factor Mgm101 in *S. cerevisiae* [3,4]. The susceptibility of most of these proteins to Lon is altered by their binding to a nucleic acid, which might have a profound effect on mtDNA replication, transcription, and translation. Moreover, it was shown that Abf2 could be succinylated *in vivo* that affects not only its DNA-binding properties, but also its digestion by Lon representing an important mechanism involved in stabilization and maintenance of yeast mtDNA [5]. Such regulation might facilitate dynamic changes to mitochondrial nucleoids and ribosomes, which are crucial for conducting mitochondrial functions and maintaining mitochondrial homeostasis *in vivo* and could also have significant implications for human medicine.

ACKNOWLEDGEMENT

This work was supported by Slovak Grant Agency (VEGA 2/0069/23 and 1/0061/20), Slovak Research and Development Agency (APVV-15-0375, APVV-19-0298, APVV-15-0022 and APVV-19-0068) and Interreg V-A Slovakia-Austria program (www.sk-at.eu) for the StruBioMol project, ITMS: 305011X666 and Operational Program Integrated Infrastructure for the project: Center for Biomedical Research - BIOMEDIRES - II. stage, ITMS: 313011W428, co-financed by the European Regional Development Fund.

REFERENCES

E. Kutejová (2008). ATP-Dependent Proteases. Research Signpost ISBN: 978-81-308-0282-4

S. Kereïche, L. Kováčik, J. Bednár, V. Pevala, N. Kunová, G. Ondrovičová, J. Bauer, Ľ. Ambro, J.Bellová, E. Kutejová, I. Raška (2016). *Sci. Rep.* **6**, 33631

G. Ondrovicova, T. Liu, K. Singh, B. Tian, H. Li, O. Gakh, D. Perecko, J. Janata, Z. Granot, J. Orly, E. Kutejova, C.K. Suzuki (2005). *J Biol Chem.* **280**, 25103-25110

N. Kunová, G. Ondrovičová, J.A. Bauer, J. Bellová, Ľ. Ambro, L. Martináková, V. Kotrasová, E. Kutejová, V. Pevala (2017). *Sci. Rep.* **7**, 631

J. Frankovský, B. Keresztesová, J. Bellová, N. Kunová, N. Čanigová, K. Hanakova, J.A. Bauer, G. Ondrovičová, V. Lukáčová, B. Siváková, Z. Zdrahal, V. Pevala, K. Procházková, J. Nosek, P. Baráth, E. Kutejova, L. Tomaska (2021). *J Biol Chem.* **297** (4):101155

IL-10

Degradation-free intracellular delivery of nanomolar protein cargoes with ganglioside-specific recognition tags

Anasztázia Hetényi,[1] Norbert Imre,[1] Enikő Szabó,[2] Brigitta Bodnár,[1] Abel Szkalisity,[4] Ilona Gróf,[3] Alexandra Bocsik,[3] Mária A. Deli,[3] Peter Horvath,[4] Ágnes Czibula,[2] Éva Monostori,[2] Tamás A. Martinek[1]

[1]Department of Medical Chemistry, University of Szeged, Szeged, Hungary,
[2]Institute of Genetics, Biological Research Center, Szeged, Hungary
[3]Institute of Biophysics, Biological Research Center, Szeged, Hungary
[4]Synthetic and Systems Biology Unit, Biological Research Center, Szeged, Hungary

There is a pressing need to develop ways to deliver therapeutic macromolecules to their intracellular targets. Certain viruses and bacterial toxins exploit lipid raft-mediated endocytosis to deliver macromolecules in their functional form without degradation. We aimed to mimic this entrapment- and lysosomal degradation-free endocytic pathway. These proteins of viral and bacterial origins trigger the desired intracellular delivery through binding to gangliosides at the caveolar entry points. We found a pentapeptide sequence WYKYW and its α,β-peptidic derivatives that specifically capture the glycan moiety of ganglioside GM1 (K_D = 24 nM), which is the most abundant glycolipid in the caveolar pits and overexpressed in cancer cells. Tested on live HeLa, D3, Jurkat, and CaCo cells, the WYKYW-tag facilitated the GM1-dependent selective caveolar endocytosis of proteins in which the cargo-loaded caveosomes did not fuse with lysosomes [1]. An immunoglobulin G complex (580 kDa) was successfully delivered into live cells at extracellular concentrations ranging from 20 to 160 nM. The antibodies' molecular recognition regions (Fv and Fc) remained functional after 24 hours, and the functional cargo protein escaped to the cytosol. We found that a single pentapeptidic segment was sufficient to trigger the lipid raft-mediated/caveolar endocytosis. The endocytosis routing tag WYKYW was not toxic even in the high micromolar region. Our receptor-based approach is a valuable alternative cell delivery method because the very short, easily applicable, and non-toxic tag facilitates the advantageous lipid raft-mediated/caveolar endocytosis in a carrier-triggered manner. It works at therapeutically relevant concentrations for many cell types expressing ganglioside GM1. The endocytosis routing tag can be attached with the already existing chemistry for the PEGylation protocols.

ACKNOWLEDGEMENT
This research was funded by the National Research, Development and Innovation Office of Hungary, grant number GINOP-2.2.1-15- 2016-00007, the Hungarian Ministry of Innovation and Technology, TUDFO/47138-1/2019-ITM, and the Hungarian National Brain Research Program (MTA-SE-NAP B-BIOMAG).

REFERENCES

1. N. Imre (2020) *Advanced Science*, 7, 1902621.

IL-11

A new Slovak center for research and teaching activities in structural biology – Interreg V-A Slovakia – Austria project StruBioMol

Vladimír Pevala[1], Gabriela Ondrovičová[1], Nina Kunová[1], Barbora Stojkovičová[1], Lucia Martináková[1], Henrieta Havalová[1], Vladena Bauerová[1], Jacob A. Bauer[1], Július Košťan[2], Kristina Djinović-Carugo[2], Eva Kutejová[1]

[1]Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, Bratislava, Slovakia
[2]Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Campus Vienna Biocenter 5, A-1030 Vienna, Austria

Biomedicine and biotechnology are modern scientific disciplines, the development of which contributes to improving both, the health of the population and the environment. Structural biology identifies the nature of serious illnesses and forms the basis for a design of potential drugs. It also allows searching for potentially harmful substances and studying their impact on the environment. What was lacking in our region is a common educational and scientific research base that would train and educate professionals capable of transferring current knowledge in this field into the practice. The goal of the project - *Building teaching and research capacities in structural and functional analysis of biomolecules for the needs of biomedicine and biotechnology* - was to set up a joint cutting-edge structural biology education and research center to educate PhD students, researchers and practitioners in the Bratislava - Vienna region. When building the center, we have used the experience of both partners, as well as established international cooperation. The lectures and training seminars have been organized alternately in both workplaces to attract the widest possible community and to encourage mutual contacts between PhD students and researchers in Vienna and Bratislava with the perspective of further cooperation. The individual objectives of the project include: a) strengthening the long-term and sustainable basis for the development of structural biology in the region; b) education of university students, PhD students, researchers and practitioners; (c) building a research center of excellence in structural biology for the study of human diseases. The long-term benefits will create a base for high quality education, providing guidance and excellent research in the field of biomedicine and biotechnology, which will contribute to increasing the competitiveness of the region and will attract foreign experts' interest in cooperation and the transfer of acquired knowledge into practice.

ACKNOWLEDGEMENT
The authors have received support from the Interreg V-A Slovakia-Austria program (www.sk-at.eu) for the project StruBioMol, ITMS: 305011X666 and is cofinanced by the European Regional Development Fund.

IL-12

Mass spectrometry in glycoconjugate analysis

Zuzana Pakanová, Marek Nemčovič, Rebeka Kodríková, Maroš Krchňák, Filip Pančík, Filip Květoň, Ján Mucha, Peter Baráth

Institute of Chemistry, Slovak Academy of Sciences, Bratislava

Post-translational modification of proteins by glycosylation significantly affects their structure, folding, activity, recognition, and their overall function [1]. Structurally, glycosylation is also known to affect the three-dimensional configuration of proteins and their protein-protein interactions [2]. Comprehensive knowledge of glycosylation is crucial for biopharmacological products (such as therapeutic antibodies and enzymes) or other recombinant proteins expressed by hosts different from the protein's primary origin. Furthermore, protein glycosylation has been implicated in many human diseases and there is still a large effort for improving their reliable diagnostics based also on glycobiomarker discovery. However, analysis of glycoconjugates by mass spectrometry (MS), due to their low ionization efficiency and lability in the ion sources, remains a challenge. The most popular approaches are based on i.) the determination of intact glycoprotein mass; ii.) analysis of released *N*-glycans by (LC)-MS after their derivatization; and iii.) identification of glycosylation sites and their occupancy by proteomic analysis of heavy atom labelled peptides by nanoLC-MS/MS. In this work, successful application of all above-mentioned workflows in the diagnostics of rare diseases, non-invasive determination of specific glycobiomarkers and determination of post-translational modifications of recombinant proteins as the part of basic research is presented.

ACKNOWLEDGEMENT

This work was supported by Ministry of Health of the Slovak Republic under the project registration number 2019/7-CHÚSAV-4 and VEGA 2/0060/21 from Slovak Grant Agency for Science. This publication was created with the support of the Operational Program Integrated Infrastructure for the project ITMS: 313021Y920, co-financed by the European Regional Development Fund.

REFERENCES

1. A. Sarkar, P.L. Wintrode (2011) *Int J Mass Spectrom*, 302 (1-3), 69-75.
2. B.J. Arey (2012) In *Glycosylation*, ed. Stefana Petrescu. London: IntechOpen.

IL-13

Transition metal complexes for cancer therapy

Pavel Štarha

Department of Inorganic Chemistry, Faculty of Science, Palacký University Olomouc, 17. listopadu 1192/12, 779 00 Olomouc, Czech Republic

Bioinorganic chemistry is a field of chemistry that deals with the functions of metals (metal ions) in biological systems. It covers various topics, such as metal ion transport and storage, enzymology or metals in medicine. Regarding inorganic medicines, platinum-based anticancer metallodrugs (e.g. cisplatin or oxaliplatin) represent a best-known example, since they are used world-wide for more than forty years for the treatment of various types of cancer [1]. Pt-based drugs were followed by numerous complexes of other transition metals, which have been reported in the literature as having some kind of biological activity [2]. Some of these complexes have entered the clinical trials on human patients, which could be exemplified by currently clinically tested anticancer ruthenium (IT-139) and palladium (TLD1433) compounds [3,4].

Generally speaking, unique properties of transition metal complexes offer the chemists the choice of metal and its oxidation state, as well as the choice of ligand and its coordination mode and derivatization. In combination with various types of cytotoxic action (DNA interaction, protein targeting, redox status mediation etc.) and constantly increasing possibilities of biological analysis (animal xenografts, proteomics etc.) we obviously get an infinite number of works dealing with bioactive metal complexes.

In our laboratory, we deal with the development of new biologically active complexes of various transition metals. In this contribution, some recently investigated complexes derived from various metals (e.g. Ir, Os, Ru, Rh, Ta or Pt) will be discussed [5–8]. An attention will be paid to their anticancer activity as well as to their in-solution behaviour in the presence of various relevant naturally-occurring biomolecules, such as reduced nicotinamide adenine dinucleotide (NADH), glutathione (GSH) or ascorbate.

ACKNOWLEDGEMENT

This work was financed the institutional sources of the Department of Inorganic Chemistry, Palacký University in Olomouc (Czech Republic) is acknowledged.

REFERENCES

1. L. Kelland (2007) *Nat. Rev. Cancer*, 7, 573-584.
2. E.J. Anthony, E.M. Bolitho, H.E. Bridgewater, O.W.L. Carter, J.M. Donnelly, C. Imberti, E.C. Lant, F. Lermyte, R.J. Needham, M. Palau, P.J. Sadler, H. Shi, F.-X. Wang, W.-Y. Zhang, Z. Zhang (2020) *Chem. Sci.*, 11, 12888-12917.
3. M.R. Berger, F.T. Garzon, B.K. Keppler, D. Schmahl (1989) *Anticancer Res.*, 9, 761–765.
4. S. Monro, K.L. Colón, H. Yin, J. Roque III, P. Konda, S. Gujar, R.P. Thummel, L. Lilge, C.G. Cameron, S.A. McFarland (2019) *Chem. Rev.*, 119, 797-828.
5. P. Štarha, Z. Trávníček, Z. Dvořá (2018) *Chem. Commun.*, 54, 9533.
6. L. Masaryk, D. Muthná, P. Halaš, P. Zoufalý, E. Peterová, R. Havelek, B. Drahoš, D. Milde, A. Mrkvicová, P. Štarha (2022) *Dalton Trans.*, 51, 9213.
7. L. Masaryk, J. Orvoš, K. Słoczyńska, R. Herchel, J. Moncol, D. Milde, P. Halaš, R. Křikavová, P. Koczurkiewicz-Adamczyk, E. Pękala, R. Fischer, I. Šalitroš, I. Nemec, P. Štarha (2022) *Inorg. Chem. Front.*, Advance Article, DOI: 10.1039/D2QI00535B.
8. L. Masaryk, D. Weiser Drozdková, K. Ondrušková, I. Nemec, K. Smešný Trtková, P. Štarha, patent application PV 2021-444 (2021).

IL-14

Bioinformatics analysis of the family GH13 trehalose synthases with focus on their maltokinase-like domain

Ľubica Urbániková, Štefan Janeček

Laboratory of Protein Evolution, Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, SK-84551 Bratislava, Slovakia

The Carbohydrate-Active enZymes Database (CAZy; http://www.cazy.org) is a specialized sequence-based database classifying the enzymes that degrade, modify or create glycosidic bonds into families [1]. Glycoside hydrolases (GHs), which catalyze the hydrolysis and/or rearrangement of glycosidic bonds, represent one of the enzyme classes included in CAZy with currently established 183 GH families. The family GH13, known as the main α-amylase family [2,3], is one of the largest GH families [1]. Currently, it counts ~130,000 members covering more than 30 different specificities [4], that have been until now divided into 44 official GH13 subfamilies [5]; the additional subfamilies being still awaited [6]. The present *in silico* study has been focused on trehalose synthases (TreSs) from the subfamily GH13_16 [1], which is – in a wider sense – a member of the so-called oligo-1,6-glucosidase subfamily [7]. TreS converts maltose to trehalose and also vice versa [8]. Typically, it consists of the three-domain family GH13 canonical arrangement with a catalytic $(\beta/\alpha)_8$-barrel domain A, domain B (mostly of irregular structure) protruding out of the barrel in the place of the loop 3 and domain C (a 7-stranded antiparallel β-sandwich) at the C-terminus [3]. In some GH13_16 enzymes, however, the domain C is succeeded by a C-terminal extension exhibiting clear sequence features of a maltokinase (MaK) [9,10]. Thus of total 5,933 GH13_16 members available (October 14, 2021), fragments and duplicated/identical sequences were first excluded yielding a set of 3,365 sequences. These were subsequently divided into two groups: (i) “single” TreSs – a group of 1,922 members with a C-terminal extension shorter than 110 residues and where no obvious additional MaK-like domain was found; and (ii) “fused” TreS-MaK – a group of 1,430 members with a C-terminal extension longer than 400 residues, where the full-length MaK domain was detected. The remaining 13 TreSs with a C-terminal extension varying between 110-540 residues were excluded because none or only a fragment of a MaK was detected there. MaKs are single-domain enzymes that catalyze ATP-dependent phosphorylation of maltose at position 1 [10]. The sequences of MaK domains from both fused TreS-MaK enzymes and single MaKs (i.e. those without a GH13_16 TreS) were aligned and conserved sequence regions (CSRs) were defined. Enzymes with identical CSRs were then excluded to give a final set of 222 MaK domain sequences from the fused TreS-MaK group. Finally, 17 characterized MaKs were added to the studied set of sequences. The analysis revealed that only 99 MaK domains from fused TreS-MaKs may represent standard MaKs with conserved catalytic machinery and maltose-binding residues. In contrary, 71 domains possess mutations in maltose-binding residues (their catalytic function being also questionable) and 52 domains cannot be enzymatically active because either one or both catalytic aspartates are absent. Since the presented work delivers a detailed bioinformatics analysis of fused TreS-MaK enzymes for the

first time, it might contribute to a better characterization of both TreSs and MaKs, and thus help in the study of the role of MaK/MaK-like domains in the subfamily GH13_16 enzymes.

ACKNOWLEDGEMENT

This work was financially supported by the grant No. 2/0146/21 from the Slovak Scientific Grant Agency VEGA.

REFERENCES

1. E. Drula E., M.L. Garron, S. Dogan, V. Lombard, B. Henrissat, N. Terrapon (2022) *Nucleic Acids Res.*, 50, 571-577.
2. E.A. MacGregor, S. Janecek, B. Svensson (2001) *Biochim. Biophys. Acta*, 1546, 1-20.
3. S. Janecek, B. Svensson, E.A. MacGregor (2014) *Cell. Mol. Life Sci.*, 71, 1149-1170.
4. S. Janecek, B. Svensson (2022) *Amylase*, 6, 1-10.
5. M.R. Stam, E.G. Danchin, C. Rancurel, P.M. Coutinho, B. Henrissat (2006) *Protein Eng. Des. Sel.*, 19, 555-562.
6. F.D. Sarian, S. Janecek, T. Pijning, Ihsanawati, Z. Nurachman, O.K. Radjasa, L.Dijkhuizen, D. Natalia, M.J. van der Maarel (2017) *Sci. Rep.*, 7, 44230.
7. A. Oslancova, S. Janecek (2002) *Cell. Mol. Life Sci.*, 59, 1945-1959.
8. T. Sugimoto, M. Kurimoto, Y. Tsujisaka (1995) *Biosci. Biotechnol. Biochem.*, 59, 2189-2190.
9. Y. Wang, J. Zhang, W. Wang, Y. Liu, L. Xing, M. Li (2012) *Extremophiles*, 16, 377-385.
10. A. Drepper, K. Peitzmann, H. Pape (1996) *FEBS Lett.*, 388, 177-179.

IL-15

Materials for medicine - bioactive composites and coatings

Agnieszka Sobczak-Kupiec, Dagmara Słota, Wioletta Florkiewicz, Karina Piętak, Mateusz Dyląg, Agnieszka Tomala, Magdalena Głąb, Anna Drabczyk, Sonia Kudłacik-Kramarczyk, Bożena Tyliszczak

Department of Materials Science, Faculty of Materials Engineering and Physics, Cracow University of Technology, 37 Jana Pawła II Av., 31-864 Krakow, Poland

Biomaterials, also known as biomedical materials, which can be used to manufacture devices and components that come into direct contact with the body's tissues. They play an integral role in today's medicine - restoring function and facilitating the treatment of patients after injury or disease [1]. They can be of natural or synthetic origin and are used in medicine to support, strengthen or replace damaged tissues or biological functions. Metals, ceramics, polymers and even living cells as well as tissues can be used to create biomaterials. Materials engineering provides opportunities to design new and innovative composite materials by combining one or more of them [2]. Appropriate selection makes it possible to compensate for the disadvantages of each or to provide additional functions.

In the aspect of bone tissue, the polymer-ceramic combination is an interesting solution. The polymer, by itself, is not able to transmit stress, however, suspending the bioactive ceramic in it improves mechanical strength, and adds bioactivity to the whole system towards osteointegration properties [3]. Moreover, with the impressive properties of the polymer network, additional modification with drugs, proteins or other biomolecules is possible, creating a highly bioactive material with the character of an active substance carrier [4]. Such systems can be used either as implants, fillers or coatings, applied to coat other implants (such as metallic endoprosthesis).

ACKNOWLEDGEMENT

The "Multifunctional biologically active composites for applications in bone regenerative medi-cine" project is carried out within the TEAM-NET program of the Foundation for Polish Science financed by the European Union under the European Regional Development Fund. The authors gratefully acknowledge financial support. POIR.04.04.00-00-16D7/18.

REFERENCES

1. L.L. Hench, I. Thompson, Twenty-first century challenges for biomaterials (2010) *The Royal Society Publishing*, 7, S379-S391.
2. B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons, Biomaterials Science: An Introduction to Materials in Medicine, (2006) *MRS Bulletin*, 31, 58-60.
3. K.P. Nobles, A.V. Janorkar, R.S. Williamson, Surface modifications to enhance osseointegration–Resulting material properties and biological responses (2021), *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 109, 1909-1923.
4. S.P. Adithya, D.S. Sidharthan, R. Abhinandan, K. Balagangadhara, N. Selvamurugan, Nanosheets-incorporated bio-composites containing natural and synthetic polymers/ceramics for bone tissue engineering (2020) *International Journal of Biological Macromolecules*, 164, 1960-1972.

OP-1

Cryo-EM structure of acylpeptide hydrolase: substrate selection by multimerization and a multi-state serine-protease triad

Anna J. Kiss-Szemán[1], Pál Stráner[2], Imre Jákli[1,2], Naoki Hosogi[3], Veronika Harmat[1,2], Dóra K. Menyhárd[1,2], András Perczel[1,2]

[1]*Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Budapest – 1117, Hungary.*

[2]*ELKH-ELTE Protein Modelling Research Group, Budapest – 1117, Hungary.*

[3]*EM Application Department, EM Business Unit, JEOL Ltd., Tokyo 196-8556, Japan.*

The first structure of tetrameric mammalian acylaminoacyl peptidase (AAP) was determined by cryo-EM at 3.27 Å resolution and was further elucidated by MD simulations [1]. AAP functions as an upstream regulator of the proteasome [2] through the removal of terminal *N*-acetylated residues from its protein substrates [3]. Tetramerization guided by an interaction-prone beta-edge and unique inserts leads to self-compartmentalization that equips the enzyme with its "channels-and-shutters" substrate-selection system. The active site is unique also: with a *Pro* inserted into the central beta-sheet of the hydrolase domain, conformational freedom is awarded the active Ser587, so that the classical serine protease catalytic triad alternates between active and inactive conformations. Active site flexibility suggests that the dual function of catalysis and substrate selection are fulfilled by a novel mechanism: substrate entrance is regulated by flexible loops of the double-gated channel system, while binding of the substrate to the active site is required for stabilization of the catalytic apparatus - as a second filter before hydrolysis. In case of mammalian AAP, multimerization is a prerequisite of controlled catalytic function. The determined structure of *p*AAP not only provides a sufficient model of the human enzyme that will allow drug design efforts, but also contributes to our understanding of the significance and mechanisms of protein multimerization.

ACKNOWLEDGEMENT

This work was supported by project no. 2018-1.2.1-NKP-2018-00005 of the National Research Development and Innovation Fund of Hungary; No. VEKOP-2.3.2-16-2017-00014 and VEKOP-2.3.3-15-2017-00018 of the European Union and the State of Hungary, co-financed by the European Regional Development Fund; by MedInProt Grants from the Hungarian Academy of Sciences; and within the framework of the Thematic Excellence Program 2019 by the National Research, Development and Innovation Office under project "Synth+" as well as the Hungarian Scientific Research Fund (NKFIH-OTKA) grant K116305.

REFERENCES

1. A.J. Kiss-Szemán et al. Cryo-EM structure of acylpeptide hydrolase reveals substrate selection by multimerization and a multi-state serine-protease triad. (2022) *Chem. Sci.,* published online https://doi.org/10.1039/D2SC02276A.
2. G. Palmieri et al. Acylpeptide hydrolase inhibition as targeted strategy to induce proteasomal down-regulation. (2011) *PLoS ONE,* 6 (10), e25888.
3. R.G. Krishna, F. Wold. Specificity determinants of acylaminoacyl-peptide hydrolase. (1992) *Protein Sci.*, 1 (5), 582-589.

OP-2

A study of the photochemical behaviour and relaxation mechanisms of *anti-syn* isomerisation around quinazolinone –N–N= bonds

James R. Asher,[1] Michal Hricovíni,[2] and Miloš Hricovíni,[2]

[1]*Institute of Inorganic Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 36 Bratislava, Slovakia*
[2]*Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 36 Bratislava, Slovakia*

High-resolution NMR experiments revealed that differently substituted quinazolinone-based Schiff bases undergo anti to syn isomerisation on exposure to ultraviolet light in DMSO solution [1]. The anti to syn conversion varied significantly upon substitution (between 5% and 100%) and also showed two noteworthy features: that relaxation back to the anti-form goes far faster (by at least 3 orders of magnitude) when the C6 rings B and C have ortho-OH substituents, and that relaxation can also be significantly sped up by addition of acid. Two possible mechanisms explaining the differences in relaxation process have been proposed: (I) the interaction of the azomethine hydrogen with the carbonyl oxygen results in slowing down reversion to the anti-form and/or (II) suppression of conjugation of the N3 lone pair with the N=CH double bond by protonation and/or internal H-bonding. Both these mechanisms have been analysed theoretically.

ACKNOWLEDGEMENT
The authors acknowledge financial support from the Slovak Grant Agency VEGA, grants no. 2/0135/21 and 2/0071/22, and the Slovak Research and Development Agency, grant APVV-19-0516.

REFERENCES
1. M. Hricovíni, J. Asher and M. Hricovíni (2020) *RSC Adv.*, 10, 5540–5550.

OP-3

Towards a new biomarker for Diabetic Retinopathy: exploring RBP3 structure and retinoids binding for eyes functional imaging in vivo

Vineeta Kaushik, Luca Gessa, Nelam Kumar, Humberto Fernandes

Integrated Structural Biology Lab, International Centre for Translational Eye Research, Institute of Physical Chemistry, Warsaw, Poland

Diabetic Retinopathy (DR) is a complication of diabetes, affecting a growing number of people, and is a significant cause of blindness in developed and developing countries. Currently, treatment is limited and applied at later stages of the disease and consists of injection of anti-VEGF (vascular endothelial growth factor) agents directly in the eye. Before such drastic options, efficient control of the disease can be achieved by strict diet and blood sugar control. Current diagnostics are based on Fluorescein angiography and OCT (Optical coherence tomography), with AI (Artificial Intelligence) gaining ground and promising more accurate diagnostics. But all of them are based on physical blood vessel alterations or lipofuscin deposits, and the search for other biomarkers that could potentially allow an early detection is important. It would allow an early lifestyle correction and thus minimize DR consequences by delaying or slowing down its progression. A link has been revealed between retinal binding protein 3 (RBP3) and the severity of DR [1, 2]. The studies indicate that patients with decreased levels of RBP3 have more severe DR, and have shown that supplementation of RBP3 confers DR protection [2]. RBP3 is a protein located in the interphotoreceptor matrix in the retina and supports the shuttling of retinoids between photoreceptors and RPE (retinal pigment epithelium) cells in the retina, thus allowing the visual cycle. It is composed of 4 modules, each able to bind different retinoids.

The ophthalmologic field has recently been reporting exciting new functional imaging capacities, such as two-photon excitation (TPE) fluorescence (TPEF) imaging. The technique overcomes tissue penetration and prohibitive excitation wavelength by simultaneous excitation by two photons with longer wavelengths, resulting in shorter wavelength emission light. It was validated in mouse models, proven safe in human subjects [3], and already used to detect different retinoids in retinas [4]. We are characterizing RBP3 and its ligand binding structurally and biophysically as a starting point to prepare complexes with modified ligands (or specific antibodies). The modified reagents will have unique excitation signatures that will allow detection and quantification of RBP3 levels using TPEF imaging. Thus, opening the possibility for early detection of DR.

ACKNOWLEDGEMENT

This work was financed by the International Research Agendas programme of the Foundation for Polish Science (MAB/2019/12) co-financed by the European Union under the European Regional Development Fund, and by the PASIFIC Fellowship programme of the Polish Academy of Sciences.

REFERENCES

1. M. Garcia-Ramirez, C. Hernandez et al. (2009) *Diabetologia,* 52 (12), 2633-2641.
2. H. Yokomizo, Y. Maeda et al. (2019) *Sci. Transl. Med.*,11 (499), eaau6627.
3. J. Boguslawski, G. Palczewska et al. (2022) *J. Clin. Invest.*, 132 (2), e154218.
4. G. Palczewska, P. Stremplewski et al. (2018) *JCI Insight*., 3 (17), e121555.

OP-4

Epitachophoresis - new tool for nucleic acid purification/concentration

[1]František Foret, Ivona Voráčová, Jan Přikryl, Jakub Novotný
[2]Datinska, V., Gheibi, P., Jefferson, K., Yang, J., Paladugu, S., Dallett, C., Astier, Y.

[1]Czech Academy of Sciences, Institute of Analytical Chemistry, Brno, Czech Republic
[2]Roche Sequencing Solutions, Inc., Pleasanton, USA

Most bioanalytical applications require sample pre-separation. Non-affinity-based separation methods such as electrophoresis take advantage of differences in electro-migration to separate and concentrate selected analytes from crude samples. Recently, we have introduced epitachophoresis as a new electrophoretic technique for the concentration and separation of nucleic acids from milliliter sample volumes.
This communication reports on new instrumental systems for processing the crude samples by discontinuous electrophoresis in a circular arrangement – epitachophoresis (ETP) with almost unlimited concentration factor. Several experimental parameters have been studied, including the size, shape, and size of the zone stabilizing media and devices for large samples. Polyacrylamide or agarose gels are the most frequently used sieving and stabilizing media in slab gel electrophoresis; however, such sieving materials limit the size of the concentrated nucleic acids. In part of this work, we have also explored large pore materials and 3D printing to form rigid stabilizing manifolds to minimize liquid flow during the epitachophoresis. The device was printed using the stereolithography technique from a low water-absorbing resin. Different geometries of the 3D printed stabilizing manifolds were tested to concentrate ionic sample components in the anionic or cationic mode. Depending on its geometry, the devices can focus analytes from 1 to 50 ml of the sample into the collection cup with a size of 150 µL or less. Depending on the stabilization media and power used, the concentration time ranges from minutes to one hour. We have used the ETP to isolate DNA and RNA from biologically relevant samples in a single run, including formalin-fixed paraffin-embedded tissue. While the system was initially designed for extraction and focusing of nucleic acids, this presentation will also discuss the potential of the ETP for the separation and concentration of other analytes, including peptides and proteins.

ACKNOWLEDGEMENT
This work was financed by Roche, Inc., Pleasanton, USA.

REFERENCES
1. F. Foret, V. Datinska, I. Voracova, J. Novotny, P. Gheibi, J. Berka, Y. Astier (2019) *Anal. Chem.*, 111, 7047-7053.

OP-5

Theoretical chemistry approaches to biologically relevant problems in the molecular systems containing glycosaminoglycans

Sergey A. Samsonov

Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308 Gdańsk, Poland

Glycosaminoglycans (GAGs) represent a chemically highly heterogeneous class of linear anionic periodic polysaccharides participating in a number of biochemically crucial interactions in the extracellular matrix via interactions with their protein partners [1]. Due to their involvement in processes of cell signaling, cell prolifiration, cell adhesion and pathological onsets of such diseases as Alzheimer's, Parkinson's, cancer or viral infections including SARS-CoV2, they are especially attractive targets for regenerative medicine and novel pharmacological approaches [2]. However, due to their intrinsic properties as flexibility, broad conformational space, chemical heterogeneity and periodicity, they are very challenging both for experimental and computational approaches. In our lab, we perform molecular modeling of GAGs containing systems within frameworks of indisciplinary research [3], and together with the experimental groups, who use NMR, SAXS, SPR, MS and biochemical assays, we complement each other in order to understand the molecular mechanisms underlying the effects of GAGs in biologically relevant systems. We also contribute to the developement of the theoretical chemistry tools, based on the approaches including molecular docking, molecular dynamics and free energy calculations, to design them specifically for these challenging systems [4, 5].

ACKNOWLEDGEMENT

This work was financed by the National Science Centre of Poland, grant numbers UMO-2016/21/P/ST4/03995 (This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 665778.), UMO-2018/30/E/ST4/00037 and UMO-2018/31/G/ST4/00246.

REFERENCES

1. S. Ricard-Blum, S. Perez. Glycosaminoglycan interaction networks and databases. (2022) *Current Opinion in Structural Biology*, 74, 102355.
2. D. Scharnweber, L. Hübner, S. Rother, U. Hempel, U. Anderegg, S.A. Samsonov, M.T. Pisabarro, L. Hofbauer, M. Schnabelrauch, S. Franz, J. Simon, V. Hintze. Glycosaminoglycan derivatives: promising candidates for the design of functional biomaterials. (2015) *J Mater Sci Mater Med*., 26, 232.
3. G. Künze, D. Huster, S.A. Samsonov. Investigation of the structure of regulatory proteins interacting with glycosaminoglycans by combining NMR spectroscopy and molecular modeling – the beginning of a wonderful friendship. (2021) *Biological Chemistry*, 402, 1337-1355.
4. M.M. Kogut, M. Marcisz, S.A. Samsonov. Modeling glycosaminoglycan-protein complexes. (2022) *Current Opinion in Structural Biology*, 73, 102332.
5. B. Nagarajan, S.G. Holmes, N.V. Sankaranarayanan, U.R. Desai. Molecular dynamics simulations to understand glycosaminoglycan interactions in the free- and protein-bound states. (2022) *Current Opinion in Structural Biology*, 74, 102356.

OP-6

New docking strategy to enhance protein interactions with an application to the RAS G12D mutations

Vince Grolmusz*[1,5], József Tóvári[2], Attila Kigyós[2], Beáta Vértessy[3], József Tímár[4], Kinga Nyíri[3], Iván Randelovic [2]

[1]*Eötvös University, Budapest*
[2]*Institute of Oncology, Budapest*
[3]*ELKH-TTK, Institute of Enzymology, Budapest*
[4]*Semmelweis University, Budapest*
[5]*Uratim Ltd., Budapest*

Mutated genes are causing several serious illnesses, including different cancers. More than 600 cancer causing genes are listed in in the Catalogue of Somatic Mutations In Cancer. Some of these mutations change the steric properties of the proteins, which modify their function. One important example is the RAS gene, whose mutations are found in more than 25% of human tumors. The mutations of RAS are connected to some of the hardest-to-treat, most lethal cancers. The RAS protein was tagged “undruggable” until the most recent times, since it escaped the molecular docking efforts, because of the lack of the molecular docking cavities on its surface. It is known that the main reason for the oncogenic effects of the RAS is that they prevent the formation of the protein complex, consisting of the RAS and the GTPase-activating protein (GAP). The oncogene mutations have a steric structure that does not allow the RAS-GAP binding. Here we show a general method for the enhancement of molecular binding of macromolecules, through the application of molecular docking to the specific conformations of those macromolecules. As a demonstration of the power of the new method, we show two new molecules which have high differential activity in the nM concentration for G12D mutated vs. wild type RAS molecules.

ACKNOWLEDGEMENT
The work and the conference participation was partially supported by the NKFIH OTKA NKFI-127909 grant of the National Research- and Innovation Office of Hungary.

OP-7

Thermal analysis of tendon, cartilage and bone samples of patients underwent shoulder replacement with differential scanning calorimetry (DSC) and thermogravimetry (DTA/TG)

Dénes Lőrincy[1], András Bata[2], Zoltán Lábas[2], Hajnalka Szabó[2], János Cifra[2], László G. Nöt[2,3]

[1]Department of Biophysics, Medical School, University of Pécs, 12 Szigeti street, Pécs, 7624, Hungary
[2]Department of Traumatology and Orthopaedics, Balassa János Teaching Hospital of Tolna County, 5-7 Béri Balogh Ádám street, Szekszárd, 7100, Hungary
[3]Institute of Physiotherapy and Sport Science, Faculty of Health Sciences, University of Pécs, 4 Vörösmarty street, Pécs, 7621, Hungary

Background: There is a growing number of shoulder replacements every year with orthopaedic and trauma indications. Despite advanced imaging technologies, there is still a need for more details about the degree of joint degeneration and changes of the collagen content of tendons, to select the proper implant type. **Aims:** The purpose of the study was to compare the thermal parameters of various tissue samples collected from patients undergoing shoulder arthroplasty. We also aimed to correlate the thermodynamic changes with the results of radiological and histopathological evaluations. **Patients and methods:** rotator cuff (ROC) tendon, humerus head cartilage and subchondral bone samples were collected from patients during surgery, performed due to comminuted 4-part proximal humerus fracture or cuff tear arthropathy. Thermal parameters were detected by differential scanning calorimetry (DSC) and thermogravimetry (DTA/TG). The degenerative changes were confirmed by radiological evaluation and histopathology. **Results:** ROC tendons: in the orthopaedic samples, consequence of advanced collagen damage was demonstrated by the denaturation curves. The significant decrease of the calorimetric enthalpy (ΔH_{cal}) indicated the structural consequence of the medical abnormality. The moderately degenerated tendons of trauma patients exhibited only mild thermal differences from the controls [1]. Cartilage: the denaturation temperature range and the half-width of the heat flow curves were significantly wider in the orthopaedic samples compared to the control and traumatic ones. The melting temperatures (T_m) showed that structural change caused by degenerative condition is greater than the effect of trauma [2]. Bone: calorimetric parameters of orthopaedic samples exhibited higher level of structural damage and loss of mineralization compared to trauma samples. These differences indicate more progressed osteoarthritis (OA) in orthopaedic patients. In addition, correlation was found between the degree of OA and calorimetric enthalpy [3]. **Conclusion:** Authors suggest that thermal analysis could be useful in differential diagnosis of orthopaedic and posttraumatic shoulder diseases in future studies.

ACKNOWLEDGEMENT
This work was financed by CO-272 (OTKA) grant (D.L.).

REFERENCES

1. L.G. Nöt, A. Bata,H. Szabó, J. Cifra, D. Lőrinczy: DSC examination of rotator cuff damage in patients with total shoulder arthroplasty. (2021) *Journal of Thermal Analysis and Calorimetry,* 146, 165-170.
2. A. Bata, L.G. Nöt, H. Szabó, J. Cifra, D. Lőrinczy: DSC examination of cartilage damage of patients undergoing shoulder replacement. (2022) *Journal of Thermal Analysis and Calorimetry,* 147, 1275-1280.

3. A. Bata, L.G. Nöt, H. Szabó, J. Cifra, D. Lőrinczy: Thermogravimetric analysis of cancellous bone of humerus head in patients undergoing total shoulder arthroplasty. (2022) *Journal of Thermal Analysis and Calorimetry,* 147, 3107-3115.

OP-8

Computational and experimental analysis of the interactions between glycosaminoglycans and small molecules

Martyna Maszota-Zieleniak[1], Ferenc Zsila[2], Sergey Samsonov[1]

[1]Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland
[2]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Budapest, Hungary

Glycosaminoglycans (GAGs), long unbranched periodic anionic polysaccharides composed of repeating disaccharide units [1], are a class of chemical compounds involved in cellular communication, adhesion and proliferation in the extracellular matrix of the cell through interactions with their protein targets [2,3]. Alterations in protein-GAG interactions can cause a variety of pathologies including cancer, Alzheimer's and Parkinson diseases [4]. GAGs also increase the ability of peptides to integrate into membranes and modify drug activity [5,6].
Despite the great pharmacological potential of GAGs, the understanding of the molecular mechanisms underlying their interactions with small molecules is in particular far from being complete. Experimental methods are not always able to provide sufficient information about the nature of interactions in GAG-small molecule systems, and the corresponding available structural data are limited. In our work, we applied computational chemistry approaches such as molecular docking, molecular dynamics as well as free energy analysis to gain insights into these biologically relevant and theoretically scarcely investigated systems. We selected more than 20 drug molecules (ellipticine, berenil, pentamidine, surfen, tacrine, thioflavin T and others) and performed rigorous in silico analysis of their complexes with GAGs to expand our knowledge about the molecular aspects of the interactions between GAGs and small molecules. Our results were complemented by the experimental data [7-11].

ACKNOWLEDGEMENT
This work was funded by the National Science Centre (grant number UMO-2018/30/E/ST4/00037).

REFERENCES

1. J.D. Esko, K. Kimata, U. Lindahl (2009) In Essentials of Glycobiology. 2nd edition. Cold Spring Harbor Laboratory Press.
2. N. Perrimon, M. Bernfield (2000) *Nature*, 404 (6779), 725-728.
3. D. Xu, J.D. Esko (2014) *Annu. Rev. Biochem,* 83, 129-157.
4. K. Nishitsuji (2018) *Glycoconjugate Journal*, 35 (4), 387-396.
5. F. Zsila (2015) *Phys. Chem. Chem. Phys.*, 17, 24560-24565.
6. F. Zsila, T. Juhász, G. Kohut, T. Beke-Somfai (2018) *J. Phys. Chem. B.*, 122, 1781-1791.
7. F. Zsila, S.A. Samsonov (2018) *Carb. Res.*, 462, 28-33.
8. S.A. Samsonov, S. Freza, F. Zsila (2019) *Carb. Res.*, 482, 107742.
9. F. Zsila, S.A. Samsonov, M. Maszota-Zieleniak (2020) *J. Phys. Chem. B.*, 124, 11625-11633.
10. M. Maszota-Zieleniak, F. Zsila, S.A. Samsonov (2021) *Carb. Res.*, 507, 108390.
11. M. Maszota-Zieleniak, F. Zsila, S.A. Samsonov (2022) *Molecules,* 27, 2654.

OP-9

Residue Networks – protein structure, function and computational analysis

Vladimir Sladek

Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 84538 Bratislava, Slovakia

The knowledge of the role of particular residues in large biomolecules or their complexes is desirable in several tasks in molecular biology. A good example would be the assessment of residue importance in the formation of protein complexes. Non-trivial experimental assays such as alanine scans combined with e.g. surface plasmon resonance measurements can be used for this purpose. On the other hand, having a theoretical model of the protein that would allow to carry out such assessment in a reproducible, qualitative and quantitative way *a priori* of experimental work would certainly prove beneficial in many cases. Protein Residue Networks (PRN) are network models of proteins wherein each residue is a node in the network and their interactions, i.e. connections, are edges. Recent advances in this field [1] allow us to build PRN models based on modern (quantum) chemical calculations. The capabilities of specialised computational techniques such as Fragment Molecular Orbital (FMO) [2] methods are particularly well suited to obtain all residue-residue pair interaction energies (PIE). These are used to assign weights to the network edges. Subsequently, a wide range of network analytical approaches can be carried out on the model. The results can be used to asses/rank the importance of nodes in the networks topology and draw conclusions on the structural role of the residues in e.g. secondary, tertiary and quaternary protein structure. In this contribution we will focus on our own Network Differential Analysis (NDA) method as well as on variants of singular value decomposition (SVD) techniques suited to accomplish these tasks. Both were recently implemented into the free, open source PyMOL plugin pyProGA [3]. Our model for the case study will be the protein complex TR-2 – UL141 [4] and we will show how the network predictions correlate with experimental data.

ACKNOWLEDGEMENT

This work was financed by APVV 20-0230 and 19-0376, VEGA 2/0031/19 and SAS-MOST/JRP/2019/882/GM-INHIB.

REFERENCES

1. V. Sladek, H. Tokiwa, H. Shimano, Y. Shigeta, Protein Residue Networks from Energetic and Geometric Data: Are They Identical? (2018) *J. Chem. Theory Comput*.,4 (12), 6623-6631.
2. D. G. Fedorov, The fragment molecular orbital method: theoretical development, implementation in GAMESS, and applications. (2017) *WIRES,* **7**(6), e1322.
3. V. Sladek, Y. Yamamoto, R. Harada, M. Shoji, Y. Shigeta, V. Sladek, pyProGA – A PyMOL plugin for protein residue network analysis, (2021) *Plos ONE,* 16 (7), e0255167. https://gitlab.com/Vlado_S/pyproga.
4. I. Nemčovičová, C. A. Benedict, D. M. Zajonc, Structure of Human Cytomegalovirus UL141 Binding to TRAIL-R2 Reveals Novel, Non-canonical Death Receptor Interactions. (2013) *PLOS Pathogens,* **9** (3), 1-14.

OP-10

Interpretation of Single-Molecule Force Microscopy Experiments Using Normal Mode Analysis

Jacob Bauer[1], Gabriel Žoldák[2]

[1]Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Center for Interdisciplinary Biosciences, P. J. Šafárik University, Technology and Innovation Park, Košice, Slovakia

Protein folding and unfolding processes can be experimentally examined in the presence of an applied mechanical force using single-molecule force spectroscopy. Steered molecular dynamics is probably the most informative method for interpreting the results at the molecular level. These simulations are quite computationally expensive, however, and take so long to run that they can become the limiting step of any force-microscopy study that uses them. I describe the use of normal mode analysis, a computationally inexpensive technique, as an alternative method for interpreting these experiments. By applying NMA to three proteins previously studied by force spectroscopy and interpreted with the help of steered MD, we find that we can largely recover the results of the MD analysis, though only in a qualitative way. We were also able to provide tentative answers to three outstanding questions surrounding the fourth protein, which had not been analyzed using MD simulation. We conclude that NMA can be a useful addition to the single-molecule force spectroscopy analysis it [1].

ACKNOWLEDGEMENT
This work was financed by VEGA 2/0131/20 and 1/0175/19 and APVV-18-0285.The conference participation was covered by Interreg SK-AT StruBioMol ITMS: 305011X666.

REFERENCES
1. J. Bauer, G. Žoldák (2021) *Nanomaterials (Basel)*, 11, 2795.

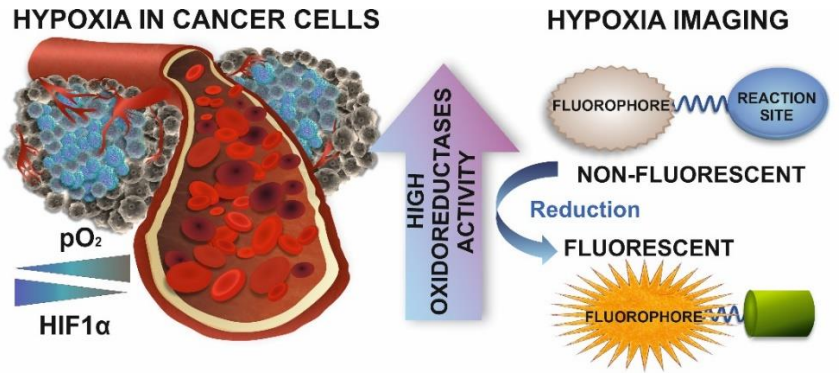
OP-11

Design new fluorescent turn off-on probes for hypoxia imaging

Małgorzata Brindell[1], Ewelina Janczy-Cempa[1], Olga Mazuryk[1], Franck Suzenet[2]

[1]Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland
[2]Institute of Organic and Analytical Chemistry, University of Orléans, UMR-CNRS 7311, rue de Chartres, BP 6759, 45067 Orléans Cedex 2, France
Malgorzata.brindell@uj.edu.pl

Hypoxia generally refers to a low oxygen levels in the tissue and very often occurs in solid tumors. Hypoxia can lead to the development of a more aggressive tumor phenotype and was identified as a negative prognostic indicator of patient outcome. Recognizing the localization and extent of hypoxia in the tumor is of great importance in the selection of an appropriate treatment to improve the efficacy. Recently, optical imaging has become one of the most widely developing methods of quantifying hypoxia. It offers the high sensitivity and low cost of the probes, real-time monitoring, nanometric resolution, and the possibility of direct intraoperative visualization. One of the approaches in the design of hypoxia sensors is to use the reducing tumor microenvironment for irreversible reduction catalyzed by the overexpressed oxidoreductases [1]. To contribute to the development of new imaging agents, we synthesized an entirely new type of sensors obtained by conjugation of the pyridazino-1,3a,6a-triazapentalene to a nitrophenyl moiety [2]. The main goal of this work was to provide a probe that in a relatively easy manner enables one to distinguish hypoxic from normoxic cells with the application of various devices such as flow cytometry, plate reader, or fluorescent microscope. We assumed that nitroreductase (NTR) can serve as a good indicative biomarker involved in the transcriptional response to low oxygen tension and is directly correlated with the level of hypoxia in cells. We proved this assumption by showing that indeed human melanoma A2058 cells produced a higher amount of nitroreductase in a hypoxic environment. Furthermore, the designed probes showed strong enhancement of the fluorescence intensity in the presence of NTR and it was proportional to the level of NTR. We have shown that there is a direct correlation between the level of NTR in cells and conditions i.e., hypoxia vs. normoxia.



ACKNOWLEDGEMENT
This work was financed by National Science Center (DEC-2019/33/B/NZ7/02980).

REFERENCES

1. E. Janczy-Cempa, O. Mazuryk, A. Kania, M. Brindell (2022) *Cancers*, 14, 2686.
2. E. Janczy-Cempa, O. Mazuryk, D. Sirbu, N. Chopin, M. Żarnik, M. Zastawna, C. Colas, M-A. Hiebel, F. Suzenet, M. Brindell (2021) *Sensors & Actuators B Chem*., 346, 130504.

OP-12

Photodynamic therapy „pre-quided by“ organic chemistry & synthesis

Zita Tokárová[1], Dominika Kendrová[1], Falko Pippig[2], Anita Andicsová-Eckstein[2], Natália Kabaňová[1], Marcela Gašparová[1]

[1]Department of Chemistry, Faculty of Natural Sciences, University of ss Cyril and Methodius in Trnava, Nám. J Herdu 2, 917 01 Trnava, Slovakia, zita.tokarova@ucm.sk

[2]Polymer Institute, Slovak Academy of Sciences, Dúbravská cesta 9, 845 41 Bratislava, Slovakia

Photodynamic therapy (PDT) is an emerging and promising way in a cancer therapy. Particular therapeutic technique is based on the interaction of photosensitizer (PS) with light [1]. Upon irradiation the reactive oxygen species (ROS) are released affecting the cytotoxicity on the cancerous cell [2]. During the process of irradiation the PS from the ground singlet state (S^0) is excited to the first excited singlet state (S^1) and further can reach first excited triplet state (T^1). PS in T^1 can directly interact with the substrate to generate ROS ($O_2^{\bullet-}$, $^{\bullet}OH$) inducing the tumor tissue destruction. Clinically, the treatment is being recently performed for skin therapy, i.e. complete elimination of lesions [3]. However, such repeated treatment is often accompanied by poor clinical prognosis, tumor recurrence, and eventually PDT resistance. For that reason, the development of class of an active photosenzitisers is key issue in the field of photodynamic therapy and related medicinal fields (i.e. photoactivated chemotherapy PACT). Traditional PSs are small organic molecules and organometallic compounds with π-conjugated backbone affecting the unique conductive and photophysical properties. Actually, the relation between the PSs and organic semiconductors (OSCs), in terms of properties, have resulted in our current research. The electronic bands of PSs are analogous to conductive metals. Either the OSCs or the PSs have to show the contiguity of the energy levels of frontier molecular orbitals (HOMO /LUMO) to produce charge or ROS release, respectively. Our research was, until now, predominantly focused on π-conjugated small-molecules as building blocks for organic semiconducting materials and organic photovoltaic devices. Beyond thiophene-based and thiazolo[5,4-*d*]thiazole-based compounds, also the phthalocyanines (PC) are of our interests.

Concerning the PDT, phthalocyanines have been proposed as on of the promising photosensitizers for the treatment of several microbial infections [4]. Generally, phthalocyanines absorb strongly at phototherapeutic window, emitt red fluorescence, and efficiently produce the formation of reactive oxygen species.

We wish to present our results achieved during the design, synthesis and characterization process on a series of a novel type of phthalocyanines and their precursors as attractive for further research as photosensitizers in PDT. The view, comments and advices of the experts from non-chemist fileds of research will be gratefully apprecitated.

ACKNOWLEDGEMENT

This work was financed by VEGA 1/0086/21, VEGA 1/0191/22 and APVV 19-0087.

REFERENCES

1. L. Gourdon, K. Cariou, G. Gasser (2022) *Chem. Soc. Rev.*, 51, 1167-1195.
2. N. Sun, X. Wen, S. Zhnag (2022) *Int. J. Nanomedicine*, 17, 247-271.
3. A. M. Mackay (2022) *Photochem. Photobiol. Sci*. 21, 385 – 395.
4. K. L. Macena Santos, {...}, J. A. Oshiro Jr. (2020) *Photodiagnosis and Photodynamic Therapy* 32, 102032.

OP-13

Air-pollution-derived metal ions induced decomposition of S-nitrosothiols

Maria Oszajca, Anna Wądołek

Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, 30-387 Krakow, Poland
maria.oszajca@uj.edu.pl

Particulate matter (PM) air pollution is recognised as the most severe environmental threat to human health. Chronic exposure to air pollutants is linked to an enhanced risk of developing numerous diseases, though the molecular mechanisms related to PM-induced disease development or their exacerbation remain unclear. Inorganic, aqueous soluble constituents of PM upon reaching the respiratory tract lining fluids and consequently the bloodstream, may result in a dysregulation of physiological redox-metabolism. One of the potentially vulnerable pathways relies on the deregulation of nitric oxide signaling processes occurring through S-nitrosation. Since metal-ion-induced reductive decomposition of S-nitrosothiols is well known, urban PM as a rich source of metal ions could disturb the physiological balance between NO and low- and high-molecular-weight thiols.

Considering the above, we investigated whether PM can affect the stability of S-nitrosoglutathione (GSNO) and human S-nitrosoalbumin (HSA-NO), two important S-nitrosothiols involved in keeping S-nitrosation balance [1,2]. In our studies, we applied two types of urban PM of different origins (Standard Reference Material 1648a and PM collected in Krakow) [1,2]. The influence of PM on the stability of S-nitrosothiols was studied electrochemically by following the released NO with the application of a selective NO-sensor. Our studies revealed that both GSNO and HSA-NO release NO upon treatment with PM aqueous extract, whereas particle interfaces had no direct effect. Biologically relevant reductants such as GSH or ascorbic acid effectively promote PM-induced decomposition of S-nitrosothiols. Analysis of the wide range of metal ions identified as constituents of PM extracts indicated copper ions as the main decomposing species [1,2].

Long-term exposure to air pollutants increases mortality and deterioration of life parameters among people diagnosed with cardiovascular diseases (CVD). Therefore we decided to compare the blood plasma's protective ability against GSNO decomposition under the influence of PM extracts in patients with exacerbation of heart failure and coronary artery disease versus healthy volunteers. Our studies revealed that NO release from GSNO is facilitated in the environment of CVD patients' plasma indicating diminished protection against PM extract-induced GSNO degradation [3].

ACKNOWLEDGEMENT
This work was financed by the National Science Center in Poland, OPUS Project no. 2019/35/B/ST4/04266.

REFERENCES

1. M. Oszajca, A. Wądołek, J. Hooper, M. Brindell, R. van Eldik, G. Stochel (2019) *ChemSusChem,* 12, 661-671.
2. A. Wądołek, M. Oszajca, W Pęcak, M. Brindell, G. Stochel (2021) *Dalt. Trans.,* 50, 9923-9933.
3. A. Wądołek, D. Drwiła, M. Oszajca, G. Stochel, E. Konduracka, M. Brindell (2021) *Int. J. Mol. Sci.,* 22, 10500.

OP-14

Antimetastatic activity of polypyridyl ruthenium(II) complexes – in vitro functional and molecular studies

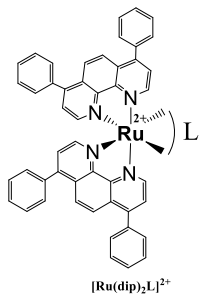
Olga Mazuryk, Ilona Gurgul, Ewelina Janczy-Cempa, Przemysław Gajda-Morszewski, Małgorzata Brindell

Faculty of Chemistry, Jagiellonian University in Krakow, Gronostajowa 2, 30-387 Krakow, Poland

While cancer death rates continue to decline due to modern medical advances, patients with metastatic disease have not seen such improvements. The diagnosis of metastatic lesions indicates a negative prognosis in the vast majority of cancer patients. An urgent need remains for novel therapeutic strategies and agents that prevent metastasis formation and development.

The pioneer in the research on antimetastatic properties of metal compounds is imidazolium trans-[tetrachlorido(1H-imidazole)(S-dimethylsulphoxide)ruthenate(III)] known as NAMI-A. NAMI-A was the first metal complex that had entered the clinical trials as a potential nontoxic antimetastatic agent. It prevents the development and growth of metastases. Unfortunately, NAMI-A clinical studies were terminated due to the absence of satisfactory results.

Ru polypyridyl complexes have gained a lot of attention as promising anticancer agents during the last decade. Ru complexes containing two 4,7-diphenyl-1,10-phenanthroline ligands exhibit high stability resulting from substitution inertness and resistance to oxidation. The compounds were found to be cytotoxic toward a broad panel of cancer cell lines with low micromolar IC_{50} values [1].



$[Ru(dip)_2L]^{2+}$

Figure 1. The studied ruthenium(II) polypyridyl complexes.

We explored the antimetastatic properties of Ru complexes and were able to show that they modulate cancer and endothelial cells' adhesion properties [2, 3, 4]. Ru polypyridyl complexes inhibited cell detachment either from plastic or collagen-coated surfaces. On the other hand, cells pre-treated with the compounds were less able to re-adhere to new sites after detachment. Additionally, Ru complexes inhibited invasion, migration and transmigration of cancer cells as well as influenced endothelial cells' functions. Furthermore, to get a better insight into the molecular basis of the observed cellular functional changes induced by Ru(II) polypyridyl complexes, the expression of focal adhesion components such as vinculin and paxillin and the resulting number of focal adhesion contacts as well as cells' mechanical properties were investigated. In order to identify the potential molecular targets for studied Ru(II) compounds, the activity of several matrix metalloproteinases as well as selected integrins was additionally investigated [4, 5].

ACKNOWLEDGEMENT

This work was financed by the National Science Center in Poland for the OPUS Project no. 2016/21/B/NZ7/01081.

REFERENCES

1. O. Mazuryk, F. Suzenet, C. Kieda and M. Brindell (2015) *Metallomics*, 7, 553-566.
2. I. Gurgul, O. Mazuryk, M. Łomzik, et al. (2020) *Metallomics*, 12, 784-793.
3. I. Gurgul, O. Mazuryk, K. Stachyra, et al. (2022) *Int. J. Mol. Sci.,* 23, 7708.
4. I. Gurgul, E. Janczy-Cempa, O. Mazuryk, et al. (2022) *J. Med. Chem.,* accepted.
5. P. Gajda-Morszewski, I. Gurgul, E. Janczy-Cempa, et al. (2021) *Pharmaceuticals*, 14, 1014.

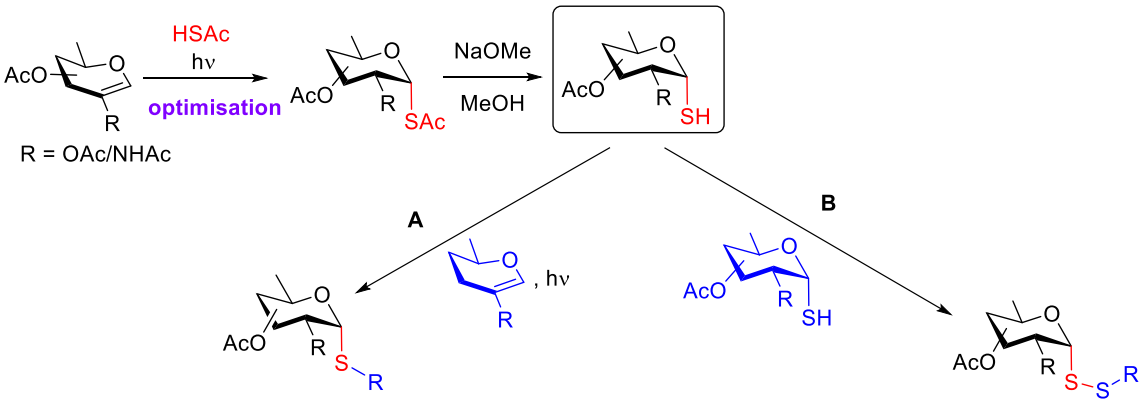
OP-15

Stereoselective synthesis of 1,2-*cis*-α-thioglycosides by two sequential photoinitiated thiol-ene additions

Viktor Kelemen[1], Nóra Debreczeni[1,2], Miklós Bege[1,2,3], Anikó Borbás[1]

[1]*Department of Pharmaceutical Chemistry, University of Debrecen, Debrecen, Egyetem tér 1, Hungary*
[2]*Institute of Healthcare Industry, University of Debrecen, Debrecen, Nagyerdei körút 98, Hungary*
[3]*MTA-DE Molecular Recognition and Interaction Research Group, Egyetem tér 1, Debrecen, Hungary*
kelemen.viktor@pharm.unideb.hu

The formation of 1,2-*cis*-α-thiols is a notoriously challenging task, so far, no general method was described. Photoinitiated thiol-ene addition reaction of 2-substituted glycals has already been used by our group to synthesize 1,2-*cis*-α-*S*-linked thioglycosides. [1-3] Here, we present that the addition of thioacetic acid to 2-substituted hexopyranosyl D- and L-glycals results in the selective formation of the desired 1,2-*cis*-α-1-thioacetates, which can then be selectively converted into the corresponding thiols. Those compounds were then used in a second photoinitiated thiol-ene coupling reaction to furnish trehalose-type 1,2-*cis*-α,α-thiodisaccharides (**A**), or in a non-photochemical oxidation reaction to furnish protected or deprotected disulfides (**B**).



Scheme 1. Synthesis of 1,2-*cis*-α carbohydrate thiols and thioconjugates.

ACKNOWLEDGEMENT

The research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.4-15-2020-00080, and by the National Research, Development and Innovation Office of Hungary (OTKA K 132870).

REFERENCES

1. L. Lázár, M. Csávás, M. Herczeg, P. Herczegh, A. Borbás (2012) *Org. Lett.*, 14, 4650-4653.
2. D. Eszenyi, V. Kelemen, F. Balogh, M. Bege, M. Csávás, P. Herczegh, A. Borbás (2018) *Chem. Eur. J.*, 24, 4532-4536.
3. V. Kelemen, M. Bege, D. Eszenyi, N. Debreczeni, A. Bényei, T. Stürzer, P. Herczegh, A. Borbás (2019) *Chem. Eur. J.*, 25, 14477-14477.

OP-16

Selected applications of Langmuir balance in pharmaceutical sciences

Witold Musiał, Iwona Golonka, Remigiusz Zapolski

Department of Physical Chemistry and Biophysics, Wroclaw Medical University, Borowska 211A, 50–556 Wrocław, Poland; witold.musial@umw.edu.pl

The study of surface tension and the plotting of isotherms of the dependence of surface pressure on the size of the surface on which the monomolecular layer of surfactant is dispersed dates back to the pioneering works of Wilhelmi and Langmuir. The development of modern isothermal research techniques allows them to be used in numerous works on the development or analysis of new drugs or medicinal products.

The aim of the study was to indicate the most important and interesting applications of the Langmuir balance in research on new medicinal substances or medicinal products. The available English-language literature on the applications of compound monolayer research in such fields of applied science as: drug analysis, drug chemistry, pharmaceutical chemistry, drug synthesis and technology, drug form technology, pharmacokinetics, biopharmacy and pharmacology was analyzed. Among the series of studies carried out with the use of the Langmuir balance, one can distinguish a group of studies on pure drug substances, and a group of studies in which the interaction or influence of a drug substance on the properties of the monolayer is assessed, as was done on the example of phenothiazine derivatives [1], and with the use of dibucaine [2]. A separate group are the interactions of nano-scale polymer particles - potential drug carriers - with the lipid layer [3]. The influence of radiation, pH and / or electrolytes on the properties of a monolayer obtained from a specific substance with surface-active properties, eg with the use of dipalmitoylphosphatidylcholine [4] or naphthenic acid [5], is also investigated. An interesting area of research are the attempts to use the monolayer as a model of the cell membrane, eg in the study of violacin [6], or as a model of tissue fluid, eg tear fluid [7]. Studies using the Langmuir balance remain an important option for the modeling and development of substances and medicinal products with a component having a pronounced effect on the surface tension of the solution.

ACKNOWLEDGEMENT

This work was financed in the frames of the project of Wroclaw Medical University, No SUBK.D060.22.078.

REFERENCES

1. A.A Hidalgo et al. (2004) *Biophysical Chemistry*, 109 (1), 85-104.
2. G.S. Lorite et al. (2009) *Biophysical Chemistry,* 139 (2-3), 75-83.
3. L. Peltonen, J. Hirvonen (2008) *Current Nanoscience,* 4(1), 101-107.
4. T. Havre et al. (2002) *Colloid and Polymer Science*, 280 (7), 647-652.
5. H.M.K. Mohd et al. (2013) *Malaysian Journal of Analytical Sciences.*
6. K.D. de Souza et al. (2017) *Colloids and Surfaces B: Biointerfaces*, 160, 247-253.
7. T.F. Svitova, C. L. Meng (2016) *Advances in Colloid and Interface Science,* 233, 4-24.

PhD-1

The use of K562 aptasenzor as detection method for chronic myeloid leukemia

Michaela Domšicová[1], Tomáš Kyca[1], Andrea Bábelová[2], Michal Šelc[2], Kristína Jakič[2], Veronika Némethová[3], Filip Rázga[3], Alexandra Poturnayová[1]

[1]Institute of Molecular Physiology and Genetics CBs SAS, Bratislava
[2]Cancer Research Institute BMC SAS, Bratislava
[3]Faculty of Medicine, Comenius University, Bratislava

The high prevalence and mortality of oncological diseases make them one of the most studied topics in clinical research. Chronic myeloid leukemia is a form of cancer affecting white blood cells, which mainly affects the adult population and accounts for 15-20% of all leukemic diseases [1]. When diagnosing leukemic diseases, patients often undergo time-consuming and painful procedures, which is why research into new diagnostic methods that are fast, accurate, non-invasive and highly specific is highly emphasized and in demand.

In the presented work, we used DNA aptamers (T2-KK1B10), which can recognize chronic myeloid leukemia cells [2]. A highly sensitive acoustic method - quartz crystal microbalance - was used to monitor the interactions between the aptamer and different leukemic cell lines (BV-173, K562) or control THP cells and healthy lymphocytes. These interactions were monitored in real time. We compared 2 ways of sensor modification and compared their sensitivity, while the system based on the self-assembly of thiol-labeled aptamer molecules proved to be more sensitive than the system based on the interaction of neutravidin and biotin-labeled aptamer. We optimized the detection conditions, such as the composition of the washing solutions, the concentration of the aptamer used for detection and its pretreatment. By non-specific reactions of the aptamer with other cells and the K562 cell line with a non-specific aptamer, we confirmed that the used T2-KK1B10 aptamer is specific to the used cell line. We also verified its affinity using confocal microscopy, where we visualized fluorescently labeled aptamers on the surface of K562 cells.

An important point is to confirm the ability of the biosensor to regenerate the sensitive layer for the purpose of multiple use in practice. We demonstrated this ability by using SDS regeneration reagent, when the layer of cells was washed off the surface of the sensitive aptamer layer and the aptamer was accessible to new interactions. We were able to regenerate both types of aptasensors 2 to 3 times with an efficiency of 88 - 122 % for HS-T2-KK1B10 and 78 - 107 % for biotin T2-KK1B10.

The obtained results show that the combination of aptamers and sensors can provide a highly sensitive biosensing system and target-specific imaging of cancer cells. The benefit of our results is the perspective of shifting the detection of oncological diseases towards faster, more sensitive and less invasive diagnostic methods. They lay the foundations for the future, when diagnostics using aptasensors could offer an alternative to the usual methods.

ACKNOWLEDGEMENT
The presented work was supported by the grant agency VEGA (2/0160/21 and 1/0069/20).

REFERENCES

1. D. Vetrie, G. Helgason, M. Copland, The leukaemia stem cell: similarities, differences and clinical prospects in CML and AML. (2020) *Nature Reviews Cancer*, 20, 158-173.

2. T. Yu, et al., A Simple Electrochemical Aptamer Cytosensor for Direct Detection of Chronic Myelogenous Leukemia K562 Cells. (2016) *Electroanalysis*, 29, 828-834.

PhD-2

Identifying druggable sites of the Mg^{2+}-free intermediate state of the catalytic cycle of K-Ras^{G12C} oncogenic protein by NMR spectroscopy

Márton Gadanecz[1], Gyula Pálfy[2], András Perczel[1,2]

[1]*Laboratory of Structural Chemistry and Biology, Eötvös Loránd University, Institute of Chemistry, Budapest, Hungary*
[2]*MTA-ELTE Protein Modelling Research Group, Eötvös Loránd Research Network, Budapest, Hungary*

About 30% of all human oncogenic diseases initiated by mutations in one of the three RAS genes, K-RAS, H-RAS and N-RAS. K-Ras is a membrane-bound small G-protein that plays a key role in sevaral signal transduction pathways as a molecular switch, regulating cell growth, proliferation and survival. Normally, K-Ras alternates between a GTP-bound active and a GDP-bound inactive form, by the assistance of guanine nucleotide exchange factor proteins (GEFs) and GTPase activation proteins (GAPs). K-Ras also binds a Mg^{2+} cofactor in both of its active and inactive forms. Mutations on critical positions, such as G12, G13, Q61 and A146, lead to a shift toward the active form and consequently to the excessive activation of the signal transduction pathways. Over 75% of K-Ras oncogenic mutations are found at the G12 site (the most frequent ones are G12D, G12V and G12C in order of incidence) [1-3].
During the GDP→GTP nucleotide exchange step of the K-Ras catalytic cycle (this step is typically assisted by GEFs, e.g. Sos) the Mg^{2+} cofactor is released. Knowing the exact structure of the Mg^{2+}-free but nucleotide-bound form (a probable intermediate along the nucleotide exchange pathway) could aid the development of new, allosteric inhibitors, targeting K-Ras signaling [4].
The spectra of the Mg^{2+}-free G12C mutant has been assigned partly by the program 4D-CHAINS [5] and partly manually by using 4D spectra (4D HC(CC-TOCSY(CO))NH and 4D ^{13}C, ^{15}N edited SFHMQC-NOESY-SFHMQC (HCNH)). A fragment library has been built, using the sequence and chemical shift information, containing many possible backbone conformations, to drive the structure building process. The structure determination was completed by the program autoNOE-Chemical-Shift-Rosetta, which is an iterative protocol, that uses the low-energy pool of previously picked fragments to improve the NOE crosspeak assignments [6]. Now using this method we can identify G12C-specific, otherwise “hidden” surfaces of potential drug target sites.

ACKNOWLEDGEMENT

This work was supported by project no. 2018-1.2.1-NKP-2018-00005 of the National Research Development and Innovation Fund of Hungary; no. VEKOP-2.3.2-16-2017-00014 and VEKOP-2.3.3-15-2017-00018 and GINOP-2.3.3-15-2016-00004 of the European Union and the State of Hungary, co-financed by the European Regional Development Fund; by Hungarian Scientific Research Fund (NKFIH-OTKA) grant K116305; by MedInProt Grants from the Hungarian Academy of Sciences; and within the framework of the Thematic Excellence Program 2019 by the National Research, Development and Innovation Office under project “Synth+”, by the Ministry for Innovation and Technology from the Hungarian NRDI Fund (2020-1.1.6-JÖVŐ-2021-00004), by iNEXT (grant number 13629) funded by the Horizon 2020 program of the European Commission. Dr. Frank Löhr and Dr. Christian Richter are acknowledged for their help in measurement of 4D NMR experiments under the iNEXT program.

REFERENCES

1. L. Herdeis, D. Gerlach, D.B. McConnell, D. Kessler, Stopping the beating heart of cancer: KRAS reviewed. (2021) *Current Opinion in Structural Biology*,71, 136-147.

2. D.K. Menyhárd et al., Structural impact of GTP binding on downstream KRAS signaling. (2020) *Chemical Science*, 34. doi:10.1039/d0sc03441j.
3. G. Pálfy, D.K. Menyhárd, A. Perczel, Dynamically encoded reactivity of Ras enzymes: opening new frontiers for drug discovery. (2020) *Cancer and Metastasis Reviews*, 39, 1075-1089.
4. G. Pálfy et al., The Importance of Mg^{2+}-free State in Nucleotide Exchange of Oncogenic K-Ras Mutants. (2022) *Chemistry – A European Journal*. doi:10.1002/CHEM.202201449.
5. T. Evangelidis et al., Automated NMR resonance assignments and structure determination using a minimal set of 4D spectra. (2018) *Nature Communications*, 9, 1-13.
6. S. Nerli, N.G. Sgourakis, CS-ROSETTA. (2019) *Methods Enzymol*, 614, 321.

PhD-3

The new quantum dot luminescent probe for caspase-3/7 imaging inside cells

Markéta Procházková[1,2], Karel Klepárnik[1]

[1]*Institute of Analytical Chemistry of the Czech Academy of Science, v. v. i., Brno, Czech Republic*
[2]*Faculty of Science, Masaryk University, Brno, Czech Republic*
prochazkova@iach.cz

High-sensitivity and high-selectivity analyses are more widely used not only as a tool of investigation in biology and medicine but also in diagnostic practice. Modern technologies and instrumentations of laser-induced fluorescence or bioluminescence offer the possibility to study biological phenomena at a cellular or even molecular level. The progress of bioanalytical techniques has accelerated the deep understanding of cellular processes [1,2].

Our research is focused on biologically active molecules, such as caspases, which play important roles in cell signaling regulation in normal and diseased states and are attractive targets for biological diagnosis and also for medical therapy [2,3].

Recent progress of bioanalytical techniques has accelerated the deep understanding of cellular states and development of novel drugs as well as medical diagnosis. To analyze the biological events in single cells, technologies related to fluorescence and luminescence imaging advanced rapidly in the past two decades. Three prominent types of fluorescent molecules have been used for bioimaging: fluorescent proteins, artificially synthesized organic dyes, and fluorescent nanoparticles [1,4-6].

During the past two decades, quantum dot (QD) nanoparticles have become frequent components of highly luminescent tags, probes, and sensors with a broad application in bioanalytical chemistry [7,8]. Compared with traditional luminescent organic dyes, QDs exhibit excellent photophysical properties, for example, high photostability, broad excitation, and narrow symmetric emission bands. The emission wavelengths of QDs, dependent on their sizes, are tunable by particle synthesis. Moreover, their high extinction coefficients make them ideal for absorption and transfer of relatively large amounts of energy [9]. Water-dispersed QD particles are usually charged. Therefore, capillary electrophoresis with laser induced fluorescence (CE-LIF) is a method of choice for analyses of the QD surface modifications including determination of particle effective charge [10,11]. Bioanalytical applications of QDs include detection and quantification of biologically relevant molecules, cellular imaging, cell tracking, or pathogen and toxin detection [12]. Recently luminescent semiconductor QDs were widely applied in different areas. QDs can be used as photoluminescent labels with excellent possibilities for high-throughput detection and diagnostics. Although QDs have a lot of advantages over organic dyes, most of the techniques that have been developed for QD functionalization and bioconjugation are more complicated than the corresponding techniques for organic fluorescent dyes [13].

Recent studies have shown that electron transfer between quantum dots and attached fluorophores increases the FRET efficiency in this system [14]. This enables real-time monitoring of enzymatic activity of peptide cleaving enzymes (**Figure 1**).

The aim of our research was to synthesize a new quantum dot luminescent probe for a longtime monitoring of caspase 3/7 distribution in apoptotic and nonapoptotic osteoblastic cells. The two step synthesis of luminescent probe based on ligand-exchange in the first step and the specific reaction of amino group in the second step was optimized. The QDs and their

conjugates after first step of synthesis were analyzed by the laboratory built CE-LIF system. Optimal conditions for quantitative separation of the native quantum dots and their conjugates with peptide by using capillary electrophoresis were found and checked (**Figure 2**). In this work, testing of the novel quantum dot luminescent probe will be presented. The luminescence properties of the novel quantum dot luminescent probe were checked by monitoring of the reaction inside the MC3T3-E1 cells under microscope Olympus IX 71 with Xe-lamp (**Figure 3**). The fluorescent probe reaction inside the cells treated with camptothecin was monitored by the fluorescence emission at 600 nm with excitation light at 530 nm. The cells were incubated with the fluorescence probe for 24 hours at 37°C and 5% CO_2. The synthetized luminescent probe proved to enable much longer monitoring of active caspases than commercially available probes. Stability of the fluorescence signal inside the cells is more than 8 days.

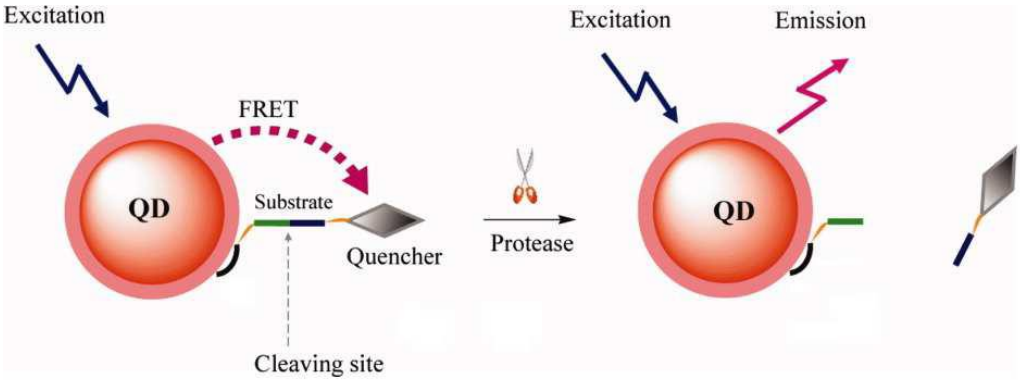


Figure 1 Scheme of the quantum dot FRET-based luminescent probe reaction with cleaving enzyme.

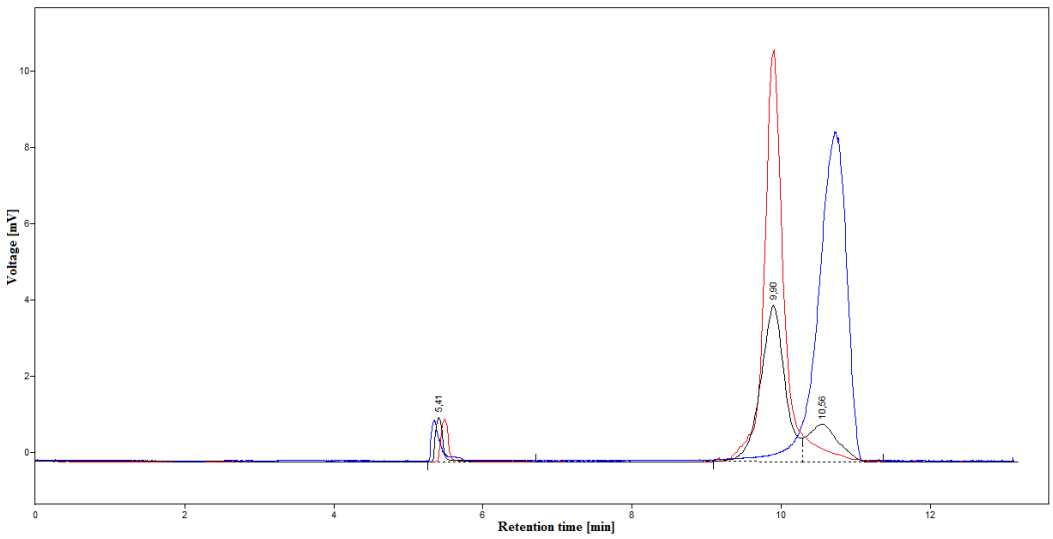


Figure 2 Electropherogram of the quantum dots conjugate with peptide (red line), native quantum dots (blue line) and their mixture (black line). The velocity of the EOF was determined using coumarin, a neutral fluorescent marker, peak with retention time 5.4 min. Excitation wavelength 405 nm and emission wavelength 607 nm.

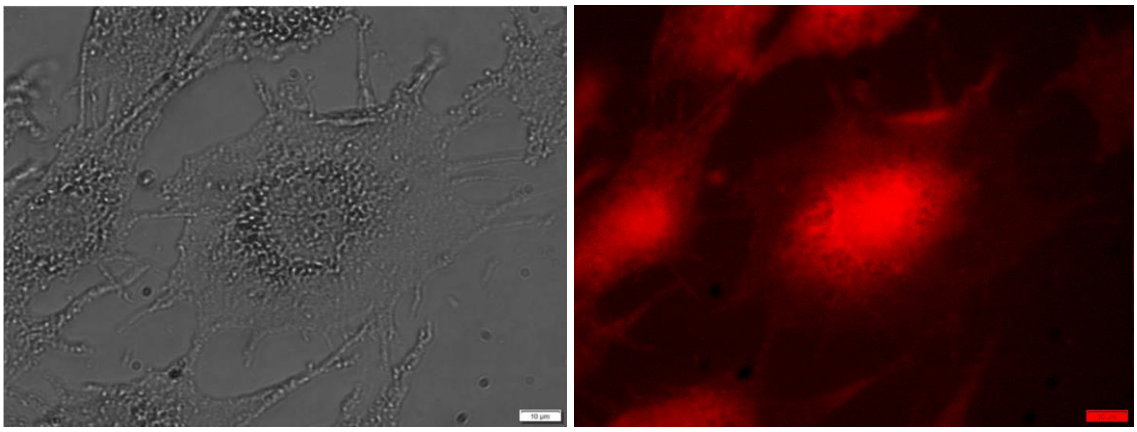


Figure 3 Comparison of the white light microscope picture (in the left) and the fluorescence microscope picture (in the right) of the MC3T3-E1cells treated with camptothecin 24 hours after incubation with fluorescent probe.

ACKNOWLEDGEMENT
This research was financed by the Grant Agency of the Czech Republic, project no. GA20-00726S.

REFERENCES

1. T. Ozawa, H. Yoshimura, S. B. Kim (2013) *Analytical chemistry*, 85, 590-609.
2. B. Turk (2006) *Nature Reviews Drug Discovery*, 5, 785-799.
3. A. Hamer, P. Dierickx, R. Arts, J. S. P. M. de Vries, L. Brunsveld, M. Merkx (2017) *American Chemical Society Sensors*, 2, 729-734.
4. J. Cheng,, L. Tian, J. Ma, Y. Gong, Z. Zhang, Z. Chen, B. Xu, H. Xiong, C. Li, Q. Huang (2015) *Mol. Oncol.,* 9, 105-114.
5. Y. Shaulov-Rotem, E. Merquiol, T. Weiss-Sadan, O. Moshel, S, Salpeter, D. Shabat, F. Kaschani, M. Kaiser, G. Blum (2016) *Chem. Sci.,* 7, 1322-1337.
6. V. G. Ostapchenko, J. Snir, M. Suchy, J. Fan, M. R. Cobb, B. A. Chronik, M. Kovacs, V. F. Prado, R. H. E. Hudson, S. H. Pasternak, M. A. M. Prado, R. Bartha (2019) *Contrast Media Mol. Imaging*, 2, 17 pages.
7. J. B. Blanco-Canosa, M. Wu, K. Susumu, E. Petryayeva, T. L. Jennings, P. E. Dawson, W. R. Algar, I.L. Medintz (2014) *Coordin. Chem. Rev.*, 263, 101-137.
8. E. Petryayeva, W. R. Algar, I. L. Medintz, I. L. (2013) *Appl. Spectrosc*., 67, 215-252.
9. J. M. Klostranec, W. C. W. Chan (2006) *Adv. Mater*., 18, 1953-1964.
10. F. M. Sang, X. Y. Huang, J. C. Ren (2014) *Electrophoresis,* 35, 793-803.
11. M. Liskova, I. Voracova, K. Kleparnik, V. Hezinova, J. Prikryl, F. Foret (2011) *Anal. Bioanal. Chem*., 400, 369–379.
12. T. Jamieson, R. Bakhshi, D. Petrova, R. Pocock, M. Imani, A. M. Seifalian (2007) *Biomaterials,* 28, 4717–4732.
13. A. Foubert, N. V. Beloglazova, A. Rajkovic, B. Sas, A. Madder, I. Y. Goryacheva, S. D. Saeger (2016) *Trends in Analytical Chemistry*, 83, 31–48.
14. L. Yuan, W. Lin, K. Zheng, S. Zhu (2013) *Accounts of Chemical research,* 46, 1462-1473.

PhD-4

Separation and identification of the α- and β-anomers of sugar amino acids in chimera peptides

Kim Hoang Yen Duong[1,2], Gitta Schlosser[3], Viktória Goldschmidt Gőz[4], Viktor Farkas[4], András Perczel[2,4]

[1]Hevesy György PhD School of Chemistry, Institute of Chemistry, ELTE Eötvös Loránd University – Pázmány P. stny. 1/A, 1117 Budapest, Hungary

[2]Laboratory of Structural Chemistry and Biology, Institute of Chemistry, ELTE Eötvös Loránd University – Pázmány P. stny. 1/A, 1117 Budapest, Hungary

[3]MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group, ELTE Eötvös Loránd University – Pázmány P. stny. 1/A, 1117 Budapest, Hungary

[4] MTA-ELTE Protein Modeling Research Group, Eötvös Loránd Research Network (ELKH) ELTE Eötvös Loránd University – Pázmány P. stny. 1/A, 1117 Budapest, Hungary

β-Sugar Amino Acids (β-SAAs) have received a considerable interest as building blocks of chimera peptides. Fmoc-protected β-SAA (Fmoc-RibAFU(ip)-OH) as a structural Lego-element was successfully incorporated into biologically relevant oligo- and polypeptides. The furanoid β-SAA building block was a good alternative to α-amino acid, achieving tunable hydrophilicity as the 1,2-*O*-isopropylidene protecting group was removable [1,2]. The α- and β- anomers of fully unprotected furanoid β-SAA-containing peptides were in equilibrium. Cyclic ion mobility-mass spectrometry (cIM-MS) [3] and nuclear magnetic resonance spectroscopy (NMR) [4] techniques were applied for distinguishing our anomers. To block the mutarotation in solution of -RibAFU(α/β)-chimeras, the methylation was executed to obtain corresponding -RibAFU(α/β,Me)-peptides. Using the high-resolution cIM separation capability, we resolved the two anomeric components of chimera peptides. The α- and β-anomers were identified based on the NMR. Each anomer was assigned by comparison with the spectra of the corresponding methylated anomeric component. This revealed the combination of cIM-MS and NMR to effectively separate and identify the anomers of chimera peptides containing -RibAFU(α/β)- building block.

ACKNOWLEDGEMENT

This research project was supported by the European Union and the State of Hungary and financed by the European Regional Development Fund (VEKOP-2.3.2-16-2017-00014) grant of the NKFIH of the Hungarian Academy of Sciences. This work was completed in the ELTE Thematic Excellence Programme supported by the Hungarian Ministry for Innovation and Technology. Thematic Excellence Programme 2020, National Challenges Subprogramme - TKP2020-NKA-06.

REFERENCES

1. K. H. Y. Duong, V. Goldschmidt Gőz, I. Pintér, A. Perczel (2021) *Amino Acids*, 53, 281-294.
2. V. Goldschmidt Gőz, K. H. Y. Duong, D. Horváth, K. Ferentzi, V. Farkas, A. Perczel (2021) *Eur. J. Org. Chem.*, 44, 6071-6083.
3. J. Ujma, D. Ropartz, K. Giles, K. Richardson, D. Langridge, J. Wildgoose, M. Green, S. Pringle (2019) *J. Am. Soc. Mass Spectrom.*, 30, 1028-1037.
4. W. A. Bubb (2003) *Concepts Magn. Reson. A*, 19A, 1-19.

POSTERS

P-01

Electrical pulse stimulation as an in vitro model of exercise – comparison of the two protocols in differentiated human skeletal muscle cells

Klára Gabrišová[1], Tímea Kurdiová[1], Jozef Ukropec[1], Barbara Ukropcová[1,2]

[1]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences
[2]Institute of Pathological Physiology, Faculty of Medicine, Comenius University; Bratislava, Slovakia

INTRODUCTION
Regular exercise has a positive effect on metabolic health and is associated with adaptive changes of skeletal muscle. The molecular mechanisms related to these adaptations are still not fully understood. To observe exercise-like effects in skeletal muscle cells, we use electrical pulse stimulation (EPS) to induce controlled contractions of differentiated human skeletal muscle cells (myotubes) in vitro.

AIM
The aim was to compare the effectiveness of the two distinct EPS protocols in primary human skeletal muscle cell cultures in modulating: (i) release of specific myokines into the media, (ii) cellular energy sensor AMP-kinase (AMPK) activation, and (iii) mitochondrial respiratory chain protein content.

MATERIALS AND METHODS
Skeletal muscle cells derived from healthy, non-obese men (n = 4, age: 31.0 ± 4.0 years; BMI: 23.3 ± 2.4 kg/m^2) were exposed to EPS (24 h, voltage 11.5V; Ionoptix, USA), using two EPS protocols: (i) continuous 24 h stimulation aimed at mimicking low-intensity exercise (frequency 1 Hz, pulse duration 2 ms), and (ii) intermittent 24 h stimulation where higher frequency stimulation was preceded and followed by subthreshold frequency stimulation (0.2 – 5 – 0.2 Hz) lasting for 2.0 – 0.5 – 2.0 hours in one cycle (pulse duration 4 ms). Myokines released into the media (ELISA), magnitude of AMPK activation (phosphorylation) and mitochondrial respiratory chain proteins (immunoblotting) were quantified.

RESULTS
Both EPS protocols increased myokine release (IL-6, IL-8) into the media. Chronic low-intensity stimulation tended to increase IL-6 in media (3.5-fold, p=0.19, n=3), while intermittent electrical pulse stimulation was even more effective in increasing IL-6 media content (6.5-fold, p=0.02, n=4) when compared to unstimulated cells. Similarly, chronic stimulation tended to increase media content of IL-8 (2.5-fold, p=0.136, n=3), while intermittent electrical pulse stimulation led to a significant increase in IL-8 (3-fold, p=0.025, n=4). Activation of AMPK (+39%, p=0.287, n=2) and increases of specific mitochondrial respiratory chain proteins (+11-20%) were observed only after the intermittent EPS.

CONCLUSION
Using an electrical pulse stimulation protocol allowing intermittent dosing of higher-frequency EPS lasting in total for 4,5 h during the 24 h period and combined with low-frequency subthreshold stimulation in the rest of the experimental period elicited strong physiological adaptive response in cultured human muscle cells, manifested by the release of contraction-

regulated myokines, activation of AMP-kinase and an increase of mitochondrial respiratory chain proteins.

KEY WORDS
AMPK, electrical pulse stimulation, in vitro model of exercise, myokines, primary human skeletal muscle cells

ACKNOWLEDGEMENT
This work was financed by APVV 20-0466, MZCR NU21-05-00322, VEGA 2/0076/22, European Regional Development Fund - OP Integrated Infrastructure [ITMS: 313011V344].

P-02

Graphene Oxide derivatives as potential drug nanocarriers

Marlena Paździor, Aleksandra Świetlicka, Agata Hadryś, Andrzej Bąk, Violetta Kozik

Institute of Chemistry, University of Silesia in Katowice, Poland

Nanotechnology is a multidisciplinary research field drawing from chemistry, engineering, physics as well as materials science. Creation and development of novel nanomaterials with unique properties result in the increased numbers of their applications in biology and medicine [1]. Since, transporting of drug to its target(s) has been a challenge in pharmacology for at least the several decades, the new drug delivery systems are being extensively scrutinized. It was noticed, that graphene modifications, mainly graphene oxide (GO) derivatives are well suited to serve as the carriers of anti-cancer drugs. In fact, it is possible to attach drugs to GO through chemical reactions or physical sorption, while high hydrophilicity and biocompatibility make GO a nanocarrier with the high potential drug loading capacity. In particular, the presence of COOH and OH groups enables to combine graphene oxide with other structures, e.g., polymers, Fe_3O_4 nanoparticles, and biomolecules, respectively. The modification of GO with biodegradable polymer material, such as chitosan (CS), can noticeably improve the drug-releasing feature of the parent matrix. Practically, graphene oxide can be linked to chitosan using the hydrogen bonds (HBs) between epoxy and amine groups, respectively [2].

Synthesis of the GO- selected amino acids was based on nucleophilic substitution of amino acids on GO nanoparticles. In the first step, the selected amino acids (AAs) were transformed into amino acids methyl ester hydrochlorides in order to protect the C-terminal group of the corresponding amino acid. As a matter of fact, we focused mainly on refining the syntheses and modifications of graphene oxide with amino acids, peptides, chitosan. Moreover, the carrier combinations with drugs were investigated as well. The chemical structures of GO conglomerates were confirmed by Fourier transform infrared spectroscopy (FT-IR), whereas ^{1}HNMR and ^{13}CNMR spectra were recorded using a spectrometer (400 MHz).

It should be emphasised that, GO derivatives might be used to detect tumor-related changes and destroy melanoma cells - a type of cancer that is resistant to chemotherapy so far. The synthesized GO derivatives with the promising cytotoxic profiles will be ultimately implemented in the pre-clinic survey.

ACKNOWLEDGMENTS

This work was financed by a subsidy from the Bioorganic research group, Institute of Chemistry, Faculty of Exact and Technical Sciences, the University of Silesia in Katowice, and the funds for the scientific activity of doctoral students of the Doctoral School at the University of Silesia in Katowice.

REFERENCES

1. C. Liao, Y. Li, S. Tjong, Graphene Nanomaterials: Synthesis, Biocompatibility, and Cytotoxicity. (2018) *Int. J. Mol. Sci.*, 19 (11), 3564.
2. S. Naahidi, M. Jafari, M. Logan, Y. Wang, Y. Yuan, H. Bae, B. Dixon, P. Chen, Biocompatibility of hydrogel-based scaffolds for tissue engineering applications. (2017) *Biotechnol. Adv.*, 35, 530-544.

P-03

The effect of acute aerobic exercise on extracellular vesicles/EVs in serum and cerebrospinal fluid in humans: the comparison of two methods of EVs isolation

Oksana Mytiai[1,2], Kristína Balčoková[1], Kristína Ukropcová[1], Nikoleta Alchus Laiferová[1,2], Martin Schön[1], Mária Tomková[1], Jozef Ukropec[1], Barbara Ukropcová[1,2]

[1]*Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences*
[2]*Institute of Pathological Physiology, Faculty of Medicine, Comenius University; Bratislava, Slovakia*

INTRODUCTION

Health benefits of regular physical exercise are mediated by multiple mechanisms that act in parallel. Extracellular vesicles (EVs), released into circulation in response to each exercise bout, are known to transport large spectrum of bioactive molecules [1], potential mediators of exercise-induced adaptive response [2]. The dynamics of circulating EVs production and release by exercise as well as the impact of different isolation methods on the EVs biophysical characteristics is largely unexplored.

AIM

The aim of this work is (i) to assess the effect of acute bout of intense aerobic exercise on EVs secretion/release into the blood and cerebrospinal fluid (CSF) of healthy humans, and (ii) to compare two EVs isolation methods i.e size exclusion chromatography (SEC) and ExoQuick™.

MATERIALS AND METHODS

The EVs were isolated from serum and CSF of young, healthy, physically active adults (age 26.0 ± 4.0 yrs., VO_2max 50.1 ± 10.5 mlO_2/kgBW/min) by SEC and ExoQuick™. Biological material was obtained in three different experimental timepoints (i) before the 90-minute monitored outdoor run (75-85% HRmax, serum and CSF), (ii) immediately after the run (serum), and (iii) 60 minutes after the run (serum and CSF). Maximal aerobic capacity (VO_2max) was determined by cycle ergometry. Concentration and size of EVs was assessed using Nanoparticle Tracking Analysis (NTA, Nanosight. UK).

RESULTS

Examination of EVs isolated from serum using ExoQuick™ revealed that smaller nanoparticles (size range Ø 30 to 80 nm) increased after exercise by approximately 20% while a more significant increase in concentration (30-50%) was found for larger EVs population (Ø 80 - 150 nm) ($p<0.05$). EVs isolated from serum by SEC displayed >60% increase in small nanoparticles (Ø 30 to 50 nm), while the number of nanoparticles larger 50 nm increased by about 40% ($p<0,01$). Our results clearly show a significant increase in the number of EVs of the size of exosomes in serum immediately after acute endurance exercise, which was followed by almost complete normalization within a 60-minute recovery period, while increase in concentration and size of EVs derived from CSF remained increased at least 60 minutes after exercise (CSF). Compared to SEC, isolation of EVs with ExoQuick™ provides more concentrated but more heterogenous (in size) sample of smaller exosomal particle population (Ø 70.8 ± 22.1 nm) when compared to those isolated by SEC (Ø 101.5 ± 7.9 nm). However, nanoparticles isolated from CSF were bigger in size when compared with those from serum and both isolation methods provided nanoparticle populations of similar size (Exoquick™: Ø

153.9 ± 17.8 nm) and (SEC: Ø 153.8 ± 18.5 nm) from CSF. In CSF, twenty and fifty percent post-exercise increase in EVs/exosomes concentration was found when isolated by SEC and ExoQuick™, respectively.

CONCLUSION

Acute endurance exercise increases the number of exosome-sized particles in systemic circulation and in CSF. Compared to ExoQuick™, isolation of EVs from serum by size exclusion columns yields more homogenous population of larger nanoparticles. However, both methods provided EVs/exosome populations of similar size and concentration when applied in CSF (less complex biological sample).

KEY WORDS

Extracellular vesicles, exosomes, acute exercise, cerebrospinal fluid, serum, nanoparticle tracking analysis, ExoQuick™, size exclusion chromatography

ACKNOWLEDGEMENT

This work was supported by APVV20-0466, APVV 15-0253, VEGA-2/0076/22.

REFERENCES

1. B. Yue et al., Exosome biogenesis, secretion and function of exosomal miRNAs in skeletal muscle myogenesis. (2020) *Cell Proliferation*, 53, no. 7. doi: 10.1111/cpr.12857.
2. A. E. Rigamonti et al., Effects of an acute bout of exercise on circulating extracellular vesicles: tissue-, sex-, and BMI-related differences. (2020) *International Journal of Obesity*, 44, no. 5. doi: 10.1038/s41366-019-0460-7.

P-04

Zoom in the brain sphingomyelin

Petra Maleš[1], Jana Munivrana[2], Danijela Bakarić[1]

[1]*Ruđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Bijenička 54, 10000 Zagreb, Croatia*
[2]*University of Zagreb, Faculty of Science, Department of Chemistry, Horvatovac 102A, 10000 Zagreb, Croatia*
**Corresponding author email address:* pmales@irb.hr

The biological membranes are multicomponent systems with two compositionally specific leaflets. Due to the extremely complex structure, their characterization is often performed on simpler models, which are dominated by membranes made only of lipids, such as sphingomyelin (SM) [1]. With the main phase transition temperatures in the range of 30-45 °C, depending on the length of hydrophobic chains, SM might have the potential to introduce lateral heterogeneity and immutability in the membrane plane. The role of SM in biomembranes is believed to participate in the formation of a phase domain structure, although there is no agreement about the molecular basis of this domain structure [2]. Since they possess several hydrogen bond-accepting and -donating groups at the polar/non-polar interface, hydrogen bonding seems particularly likely in the case of sphingomyelin [3]. Using the brain SM, which is a mixture of SM lipids with different lengths of hydrophobic chains, we examined the effect of buffers of different pH values on hydrogen bond network with calorimetric and spectroscopic techniques. The analysis of the brain SM multilamellar liposomes with FTIR ATR and fluorescence spectroscopy, UV/Vis spectrophotometry and DSC calorimetry will try to clarify influence of different pH on SM structural domains [4].

ACKNOWLEDGEMENT
This work was financed by Croatian Science Foundation within the project „Model of demyelination on a molecular scale at physiological and pathological conditions“ (HrZZ UIP-2020-02-7669).

REFERENCES
1. T. Heimburg (2007) *Thermal Biophysics of Membranes*, Wiley, Weinheim, 29-40.
2. L. K. Bar, Y. Barenholz, T. E. Thompson (1997*) Biochemistry*, 36, 2507-2516.
3. Z. Arsov, L. Quaroni (2008) *BBA*, 1778, 880–889.
4. P. Maleš, Z. Brkljača, D. Domazet Jurašin, D. Bakarić (2022) *SAA*, 272, 121013, 7.

P-05

Novel carbohydrate-based amphiphiles for medical and pharmaceutical applications: Structure-biological activity relationships

Zuzana Hricovíniová[1*] and Wojciech Smułek[2]

[1]*Institute of Chemistry, Slovak Academy of Sciences, SK-845 38 Bratislava, Slovakia*
[2]*Institute of Chemical Technology and Engineering, Poznan University of Technology, 60-965 Poznan, Poland*

Carbohydrate-based amphiphiles represent a class of structurally diverse, low toxic and biodegradable nonionic surfactants. They are considered as environmentally friendly molecules and their use became widespread in recent years [1-2].

Structurally different alkyl glycosides were prepared in very good yields and new derivatives varying in chain structure (C_4-C_{20}) were characterized by NMR spectroscopy. Molecular geometry optimizations of different ring forms ($^1C_4$ and $^4C_1$) and anomeric configurations were carried out using DFT calculations [3]. A series of structurally variable alkyl L-rhamnosides and D-lyxosides has been investigated to accomplish a better understanding of their physico-chemical properties as well as biological activities. The impact of the compounds on biofilm formation, cell surface hydrophobicity, cell membrane permeability together with their toxicity on the selected microorganisms have been determined [4]. Antimicrobial activity of the target compounds was tested against bacteria of the genus *Pseudomonas* and pathogenic fungi *Candida albicans* and *Aspergillus niger*. The data presented in this study demonstrate that well-defined alkyl-glycoside surfactants varying in the carbohydrate head-group, anomeric configuration and length of the alkyl chain are ideal models for investigating how small changes in the structure affect their amphiphilic and biological properties.

Overall, the results indicate that long-chain alkyl D-lyxosides and alkyl L-rhamnosides can be considered as inexpensive, biocompatible, nontoxic agents and serve for the surface design to avoid bacterial adhesion as an alternative solution to antibiotic treatment. The application of this approach for the synthesis of new family of non-ionic amphiphiles makes the method attractive because of their potential use in biomedical and pharmaceutical chemistry.

ACKNOWLEDGEMENT

This work was supported by the Scientific Grant Agency VEGA of the Slovak Republic Project 2/0071/22.

REFERENCES

1. H. Razafindralambo, C. Blecker, M. Paquot (2012) *Adv. Chem. Eng.,* 215–228.
2. M. J. Rosen, Surfactants and Interfacial Phenomena, 3rd ed.; Wiley Interscience, 2004, 1–33.
3. Z. Hricovíniová, M. Hricovíni (2014) *Tetrahedron: Asymmetry,* 25, 1008–1014.
4. W. Smułek, N. Burlaga, M. Hricovíni, A. Medveďová, E. Kaczorek, Z. Hricovíniová (2021) *Chemosphere*, 271, 129818.

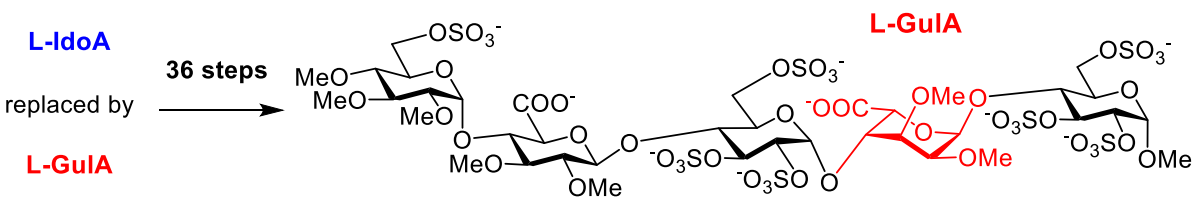
P-06

Synthesis and anticoagulant activity of an L-guluronic acid containing idraparinux analogue pentasaccharide

Mihály Herczeg[1], Fruzsina Demeter[1], István Timári[2], Katalin E. Kövér[3,4], Anikó Borbás[1]

[1]*Department of Pharmaceutical Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary,*
[2]*Department of Organic Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary*
[3]*Department of Inorganic and Analytical Chemistry, University of Debrecen, Egyetem tér 1, Debrecen, H-4032, Hungary*
[4]*MTA-DE Molecular Recognition and Interaction Research Group, University of Debrecen, Egyetem tér 1, Debrecen, H-4032, Hungary*
E-mail: demeter.fruzsina@science.unideb.hu

One of the most problematic parts of the synthesis of heparinoid anticoagulants is the efficient preparation of the L-iduronic acid unit [1]. In the case of idraparinux, a heparin-related anticoagulant pentasaccharide, we demonstrated that L-iduronic acid can be replaced by an easier-to-produce L-sugar without significant change in biological activity. From the inexpensive D-mannose, through a highly functionalized phenylthio-mannoside, L-gulose donor was prepared by C-5 epimerization [2] in 10 steps. This unit was incorporated into the pentasaccharide by α-selective glycosylation and oxidized to L-guluronic acid. The complete synthesis required only 36 steps. The guluronate-containing pentasaccharide was able to inhibit coagulation factor Xa with high potency, indicating that L-iduronic acid is interchangeable without loss of bioactivity.



ACKNOWLEDGEMENT
The synthetic work was supported by the National Research and Development and Innovation Office of Hungary (K 132870, A. B.; FK 137924 M. H.), Premium Postdoctoral Program of HAS (PPD 461038, M. H.) and the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.4-15-2020-00008.

REFERENCES

1. S. Mohamed, V. Ferro (2015) *Adv. Carbohydr. Chem. Biochem*., 72, 21-61.
2. F. Demeter, F. Veres, M. Herczeg, A. Borbás (2018) *Eur. J. Org. Chem.*, 48, 6901-6912.

P-07

From nano- to milimolar: dimerization dissociation constant determination

Alexandra Náplavová[1,3], Aneta Kozeleková[1,3], Jozef Hritz[1,2*]

[1]Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czechia
[2]Department of Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia
[3]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, Brno, Czechia
**jozef.hritz@ceitec.muni.cz*

The self-association of proteins is the cornerstone of protein regulation, aiding proper functionality and interactome [1]. To properly understand the role of dimerization of protein, only the detection of such dimer is not sufficient, but a quantitative analysis is crucial.
A parameter widely used to quantify the self-association is the dissociation constant K_D [2]. The K_D describes equilibrium between monomers and higher oligomers - in general, the lower the K_D, the higher the propensity for oligomerization. However, K_D of various proteins differs in several orders of magnitude, providing a challenge in its determination [3].
Here we showcase an array of biophysical methods for coverage of the whole relevant concentration range. For proteins with K_D in nM region we optimised Förster resonance energy transfer assay [4]. In µM range an analytical size exclusion combined with multiple angle light scattering can be employed [3]. Finally, we used ^{19}F Trp NMR for evaluation in higher concentrations. Moreover, we show application of such methods on the example of 14–3–3 proteins – cellular regulators connected to oncologic and neurodegenerative diseases [5,6].

ACKNOWLEDGEMENT
This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic within programme INTER-ACTION (project no. LTAUSA18168). AN acknowledges the Grant Agency of Masaryk University (MU) for the support of an excellent diploma thesis (no. MUNI/C/0091/2022). We acknowledge Josef Dadok National NMR Centre and Biomolecular interactions and crystallization and Proteomics Core Facilities of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127).

REFERENCES
1. N.J. Marianayagam, M. Sunde et al. (2004) *Trends in Biochemical Sciences*, 29, 618-625.
2. I. Jarmoskaite, I. AlSadhan et. al. (2020) *Elife* 9.
3. A. Kozeleková, A. Náplavová et. al. (2022) *Frontiers in Chemistry*, 10.
4. Z. Trošanová, P. Louša P et. al. (2022) *Journal of Molecular Biology*, 434, 167479.
5. P. Steinacker, A. Aitken et. al. (2011) *Seminars in Cell & Developmental Biology*, 22, 696-704.
6. N.N. Sluchanko, D.M. Bustos (2019) *Progress in Molecular Biology and Translational Science*, 19-61.

P-08

Evaluation of the effect of hydrogel substrate components on the stability of tetracycline hydrochloride

Agnieszka Kostrzębska, Karolina Pączek, Angelika Weselak, Witold Musiał

Department of Physical Chemistry and Biophysics, Faculty of Pharmacy, Wroclaw Medical University, ul. Borowska 211A, 50-556 Wrocław, Poland

Tetracyclines belong to a group of broad-spectrum antibiotics that prevent the proliferation of bacteria by inhibiting bacterial protein synthesis. Among its numerous indications, tetracycline is one of the primary antibiotics used in oral anti-acne therapy [1]. In addition to their antibacterial activity, tetracyclines exhibit non-antibiotic activities, such as anti-inflammatory activity, which can be used effectively in the topical treatment of acne [2]. Tetracyclines are also unstable compounds, with a very high sensitivity to external factors and under unfavorable conditions they degrade with the formation of numerous decomposition products [3].

The subject of our analysis was to evaluate the effect of varying hydrogel compositions on the stability of tetracycline hydrochloride contained in these formulations. Its results will allow the design of hydrogel formulations with an optimal composition that ensures both antimicrobial efficacy and long-term stability of the antibiotic contained in them. The research was conducted in two parts. The content of tetracycline hydrochloride in both parts was the same. In the first part, hydrogel formulations were developed with a fixed content of the anionic acrylic acid polymer and with a variable content of TRIS alcoholamine physically cross-linking the system. The prepared formulations differed in pH values reaching values from 5.00 to 8.36. Stability evaluation of tetracycline hydrochloride contained in the hydrogels was carried out based on HPLC analysis for a period of 28 days taking samples at equal intervals once a week. The 28-day analysis showed that the antibiotic degraded rapidly in the alkaline formulations. The concentration of the drug in the samples analyzed by HPLC at the time t_0 was about 9 μg/ml. After 28 days, a slight decrease in the tetracycline content was demonstrated in slightly acidic and neutral hydrogels, where the drug concentration reached the value of about 7.5 μg/ml. In the case of the formulations with pH values of 8.04 and 8.36, the concentration reached the value of 1 μg/ml and below 1 μg/ml. The progressive decomposition process and the appearance of new products were also demonstrated by evaluating the change in color of the hydrogels. In the second part, formulations with a constant amount of TRIS alcoholamine were prepared. Likewise, the polymer content was constant, however, three different polymers of acrylic acid were selected for the study to assess whether the type of carbomer selected had an impact on the stability of tetracycline hydrochloride. The pH value of all formulations was about 6.5. As with the first part of the study, the formulations were subjected to HPLC analysis lasting 28 days. After this time, high stability of the antibiotic was demonstrated in all formulations regardless of the type of polymer used. The drug concentration values were close to the concentrations at t_0. Also, optical evaluation of color changes in the tested formulations showed no significant changes. The conducted tests showed that the type of selected polymer did not affect the stability of the tetracycline hydrochloride contained in the formulation. However, the mutual proportion of the polymer and the alcoholamine used, and thus the pH value of the entire developed system, is extremely important. An excessively alkaline pH leads to a very rapid degeneration of the antibiotic used.

ACKNOWLEDGEMENT
This work was financed by SUBZ.D060.22.034 grant of Wroclaw Medical University.

REFERENCES
1. I. Chopra, M. Roberts, Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. (2001) *Microbiol. Mol. Biol. Rev.*, 65, 232-260.
2. A.N. Sapadin, R. Fleischmajer, Tetracyclines: Nonantibiotic properties and their clinical implications. (2006) *J. Am. Acad. Dermatol.*, 54, 258-265.
3. A. Kostrzębska, A. Złocińska, W. Musiał, Evaluation of the influence of a hydrogel containing AMPD on the stability of tetracycline hydrochloride. (2021) *Pharmaceutics*, 13, 1-15.

P-09

Co-precipitation of viral glycoprotein HCMV UL144 and human NK cell activating ligand CD160 has revealed their mutual engagement

Andrej Bitala[1], Simona Lenhartová[1], Mário Benko[1], Marek Nemčovič[2], Ivana Nemčovičová[1]

[1]Biomedical Research Center, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

Many receptors and surface-exposed ligands acting on immune cells are being considered as a starting point in drug development applications. As they are dedicated to manipulating a wide range of immune responses, accurately predicting their molecular interactions will be necessary for the development of safe and effective therapeutics to enhance immune responses and vaccination. Human cytomegalovirus (HCMV, β-herpesvirus) with a linear double-stranded DNA, is the leading viral cause of congenital birth defects and is responsible for morbidity and mortality in immunosuppressed individuals. In such case, HCMV can replicate to high levels and cause serious end-organ disease [1]. Currently, an effective vaccine for HCMV has not been licensed, although it has been considered a global health priority. In recent years, several immunoreceptors were identified as candidate target for immunotherapy, however human CD160 stands from the crowd because its specificity for HCMV. HCMV within its unique long (UL)/b' locus, encodes the key immunomodulatory proteins [2], such as UL144 which is highly orthologous to tumor necrosis factor receptor HVEM (TNFR/SF14, herpesvirus entry mediator) thus resembling some of its promiscuity on the cell surface. HVEM binds the TNF ligands, LIGHT and LTa; the immunoglobulin inhibitory receptor, B and T lymphocyte attenuator (BTLA); and the natural killer cell-activating receptor CD160. However, the initial studies showed that UL144 selectively binds only BTLA [3], while avoiding activation of inflammatory signaling initiated by CD160 in natural killer (NK) cells. This molecular network is quite well described, however, the engagement of CD160 by UL144 has not yet been satisfactorily studied. Our recent data suggest that HCMV UL144, the viral mimic of the HVEM, does not bind CD160, we hypothesize this is due to altered N-linked glycosylation. Mutants and species variants of UL144 lacking these glycosylation sites were generated and it was shown by co-IP assay and other methods the CD160 binding was impacted.

ACKNOWLEDGEMENT

This research was funded by the contribution of the Slovak Research and Development Agency under the project APVV-19-0376; and the contribution of the Scientific Grant Agency of the Slovak Republic under the grant VEGA-02/0026/22 and VEGA-02/0060/21. IN is Marie Curie Fellow and was financed by program SASPRO co-funded by the European Union and the Slovak Academy of Sciences.

REFERENCES

1. P. Griffiths, M. Reeves (2021) *Nat. Rev. Microbiol*., 19, 759-773.
2. T. L. Murphy, K. M. Murphy (2010) *Annu. Rev. Immunol*., 28, 389-411.
3. C. F. Ware, J. R. Šedý (2011) *Curr. Opin. Immunol*., 23, 627-31.

P-10

Physicochemical and mechanical behavior analysis of composite coatings designed for bone regeneration

Dagmara Słota, Wioletta Florkiewicz, Karina Piętak, Mateusz Dyląg, Agnieszka Sobczak-Kupiec

Department of Materials Science, Faculty of Materials Engineering and Physics, Cracow University of Technology, 37 Jana Pawła II Av., 31-864 Krakow, Poland

The coating technique requires covering the surface of the implant with a layer of another biomaterial to give it additional properties like a specific biological response of the host tissue in the peri-implant area. Particularly interesting in the case of bone implants in this aspect are bioactive ceramic materials, such as hydroxyapatite (HA), which in a living organism spontaneously form a layer of bone apatite on its surface and through this layer fuse to the natural bone. These kinds of materials are of great clinical importance as bone reconstruction materials [1-2]. The apatite formed is highly similar to bone mineral in its composition and structure. Therefore, osteoblasts preferentially proliferate and differentiate to produce apatite, as well as collagen on this layer of apatite. When an inert material is coated with HA, bone cells adhere to the surface of the apatite coating without intermediate layers. The HA matrix of bone cells later becomes integral with the coating, resulting in excellent adhesion of the coated implant to the bone [3].

The aim of the study was to evaluate the mechanical as well as physicochemical properties of materials composed of polymeric matrix (polyvinylpyrrolidone and polyglycol ethylene) enriched with glutathione, collagen and reinforced with HA. It should be noted that the developed materials have great potential due to the high biological value of the components used in their synthesis, such as glutathione and hydroxyapatite, which promote osteogenesis. An analysis of sorption capacity was carried out which confirmed the effect of ceramic content on this parameter. A higher amount of HA resulted in less swelling. It was also observed that coatings with HA demonstrated higher hardness parameter.

So far, no other solution of this type has been found.

ACKNOWLEDGEMENT

The "Multifunctional biologically active composites for applications in bone regenerative medi-cine" project is carried out within the TEAM-NET program of the Foundation for Polish Science financed by the European Union under the European Regional Development Fund. The authors gratefully acknowledge financial support. POIR.04.04.00-00-16D7/18.

REFERENCES

1. T. Kokubo, T. Himeno, H.M. Kim, M. Kawashita, T. Nakamura, Process of Bonelike Apatite Formation on Sintered Hydroxyapatite in Serum-Containing SBF. (2004) *Key Engineering Materials,* 254-256, 139-142.
2. N. Saba, M. Jawaid, M.T.H. Sultan, An overview of mechanical and physical testing of composite materials (2018) *Elsevier*.
3. K. Pluta, W. Florkiewicz, D. Malina, K. Rudnicka, S. Michlewska, J.B. Królczyk, A. Sobczak-Kupiec, Measurement methods for the mechanical testing and biocompatibility assessment of polymer-ceramic connective tissue replacements. (2021) *Measurement: Journal of the International Measurement Confederation,* 171, 108733.

P-11

Polymorphic amyloid-like crystal structures of proglucagon derived hexapeptides demonstrate pH-dependent reversible amyloid formation

Dániel Horváth[1], Zsolt Dürvanger[2], Nóra Taricska[2], Máté Sulyok-Eiler[2], András Perczel[1*]

[1]ELKH-ELTE Protein Modelling Research Group, Budapest, Hungary
[2]Laboratory of Structural Chemistry and Biology, Budapest, Hungary
**perczel.andras@ttk.elte.hu*

A large group of hormone peptides are functionally stored as amyloid fibrils in acidic (*pH*= 5.5) secretory vesicles.[1] Granular storage of hormones in phase-separated form is advantageous because large amounts of protein can be stored in an intact, stabilised form.[2] As secretion signal triggers, the granules release instantaneously the stored hormones into the bloodstream allowing an acute export beyond the biosynthetic capacity. Aggregates dismantle due to the changing chemical environment, and the monomers readopt their helical structure. Glucagon, glucagon-like peptide 1 and 2 (GLP-1 and 2) glucose-dependent insulinotropic peptide (GIP), and orthologue of exendins are members of a family of secretin-like gastrointestinal hormones, their synthetic derivatives have been successfully used in medicine. We identified an evolutionarily conserved hexapeptide sequence (xFxxWL) as a major aggregation-prone region, which is also responsible for receptor binding. We determined the amyloid-like crystal structures of the aggregation core of GLP-1, GLP-2, exendin-4 and the tc5b model peptide. The latter two one exhibit significant polymorphism. Based on combined ECD and crystallographic results, we provide the molecular basis for pH-dependent reversible amyloid formation and show that the "gatekeeper" glutamate/aspartate side chains in the aggregation core act as molecular switches between the amyloid and native states depending on their protonation state. We demonstrate that in hexapeptide lacking this molecular switch, amyloid aggregation occurs over a much wider pH range, even under physiological conditions. The aggregation of peptide-based drugs poses a major threat in manufacturing processes, on the other hand, the controlled and reversible self-assembly of hormone derivatives as nanofibrillar amyloid depots may open new opportunities for the development of sustained release drug delivery systems.

ACKNOWLEDGEMENT

This research project was supported by the European Union and the State of Hungary and co-financed by the European Regional Development Fund (VEKOP-2.3.2-16-2017-00014 and VEKOP-2.3.3-15-2017-00018*). Project no. 2018-1.2.1-NKP-2018-00005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2018-1.2.1-NKP funding scheme. This work was completed in the ELTE Thematic Excellence Programme supported by the Hungarian Ministry for Innovation and Technology. Thematic Excellence Programme 2020, National Challenges Subprogramme - TKP2020-NKA-06.*

REFERENCES

1. S.K. Maji, M.H. Perrin, M.R. Sawaya, S. Jessberger, K. Vadodaria, R.A. Rissman, P.S. Singru, K.P.R. Nilsson, R. Simon, D. Schubert, D. Eisenberg, J. Rivier, P. Sawchenko, W. Vale, R. Riek, Functional Amyloids as Natural Storage of Peptide Hormones in Pituitary Secretory Granules. (2009) *Science*, 325 (5938), 328-332.
2. P. Arvan, D. Castle, Sorting and Storage during Secretory Granule Biogenesis: Looking Backward and Looking Forward. (1998) *Biochem. J.*, 332 (Pt 3), 593-610.

P-12

The interaction of sodium hyaluronate with lidocaine hydrochloride and sodium naproxen

Dorota Wójcik-Pastuszka, Karolina Stawicka, Witold Musiał

Wroclaw Medical University, Faculty of Pharmacy, Department of Physical Chemistry and Biophysics
ul. Borowska 211A, 55-556 Wroclaw, Poland
e-mail: dorota.wojcik-pastuszka@umw.edu.pl; witold.musial@umw.edu.pl

Hyaluronic acid is a natural component of the intercellular matrix of the dermis. This biomolecule has strong hygroscopic, regenerating and nourishing properties. Biocompatibility, rheological properties and biodegradability make this biopolymer widely used in the cosmetics industry, as well as a drug carrier in the pharmaceutical industry [1,2].
The aim of the research was to study the interaction between the carrier, that was sodium hyaluronate (HA), and the drug- lidocaine hydrochloride (L) or sodium naproxen (N). For this purpose gels based on HA doped with L or N or both of them were obtained.
Formulations were prepared by addition of the drug solution to HA obtaining the gels with the carrier concentration of 1,5%, 2%, 2,5%. The viscosity of the formulations were measured at the temperature of 37°C employing the rotational viscometer. The gels samples were dried at 6°C, grinded in a mortar and subjected to FTIR and DSC tests. The spectra of pure ingredients, formulations and the physical mixtures consists of the corresponding components were recorded using the FTIR spectrometer coupled to the ATR mode in the wavenumber range of 500-4000 cm^{-1}. The DSC study was carried out in the nitrogen atmosphere in the temperature range from -10 to 300°C using the calorimeter.
The viscosity tests showed that the incorporation of L or N or both of them to the formulation increases the viscosity of the appropriate gel relative to the drug-free formulation. The FTIR spectra analysis revealed that there is no interaction between composition ingredients in their physical mixtures. All characteristic bands of the components in the spectra of physical mixtures were present. However, in the case of formulations doped with L the maximum of L at 3451 cm^{-1}, 3383 cm^{-1}, 1654 cm^{-1} belonging to N-H and C=O groups disappeared. Similar observation was in preparations containg N. The COO- group signal belonging to N was shifted towards the lower 1541 cm^{-1} wave frequency. The mentioned modifications were also noticed in the spectrum of the formulation containing both drugs. The FTIR results were consistent with data obtained from the DSC study.
It may be concluded that in the obtained formulations the interaction between the group C-O belonging to HA and N-H group from L was founded as well as between the deprotonated hydroxyl group coming from from HA and the acid groups COO- belonging to N. These interaction may explain the increase of the viscosity of the gels dopped with L, N or both of them.

ACKNOWLEDGEMENT
This work was financed by the grant of Wroclaw Medical University and Ministry of Science and Higher Education number SUBZ.D060.22.034.

REFERENCES
1. A. M. Juncan, D. G. Moisă, A. Santini, C. Morgovan, L. L. Rus, A. L. Vonica-țincu, F. Loghin, (2021) *Molecules*, 26, 1–43.
2. X. Zhang, D. Wei, Y. Xu, Q. Zhu, (2021) *Carbohydr. Polym.*, 264, 118006.

P-13

Semisynthetic teicoplanin derivatives against SARS-CoV-2 and multiresistant bacteria

Eszter Boglárka Lőrincz[1,2,3], Erzsike Rőth[1], Herczegh Pál[1], Borbás Anikó[1,4], Bakai-Bereczki Ilona[1,4]

[1]*Department of Pharmaceutical Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary*
[2]*Institute of Healthcare Industry, University of Debrecen, H-4032, Debrecen, Nagyerdei körút 98, Hungary*
[3]*Doctoral School of Pharmaceutical Sciences, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary*
[4]*National Laboratory of Virology, Szentágothai Research Centre, Ifjúság útja 20, H-7624 Pécs, Hungary*

Glycopeptide antibiotics can be used as a last resort against Gram-positive bacteria that cause serious, often fatal infections. In addition, several natural and semi-synthetic derivatives have significant antiviral activity against viruses, for example Teicoplanin was discovered to potently prevent the entry of Ebola and coronaviruses (MERS and SARS) into the cytoplasm of the host cell.

Our research group has been working with glycopeptide antibiotics for a long time. Some of the previously prepared teicoplanin pseudoaglycone derivatives has activity against human coronavirus [1]. Moreover, some derivatives containing fluorinated side chains have been shown to have excellent anti-SARS-CoV-2 activity [1]. Biological studies shown that the length of the linker between the glycopeptide core and the fluorinated side chain and the length of the perfluoroalkyl chain significantly affect the antiviral and antibacterial activity. Therefore, by further modifications of the linker and the fluorinated side chain, we have attempted to produce derivatives with excellent SARS-CoV-2 activity while maintaining or enhancing antibacterial activity. This would also be advantageous because SARS-CoV-2 infection often results in bacterial over-infection, which can lead to the death of the patient.

REFERENCES

1. I. Bereczki, M. Csávás, Z. Szűcs, E. Rőth, G. Batta, E. Ostorházi, L. Naesens, A. Borbás, P. Herczegh, Synthesis of Antiviral Perfluoroalkyl Derivatives of Teicoplanin and Vancomycin. (2020) *ChemMedChem*, 15 (17), 1661-1671.

P-14

Indirect Nuclear Spin-Spin & Hyperfine Coupling pathway visualisation in heavy metal complexes

Florian Lemken, Olga L. Malkina, Vladimir Malkin, Stanislav Komorovský

Institute of Inorg. Chem., Slovak Academy of Sciences, Dúbravská cesta 9, 84536 Bratislava, Slovakia
Corresponding author: florian.lemken@savba.sk

Metal complexes (including many enzymes) serve a plethora of essential roles in biological systems far beyond the scope of this abstract. Besides the well-established effect of the conformation of various enzymes on their biological functionality, the activity of enzymes such as cytochrome P450 is highly dependent on spin-states [1]. The same holds for various synthetic, bioactive compounds such as the new antimetastasis inhibitor (NAMI) [trans-$Ru(III)Cl_4(DMSO)Im]^{-1}$ (cf. fig. 1).

Magnetic resonance techniques provide an essential tool for the investigation of spin-states and in some cases even conformation (e.g. ref. [2]) and are in addition potentially applicable to in vitro and in vivo systems a like due to their non-invasive nature. Therein, the internal magnetic couplings (indirect nuclear spin-spin and electronic hyperfine coupling) are essential observables. Theoretical studies centred around the visualisation of the pathway those coupling follow throughout a given molecule have significantly aided magnetic resonance investigations (e.g. ref. [3]).

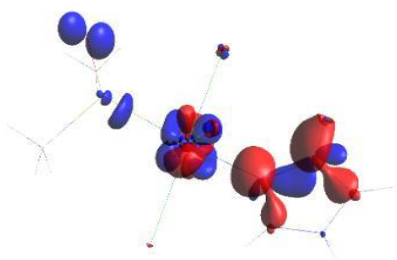


Figure 1: Hyperfine coupling pathway visualisation in NAMI.

Those investigations were generally limited to subsections of magnetic interactions (i.e. Fermi contact) between the coupling partners and neglective of relativistic effects [3]. Both are valid assumptions for molecules without heavy atoms but may not be suitable for the biologically interesting compounds detailed above. Therefore, we present a study of relativistic effects on magnetic coupling pathways in heavy metal complexes.

ACKNOWLEDGEMENT

The s upport from Slovak grant agencies APVV grant No. APVV 19 0516 and VEGA (grant No. 2/0135/21) is acknowledged.

REFERENCES

1. W.R. Scheidt, C.A. Reed (1981) *Chem. Rev. Rev*., 81, 543 555.
2. M. Dračínský, M. Buchta, M. Buděšínský, J. Vacek Chocholoušová, I. Stará, I. Starý, O. L. Malkina (2018) *Chem. Sci.*, 9, 7437 744 6.
3. M. Bühl, F. R. Knight, A. Křstkova, I. Malkin Ondík, O. L. Malkina, R. A. M. Randall, A. M. Z. Slawin, J. D. Woollins (2013) *Angew. Chem*., 125, 2555 25582558.

P-15

Understanding the intrinsic Asn-Gly isomerization reaction of polypeptides and proteins by NMR kinetics and *ab initio* calculations

Fruzsina Pilhál[1], Imre Jákli[1], Ernő Keszei[2], András Láng[1] and András Perczel[1]

[1]MTA-ELTE Protein Modeling Res. Group and Laboratory of Structural Chemistry and Biology, Institute of Chemistry, ELTE Eötvös Loránd University – Pázmány P. stny. 1/A, 1117 Budapest, Hungary,
[2]Department of Physical Chemistry and Chemical Kinetics Laboratory; Institute of Chemistry, ELTE Eötvös Loránd University – Pázmány P. stny. 1/A, 1117 Budapest, Hungary

Understanding the molecular details of the well-known reactions - deamidation, isomerization and hydrolysis - of -Asn-Gly- (NG for short) containing polypeptides and protein's subunits was the main goal of our research. Based on the literature [1] and previous NMR data [2] their spontaneous isomerization can trigger backbone rearrangements, goes *via* a succinimide intermediate and makes α- and β-aspartic acid derivatives at ~1:4 ratio. The reaction rate-determining step is the deamidation and succinimide formation. Under physiological conditions it happens within hours or days, depending on the pH, T, *c*, *t*, *etc*. It is harmful, especially to long-turnover-rate proteins *e.g.* hemoglobin, crystalline, threatening their structural integrity [3], but the exact mechanism of the isomerization is yet unclear.
We measured the NMR spectra of different tetrapeptides (Ac-NGAA-NH_2, Ac-NGRA-NH_2, Ac-NGKA-NH_2 and Ac-NGEA-NH_2) at different temperatures (T= 301, 310, 319 and 328 K) and pH (5.1, 6.3, 7.4 and 7.8) to investigate the effect of the charged amino acid in the third position, and to examine the temperature and pH dependence of the isomerization. We attempted the kinetic modeling of the reaction of selected tetrapeptides based on the relative integral values obtained from ^{1}H-NMR spectra and the calculated activation energies of the rate determining step from rate coefficients using the natural-based logarithmic form of the Arrhenius equation [4]. The positively charged amino acid, R:= Arg(+), at the third position of the selected tetrapeptides and an increase in temperature and pH accelerate, and a partly negatively charged E:= Glu, decelerate the NG-isomerization.
Based on IRC (Intrinsic Reaction Coordinate) path and NBO (Natural Bond Orbital) [5] analysis of the deamidation the reaction can be divided into 4 main steps: proton transfer, transition state, ring closure and NH_3 release. The preconditions of the reaction are: the geometry of reactants in the transition state match the *Bürgi-Dunitz* angle - the N(amide$_{n+1}$)-Asn(C$^{\gamma}$)-Asn(O$^{\delta 1}$) atoms are in the optimal ~109.5°, and the N(amide$_{n+1}$)-Asn(C$^{\gamma}$) atoms are close enough (< ~2.5Å). The IRC and NBO calculations were carried out at B3LYP/6-31G(d) level of theory. All other calculations were completed with the Gaussian 16 C01 software [6]. Individual points of the IRC paths were collected, and a frequency calculation was completed at each point to obtain Δ*G* values. Endpoints of the IRC paths and transition states were further optimized at DFT B3LYP/6-31++G(d,p) level of theory both in vacuum and using IEFPCM water model.
According to the results based on NMR measurements and *ab initio* calculations a more comprehensive view of the NG isomerization was determined.

ACKNOWLEDGEMENT
The authors thank to Gábor Mező for the oligopeptide synthesis. This work was completed in the ELTE Thematic Excellence Programme supported by the Hungarian Ministry for Innovation and Technology. Thematic Excellence Programme 2020, National Challenges Subprogramme - TKP2020-NKA-06. ELTE Institutional Excellence Program

(1783-3/2018/FEKUTSTRAT) supported by the Hungarian Ministry of Human Capacities and grants from the European Union and the State of Hungary, co-financed by the European Regional Development Fund (VEKOP-2.3.3-15-2017-00020, VEKOP-2.3.2-16-2017-00014). Project no. 2018-1.2.1-NKP-2018-00005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2018-1.2.1-NKP funding scheme.

REFERENCES

1. M. Xie, R.L. Schowen, Secondary Structure and Protein Deamidation. (1999) *Journal of Pharmaceutical Sciences*, 88 (1), 8-13.
2. A. Láng, I. Jákli, K. Enyedi, G. Mező, D. Menyhárd, A. Perczel, Off-pathway 3D-structure provides protection against spontaneous Asn/Asp isomerization: Shielding proteins Achilles heel. (2020) *Quarterly Reviews of Biophysics, 53*, E2. doi:10.1017/S003358351900009X.
3. X. Lu, L.A. Machiesky, N. De Mel, Q. Du, W. Xu, M. Washabaugh, X.R.Jiang, J. Wang, Characterization of IgG1 Fc Deamidation at Asparagine 325 and Its Impact on Antibody-dependent Cell-mediated Cytotoxicity and FcγRIIIa Binding. (2020) *Scientific reports*, 10 (1), 383. https://doi.org/10.1038/s41598-019-57184-2.
4. E. Keszei, A reakciókinetika alapjai, (2016).
5. NBO Version 5.9, E. D. Glendening, A. E. Reed, J. E. Carpenter, and F. Weinhold.
6. Gaussian 16, Revision C.01, M. J. Frisch et al., Gaussian, Inc., Wallingford CT, (2016).

P-16

Influence of the poly(ethyleneglycol) dimethacrylates on the selected physicochemical properties of thermally sensitive polymeric particles of NIPA derivatives

Agnieszka Gola, Borys Podżus, Witold Musiał

Department of Physical Chemistry and Biophysics, Pharmaceutical Faculty, Wroclaw Medical University, Borowska 211, 50-556 Wroclaw, Poland

Potential materials meeting the requirements of modern pharmacotherapy are three-dimensional polymer networks reacting to environmental stimuli in a reversible manner. The multiplicity of possible modifications to their structure, which is related to the influence on their physicochemical properties, speaks in favor of their use as a carrier of a drugs. The synthesis of polymers with the use of cross-linking agents enables obtaining products with different reaction thresholds to external factors (e.g. temperature, pH, ionic strength) compared to the basic linear structure of the polymer. Currently, the aim is to obtain polymers with selected controlled parameters that would enable release of the drug substance at the target site. The value of phase transition temperature equal 32°C of the poly *N*-isopropylacrylamide (PNIPA), similar to the physiological temperature of the human body, makes PNIPA an object of interest medical researchers [1,2]. The subject of research was synthesis of the chemically cross-linked polymeric structures of *N*-isopropylacrylamide (NIPA) derivatives P1-P7 and determine the impact of cross-linking agents on the physicochemical properties of the synhesised products. Thermosensitive polymers P1-P7 of NIPA and poly(ethylene glycol) dimethacrylates (PEGDMAs), average Mn 200-20000, were synthesized via surfactant free precipitation polymerization (SFPP) using ammonium persulfate (KPS) at 70 °C. The polymerization course was evaluated by the conductivity. The hydrodynamic diameters (HD) and the polydispersity indexes (PDI) of aqueous dispersion of synthesized polymers P1-P7 in 18-45°C range, assesssed via dynamic light scattering (DLS) were at 18° (nm): 586.70 $\pm$ 19.51 (PDI 0.57 $\pm$ 0.08), 74.62 $\pm$ 0.76 (PDI 0.56 $\pm$ 0,01), 69.45 $\pm$ 1.47 (PDI 0.57 $\pm$ 0.03), 196.2 $\pm$ 2.50 (PDI 0.53 $\pm$ 0.04), 194.30 $\pm$ 3.36 (PDI 0.56 $\pm$ 0.04), 81.99 $\pm$ 0.53 (PDI 0.56 $\pm$ 0.01), 76.87 $\pm$ 0.30 (PDI 0.54 $\pm$ 0.01), respectively. The electrophoretic mobilities estimated the zeta potential (ZP) in 18–45°C range, and at 18 °C were (mV): -2.57 $\pm$ 0.10, -4.32 $\pm$ 0.67, -5.34 $\pm$ 0.95, -3.02 $\pm$ 0.76, -4.71 $\pm$ 2.69, -2.30 $\pm$ 0.36, -2.86 $\pm$ 0.42 for polymer suspensions P1-P7. The polymers were characterized by Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR), H nuclear magnetic resonance (^{1}H NMR), thermogravimetric analysis (TG/DTA), differential scanning calorimetry (DSC), powder X-ray diffraction analysis (PXRD). The amorphous polymers were obtained. The analysis of the results made it possible to determine the influences of the length of the co-monomer chain on physicochemical properties and estimate the usefulness of the obtained product as a potential carrier of the drugs.

ACKNOWLEDGEMENT

This work was financed by SUBZ.D060.22.034 grant of Wroclaw Medical University.

REFERENCES

1. A. Raza, T. Rasheed, F. Nabeel, U. Hayat, M. Bilal, H. M. N. Iqbal (2019) Molecules, 24, 1117.
2. C. Alvarez-Lorenzo, V. Y. Grinberg, T. V. Burova, A. Concheiro (2020) Int. J. Pharm., 579, 119157.

P-17

Improvement of Total Phenanthrene Quantification by a Novel LC-DAD Analytical Method

Petra Chaľová[1,2*], František Csicsay[2], Jaroslav Galba[2] , Michaela Matušková[1,3], Ivana Čižmárová[1,3], Juraj Piešťanský[1,3], Ľudovít Škultéty[2]

[1]Comenius University Bratislava, Faculty of Pharmacy, Department of Pharmaceutical Analysis and Nuclear Pharmacy, Odbojárov 10, Bratislava, Slovak Republic
[2]Biomedical Research Center of the Slovak Academy of Sciences, Institute of Virology, Dúbravská cesta 9, Bratislava, Slovak Republic
[3]Toxicological and Antidoping Center, Faculty of Pharmacy, Comenius University in Bratislava, Odbojárov 10, 832 32 Bratislava, Slovak Republic
**Corresponding author: chalova2@uniba.sk*

The polycyclic aromatic hydrocarbons (PAHs) are persistent organic compounds made up of at least two aromatic rings. Anthropogenic activities are mainly responsible for the formation of PAHs. The mutagenic, carcinogenic and toxic properties of phenanthrene (Phe) make it a significant environment pollutant with a long persistence. The results of this study demonstrate how efficiently microbial bioremediation can remove or immobilize pollutants. Using liquid chromatography and DAD detection, we developed and optimized a fast and efficient sample processing method compared to commonly available extraction methods. The method was established to monitor the levels of Phe in different bacterial samples. Some of these bacteriae are capable of metabolizing Phe molecules. The linearity of the advanced method was set at 0.9994 for a linear range of 25-1000 µg/mL. With a constant wavelength (254 nm) detection by a DAD detector, the limit of detection and quantification of the novel phenanthrene method were determined to be 1.59 µg/mL and 4.83 µg/mL, respectively. Possible future applications may include biodegradation of PAH-contaminated environments by appropriate microbial cultures, followed by quantitative analysis.

Keywords: Phenanthrene, Liquid Chromatography, DAD detection, Processing Method

ACKNOWLEDGEMENT
This project was supported by grants VEGA 1/0514/22, KEGA 027UK-4/2020, VEGA 2/0052/19, VEGA 2/0057/19, APVV-19-0519, FaF/18/2022.

P-18

Effect of structures of newly synthesized peptides on the stability of the formed monolayers

Iwona Golonka[1], Patyrycja P. Petrus[1], Jakub E. Pucułek[1], Katarzyna E. Greber[2], Witold Musiał[1]

[1]Department of Physical Chemistry and Biophysics, Faculty of Pharmacy, Wroclaw Medical University, Borowska 211A, 50–556 Wrocław, Poland; iwona.golonka@umw.edu.pl
[2]Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland.

Research on the surface tension phenomenon started in the 19th century by Lord Rayleigh and Agnes Pockels, who contributed to the development of research on monolayers [1]. The nature of the surface of a given molecule may affect the dissolution and bioavailability of the active substance in the drug form, and, consequently, its potency. The Langmuir method is currently used to design monolayer models of membranes reflecting the cell membrane in order to study the interactions and physicochemical properties of monolayer components [2]. The aim of the research was to assess the impact of the peptide structure (WK) 2-KWK-NH2, (WKWK) 2-KWKWK-NH2, (WR) 2-KWR-NH2, (C12) 2-KKKK-NH2, (KWK) 2-KWWW -NH2, (KK) 2-KWWW-NH2 on the values of the surface pressure in the formed monolayers and thus on their stability.

Langmuir-Wilhelmy balance was used to obtain and analyze the properties of monomolecular layers at the liquid-gas interface. The investigations based on the performance of compression isotherms and hysteresis in which the change of surface pressure versus area per molecule was evaluated. The compressibility modulus was used to determine the physical state of the monolayers made of peptides composed of the amino acids tryptophan, lysine and arginine (Figure 1).

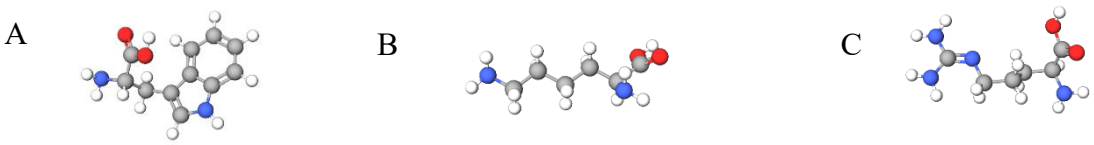


Figure 1. Structure of amino acids: A tryptophan, B lysine, C arginine.

On the basis of the compression isotherms of the studied peptides and their systems in different systems, it can be concluded that they all form Langmuir monolayers. Taking into account the surface pressure at which the secondary structure of the monolayer is destroyed, its stability can be assessed. The peptide 4 had the highest value of the π-collapse parameter 42.35 mN/m, what supports the conclusion that it creates the most durable film. The analysis of the compressibility coefficient shows that all peptide monolayers formed are in the expanded liquid phase as its value is less than 50.00 mN/m.

Key words: Langmuir monolayer, compression isotherm, cationic peptides.

ACKNOWLEDGEMENT

This work was financed by the grant of Wroclaw Medical University and Ministry of Science and Higher Education number SUBZ.DO60.22.034.

REFERENCES

1. J. E. Greene, Organic thin films: From monolayers on liquids to multilayers on solids. (2014) *Physics Today*, 67, 44-45.

2. M. D. Phan, K. Shin, A Langmuir Monolayer: Ideal Model Membrane to Study Cell. (2014) *J. Chem. Biol. Interfaces*, 2, 1-5.

P-19

Glycoprofiling of oligosaccharides from urine samples of α-mannosidosis patients using MALDI-TOF/TOF and ESI-MS/MS analysis

Filip Pančik[1], Maroš Krchňák[1], Zuzana Pakanová[1], Marek Nemčovič[1], Anna Hlavatá[2], Anna Šalingová[3], Stanislav Kozmon[1], Peter Baráth[1]

[1]Institute of Chemistry, SAS, Dúbravská cesta 9, 845 38 Bratislava, Slovakia
[2]Commenius University in Bratislava, Faculty of Medicine, National Institute of Children's Diseases, Department of Paediatrics, Limbová 1, 833 40 Bratislava, Slovakia
[3]National Institute of Children's Diseases, Centre for Inherited Metabolic Disorders, Limbová 1, 833 40 Bratislava, Slovakia

Alpha-mannosidosis (α-mannosidosis) is a rare lysosomal storage disorder with an autosomal recessive inheritance caused by mutations in the gene *MAN2B1*. This gene encodes lysosomal α-D-mannosidase [1]. Alpha-mannosidosis manifests a broad variety of symptoms, e.g. mental retardation, speech delay, coarse facial features, hypotonia, ocular manifestations etc [2]. There are many diagnostics approaches towards lysosomal storage disorders such as enzymatic assay or biochemical and molecular genetic testing [3].
Mass spectrometry (MS) analysis is useful method for α-mannosidosis diagnostics as well. Using this method, enzyme activity assays for the enzymes from dried blood spots relevant to α-mannosidosis are analysed [4].
In this work MALDI-TOF and ESI-MS analyses of oligosaccharides from urine samples were performed. Urine samples were firstly purified with SPE columns for the desalination purposes. Results obtained from MALDI-TOF and ESI-MS analyses were then complemented by the results from MALDI-TOF/TOF and ESI-MS/MS analyses respectively. The results from above mentioned analyses lead to obtaining a specific biomarkers and fingerprint of α-mannosidosis. The results were then compared for purpose of resolution and reliability of each method.

ACKNOWLEDGEMENT

This work was supported by grants from Ministry of Health of SR under the project registration number 2019/7-CHÚSAV-4; VEGA 2/0060/21; VEGA 2/0137/20; APVV-18-0336; APVV-20-0230; Operational Program Integrated Infrastructure ITMS: 313021Y920, co-financed by the European Regional Development Fund. Coauthors of this work are members of MetabERN - Project ID No. 739543.

REFERENCES

1. M. R. Ceccarini, M. Codini, C. Conte, F. Patria, S. Cataldi, M. Bertelli, E. Albi, T. Beccari (2018) *Int. J. Mol. Sci.*, 19 (5), 1500.
2. N. Guffon, A. Tylki-Szymanska, L. Borgwardt, A. M. Lund, M. Gil-Campos, R. Parini, J. B. Hennermann (2019) *Mol. Genet. Metab.*, 126, 470-474.
3. S. Mattosova, A. Hlavata, P. Spalek, L. Kotysova, D. Macekova, J. Chandoga (2015) *Bratisl. Med. J.*, 116 (8), 502-505.
4. A. B. Kumar, X. Hong, F. Yi, T. Wood, M. H. Gelb (2019) *Mol. Genet. Metab.*, 127 (3), 207-211.

P-20

Bioconjugation of a fluorescent turn off-on probe to holo-transferrin for the selective delivery and imaging of hypoxic cancer cells

Ewelina Janczy-Cempa[1], Olga Mazuryk[1], Franck Suzenet[2], Malgorzata Brindell[1]

[1]Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland
[2]Institute of Organic and Analytical Chemistry, University of Orléans, UMR-CNRS 7311, rue de Chartres, BP 6759, 45067 Orléans Cedex 2, France
ewelina.janczy@doctoral.uj.edu.pl

One of the crucial microenvironmental factors in most solid tumors is the chronic lowered level of oxygen in cancer tissue called hypoxia. Hypoxia is responsible for the modification of cellular metabolism that can result in the development of more aggressive tumor phenotypes; therefore, the hypoxic state is considered the primary indicator of a poor prognosis. Consequently, monitoring the area of hypoxia and its severity in the tumor is of key importance in selecting the appropriate treatment method. Reduced oxygen concentration in hypoxic tumor cells leads to increased oxidoreductase activity, which can be used to design hypoxia-sensitive sensors. Under these conditions, the reducing enzymes convert non-fluorescent compounds into fluorescent ones (fluorescent turn off-on probes) through irreversible reduction, enabling them to be used for selective imaging of hypoxic cancer cells. To further increase the selectivity of the fluorescence probe to hypoxic neoplastic tissue, we proposed a targeted delivery of a new probe using transferrin receptors (TfR), which are often overexpressed in cancer cells [1]. Transferrin, as a natural TfR ligand, is often used for conjugation to drugs or diagnostic molecules, which facilitate its internalization and, in this way, improve its efficiency and selectivity toward cancer tissue, while minimizing side effects [2]. In this work, we present the use of the transferrin receptor for the selective imaging of hypoxic neoplastic cells. The newly synthesized holo-transferrin conjugate with an optical probe based on the nitro-pyrazinotriazapentalene derivative [3] sensitive to the concentration of nitroreductase (NTR) in cancer cells is a novel selective sensor for hypoxia, which could be delivered to cells by the TfR-mediated endocytosis pathway. The main aim of the research was to optimize the bioconjugation of the probe with the protein and to investigate the effectiveness of conjugate transport to cancer cells in normoxia and hypoxia. It was shown that the conjugated probe was efficiently reduced to a highly fluorescent form by NTR, which was used as a biomarker of low tissue oxygen pressure. Moreover, the designed probe showed rapid accumulation in tumor cells and significantly higher fluorescence intensity under hypoxic conditions compared to normoxia. We have also shown that the conjugate is delivered to cancer cells through the endocytosis pathway by the transferrin receptor, the same as unmodified transferrin.

ACKNOWLEDGEMENT
This work was financed by National Science Center (DEC-2019/33/B/NZ7/02980).

REFERENCES

1. D.H. Manz, N.L. Blanchette, B.T. Paul, F.M. Torti, S.V. Torti (2016) Acad. Sci., 1368, 149.
2. S. Tortorella, T.C. Karagiannis (2014) *Curr. Drug Deliv*. , 11, 427.
3. E. Janczy-Cempa, O. Mazuryk, D. Sirbu, N. Chopin, M. Żarnik, M. Zastawna, C. Colas, M-A. Hiebel, F. Suzenet, M. Brindell (2021) *Sensors & Actuators B Chem*., 346, 130504.

P-21

Effect of co-solvents on the solubility and dissolution rate of cryptotanshinone from alcohol gels

Justyna Kobryń, Patryk Demski, Witold Musiał

Department of Physical Chemistry and Biophysics, Faculty of Pharmacy, Wroclaw Medical University,ul. Borowska 211A, 55-556 Wroclaw, Poland

Cryptotanshinone (CT), also called tanshinone C, is a compound derived naturally from a number of tanshinones - a group of compounds with the structure of diterpene quinones [1]. CT is an orange, water-insoluble powder. However, it is well soluble in chloroform, methanol or ethanol, among others [2]. In our previous study the increased solubility of CT was demonstrated in alkaline solvents with ethanol as cosolvent [3]. The present study concerned selected substances of alkaline nature that showed sufficient solubility and, at the same time, stability of CT – 2-amino-2-methyl-1,3-propanediol (AMPD) and sodium hydroxide (NaOH) were chosen. CT dissolved in alkaline solutions with ethanol was incorporated in anionic polymeric hydrogel – Carbopol 980 NF. Hydrogel formulations were prepared for potential dermal application. Therefore, the weight ratio of ingredients was adjusted to the physiological pH of the skin (pH 5 – 6) and hydrogels viscosity was measured. The CT release was evaluated by employing the apparatus paddle over a disc. CT was released into the medium which was a phosphate buffer pH 5.5 with 40% PEG 400 content. The CT release amount was obtained using high-performance liquid chromatography (HPLC) test. The release was analyzed on the basis of different kinetic models: zero-, first-, and second-order kinetics, as well as Higuchi and Ritger–Peppas equations. The best model presenting the observed process was selected according to the value of the coefficient of the determination. The effect of ultrasound at 60 °C on the CT structure was observed. The stability of CT stored in gels up to 9 weeks was tested using 96% ethanol as a solvent. The concentration of CT remaining in the gels was detected by HPLC. The effect of NaOH and AMPD on the structure of CT was investigated using the electrospray ionization mass spectrometry (ESI-MS), Fourier-transform infrared spectroscopy (FTIR) analysis and differential scanning calorimetry (DSC). The release study revealed the best correlation to Higuchi model. Relatively small amounts of released CT from the gels as well as the results of DSC and FTIR studies indicated interactions between CT and gel components. ESI-MS measurement additionally showed conversion CT to tanshinone V and its sodium salt in the presence of AMPD and 0.1 mol/L NaOH solution. 1.0 mol/L NaOH using resulted in CT transition only to sodium salt of tanshinone V.

ACKNOWLEDGEMENT

This work was financed by grant of Wroclaw Medical University SUBZ.D060.22.034.

REFERENCES

1. A. Nagappan, J. H. Kim, D. Y. Jung, and M. H. Jung, Cryptotanshinone from the salvia miltiorrhiza bunge attenuates ethanol-induced liver injury by activation of AMPK/SIRT1 and Nrf2 signaling pathways. (2020) *International Journal of Molecular Sciences*, 21, 265.
2. W. Chen, Y. Lu, G. Chen, and S. Huang, Molecular Evidence of Cryptotanshinone for Treatment and Prevention of Human Cancer. (2013) *Anti-Cancer Agents in Medicinal Chemistry*, 13, 979–987.
3. J. Kobryń, J. Dałek, and W. Musiał, The Influence of Selected Factors on the Aqueous Cryptotanshinone Solubility. (2021) *Pharmaceutics*, 13, 992.

P-22

Preparation and characterization of pullulan-enriched polymer-ceramic composites

Karina Piętak, Dagmara Słota, Wioletta Florkiewicz, Agnieszka Sobczak-Kupiec

Department of Materials Science, Faculty of Materials Engineering and Physics, Cracow University of Technology, 37 Jana Pawła II Av., 31-864 Krakow, Poland

The size of the global biomaterials market is growing steadily, which is related to the search for new, functional materials for various implants. However, they must meet certain parameters such as biocompatibility, nontoxicity, mechanical properties, or technological properties in order to be used in regenerative medicine. In order to achieve these parameters, polymer-based composites and ceramics are being developed, which can be modified to be personalized for a given patient. Synthetic polymers such as polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) are the most commonly used. They are characterized by good biocompatibility, flexibility and high tensile strength. They can form hydrogel matrices for controlled release of active ingredients [1]. The ceramic phase in composites for medical applications is calcium phosphate ceramics. Of growing interest for its biological and mechanical properties is brushite. Brushite-based materials are biocompatible with soft tissues and bone [2]. In purpose of giving more functionality to the material, modifications are made to the composition of the composite with polysaccharides, for example, pullulan. It can provide a good carrier of various drugs to specific tissues [3].

In the present study, the aim was to design a polymer-ceramic biomaterial modified with polysaccharides. The polymer phase consisted of polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG), while the ceramic phase was synthesized brushite (DCPD). The formed polymer-ceramic composite was modified with pullulan. The obtained materials were incubated in fluids simulating the internal environment of the organism. After 14 days of incubation, morphology was examined using Scanning Electron Microscopy (SEM). In addition, the swelling ability of the incubated composites in distilled water was examined.

During the 14-day incubation of the composites, the greatest changes were seen in the simulated body fluid (SBF), which is associated with the newly formed apatite layers in the composites, as observed by SEM microscopy. As the amount of PVP in the composite matrices increases, the swelling ratio decreases. The obtained composite biomaterials could be a carrier of active substances for applications in bone regenerative medicine.

ACKNOWLEDGEMENT

The "Multifunctional biologically active composites for applications in bone regenerative medi-cine" project is carried out within the TEAM-NET program of the Foundation for Polish Science financed by the European Union under the European Regional Development Fund. The authors gratefully acknowledge financial support. POIR.04.04.00-00-16D7/18.

REFERENCES

1. A.H.O. Alkhayatt, A.H. Al-Azzawi, Z. Alakayashi, Rheological and optical characterization of Polyvinylpyrrolidone (PVP) - Polyethylene glycol (PEG) polymer blends. (2019) *Journal Of Kufa–Physics*, 11 (02).
2. K. Issa, A. Alanazi, K.A. Aldhafeeri, O. Alamer, M. Alshaaer, Brushite: Synthesis, Properties, and Biomedical Applications. (2022) *Crystallization and Applications*, 145.
3. Y. Wang, Z. Guo, Y. Qian, Z. Zhang, L. Lyu, Y. Wang, F. Ye, Study on the electrospinning of gelatin/pullulan composite nanofibers. (2019) Polymers, 11 (9), 1424.

P-23

Synthesis of an effective MRSA inhibitor based on trifluoromethyl diamide scaffold

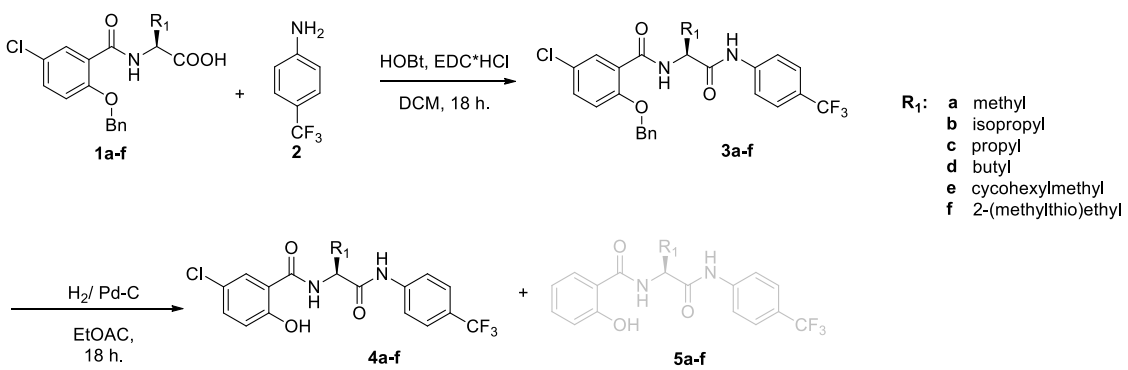
Eliska Pilarova[1], Karel Pauk[1], Pratibha Magar[1], Ales Imramovsky[1], Dominika Pindjakova[2], Alois Cizek[3], Josef Jampilek[2]

[1]Faculty of Chemical Technology, Institute of Organic chemistry and Technology, University of Pardubice, Studentská 95, CZ-530 09 Pardubice, Czech Republic
[2]Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Ilkovicova 6, 84215 Bratislava, Slovakia; pindjakova.dominika@gmail.com (D.P.); josef.jampilek@gmail.com (J.J.)
[3]Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Palackeho 1946/1, 61242 Brno, Czech Republic; cizeka@vfu.cz (A.C.)

Salicylanilides (compounds derived from salicylic acid) are a group of substances that have good antibacterial and antifungal properties and can also be used in the treatment of cancer. Their effect against staphylococci and *Mycobacterium Tubrerculosis* has been described previously and, more recently, for example on Onchocerca volvulus or Toxoplasma gondii parasite. Their activity is also related to the presence of halogen in the molecule. According to the literature, fluorine-containing substances showed the best properties and it is likely that electronegativity is directly related to antibacterial activity (F> Cl> Br> I). In most cases, these substances are more effective against gram-positive bacteria than against gram-negative bacteria [1, 2, 3].

This research builds on previous work where compounds containing 4-(trifluoromethyl) aniline were prepared. These substances were highly active against 4 strains, namely *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Bacillus cereus* and *Clostridium perfringens*. Based on this, we decided to expand this series of substances with other amino acids [4].

Monopeptide acid was used a a starting material, which was coupled with 4-trifluoromethyl aniline, followed by debenzylation with hydrogen. Both benzylated (**3a-f**) and debenzylated (**4a-f**) substances were tested for antibacterial activities.



ACKNOWLEDGEMENT

The authors wish to acknowledge the financial support of the Faculty of Chemical Technology, University of Pardubice (Support of Excellent teams); The Slovak Research and Development Agency (project APVV-17-0373) and grants of Comenius University (UK320).

REFERENCES

1. A.L. Garner, C. Gloeckner, N. Tricoche, J.S. Zakhari, M. Samje, F. Cho-Ngwa, S. Lustigman, K.D. Janda (2011) *J. Med. Chem*., 54, 3963-3972.

2. A. Fomovska, R.D. Wood, E. Mui, J.P. Dubey, L.R. Ferreira, M.R. Hickman, P.J. Lee, S.E. Leed, J.M. Auschwitz, W.J. Welsh *et al.* (2012) *J. Med. Chem*., 55, 8375-8391.
3. M. Krátký, J. Vinšová, E. Novotná, J. Mandíková, F. Trejtnar, J. Stolaříková (2013) *Molecules*, 18, 3674-3688.
4. K. Pauk, I. Zdražilová, A. Imramovský, J. Vinšová, M. Pokorná, M. Masaříková, A. Čížek, J. Jampílek (2013) *Bioorg. Med. Chem*., 21, 6574-6581.

P-24

Advances and challenges of liposome-assisted drug release in the presence of serum albumin molecules. Influence of surrounding pH

Danuta Pentak[1]*, Anna Ploch-Jankowska[1], Andrzej Zięba[2], Violetta Kozik[3]*

[1]Faculty of Chemistry, University of Opole, Oleska 48, 45-052 Opole, Poland
[2]Department of Organic Chemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland
[3]Institute of Chemistry, University of Silesia, Szkolna 9, 40-006 Katowice, Poland
**Correspondence: Danuta Pentak, E-mail address: danuta.pentak@uni.opole.pl Tel.: +48 774527149*
**Violetta Kozik, e-mail address: violetta.kozik@us.edu.pl*

The aim of this study was to prepare a liposomal delivery system for 5-methyl-12 (H)-quino[3,4-b]-1,4-benzothiazine chloride (5-MBT) and study *in vitro* release characteristics. Release of 5-MBT from liposomal complex with human serum albumin (HSA) [$L_{DPPC/5\text{-}MBT}$]:HSA was examined using spectrophotometric method and differential scanning calorimetry (DSC). Electronic paramagnetic resonance was used to assess the influence of the pH of the environment on the conformation of phospholipids, the latter determining the degree of release of the encapsulated compound. The applied mathematical models made it possible to determine the necessary analytical parameters to facilitate the process of potential drug release from liposomes. The complexes formed by liposomal 5-MBT with serum albumin (HSA) particles allowed the description of the Fick process. The change in the polarity of the phospholipid membrane resulting from the changes in the pH of the surroundings significantly influenced the percentage of 5-MBT entrapment in the liposomes. It also affected the release percentage.

REFERENCES

1. D. Pentak, A. Ploch-Jankowska, A. Zięba, V. Kozik, Advances and challenges of liposome-assisted drug release in the presence of serum albumin molecules. Influence of surrounding pH. (2022) *Materials*, 15 (4), 1586. https://doi.org/10.3390/ma15041586.

P-25

Homology modelling of α4β1 integrin and its interactions with ligands

Stanislav Kozmon[1,2], Barbora Stratilová[1]

[1]Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9 SK-84538 Bratislava, Slovakia
[2]Medical Vision o.z., Záhradnícka 4837/55, 821 08 Bratislava, Slovakia

Integrins are family of cell adhesion molecules mediating cell-cell and cell-matrix interactions. They are heterodimers, each consisting of non-covalently associated α and β subunits. α4 integrin family is of a great interest to pharmaceutical research due to its role in inflammatory processes.

In this work we have constructed two models of α4β1 integrin complex. First model was created using Modeller 10.2 [1]. Crystal structures of α5β1 integrin headpiece (3VI3.pdb and 3VI4.pdb) were used as a template for β1 part of the heterodimer and structures of α4β7 integrin headpiece (3V4P.pdb and 3V4V.pdb) were selected as templates for the α4 part. Second 3D model was created using AlphaFold [2] protein structure prediction software. This model was selected for further study by means of molecular dynamics as well as molecular docking of RGD ligand using Schrödinger Glide [3] and study of its interactions. Subsequently, search for structures similar to RGD ligand was conducted followed by molecular docking and evaluation of binding affinity of selected compounds.

ACKNOWLEDGEMENT

This publication is the result of the project implementation CEMBAM – Center for Medical Bioadditive Research and Manufacturing, ITMS2014+: 313011V358 supported by the Operational Programme Integrated Infrastructure funded by the European Regional Development Fund.

REFERENCES

1. B. Webb, A. Sali (2016) *Curr. Protoc. Bioinform*., 54, 5.6.1-5.6.37.
2. J. Jumper et al. (2021) *Nature,* 596, 583-589.
3. Schrödinger Release 2022-2: Glide, Schrödinger, LLC, New York, NY, 2021.

P-26

Effect of carotid endarterectomy on induction of ischemic conditioning in stroke

Marek Furman[1], Miroslava Nemethova[1], Lubica Macakova [1], Vladimir Sihotsky[3], Ivan Kopolovets[2], Michal Virag[2], Rastislav Mucha[1]

[1]Institute of Neurobiology, Biomedical Research Center, Slovak Academy of Sciences, Šoltésovej 4, 040 01 Košice, Slovakia (furman@saske.sk, nemethova@saske.sk, macakova@saske.sk, mucha@saske.sk)
[2]Eastern Slovak Institute of Cardiovascular Diseases and Faculty of Medicine, Pavol Jozef Safarik University, Ondavska 8, 040 01 Kosice, Slovakia (vsihotsky@vusch.sk, i.kopolovets@gmail.com, mvirag@centrum.sk)
[3] Faculty of Medicine, Pavol Jozef Safarik University, Trieda SNP 1, 040 11 Kosice, Slovakia (sihotskyv@gmail.com)

The most common reason of ischemia is atherosclerotic plaque which could block a cerebral artery. Atherosclerotic plaque accumulated on the walls of the carotid arteries can be surgically removed using a procedure called carotid endarterectomy (CEA). In this procedure, the artery from which the atherosclerotic plaque is removed is temporarily occluded and blood is delivered to the brain by only one carotid artery [1]. Such application of a mild ischemic attack could also be classified as some form of ischemic conditioning. Ischemic conditioning is the process of applying stressors at the sublethal level to induce ischemic tolerance (IT). The neuroprotective effect of IT reduces ischemic damage [2]. There are certain specific markers such as ADM, which provide an anti-hypoxic effect and plays role in the induction of the cells to tolerate oxidative stress, CDKN1A, which is specific marker of brain ischemia, GADD45G which is the marker of cell stress, IL6, which is the pleiotropic factor initiating pro-inflammatory immune response resulting in vasculature disruption but also promoting angiogenesis. TM4SF1, which level of expression in the brain and blood quantitatively and qualitatively correlates, play role in cell cycle regulation, apoptosis and ROS metabolism [3]. The aim of this work is to elucidate the effect of carotid endarterectomy on IT induction. Detection of the IT induction rate was determined by changes in gene expression of the genes mentioned above. Our testing group was consisted of patients overcame an ischemic stroke within 7 to 180 days before the planned surgical removal of the atherosclerotic plaque (CEA). Whole peripheral blood was collected before CEA and 48 hours after CEA. RNA, isolated from blood cells, was transcribed into cDNA and subsequently quantitatively analyzed. Our results suggest significantly increased expression of ADM, TM4SF1 and GADD45G, and reduced expression of CDKN1A in the blood of patients after CEA compared to the group before surgery. Expression level of IL6 remains after CEA without significant changes. Based on these results, we can suggest that CEA is able to induce activation of IT at statistically significant level.

ACKNOWLEDGEMENT

This work was financed by research grants: SK-VEGA 2/0054/20 grant and Operational Programme Integrated Infrastructure, IMTS: 313011V344, co-financed by the European Regional Development Fund.

REFERENCES

1. J. J. Earnshaw (2002) *J R Soc Med*, 95(4), 168-170.
2. A. Vinciguerra, O. Cuomo, P. Cepparulo, S. Anzilotti, P. Brancaccio, R. Sirabella, N. Guida, L. Annunziato, G. Pignataro (2018) *Journal of neuroscience methods*, 310, 63-74.
3. M. Furman, M. Nemethova, L. Macakova, V. Sihotsky, I. Kopolovets, P. Berek, M. Virag, R. Mucha (2022) *Mol. Biol. Rep.*, 49(1), 477-485.

P-27

Natural protection against aggregation: the role of the gatekeeper amino acids

Nóra Taricska[1],Dániel Horváth[2], András Perczel[1,2]

[1]Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Pázmány Péter sétány 1/A, Budapest, 1117 Hungary
[2]MTA-ELTE Protein Modelling Research Group, Institute of Chemistry, Eötvös Loránd University, Pázmány Péter sétány 1/A, Budapest, H-1117 Hungary
E-mail: nora.taricska@ttk.elte.hu.

Nowadays the protein aggregation and amyloid formation has become important research area. The conformational change of proteins is in the background of many diseases [1]. Conditions of amyloid formation have to be fine-tuned as those of crystallization. The conformational hyperspace, ΔG= $f(T, pH, c_{protein}, c_{ion}, t, \zeta)$, associated with such transition has key minima, F-, U-, I- and Amy-states.

Our research targeting the amyloid formation of miniproteins (e.g. E5: EEEAVRLYIQWLKEGGPSSGRPPPS) which is the C-terminus 25 residue-long part of Exenatide-4, a drug daily used to treat type II *Diabetes Mellitus* [2,3] is focusing on the transformation of otherwise well folded proteins. In the previous CTB8 conference we presented the details of its amyloid formation path traced by spectroscopy (ECD and NMR) on a 7D-hypersurface. We have identified several points of no return on the F↔U equilibrium path (e.g. T= 37 °C, pH= 4.1, c(E5)= 250 µM, c(NaCl)= 50 mM, t> 4-6 h, ζ= stirring) concluding into amyloids of predominantly β-backbone structures.[4] Now we determined the amyloid core region of E5 miniprotein. This LYIQWL amyloidogenic segment is inside the GPCR binding site and this region adopts a helical structure in the native miniprotein. We observed that LYIQWL easily forms amyloid in a short time (pH= 2.7-10.0, c_{LYIQWL}= 0.05 mM, t~ 1 h, T= 37 °C, ζ= stirring]). In the nature the proteins have evolved numerous methods to counteract tendency to easily aggregate into amyloid-like fibrils. One of these are the gatekeeper (GK) amino acids (Asp, Glu, Lys, Arg), which can slow down or inhibit the amyloid formation. [5] We began to study this GK effect using the easily aggregating LYIQWL segment.

ACKNOWLEDGEMENT

This work was financed by the European Union and the State of Hungary and co-financed by the European Regional Development Fund (VEKOP-2.3.2-16-2017-00014). Project no. 2018-1.2.1-NKP-2018-00005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2018-1.2.1-NKP funding scheme.

REFERENCES

1. F. Chiti, M. C. Dobson (2006) *Annu. Rev. Biochem.*, 75, 333-366.
2. P. Rovó, V. Farkas, P. Stráner, M. Szabó, Á. Jermendy, O. Hegyi, K. G. Tóth, A. Perczel (2014) *Biochemistry*, 53, 3540-3552.
3. J. J. Meier (2012) *Nat. Rev. Endocrinol.*, 8, 728-742.
4. N. Taricska, D. Horváth, D. K. Menyhárd, H. Ákontz-Kiss, M. Noji, M. So, Y. Goto, T. Fujiwara, A. Perczel (2020) *A. Chemistry A European Journal*, 26(9), 1968-1978.
5. F. Rousseau, L. Serrano, J. W. H. Schymkowitz (2006) *J. Mol. Biol.,* 355, 1037-1047.

P-28

Expression, purification, partial characterization and *in-silico* modeling of hRyR2 tandem Repeat1-2

Alica Petrová[1,2], Terézia Hromádková[1,2], Konrad Beck[3], Monika Zámocká[1], Jacob A. Bauer[1], Vladena Bauerová-Hlinková[1]

[1]Institute of Molecular Biology Slovak Academy of Sciences, Department of Biochemistry and Protein Structure, Dubravska cesta 21, 845 51 Bratislava, Slovak Republic; vladena.bauerova@savba.sk
[2]Faculty of Natural Sciences Comenius University in Bratislava, Department of Genetics, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovak Republic; petrova62@uniba.sk
[3]Cardiff University School of Dentistry, Heath Park, Cardiff CF14 4XY, Wales

Ryanodine receptors (RyRs) are mainly known for their participation in excitation-contraction coupling, a key physiological process involved in muscle contraction. RyRs are found in the membrane of the sarcoplasmic reticulum in muscle cells and the endoplasmic reticulum in other cells where they particpate in the calcium cycle. RyRs are comprised of four monomers, which are further divided into 11 domains [1,2]. In this work, we focused on one of the tandem repeat domains, Repeat1-2, of the clamp domain of the human cardiac RyR2 (hRyR2). Repeat1-2 is associated with mutations that cause severe cardiac and non-cardiac illnesses [1,3]. This region also provides options for studying the insecticide resistance of *Plutella xylostella* [4]. We expressed and purified hRyR2 Repeat1-2 in an *Escherichia coli* expression system with a final yield of 2-3 mg of pure protein from a liter of culture media. The main purification steps involved IMAC and SEC, and the protein purified in a monomeric form. CD-spectroscopy showed that the domain is folded and contains ≈50% α-helices, which is in agreement with the RyR2 Repeat1-2 structure solved by cryo-electron microscopy [2]. The thermal stability of hRyR2 Repeat1-2 was investigated by nanoDSF and we found that melting point of this domain is ≈42°C. nanoDSF was also used to identify ligands that affected the thermal stability of this domain.

ACKNOWLEDGEMENT
Authors thank the Eva Kutejová for her support while performing this study. This work was financially supported by the Slovak Grant Agency VEGA, grant 2/0131/20 and Interreg SK-AT StruBioMol ITMS: 305011X666.

REFERENCES

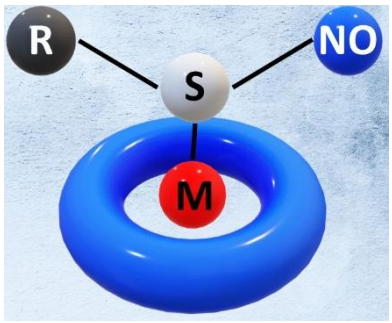
1. V. Bauerova-Hlinkova, D. Hajdúchová, J.A. Bauer. (2020) *Molecules*, 25, 4040.
2. W. Peng, H. Shen, J.P. Wu, W. Guo, X. Pan, R. Wang, S.R.W. Chen, N. Yan. (2016) *Science,* 354, 301.
3. The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk).
4. T. Xu, Z. Yuchi. (2019) *BMC Biolog,* 17, 77.

P-29

Characteristics of interactions of cobalt and iron complexes with selected glutathione and nitrosoglutathione in aqueous solutions

Dominika Porębska[1], Maria Oszajca[1], Dorota Rutkowska-Żbik[2], Grażyna Stochel[1], Łukasz Orzeł[1]

[1]Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland
[2]Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30-239 Krakow, Poland



Geriatric diseases are one of the main problems of modern medicine. A significant difficulty is the lack of precise knowledge of the pathomechanisms that contribute to the development of diseases, including neurodegenerative diseases or cancer. As already known, with age, the metabolism of metal complexes in the human body is disturbed, which can directly affect NO-dependent cell signaling. The specific interaction with ligands in the axial position makes Co and Fe porphyrinoids' derivatives play an important role in the activation of endogenous small molecules. In biological systems, this ability is of particular importance in relation to NO and its intracellular donors. Therefore, it is extremely important to fully understand the mechanisms of how Aquacobalamin, Co/Fe-Porphyrin and Co/Fe-Protoporphyrin IX react with endogenous NO-releasing compounds.[1,2] The aim of the research was to determine the effect of tetrapyrrolic Co and Fe complexes on the stability of the -SNO bonds in RSNO and hence, potentially, on the NO homeostasis. The special attention was paid to the interplay between metal and macrocyclic ligand properties in the activation of RSNO towards NO release. Reactions were monitored by the UV-VIS spectroscopy under both in oxygen-free and oxygen-saturated solutions. The experimental studies were supplemented by molecular modelling using the DFT method which provided information on the stability of both Co-(RSNO) and S-NO bonds. Possible demonstration of the influence of corrin and porphyrin complexes on stability of natural nitrosothiols (RSNO) could be used as a message for the regulation of intracorporeal NO homeostasis.

ACKNOWLEDGEMENT
This work was financed by NCN, grant OPUS nr. 2019/35/B/ST4/04266.

REFERENCES

1. M. Oszajca, A. Wądołek, J. Hooper, M. Brindell, R. van Eldik, G. Stochel, Urban Particulate Matter-Induced Decomposition of S -Nitrosoglutathione Relevant to Aberrant Nitric Oxide Biological Signaling. (2019) *ChemSusChem*, 12, 661-671.
2. M. Wolak, G. Stochel, R. van Eldik, Reactivity of Aquacobalamin and Reduced Cobalamin toward S -Nitrosoglutathione and S -Nitroso- N -acetylpenicillamine. (2006) *Inorganic Chemistr*, 45, 1367-1379.

P-30

Theoretical investigation of small molecular binding to 17β-HSD type 1,2 and AKR1C enzymes

Vivien Erzsébet Resch[1], Erzsébet Mernyák[2], Eva Hafner[3], Tea Lanisnik-Rizner[3], Gábor Paragi[1,4]

[1]Department of Medical Chemistry, University of Szeged, Dóm-tér 8, H-6720 Szeged, Hungary
[2]Department of Organic Chemistry, University of Szeged, Dóm-tér 8, H-6720 Szeged, Hungary
[3]Institute of Biochemistry and Molecular Genetics, University of Ljubljana, Ljubljana, Slovenia
[4]Department of Physics, University of Pécs Ifjúság útja 6. H-7624 Pécs, Hungary

The mechanism of action of synthetic estrone-derived antitumoral agents is mostly unknown. In order to monitor their mechanism, their fluorescent labelling is essential [1]. Here we aimed to investigate the binding of C-15-labeled BODIPY-estrone conjugates and their starting compounds to 17β-HSD 1, 2 or AKR1C1–3 enzymes [2] by computational simulations.
A specific descriptor of the binding is the free energy value. Several theoretical methods have been developed to calculate the binding free energy, such as umbrella sampling (US) [3] or generalized Boltzmann method (MM/GBSA) [4]. In this context, US, MM/GBSA methods were used to calculate the binding free energy of modified molecules to corresponding binding pockets concerning the selected target proteins.
In the first step, a 3D models of the target proteins were prepared following by docking, molecular dynamics and binding free energy calculations. The obtained ΔG values of theoretical calculations properly correlated with the experimental binding preference order. These results allowed us to investigate the molecular background of selectivity found by the experiments.

ACKNOWLEDGEMENT
This work was financed by OTKA-SNN 139323.

REFERENCES

1. Bacsa, C. Konc, A. B. Orosz, G. Kecskeméti, R. Rigó, C. Özvegy-Laczka, E. Mernyák (2018) *Molecules*, 23, 821.
2. M. Sinreih, R. Jójárt, Z. Kele, T. Büdefeld, G. Paragi, E. Mernyák, R. T. Lanisnik (2021) *J. Enzym Inhib. Med. Chem.*, 1, 1500-1508.
3. J. Kästner (2011) *Wires Comput. Mol. Sci.*,1 (6), 932-942.
4. P.A. Kollman, I. Massova, C. Reyes, B. Kuhn, S. Huo, L. Chong, M. Lee, T. Lee, Y. Duan, W. Wand, O. Donini, P. Cieplak, J. Srinivasan, D. A. Case, T. E .Cheatham (2000) *Acc. Chem. Res.*, 12, 889-897.

P-31

Host Cell Interaction with *Coxiella burnetii*

Shreein Ahmed[1,2], Katarína Palkovičová[2], Ľudovít Škultéty[2,3]

[1]*Comenius University Faculty of Natural Sciences,*
[2]*Department of Rickettsiology, Institue of Virology, Biomedical Research Center, Slovak Academy of Sciences, Slovakia*
[3]*Laboratory of Molecular Structure Characterization, Institute of Microbiology, Czech Academy of Sciences, Czech Republic*

Coxiella burnetii is a Gram-negative bacterium that causes Q fever, a life-threatening infection with both acute and chronic presentations. C. burnetii invades a variety of host cell types and replicates within a unique vacuole derived from the host cell lysosome. To understand Host cell signaling pathways altered by effectors of Coxiella burnetii, we have investigated the metabolism of intracellular cultivated bacteria [1,2].
For tagging experiment, THP-1 cells seeded at 3.5 × 105 cells/ml in RPMI + 10% FBS were differentiated with 10 Nm PMA for 3 days. Cells were then infected with C. burnetii, at MOI of 10. Metabolite extraction C. burnetii was prepared by scraping infected THP-1 cells in warm (PBS), then lysing by exposing to liquid nitrogen. Host cell debris was pelleted by centrifugation and the supernatant was centrifuged to pellet bacteria. Cultures were then rapidly exposed to liquid nitrogen to halt metabolic activity.Bacterial pellets were extracted in chloroform:methanol:water (1:3:1 v/v) containing 1 nM scylloinositol. After the initial addition of methanol to water (3:1 v/v), bacterial cells were sheared by exposure to liquid nitrogen. Chloroform was then added to the aforementioned 1:3:1 ratio. Samples were then centrifuged to remove cell debris. The supernatant was adjusted to Chloroform:methanol: water(1:3:3 v/v) centrifuging to induce phase separation. Our analysis of polar and nonpolar metabolites showed differences between infected cells and non-infected cells.

ACKNOWLEDGEMENT
This work was financed by VEGA 2/0052/19, APVV-19-0519.

REFERENCES

1. I. Häuslein, F. Cantet, S. Reschke, F. Chen, M. Bonazzi, W. Eisenreich, Multiple substrate usage of Coxiella burnetii to feed a bipartite metabolic network. (2017) *Front. Cell. Infect. Microbiol.*, 7, 285.
2. A. Omsland, D.C. Cockrell, D. Howe, E.R. Fischer, K. Virtaneva, D.E. Sturdevant et al., Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. (2009) *Proc. Natl Acad. Sci. U.S.A.*, 106, 4430-4434.

P-32

Biological response of substituted quinazolinones: Structure-activity relationship study

Jana Hricovíniová[1,2] and Zuzana Hricovíniová[3]

[1]*Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*
[2]*Comenius University Bratislava, Faculty of Pharmacy, Bratislava, Slovakia*
[3] *Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*

Heterocyclic compounds containing nitrogen atoms in their structure represent an important class of substances with pharmaceutical utility. They are present in different natural products and pharmaceuticals. Among them quinazolinones have been intensively studied as they exhibit a wide range of biological activities including antibacterial, antifungal, antimalarial, anticancer, antidiabetic, anti-inflammatory or anticonvulsant activity [1-3].
Structural modification of the quinazolinone core alters the biological activity of compounds. Evaluation of compounds that counteract the toxic effects caused by direct action of reactive oxygen species on DNA molecule is of considerable interest. Therefore, a series of 2,3-substituted quinazolinone derivatives were investigated using different assays and the relation between their biological properties and chemical structure was examined. This contribution will survey our recent results concerning biological activities of structurally different quinazolinone derivatives in terms of their antioxidant, radical-scavenging, genotoxic and DNA-protective effects, as well as their antiproliferative activity in the context of structure-biological activity relationships [4].
The results indicate that 2,3-substituted quinazolinones are excellent redox-active agents and their physico-chemical properties make them promising candidates for further biological studies. Their effectiveness towards cell-growth inhibition (normal and malignant cell lines) varies with the substitution pattern on the phenyl rings attached to the quinazolinone scaffold. Moreover, their substantial antioxidant effect, DNA-protective ability, cytotoxicity, along with significant genotoxic effect in cancerous cells suggested that studied quinazolinones might represent potential model structures for the development of pharmacologically active agents.

ACKNOWLEDGEMENT
This work was financially supported by Slovak grant agency VEGA 2/0071/22.

REFERENCES
1. A. Hameed, M. Al-Rashida, M. Uroos, S. A. Ali, K.M. Khan (2018) Expert. *Opin. Ther. Pat*. 28, 281–297.
2. P. S. Auti, G. George, A.T. Paul (2020) *RSC Advances* 10, 41353–41392.
3. S. Shagufta, I. Ahmad (2017) *Med. Chem. Commun*. 8, 871–885.
4. J. Hricovíniová, Z. Hricovíniová, K. Kozics (2021) *Int. J. Mol. Sci*. 22, 610–628.

P-33

Effects of fatty acids on pregnane X receptor

Lucia Sládeková, Petra Pečinková, Zdeněk Dvořák

Department of Cell Biology and Genetics, Faculty of Science, Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic

The pregnane X receptor (PXR) is a nuclear receptor that regulates a number of physiological processes such as lipid and carbohydrate metabolism and detoxification. Involvement of PXR in the etiology of various pathologies including hepatic steatosis, cancers, and inflammatory intestinal diseases is also well known. PXR is activated by structurally diverse chemicals (xenobiotics) comprising drugs (e.g. rifampicin), environmental pollutants (e.g. polychlorinated biphenyls), dietary compounds (e.g. hyperforin), plasticizers (e.g. phthalates), etc. Endogenous ligands of PXR include bile acids, steroids, and vitamin K2 [1]. In addition, microbial intestinal metabolites indole, indole-3-propionate, and indole-3-acetamide were identified as PXR ligands and agonists [2]. However, due to the stigma of being the xenosensor accountable for drug-drug interactions, the research on endogenous and dietary ligands of PXR was underestimated.

In the current study, we have examined the interactions between PXR and dietary and endogenous fatty acids. Using the reporter gene assays, we found that saturated fatty acids (SFAs; 20 homologs; from C3:0 to C22:0) and monounsaturated fatty acids (MUFAs; 13 compounds; from C4:1 to C22:1) are all inactive against the PXR, comprising both agonist and antagonist activity. Out of 19 tested polyunsaturated fatty acids (PUFAs), 13 compounds displayed PXR agonist activities. The strongest activation of PXR, with relative efficacy comparable to that of rifampicin, was achieved by clupanodonic, cervonic, osbond, γ-linolenic and dihomo-γ-linolenic acids. PXR-active PUFAs were further characterized for their binding at PXR and the capability to induce PXR-target genes, using TR-FRET and RT-PCR techniques, respectively.

We show that selected PUFAs are low to medium affinity PXR ligands, and low-potency and high-efficacy agonists of PXR. Our data are the first report indicating that fatty acids might be a new class of PXR dietary and endogenous ligands.

ACKNOWLEDGEMENT

This work was financed by Palacký University in Olomouc, grant #PrF-2022-009.

REFERENCES

1. Z. Dvořák, H. Sokol, S. Mani, Drug Mimicry: Promiscuous Receptors PXR and AhR, and Microbial Metabolite Interactions in the Intestine. (2020) *Trends in pharmacological sciences,* 41 (12), 900-908.
2. P. Illés, K. Krasulová, B. Vyhlídalová, K. Poulíková, A. Marcalíková, P. Pečinková, N. Sirotová, R. Vrzal, S. Mani, Z. Dvořák, Indole microbial intestinal metabolites expand the repertoire of ligands and agonists of the human pregnane X receptor. (2020) *Toxicology Letters,* 334, 87-93.

P-34

Study of acceptor substrate specificity of Xyloglucan endotransglycosylase (XET) using computational methods

Barbora Stratilová, Eva Stratilová, Stanislav Kozmon

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9 SK-84538 Bratislava, Slovakia

Xyloglucan endotransglycosylase (XET) is an enzyme which plays an important role in the reconstruction of the foundation of plant cell wall, the cellulose xyloglucan network. Beside of homotransglycosylation reactions with xyloglucan-derived acceptors and donors, XET can catalyze also heterotransglycosylation with the number of structurally diverse polysaccharides. In this work, we have studied nonspecific TmXET6.3 from *Tropaeolum majus* and specific PttXET16A from *Populus tremula x tremuloides* primarily focusing on cause of differences in their acceptor specificities. Molecular dynamics simulations followed by binding free energy calculations of selected linear acceptors in active sites of TmXET6.3 and PttXET16A proved, that linear acceptors with glucose residues connected by β-1,4- or β-1,3- glyosidic bonds were stable in active sites of both enzymes, while acceptor with s β-1,6-bond was stable only in the case of TmXET6.3. If the glucose units were replaced by mannose or xylose, the acceptor was unstable in the active site of PttXET16A which was not able to catalyze transfer of xyloglucan fragments on such substrates. Simulations with MLG-OS acceptors in complex with TmXET6.3 highlighted the importance of β-1,3-bond placement in substrates with mixed linkage β-1,4- or β-1,3- bonds, specifically the MLG-OS with β-1,3-bond between saccharide units closest to the nonreducing end, were proven unstable. Similarly, simulations with GlcMan-OS differing in position of substitution with glucose unit proved the importance of side chain placement because GlcMan-OS4 with glucose side chain on saccharide unit of nonreducing end proved to be unstable. These results can partially explain the different acceptor specificity of these enzyme observed experimentally.

ACKNOWLEDGEMENT.
This work was financed by the Slovak Grant Agency of Science (VEGA 2/0137/20) and the Slovak Research and Developmental Agency (APVV-20-0230).

P-35

Diamides and dithioamides as compounds with potential bioactivity

Aleksandra Świetlicka, Marlena Paździor, Agata Hadryś , Violetta Kozik , Andrzej Bąk

Institute of Chemistry, University of Silesia, Szkolna 9, 40 007 Katowice, Poland

In recent years the development of organic chemistry and chemoinformatics have contributed considerably to the introduction of many new drugs in pharmacy and medicine [1]. In fact, the organosulfur compounds are valued in pharmacology as antitumor, antimicrobial, anti-HIV and chemoprotective agents against a variety of carcinogenic or toxic factors. Thereby, a novel set of diamides and dithioamides of ethyl and methyl ester amino acids (AAs) was synthesised and analysed, respectively. The incorporation of AAs, either natural or their derivatives (e.g., esters or amides), as components of the parent (pro-)drugs is basically regarded as a patient-friendly approach on the route from structures to ADMET-tailored properties [2].

Conceptually, the multistep synthesis of the symmetrical α-AA-based dithioamides of terephthalic acid was proposed using the conventional heating as well as the microwave-accelerated approach. All the dithioamides were formed in a three-step synthesis. The final products were purified using column chromatography.

Moreover, the intermolecular similarity of novel terephthalic acid derivatives was estimated in the multidimensional space (mDS) of the structure/property-related *in-silico* descriptors. Hence, a number of computational methods was used in order to correlate the distance-oriented structure/property distribution of newly synthesized molecules with the experimental lipophilic data as well as the anticancer activities. As a matter of fact, the bioavailability of the selected derivatives was estimated by the calculated lipophilic clogP values, for instance, AlogPS, Molinspiration, Chemsketch, MarvinSketch, Hyperchem, Osiris, XlogP3, Kowwin and Sybyl, respectively. Moreover, the values of the calculated lipophilicity were correlated with the experimental parameters prepared using the thin-layer technique (TLC-RP18). The lipophilic studies of the tested compounds were carried out on the chromatographic plates developed using mobile phases prepared by mixing the respective amounts of the organic modifier and water. The plates were then dried and visualized in the UV light. All analyses were repeated in triplicate. The average value of RF (retardation factor) was determined in each case. A significant correlation was demonstrated between the empirical data and the theoretically calculated lipophilic parameters [3].

The proposed theoretical analysis may indicate new synthetic targets with the potential use as new biological agents. Amides and dithioamides of terephtalic acid can potentially create ADMET-friendly conglomerates with drug molecules and serve as attractive drug carriers for the poorly absorbed curative agents.

ACKNOWLEDGEMENT

This work was financed by a subsidy from the Bioorganic research group, Institute of Chemistry, Faculty of Exact and Technical Sciences, the University of Silesia in Katowice.

REFERENCES

1. A.C.L. Leite, F.F. Barbosa, M.V.d. Cardoso et al. (2014) *Med. Chem. Res.*, 23, 1701-1708.
2. J. Li, Y. Sha (2008) *Molecules*, 13, 1111-1119.
3. Z. Witkiewicz, Podstawy chromatografii. Wydawnictwo Naukowe PWN, 2017.

P-36

Flexibility and function in human ileal bile acid-binding protein

Tamara Teski[1], András Micsonai[2], József Kardos[2], and Orsolya Tőke[1]

[1]*Research Centre for Natural Sciences, Centre for Structural Science, NMR Research Laboratory, Magyar tudósok körútja 2, H-1117 Budapest, Hungary*
[2]*Department of Biochemistry, MTA-ELTE NAP B Neuroimmunology Research Group, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary*

Human ileal bile acid-binding protein (hI-BABP), expressed in the absorptive enterocytes of the distal small intestine, has a key role in the transcellular trafficking of bile salts and by stimulating the transcriptional activity of the farnesoid X receptor, contributes to the regulation of bile acid, lipid, and glucose homeostasis. Similar to other members of the family of intracellular lipid-binding proteins (iLBPs), order-disorder transitions have a major role in hI-BABP-ligand recognition. As we have shown in the past, µs-ms timescale fluctuations between a closed and a more open conformation suggest a conformational selection mechanism of bile salt entry and the occupation of the two internal binding sites. To improve our understanding of positive binding cooperativity and the accompanying site-selectivity of bile salts, we have introduced functionally impairing single-point mutations in two key regions of the protein and subjected the mutants to multiple timescale motional analysis by NMR relaxation measurements. According to our results, redistribution of motional freedom of specific protein segments has a key role in both the deterioration of the site preference of di- and trihydoxy bile salts as well as the loss of positive binding cooperativity in the protein. NMR relaxation analysis has been complemented by MD simulations, providing further evidence of a dynamic coupling between the ligand entry portal, the C/D-region, and the helical cap. The interplay of structural and dynamic effects in bile salt recognition is presented.

ACKNOWLEDGEMENT
This work was financed by the National Research, Development, and Innovation Fund of Hungary (K109035) and the MedinProt Program of the Hungarian Academy of Sciences.

REFERENCES

1. G. Horváth, Á. Bencsura, Á. Simon, G. P. Tochtrop, G. T. DeKoster, D. F. Covey, D. P. Cistola, O. Tőke (2016) *FEBS J*, 283, 541–555.
2. G. Horváth, O. Egyed, T. Changguo, M. Kovács, A. Micsonai, J. Kardos, O. Tőke, Ligand entry in human ileal bile acid-binding protein is mediated by histidine protonation. (2019) *Sci. Rep*., 9, 4825.
3. O. Tőke (2022) *Int. J. Mol. Sci.,* 23, 505.

P-37

Nostoc cf. *linckia* exopolysaccharide - a structural study

Iveta Uhliariková[1], Mária Matulová[1], Jaromír Lukavský[2], Peter Capek[1]

[1]*Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 84538 Bratislava, Slovakia*
[2]*Institute of Botany, National Centre of Competence, Academy of Sciences of the Czech Republic, Dukelská 135, CZ-37982 Třeboň, Czech Republic*

Cyanobacteria are a diverse group of the oldest photosynthetic microscopic organisms, which colonize all biotopes and territories of our planet. They synthetize many metabolites (e.g. fatty acids, toxins, pigments, peptides and proteins, polyhydroxyalkanoates, polysaccharides, etc.) of a great biotechnological and industrial importance. Metabolites can be located in cyanobacterial cells, on their surface or released into their environment [1].

Exopolysaccharides (EPSs) produced by cyanobacteria are one of the most important excreted compounds, due to their special physicochemical properties and biological activities. They protect cyanobacterial cells from environmental stresses, toxic substances or other microorganisms. The excretion and composition of EPSs depend on the individual cyanobacterial strains and environmental conditions. According to the carbohydrate content, the cyanobacterial EPSs are produced as pure polysaccharides, proteoglycans or glycoprotein conjugates. As EPSs are often very complex branched heteropolysaccharides, consisting of differently linked six and more monosaccharide units (substituted by different organic or inorganic groups), it is quite difficult to determine their primary structure [2, 3].

In this study, we have analysed water-soluble EPS isolated from a culture medium of the freshwater cyanobacterium *Nostoc* cf. *linckia* (strain Hindák 2006/20). Conventional methods of isolation, fractionation, chemical composition (protein, carbohydrates, uronic acids content), and monosaccharide compositional analysis were used. The structure of the isolated EPS was elucidated by NMR spectroscopy [4].

ACKNOWLEDGEMENT

This work was financed by the Slovak Grant Agency VEGA (Grant No. 2/0054/22), the Slovak Research and Development Agency (Grant No. APVV-19-0009), by the Research Agency under Research & Development Operational Programme Integrated Infrastructure RIS3 BSK, ITMS: 313021Y920, co-financed by the European Regional Development Fund, by COST action CA18238 (Ocean4Biotech) and Technology Agency of the Czech Republic (Grant TE 01020080 BIOCIRTECH).

REFERENCES

1. T. Encarnação, A.C.C.C. Pais, M.G. Campos, H.D. Burrows (2015) *Sci Prog,* 98, 145-168.
2. C. Delattre, G. Pierre, C. Laroche, P. Michaud (2016) *Biotechnol Adv,* 34, 1159-1179.
3. S. Pereira, A. Zille, E. Micheletti, P. Moradas-Ferreira, R. De Philippis, P. Tamagnini (2009) *FEMS Microbiol Rev,* 33, 917-941.
4. I. Uhliariková, M. Matulová, Z. Košťálová, J. Lukavský, P. Capek (2022) *Carbohydr Polym*, 276, 118801.

P-38

Order & disorder: Carbonic anhydrase IX

Vojtěch Vařečka[1,2], Krishnendu Bera[1], Jozef Hritz[1,2*]

[1]Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czechia
[2]Department of Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechia
**jozef.hritz@ceitec.muni.cz*

Carbonic anhydrase IX (CAIX) is a zinc-based metalloenzyme that catalyzes reversible hydration of carbon dioxide to bicarbonate and proton [1]. It has been reported to play a potentially significant role in tumor development and thus presents an enticing target for drug design [2]. This would seem a rather straightforward endeavor, given that structure of the enzyme's catalytic domain had been thoroughly characterized by X-ray crystallography [3,4...]. However, within this scope, CAIX shares almost all of its structural features with the other CA isoforms. Considering there is at least 15 of these, many of which play indispensable roles in native metabolism, finding a suitable selective inhibitor becomes challenging [5].
As was slightly foreshadowed, the solution might lie in extending the scope beyond catalytic domain. CAIX additionally possesses a transmembrane helix and an intrinsically disordered N-tail. The latter is unique to this isoform only and was attributed role in its cancer-related functions [6,7]. Being disordered, the domain poses an elusive subject regarding its experimental characterization.
The dynamic nature of intrinsically disordered structures matches rather computational approach of molecular dynamics [8]. Utilizing our groups experience in the field, we present such analysis of CAIXs disordered region and its interactions with the catalytic domain, which we hope to assist selective inhibitor design [9,10].

ACKNOWLEDGEMENT
Computational resources were partially supplied by the project "e-Infrastruktura CZ" (e-INFRA CZ LM2018140) supported by the Ministry of Education, Youth and Sports of the Czech Republic. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90140). VV acknowledges the Grant Agency of Masaryk University (GAMU) for the support through "excellent diploma thesis" grant (no. MUNI/C/0003/2022).

REFERENCES
1. M. Hilvo, L. Baranauskiene et al. (2008) *Journal of Biological Chemistry*, 283, 27799-27809.
2. S. Pastorekova, R.J. Gillies et al. (2019) *Cancer and Metastasis Reviews*, 38.
3. J. Kazokaitė, R. Niemans et al. (2018) *Oncotarget*, 9, 26800-26816.
4. Ö. Akgül, L. Lucarini et al. (2022) *European Journal of Medicinal Chemistry*, 227.
5. C.T. Supuran (2016) *Biochemistry Journal*, 473.
6. E. Švastová, N. Žilka et al. (2003) *Experimental Cell Research*, 290.
A. Innocenti, S. Pastorekova et al. (2009) *Bioorganic & Medicinal Chemistry Letters*, 19.
7. K. Lindorff-Larsen, N. Trbovic et al. (2012) *Journal of American Chemical Society*, 134.
8. J. Hritz, A. De Ruiter et al. (2008) *Journal of Medicinal Chemistry*, 51.
9. V. Zapletal, A. Mládek et al. (2020) *Biophysical Journal*, 118.

P-39

Towards a new biomarker for Diabetic Retinopathy: exploring RBP3 structure and retinoids binding for eyes functional imaging in vivo

Vineeta Kaushik, Luca Gessa, Nelam Kumar, Humberto Fernandes

Integrated Structural Biology Lab, International Centre for Translational Eye Research, Institute of Physical Chemistry, Warsaw, Poland

Diabetic Retinopathy (DR) is a complication of diabetes, affecting a growing number of people, and is a significant cause of blindness in developed and developing countries. Currently, treatment is limited and applied at later stages of the disease and consists of injection of anti-VEGF (vascular endothelial growth factor) agents directly in the eye. Before such drastic options, efficient control of the disease can be achieved by strict diet and blood sugar control. Current diagnostics are based on Fluorescein angiography and OCT (Optical coherence tomography), with AI (Artificial Intelligence) gaining ground and promising more accurate diagnostics. But all of them are based on physical blood vessel alterations or lipofuscin deposits, and the search for other biomarkers that could potentially allow an early detection is important. It would allow an early lifestyle correction and thus minimize DR consequences by delaying or slowing down its progression. A link has been revealed between retinal binding protein 3 (RBP3) and the severity of DR [1, 2]. The studies indicate that patients with decreased levels of RBP3 have more severe DR, and have shown that supplementation of RBP3 confers DR protection [2]. RBP3 is a protein located in the interphotoreceptor matrix in the retina and supports the shuttling of retinoids between photoreceptors and RPE (retinal pigment epithelium) cells in the retina, thus allowing the visual cycle. It is composed of 4 modules, each able to bind different retinoids.

The ophthalmologic field has recently been reporting exciting new functional imaging capacities, such as two-photon excitation (TPE) fluorescence (TPEF) imaging. The technique overcomes tissue penetration and prohibitive excitation wavelength by simultaneous excitation by two photons with longer wavelengths, resulting in shorter wavelength emission light. It was validated in mouse models, proven safe in human subjects [3], and already used to detect different retinoids in retinas [4]. We are characterizing RBP3 and its ligand binding structurally and biophysically as a starting point to prepare complexes with modified ligands (or specific antibodies). The modified reagents will have unique excitation signatures that will allow detection and quantification of RBP3 levels using TPEF imaging. Thus, opening the possibility for early detection of DR.

ACKNOWLEDGEMENT

This work was financed by the International Research Agendas programme of the Foundation for Polish Science (MAB/2019/12) co-financed by the European Union under the European Regional Development Fund, and by the PASIFIC Fellowship programme of the Polish Academy of Sciences.

REFERENCES

1. M. Garcia-Ramirez, C. Hernandez et al. (2009) *Diabetologia,* 52 (12), 2633-2641.
2. H. Yokomizo, Y. Maeda et al. (2019) *Sci. Transl. Med.*, 11 (499), eaau6627.
3. J. Boguslawski, G. Palczewska et al. (2022) *J. Clin. Invest.*, 132 (2), e154218.
4. G. Palczewska, P. Stremplewski et al. (2018) *JCI Insight*, 3 (17), e121555.

P-40

Composite materials as drug carriers for controlled release of clindamycin

Wioletta Florkiewicz, Dagmara Słota, Karina Piętak, Julia Sadlik, Agnieszka Sobczak-Kupiec

Department of Materials Science, Faculty of Materials Engineering and Physics, Cracow University of Technology, 37 Jana Pawła II Av., 31-864 Krakow, Poland

Controlled drug delivery systems (DDSs) can provide transport of active compounds directly to the target site and minimize undesirable collateral effects of antibiotics [1]. Moreover, the application of active compounds for example antibiotics as components of materials might be an effective strategy to prolong their distribution and enhance therapeutic efficiency by maintaining the appropriate concentration of the active ingredient in the locally targeted site for the whole treatment [2].

In our studies, we have focused on the preparation and characterization of ceramic-polymer composite materials based on synthetic biologically safe polymer, betaine, and hydroxyapatite enriched with clindamycin obtained by UV light crosslinking. Clindamycin is a high bioavailability antibiotic which can be used as a compound for the treatment of bone and joint infections [3]. Thus, the proposed composites can be applied as potential materials supporting bone regeneration and also providing antibacterial activity in the case of implant-related infection.

In this study, we focused on the material structure-drug releasing relationship. We perform an investigation of the swelling kinetic of composites to analyze the influence of materials composition on their sorption capacity and release of a drug. The obtained results showed that the sorption capacity of the tested materials is strongly dependent on hydroxyapatite content. Increasing content of hydroxyapatite in materials leads to a decrease in their sorption ability and also slows down the absorption of water into the structure of the materials. What is more, the sorption capacity of composites is also dependent on polymer content; the greater polymer content the greater sorption capacity. Studies of releasing clindamycin from materials were performed with HPLC chromatography. The obtained results showed that during seven days of incubation more than 50% of antibiotic is released from materials. The amount of released drug was dependent on the sorption capacity of the composites. The greater sorption capacity of the composite, the greater amount of released active substance was recorded.

ACKNOWLEDGEMENT

The "Multifunctional biologically active composites for applications in bone regenerative medi-cine" project is carried out within the TEAM-NET program of the Foundation for Polish Science financed by the European Union under the European Regional Development Fund. The authors gratefully acknowledge financial support. POIR.04.04.00-00-16D7/18.

REFERENCES

1. C. Tipa, M. T. Cidade, T. Vieira, J. C. Silva, P. I. P. Soares, J P. Borges (2021) *Nanomaterials*, 11, 25.
2. V. Mourino, J.P. Cattalini, J.A. Roether, P. Dubey, I. Roy, A.R. Boccaccini (2013) *Expert Opinion on Drug Delivery*, 10, 1353-1365.
3. J. Courjon, E. Demonchy, E. Cua, E. Bernard, P-M Roger (2017) *European Journal of Clinical Microbiology & Infectious Diseases*, 36, 2513-2518.