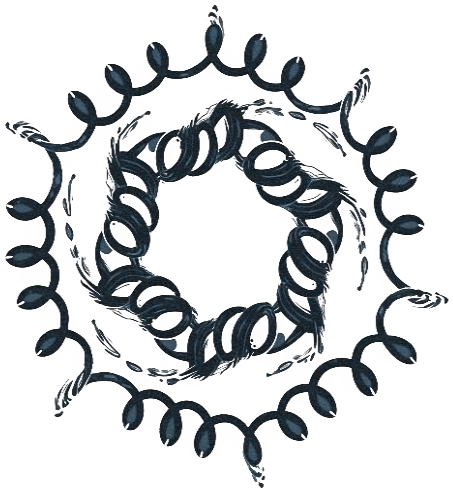
Department of Biophysics
Institute of Experimental Physics
Slovak Academy of Sciences

Slovak
Biophysical
Society

Slovak
Physical
Society



SSB 2025

13th International Conference

Structure & Stability of Biomacromolecules

9 – 12 September 2025

in Kosice, Slovak Republic

BOOK OF CONTRIBUTIONS

Institute of Experimental Physics

Slovak Academy of Sciences

Book of Contributions

13th International Conference

Structure & Stability of Biomacromolecules

September 9 – 12, 2025

Kosice, Slovakia

ISBN: 978-80-89656-27-1

Book of Contributions, 13th International Conference Structure & Stability of Biomacromolecules, September 9 – 12, 2025, Kosice, Slovakia

Editors: Z. Gazova, A. Antosova, J. Kubackova
Reviewers: G. Fabriciova, R. Varhac
Number of pages: 139

ORGANIZATION

SCIENTIFIC COMMITTEE

Diana Fedunova (IEP SAS)

Zuzana Gazova (IEP SAS)

ORGANIZING COMMITTEE

from the Department of Biophysics, Institute of Experimental Physics,
Slovak Academy of Sciences, Kosice

Andrea Antosova

Zuzana Bednarikova

Tomas Biro

Barbora Borovska

Viktoria Fedorova

Miroslav Gancar

Hanan Abdul Kareem

Jana Kubackova

Olga Parmar

Marian Reiffers

Michaela Romanonova

Cyril Slaby

Tomas Susko

Dana Svarcbergerova

Peter Zimovcak

ACKNOWLEDGEMENTS

The Organizing Committee of the 13th International Conference Structure & Stability of Biomacromolecules
would like to express appreciation and thanks to the following companies for their generous support.

Anton Paar Slovakia s.r.o.
Hattalova 12/A
831 03 Bratislava
Slovakia
https://www.anton-paar.com/sk-cs/

K-TRADE spol. s r. o.
Dubravska cesta 9 (P.O. BOX 69)
841 04 Bratislava
Slovakia
https://www.ktrade.sk/

KRD – molecular technologies s.r.o.
Dvojkrizna 49
821 06 Bratislava
Slovakia
https://www.krd.cz/

BIOTECH s.r.o.
Kresankova 12
841 05 Bratislava
Slovakia
https://www.ibiotech.sk/

SHIMADZU SLOVAKIA
Rontgenova 28
851 01 Bratislava
Slovakia
https://www.shimadzu.sk/index.html

ANAMET s.r.o.
Kovaku 3210/26
150 00 Prague 5 – Smichov
Czech Republic
https://www.anamet.cz/

Uni-Export Instruments, s.r.o.
Sultysova 642/15
169 00 Prague 6
Czech Republic
http://uniexport.co.cz/

ProScience Tech s.r.o.
Dobsinskeho 20
811 05 Bratislava
Slovakia
https://www.prosciencetech.sk/

ABL&E-JASCO Hungary Ltd.
Fehervari ut 132-144
H-1116 Budapest
Hungary
https://www.ablelab.eu/

Biotecha
Vismaliuku str. 30
10243 Vilnius
Lithuania
https://www.biotecha.lt/en/

Opre' Cidery s.r.o.
Budimír 42
044 43 Budimír
Slovakia
https://www.opre.sk/

CONTENTS

Dear colleagues, dear friends in biophysics,

On behalf of the Department of Biophysics at the Institute of Experimental Physics of the Slovak Academy of Sciences, and in collaboration with Slovak Biophysical Society and Slovak Physical Society, it is our great pleasure to welcome you to the 13th edition of the international conference *Structure and Stability of Biomacromolecules*, traditionally held in the September atmosphere of Košice.

Biophysics is, by its very nature, an interdisciplinary science that opens the way to new insights across physics, medicine, biology, pharmacology, biotechnology, and materials engineering. We are delighted that this year's program features 24 lectures and 31 poster presentations, covering a broad spectrum of biophysical research – from studies of the structure and function of biomacromolecules to novel approaches in teaching biophysics at universities.

As in previous years, the official language of both the conference and the proceedings remains English, allowing us to share our most recent results with our international colleagues and foster open scientific exchange. We hope that this symposium will stimulate new collaborations, research initiatives, and joint projects.

We wish you an inspiring and enjoyable conference, one that will spark innovative ideas, creative spirit, and fruitful scientific outcomes.

SCIENTIFIC PROGRAM

SSB 2025 PROGRAM

Tuesday, September 9, 2025

15:00 - 16:00	Registration		Lobby of IEP SAS, Watsonova 47
16:00 - 16:10		Conference Opening	
16:10 - 16:45	PL1	Niedzialek D.	Activation of the autolysin LytM from Staphylococcus aureus as a potential strategy for the development of antibacterial drugs
16:45 - 17:05	SC1	Wieczorek G.	RNA-Bender: A Novel Molecular Dynamics-Based Approach for Rapid Sequence-Based RNA Model Construction and Refinement
17:05 - 17:25	SC2	Uskoba J. BioTech a.s.	Immobilisation free in-solution kinetics using Flow Induced Dispersion Analysis (FIDA)
17:25 - 19:00		Welcome Mixer	

Wednesday, September 10, 2025

09:00 - 09:35	PL2	Sugimoto N.	*"To B or not to B" in Nucleic Acids Chemistry*
09:35 - 10:10	PL3	Viglasky V.	*Non-canonical structural motifs in DNA – their identification in the genome using the Qinder search tool and their analysis in a system mimicking mini-circular DNA*
10:10 - 10:30	SC3	Cernik M. Uni - Export Instruments, s.r.o.	*Ultimate DSC, a new tool for protein characterization*
10:30 - 11:15		*Coffee break*	
11:15 - 11:50	PL4	Zahradnikova A.	*The effect of selected RyR1 MH mutations on the interaction between the EF-hand region and S23 segment*
11:50 - 12:25	PL5	Zelenakova A.	*Magnetic nanoparticles– usefull platform for advanced biomedical applications*
12:25 - 13:00	PL6	Hritz J.	*^{19}F Tryptophan: an efficient NMR probe for protein complexes*
13:00 – 13:30		*Lunch*	
14:00 - 14:35	PL7	Jancura D.	*Cytochrome c oxidase - a key component of oxidative phosphorylation: Regulation and modulation of its catalytic activity*
14:35 - 14:55	SC4	Fabian M.	*Thermodynamics of the transition of ferryl (F) to the oxidized form of cytochrome c oxidase: implication for the proton pumping*
14:55 - 15:15	SC5	Hodosi R. SHIMADZU SLOVAKIA	*From Structure to Identification: Shimadzu Tools for Biomacromolecule Research*
15:15 - 15:50		*Flash posters*	
15:50 - 16:20		*Coffee break*	

16:20 - 18:15			*Poster Session*
19:00 - 22:00			Conference dinner

Thursday, September 11, 2025			
9:00 - 9:35	PL8	Skrabana R.	*Tiny motifs doing huge things: Side-chain Propelled Rings (SPuRs) in the aggregation domain of tau protein*
9:35 - 10:10	PL9	Cehlar O.	*Insights into the early oligomerization stages of truncated tau proteins*
10:10 - 10:30	SC6	Polak A.	*Deciphering the role of small structural motifs in aggregation of tau protein*
10:30 - 11:15			*Coffee break*
11:15 - 11:50	PL10	Bauerova V.	*Bioinformatic identification, expression, purification and structural characterization of domains in multidomain proteins*
11:50 - 12:10	SC7	Trizna L.	*Design and Characterization of Circular DNA Minicircles as a Tool for Studying Non-B DNA Structures*
12:10 - 12:30	SC8	Landl R. Anton Paar Slovakia s.r.o.	*Possibilities of measurement particle size, zeta potential and rheologic behavior with Anton Paar instruments*
12:30 - 12:50	SC9	Bednarikova Z.	*14-3-3ζ Variants as Modulators of Amyloid β Fibril Formation*
13:00 - 13:30			*Lunch*
14:15 - 21:00			Conference Trip & Dinner

Friday, September 12, 2025			
9:00 - 9:35	PL11	Stroffekova K.	*Effects of rotenone and photobiomodulation on alpha-synuclein aggregates in differentiated SH-SY5Y cells*
9:35 - 10:10	PL12	Benedetto A.	*Ionic liquids and membranes*
10:10 - 10:30	SC10	Tomkova M.	*Ribosome display selection of non-immunogenic staphylokinase with improved fibrinolytic activity*
10:30 - 11:15			*Coffee break*
11:15 - 11:35	SC10	Fedorova V.	*Programmability of protein-DNA bioconjugates self-assembly through functional ligand*
11:35 - 11:55	SC12	Ilkovicova L.	*Computational study of the tau protein aggregation*
11:55 - 12:30			Young award Ceremony and Concluding remarks

LIST OF PLENARY LECTURES

PL1 **Activation of the autolysin LytM from Staphylococcus aureus as a potential strategy for the development of antibacterial drugs**

D. Niedzialek, G. Wieczorek, M. Nowacka, E. Jagielska, I. Sabala
Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

PL2 **"To B or not to B" in Nucleic Acids Chemistry**

N. Sugimoto
Frontier Institute for Biomolecular Engineering Research, Konan University, Kobe, Japan

PL3 **Non-canonical structural motifs in DNA – their identification in the genome using the Qinder search tool and their analysis in a system mimicking mini-circular DNA**

V. Viglasky, L. Trizna, D. Pitkova, A. Varha
Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

PL4 **The effect of selected RyR1 MH mutations on the interaction between the EF-hand region and S23 segment**

[a]A. Zahradnikova, [a]I. Baglaeva, [b]A. Waheed, [b]M. Sabo
[a]Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia
[b]Bioinformatics Laboratory, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

PL5 **Magnetic nanoparticles– usefull platform for advanced biomedical applications**

A. Zelenakova
Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

PL6 **^{19}F Tryptophan: an efficient NMR probe for protein complexes**

[a, b]A. Naplavova, [a, b]A. Kozelekova, [a, b]N. Gasparik, [a, b]R. Crha, [c]A. M. Gronenborn, [a, b, d]J. Hritz
[a]National Center for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic
[b]Central European Institute of Technology, Masaryk University, Brno, Czech Republic
[c]Department of Structural Biology, University of Pittsburgh, School of Medicine, Pittsburgh, USA
[d]Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic

PL7 **Cytochrome c oxidase - a key component of oxidative phosphorylation: Regulation and modulation of its catalytic activity**

[a]D. Jancura, [b]M. Fabian

[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[b]*Center for Interdisciplinary Bioscences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*

PL8 **Tiny motifs doing huge things: Side-chain Propelled Rings (SPuRs) in the aggregation domain of tau protein**

[a]R. Skrabana, [a]K. Martonova, [a]A. Polak, [a]S. Njemoga, [a]R. Dvorsky, [b]E. Barrera, [a]O. Cehlar

[a]*Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*

[b]*Institute of Histology and Embryology (IHEM), National University of Cuyo (UNCuyo), CONICET, Mendoza, Argentina*

PL9 **Insights into the early oligomerization stages of truncated tau proteins**

[a]S. Njemoga, [b]Z. Bednarikova, [c]E. E. Barrera, [b]Z. Gazova, [a, d]O. Cehlar

[a]*Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*

[b]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*

[c]*Institute of Histology and Embryology (IHEM), National University of Cuyo (UNCuyo), CONICET, Mendoza, Argentina*

[d]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia*

PL10 **Bioinformatic identification, expression, purification and structural characterization of domains in multidomain proteins**

V. Bauerova-Hlinkova & J. A. Bauer

Department of Biochemistry and Structural Biology, Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia

PL11 **Effects of rotenone and photobiomodulation on alpha-synuclein aggregates in differentiated SH-SY5Y cells**

[a]K. Stroffekova & [b]Z. Bednarikova

[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[b]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*

PL12 **Ionic liquid to tune cell membrane's viscoelasticity & amyloid fibril's properties: A combined atomic force microscopy and neutron scattering study**

A. Benedetto

School of Physics, University College Dublin, Dublin, Ireland
Conway Institute for Biomolecular & Biomedical Research, University College Dublin, Dublin, Ireland
Department of Science, University Roma Tre, Rome, Italy

LIST OF SHORT COMMUNICATIONS

SC1 **RNA-Bender: A Novel Molecular Dynamics-Based Approach for Rapid Sequence-Based RNA Model Construction and Refinement**

[a]G. Wieczorek, [a,b]D. Niedzialek
[a]*Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*
[b]*Ensemble[3] sp. z o.o., Centre of Excellence, Warsaw, Poland*

SC2 **Immobilisation free in-solution kinetics using Flow Induced Dispersion Analysis (FIDA) – company**

[a]K. Ray, [a]H. Jensen, [a]A. C. Hundahl, presenting: [b]J. Uskoba
[a]*FIDA Biosystems ApS, Søborg, Denmark*
[b]*BioTech a.s., Prague, Czech Republic*

SC3 **Ultimate DSC, a new tool for protein characterization – company**

[a]G. Jossens, [a]Y. Ricci, [a]J. - C. Neyt, [a]M. Simond, [b]J. L. Garden, [b]G. Moiroux, presenting: [c]M. Cernik
[a]*Calneos SAS, Clermont-Ferrand, France*
[b]*Institut Neel CNRS, Grenoble, France*
[c]*Uni-Export Instruments, s.r.o., Prague, Czech Republic*

SC4 **Thermodynamics of the transition of ferryl (F) to the oxidized form of cytochrome *c* oxidase: implication for the proton pumping**

[a]A. Tomkova, [a]T. Sztachova, [a]D. Jancura, [b]M. Fabian
[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[b]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*

SC5 **From Structure to Identification: Shimadzu Tools for Biomacromolecule Research – company**

R. Hodosi
Shimadzu Slovakia, Bratislava, Slovakia

SC6 **Deciphering the role of small structural motifs in aggregation of tau protein**

[a]A. Polak, [a]K. Martonova, [a]S. Njemoga, [b]E. Barrera, [a]R. Skrabana
[a]*Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*
[b]*Institute of Histology and Embryology (IHEM), National University of Cuyo (UNCuyo), CONICET, Mendoza, Argentina*

SC7 **Design and Characterization of Circular DNA Minicircles as a Tool for Studying Non-B DNA Structures**

L.Trizna, D. Pitkova, A. Varha, V. Viglasky

Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

SC8 **Possibilities of measurement particle size, zeta potential and rheologic behavior with Anton Paar instruments – company**

R. Landl
Anton Paar Slovakia s.r.o., Bratislava, Slovakia

SC9 **14-3-3ζ Variants as Modulators of Amyloid β Fibril Formation**

[a]Z. Bednarikova, [b, c]A. Kozelekova, [b, c]J. Novotna, [b]K. Kralova, [b, c, d]J. Hritz, [a]Z. Gazova
[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
[c]*National Center for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic*
[d]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*

SC10 **Ribosome display selection of non-immunogenic staphylokinase with improved fibrinolytic activity**

M. Tomkova, M. Nemergut, I. Pilipcincova, E. Sedlak
Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

SC11 **Programmability of protein-DNA bioconjugates self-assembly through functional ligand**

[a]V. Fedorova, [b]V. Huntosova, [a]K. Siposova, [c]M. Humenik
[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[c]*Department of Biomaterials, Faculty of Engineering Science, University Bayreuth, Germany*

SC12 **Computational study of the tau protein aggregation**

[a, b]L. Ilkovicova, [a, c]J. Hritz
[a]*Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
[b]*National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic*
[c]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*

LIST OF POSTERS

PO1 **Enhanced efficiency of Fe_3O_4@SiO_2 with specific ligand layer in magnetic separation RNA**

[a]M. Barutiak, [a]A. Zelenakova, [a]P. Hrubovcak, [a]L. Nagy, [a]J. Szucsova, [a]Z. Fabriciova, [b]E. Benova, [b]N. Kiraly, [b]V. Zelenak, [c]A. Antosova

[a]Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

[b]Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

[c]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

PO2 **Spider silk eADF4(C16) nanofibrillar protein hydrogels for biomedical application**

[a]T. Biro, [b]N. Best, [c]V. Huntosova, [d]K. Paulovicova, [a]K. Siposova, [b]M. Humenik

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

[b]Department of Biomaterials, Faculty of Engineering Science, University Bayreuth, Germany

[c]Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

[d]Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

PO3 **When Structure Matters: How Linker Length and Coumarin Dimerization Shape Anti-Amyloid Activity**

[a]B. Borovska, [b]P. Krupa, [a]Z. Bednarikova, [c]S. Hamulakova, [b, d]M. S. Li, [a]Z. Gazova

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

[b]Institute of Physics, Polish Academy of Sciences, Warsaw, Poland

[c]Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

[d]Life Science Lab, Institute for Computational Science and Technology, Quang Trung Software City, Ho Chi Minh City, Vietnam

PO4 **Analysing possible interaction of mitochondrial translocator protein with chloride intracellular channel CLIC5**

[a]R. Dekhtiarenko, [b]K. Polcicova, [a]Z. Sevcikova Tomaskova

[a]Center of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia

[b]Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia

PO5 **Cancer therapy by magnetic hyperthermia: impact of cobalt ferrite nanoparticles**

[a, b]Z. Fabriciova, [a]A. Zelenakova, [a]L. Nagy, [a]P. Hrubovcak, [a]M. Barutiak, [a]D. Volavka, [a]M. Lisnichuk

[a]*Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[b]*Department of Chemistry and Industrial Chemistry & INSTM RU, nM2-Lab, University of Genoa, Italy*

PO6 **Dual effect of ionic liquids on lysozyme stability and activity**

D. Fedunova, V. Vanik, Z. Gazova

Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

PO7 **Photopolymeric microchambers for investigating the selectivity of photodynamic therapy in individual breast cancer cells**

[a]M. Galis, [b]S. Tomkova, [c]A. Migasova, [d]J. Kubackova, [c]M. Almasi, [a]G. Bano, [b]V. Huntosova

[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[b]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[c]*Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[d]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*

PO8 **Cholesteryl-anchor mediated non-covalent aptamer functionalization of β-Casein self-assemblies towards therapeutic potential**

[a]Z. Garaiova, [a]V. Subjakova, [a]A. A. Macias, [b]M. Garaiova, [b]R. Holic, [a]T. Hianik

[a]*Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia*

[b]*Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia*

PO9 **Multitarget *In Vitro* Evaluation of a Novel Quinacrine Analog: Interactions with DNA, RNA, HSA and Topoisomerase Inhibition**

[a]A. Gucky, [a]A. Mihokova, [b]E. Scalzi, [c]J. Korabecny, [a]M. Kozurkova

[a]*Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[b]*Department of Pharmacy, University of Pisa, Italy*

[c]*Biomedical Research Center, University Hospital Hradec Kralove, Czech Republic*

PO10 **Novel betulinic acid-based ionic liquids: synthesis and anti-breast cancer activity**

[a]M. Guncheva, [b]P. Ossowicz-Rupniewska, [a]Y. Raynova

[a]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[b]*Department of Organic Chemical Technology and Polymer Materials, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology, Szczecin, Poland*

PO11 **Raman characterization of the biocompatible photoresist Ormocomp**

[a]Z. Jurasekova, [a]M. Galis, [b]J. Kubackova, [b]C. Slaby, [b]Z. Tomori, [c]G. Vizsnyiczai, [c]L. Kelemen, [c]G. T. Ivanyi, [a]G. Bano
[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[b]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[c]*HUN-REN Biological Research Centre Szeged, Institute of Biophysics, Szeged, Hungary*

PO12 **Application of modular anticancer nanoparticles in Raman microscopy analysis of cells and 3D culture models**

[a]Z. Jurasekova, [a]B. G. Varchol, [b]A. Migasova, [c]X. Li, [c]M. Andreana, [c]A. Unterhuber, [b]M. Almasi, [d]V. Huntosova
[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[b]*Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[c]*Center for Medical Physics and Biomedical Engineering, Medical University, Vienna, Austria*
[d]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*

PO13 **Targeting Insulin Fibrils: Evaluating the Destruction Potential of Phytoalexins**

[a]H. A. Kareem, [a]A. Antosova, [b]M. Budovska, [a]Z. Bednarikova, [a]D. Fedunova, [a]Z. Gazova
[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

PO14 **Red Blood Cell Stiffness and Deformability as Biophysical Indicators of Chronic Lymphocytic Leukemia and Therapy Response**

[a]A. Langari, [b]V. Strijkova, [c]A. Alexandrova-Watanabe, [a]M. Ivanova, [b]V. Katrova, [d]M. Guenova, [d]L. Gartcheva, [c]E. Abadjieva, [c]T. Tiankov, [a]S. Krumova, [a]S. Todinova
[a]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria*

[b]*Institute of Optical Materials and Technologies "Acad. Yordan Malinovski", Bulgarian Academy of Sciences, Sofia, Bulgaria*
[c]*Institute of Mechanics, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[d]*National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria*

PO15 **Fluorescence study of the interaction between Silver (I), Zinc (II), and Gallium (III) thiophene-2-carboxylates and human serum albumin**

[a]H. Matajova, [b]M. Rendosova, [b]Z. Vargova, [a]M. Kozurkova
[a]*Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[b]*Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

PO16 **Hsp70-SBD as a Therapeutic Modulator: Biophysical and Structural Perspectives in Light Chain Amyloidosis**

M. Nemergut, M. Tomkova
Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

PO17 **Role of proline-, cysteine-, and poly-L-lysine coated magnetic nanoparticles in amyloid aggregation**

[a]O. Parmar, [a]A. Antosova, [c, d]P. Verducci, [c]E. Bystrenova, [a]M. Gancar, [b]I. Antal, [b]M. Kubovcikova, [a]Z. Bednarikova, [b]M. Koneracka, [b]V. Zavisova, [a]Z. Gazova
[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[c]*Consiglio Nazionale Delle Ricerche, Istituto Per Lo Studio Dei Materiali Nanostrutturati (CNR-ISMN), Bologna, Italy*
[d]*Facolta Chimica e Tecnologia Farmaceutiche, Alma Mater Studiorum-Universita di Bologna, Italy*

PO18 **Non canonical 3WJ DNA motif – helical ligand interaction**

D. Pitkova, L. Trizna, V. Viglasky
Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

PO19 **Structural characterization of the amyloid-forming protein RIPK1**

[a, b]P. Polonio, [c, d]J. P. Lopez-Alonso, [c]H. Jiang, [a]S. Andres-Campos, [a]F. Escobedo, [a]G. Titaux, [c, e]I. Ubarretxena-Belandia, [a]M. Mompean
[a]*Institute of Physical Chemistry "Blas Cabrera" (IQF-CSIC), Madrid, Spain*
[b]*Autonomous University of Madrid, Doctoral School, Madrid, Spain*
[c]*Institute of Biophysics (UPV/EHU, CSIC), Leioa, Spain*
[d] *Basque Resource for Electron Microscopy, Leioa, Spain*
[e] *Ikerbasque Foundation for Science, Bilbao, Spain*

PO20 **Exciton and electron transfer in photosynthetic complex**

M. Pudlak

Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

PO21 **Biogenic Magnetosome-mediated disassembly of protein amyloid fibrils triggered by ultrasound and magnetic field**

[a]M. Romanovova, [b]M. Molcan, [c]R. Bielas, [c]F. Ratajczak, [a]V. Fedorova, [c]A. Jozefczak, [a]K. Siposova

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

[b]Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

[c]Department of Acoustics, Faculty of Physics and Astronomy, Adam Mickiewicz University, Poznan, Poland

PO22 **Beyond Oxidative Stress: Cytochrome *c* in Alzheimer's Pathogenesis**

K. Siposova, E. Valusova, V. Fedorova, A. Musatov

Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

PO23 **Development of protocol for covalent functionalization of β-casein micelles with DNA aptamers for potential targeted delivery**

[a]V. Subjakova, [b]M. Garaiova, [b]R. Holic, [a]T. Hianik, [a]Z. Garaiova

[a]Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia

[b]Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia

PO24 **Directed Evolution in Action: Engineering Haloalkane Dehalogenase Variants for Enhanced Activity**

I. Timkova, M. Nemergut, E. Sedlak

Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

PO25 **In Vitro Characterization of the Interaction between Conventional and Novel Bruton's Tyrosine Kinase Inhibitors and Human Red Blood Cells via Atomic Force Microscopy and Biofluidic Analysis: A Pilot Study**

[a]S. Todinova, [b]A. Alexandrova-Watanabe, [c]V. Strijkova, [a]A. Langari, [d]N. Anastassova, [d]M. Argirova, [d]D. Yancheva, [a]M. Ivanova, [c]V. Katrova, [b]E. Abadjieva, [b]T. Tiankov, [e]E. Stoyanova, [a]S. Krumova, [d]M. Guncheva

[a]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

[b]Institute of Mechanics, Bulgarian Academy of Sciences, Sofia, Bulgaria

[c]Institute of Optical Materials and Technologies "Acad. Yordan Malinovski", Bulgarian Academy of Sciences, Sofia, Bulgaria

[d]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[e]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria*

PO26 **Motion Analysis of Shrimps and Microrobots: Insights into Active Matter Behavior**

[a]Z. Tomori, [b]A. Strejckova, [c]R. Galajda, [a]C. Slaby, [d]D. Horvath, [e]G. Bano
[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Department of Chemistry, Biochemistry & Biophysics, University of Veterinary Medicine & Pharmacy, Kosice, Slovakia*
[c]*Department of Biology &Physiology, University of Veterinary Medicine &Pharmacy, Kosice, Slovakia*
[d]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[e]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

PO27 **Small Au_{25} -based probe for enhanced infrared sensing of cytochrome *c***

[a]E. Valusova, [a]V. Fedorova, [a]K. Siposova, [b]M. Antalik
[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

PO28 **Detection of changes in DNA secondary structure caused by exposure to the conazole fungicide prothioconazole**

[a]V. Verebova, [a, b]J. Stanicova
[a]*Department of Chemistry, Biochemistry & Biophysics, University of Veterinary Medicine & Pharmacy, Kosice, Slovakia*
[b]*Institute of Biophysics & Informatics, First Faculty of Medicine, Charles University, Prague, Czech Republic*

PO29 **Conformation of The Ryanodine Receptor Isoform Models Created Using the AlphaFold 3 Server**

[a]A. Waheed, [b]A. Zahradnikova
[a]*Bioinformatics Laboratory, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*
[b]*Department of Cell Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*

PO30 **Structural Comparison of SERCA Conformations**

E. Wetter, M. Majekova

Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

PO31 **ProteoForce Group: Laser Tweezers Laboratory for In-Depth Analysis of Molecular Forces in Macromolecules**

M. Zoldakova, J. Pavlinska, L. Homolova, O. Ragac, N. Chomova, P. Ragan, S. Vavrakova, K. Papayova, S. Datta, S. Sovova, P. Varga, J. G. Johannesson, G. Zoldak

Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

AI-Identified Compounds Influence Aβ$_{42}$ Fibril Formation and Stability

[a]M. Gancar, [a]Z. Bednarikova, [b]C. Yue, [b]Y. Tang, [b]L. Ma, [b]B. - B. Zeng, [b]R. Wang, [a]Z. Gazova

[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*

[b]*School of Pharmacy, East China University of Science and Technology, Shanghai, China*

PLENARY LECTURES

Activation of the autolysin LytM from Staphylococcus aureus as a potential strategy for the development of antibacterial drugs

D. NIEDZIALEK, G. WIECZOREK, M.NOWACKA, E.JAGIELSKA, I.SABALA

Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Staphylococcus aureus, particularly its methicillin-resistant strain (MRSA), is recognized by the WHO as a major global health threat, which underscores the urgent need for new antibiotic strategies. This pathogen relies on peptidoglycan hydrolases (PGHs), a class of enzymes that remodel the bacterial cell wall during growth and division. The ability to control PGHs at the protein level creates the prospect of redirecting their activity against the bacteria themselves and thus opens a promising path for next-generation antibiotic development.

LytM, an important PGH from S. aureus, is naturally produced in an inactive form. The aim of this study is to elucidate the mechanism of LytM activation in vivo, focusing on the conditions and molecular events that govern its transition from the inactive to the active state. We use an integrated approach that combines experimental methods with advanced computational simulations. These computational studies are essential for revealing dynamic processes that contribute to the mechanical activation of LytM, processes that remain inaccessible to traditional experimental techniques. The insights gained through this work will provide a foundation for harnessing PGH activation as a novel strategy in antibacterial therapy.

Acknowledgment. This work was supported by research grants OPUS 26 no. 2023/51/B/NZ1/01552 funded by the National Science Centre. We gratefully acknowledge Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and support within computational grant no. PLG/2024/017439.

References

1. Razew, A., Laguri, C., Vallet, A., Morlot, C., Whitfield, R., Lazarelli, A., Lemonnier, M., Nivina, A., Ostatna, V., Bertsche, U., Vollmer, W., Simorre, J. P., Scheurwater, E., & Sauvage, E. (2023). Staphylococcus aureus sacculus mediates activities of M23 hydrolases. Nature Communications, 14, 6706.
2. Odintsov, S. G., Sabala, I., Marcyjaniak, M., & Bochtler, M. (2004). Latent LytM at 1.3A resolution. Journal of molecular biology, 335(3), 775-785.

"To B or not to B" in Nucleic Acids Chemistry

N. SUGIMOTO

Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, Kobe, Japan

In this lecture, I will provide an overview of the basic concepts, methods, and recent applications of predicting the stabilities of nucleic acid structures. I explain the theory of the most successful prediction method based on a nearest-neighbor (NN) model. To improve the versality of prediction, corrections for various solution conditions considered hydration have been investigated. I also describe advances in the prediction of non-canonical structures of G-quadruplexes and i-motifs. Finally, studies of intracellular analysis and stability prediction are discussed for the application of NN parameters for human health and diseases.

Acknowledgment. The author is grateful to the colleagues named in the cited papers from my laboratory, institute (FIBER), and others. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the Japan Society for the Promotion of Science (JSPS) (Grant No. JP17H06351, 18KK0164, 19H00928, and 20K21258), especially for Grant-in-Aid for Scientific Research (S) (22H04975), JSPS Core-to-Core Program (JPJSCCA20220005), and The Chubei Itoh Foundation.

References

1. Takahashi, S., Ghosh, S., Trajkovski, M., Ohyama, T., Plavec, J., Sugimoto, N. (2025). Twisting tetraplex DNA: A strand dynamics to regulate i-motif function in diverse molecular crowding environments. Nucleic Acids Res., 53, in press.
2. Liu, L., Takahashi, S., Ghosh, S., Endoh, T., Yoshinaga, N., Numata, K., Sugimoto, N. (2025). Predictability of environment-dependent formation of G-quadruplex DNAs in human mitochondria. Comm. Chem**.,** 8, in press.
3. Matsumoto, S., Tateishi-Karimata, H., Ohyama, T., Sugimoto, N. (2025). Controlling the local conformation of RNA G-quadruplex results in reduced RNA/peptide cytotoxic accumulation associated with C9orf72 ALS/FTD. Small Methods, in press.
4. Sarkar, S., Tateishi-Karimata, H., Ohyama, T., Sugimoto, N. (2025). Imperfect G-quadruplex as an emerging candidate for transcriptional regulation. Nucleic Acids Res., 53, 5, gkaf164.
5. Tateishi-Karimata, H., Kawauchi, K., Takahashi, S., Sugimoto, N. (2024). Development of a Pseudocellular System to Quantify Specific Interactions Determining the G-Quadruplex Function in Cells. J. Am. Chem. Soc.,146, 8005-8015.
6. Banerjee, D., Tateishi-Karimata, H., Toplishek, M., Ohyama, T., Ghosh, S., Takahashi, S., Trajkovski, M., Plavec, J., Sugimoto, N. (2023). In-Cell Stability Prediction of RNA/DNA Hybrid Duplexes for Designing Oligonucleotides Aimed at Therapeutics. J. Am. Chem. Soc., 145, 23503-23518.
7. Ghosh, S., Takahashi, S., Banerjee, D., Ohyama, T., Endoh, T., Tateishi-Karimata, H., Sugimoto, N. (2023). Nearest-neighbor parameters for the prediction of RNA duplex stability in diverse in vitro and cellular-like crowding conditions. Nucleic Acids Res., 51, 4101-4111.
8. Zhang, Y., Tateishi-Karimata, H., Endoh, T., Jin, Q., Li, K., Fan, X., Ma, Y., Gao, L., Lu, H., Wang, Z., Cho, AE., Yao, X., Liu, C., Sugimoto, N., Guo, S., Fu, X., Shen, Q., Xu, G., Herrera-Estrella, LR., Fan, X. (2022). High-temperature adaptation of an OsNRT2.3 allele is thermoregulated by small RNAs. Sci. Adv., 8, eadc9785.
9. McQuaid, K. T., Takahashi, S., Baumgaertner, L., Cardin, D. J., Paterson, N. G., Hall, J. P., Sugimoto, N., Cardin, C. J. (2022). Ruthenium Polypyridyl Complex Bound to a Unimolecular Chair-Form G-Quadruplex. J. Am. Chem. Soc., 144, 5956-5964.
10. Takahashi, S., Kotar, A., Tateishi-Karimata, H., Bhowmik, S., Wang, Z.-F., Chang, T.-C., Sato, S., Takenaka, S., Plavec, J., Sugimoto, N. (2021). Chemical Modulation of DNA Replication along G-

Quadruplex Based on Topology-Dependent Ligand Binding. J. Am. Chem. Soc., 143, 16458-16469.
11. Sugimoto, N., ed. Handbook of Chemical Biology of Nucleic Acids. Vols. 1-3. Springer Nature, (2023).

Non-canonical structural motifs in DNA – their identification in the genome using the Qinder search tool and their analysis in a system mimicking mini-circular DNA

V. VIGLASKY, L. TRIZNA, D. PITKOVA, A. VARHA

Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

There is no longer any doubt that non-canonical structural motifs (NCSs) formed by nucleic acids play a crucial role in regulating essential cellular processes. These include G-quadruplexes, i-motifs, cruciforms, triplexes, and other structures. The spatial arrangement of bases forming either Watson-Crick or Hoogsteen hydrogen bonds must be precisely defined for an NCS to form. In the case of G-quadruplexes and i-motifs, base pairing occurs exclusively between guanines and cytosines, respectively. Their stabilization requires either potassium cations or mildly acidic pH conditions. NCSs generally utilize various combinations of these hydrogen bonding types.

Our pioneering work involved introducing steric hindrance to G-G pairing within G-quadruplex structures using platinum derivatives, effectively preventing bond formation [1]. More recently, we proposed a universal intelligent system capable of spontaneously forming any NCS — even without chemical modification of the sequence. This system also mimics small plasmids [2].

Another challenge we addressed was the multimerization and tandem arrangement of G-quadruplexes. Although our key findings have been published, many uncertainties remain [3, 4]. For instance, some G-quadruplexes multimerize, but a single point mutation in a region unrelated to the G-rich domain can abolish this ability. Conversely, under dehydration conditions, the opposite effect has been observed.

Our laboratory was among the first to initiate a series of analyses of G-rich regions in viral genomes, including papillomaviruses, Ebola, and HIV [5–7]. We also explored interactions between G-quadruplexes and various ligands. For example, we demonstrated that thiazole orange and Rhodamine 6G, when complexed with G4 structures, exhibit unique induced CD spectral profiles in the visible range [8, 9].

Recently, we introduced an alternative approach to representing nucleic acid sequences in 3D space [10]. This system enables the discovery of novel, unusual structures within long sequences, and we developed a software tool for identifying sequences prone to forming NCSs. In parallel with the work of Prof. Cech et al., we predicted that $(UG)_n$ sequences can adopt a left-handed G-quadruplex conformation in DNA, but only under dehydrating crowding conditions. Furthermore, we recently showed that the $d(AG)_n$ sequence forms a new NCS with features resembling the tetrahelical VK structure [11].

Acknowledgment. This work was supported by research grants VEGA 1/0347/23 and EU NextGenerationEU through the Recovery and Resilience Plan of the Slovak Republic 09I03-03-V05-00008 – VVGS-2023-2958.

References

1. Viglasky, V. (2009). Structural features of G-quadruplexes revealed by platinum derivatives. FEBS Journal, 276(2), 401-409.
2. Trizna, L., Olajos, J., & Viglasky, V. (2024). DNA minicircles capable of forming a variety of non-canonical structural motifs. Frontiers in Chemistry, 12, 1384201.
3. Tothova, P., Krafcikova, P., & Viglasky, V. (2014). Tandem arrangement and multimerization of G-quadruplexes. Biochemistry, 53(45), 7013-7027.
4. Bauer, L., Tluckova, K., Tothova, P., & Viglasky, V. (2011). Structural diversity of G-quadruplexes. Biochemistry, 50(35), 7484-7492.
5. Tluckova, K., Marusic, M., Tothova, P., Bauer, L., Sket, P., Plavec, J., & Viglasky, V. (2013). G-rich regions in viral genomes. Biochemistry, 52(41), 7207-7216.
6. Krafcikova, P., Demkovicova, E., Halaganova, A., & Viglasky, V. (2017). Putative HIV and SIV G-Quadruplex Sequences in Coding and Noncoding Regions Can Form G-Quadruplexes. Journal of Nucleic Acids, 2017, 6513720.
7. Marusic, M., Hosnjak, L., Krafcikova, P., Poljak, M., Viglasky, V., & Plavec, J. (2017). G-quadruplexes in papillomavirus genomes. Biochimica et Biophysica Acta - General Subjects, 1861(5 Pt B), 1229-1236.
8. Trizna, L., Janovec, L., Halaganova, A., & Viglasky, V. (2021). Rhodamine 6G-ligand influencing G-quadruplex stability and topology. International Journal of Molecular Sciences, 22, 7639.
9. Krafcikova, P., Demkovicova, E., & Viglasky, V. (2017). Ligand interactions with G-quadruplexes. Biochimica et Biophysica Acta - General Subjects, 1861(5 Pt B), 1321-1328.
10. Viglasky, V. (2022). Hidden Information Revealed Using the Orthogonal System of Nucleic Acids. International Journal of Molecular Sciences, 23(3), 1804.
11. Trizna, L., Osif, B., & Viglasky, V. (2023). G-QINDER Tool: Bioinformatically Predicted Formation of Different Four-Stranded DNA Motifs from (GT)n and (GA)n Repeats. International Journal of Molecular Sciences, 24(8), 7565.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

The effect of selected RyR1 MH mutations on the interaction between the EF-hand region and S23 segment

[a]A. ZAHRADNIKOVA, [a]I. BAGLAEVA, [b]A. WAHEED, [b]M. SABO

[a]*Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*
[b]*Bioinformatics Laboratory, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*

Mutations causing malignant hyperthermia (MH) dysregulate calcium release, partially by diminishing calcium-induced inactivation of the RyR1. Several mutations in these domains cause MH and weaken the RyR1 inactivation [2]. We have recently shown [1] that the allosteric pathway responsible for the RyR1-type inactivation is dependent on the inter-monomeric interaction between the EF-hand region of one monomer and the S23 segment of the neighbor monomer. Here, we tested the effect of mutations R4736W, F4732D, and G4733E in rRyR1 on hydrogen bond formation between the EF-hand region and S23 segment, using molecular dynamics simulation.

Models of tetramers of the rRyR1 core region (rRyR1 residues 3668-5037), corresponding to the C-terminal quarter of the molecule, based on the inactivated RyR1 structure 7tdg [3] were created, and mutations were introduced in ChimeraX [4]. Models were embedded in a POPC lipid bilayer and solvated in 250 mM KCl, using the CHARMM-GUI server [5]. The systems were equilibrated and simulations performed in GROMACS [6]. 100-ns long production runs for the WT RyR1 and each of the three studied mutations were analyzed for differences in RMSD and RMSF in GROMACS. The extent of hydrogen bonding between the EF-hand region (residues 4070-4079,4100,4101) and the S23 loop (residues 4730-4736) was compared. The formation of H-bonds between residues 4736 – 4732 and 4736 – 4733 was also examined.

All four systems produced stable trajectories with RMSD stabilizing within 30 ns of simulation (Fig. 1). The steady-state RMSD increased in the order WT < F4732D = G4733E < R4736W. However, the mutation had only a minor effect on the fluctuations in the EF-hand region and S23 loop involved in the inter-monomeric interactions.

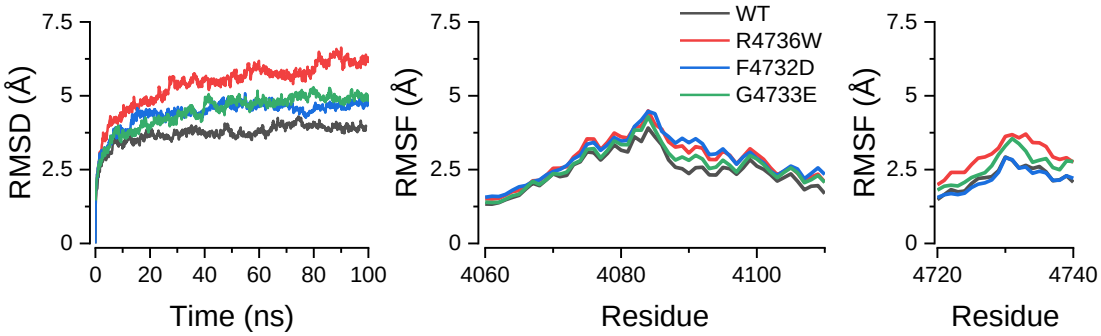


Fig. 1 Fluctuations of the Cα atoms of the RyR1 core tetramers in the course of the simulation (left) and at specific regions (center: EF-hand region; right: S23 loop).

Each mutation had a specific effect on hydrogen bonding in the examined region. Only the mutation R4736W resulted in a decrease of inter-monomer H-bond interactions. Mutations F4732D and G4733E increased H-bonding interactions between the mutated residue and arginine 4736.

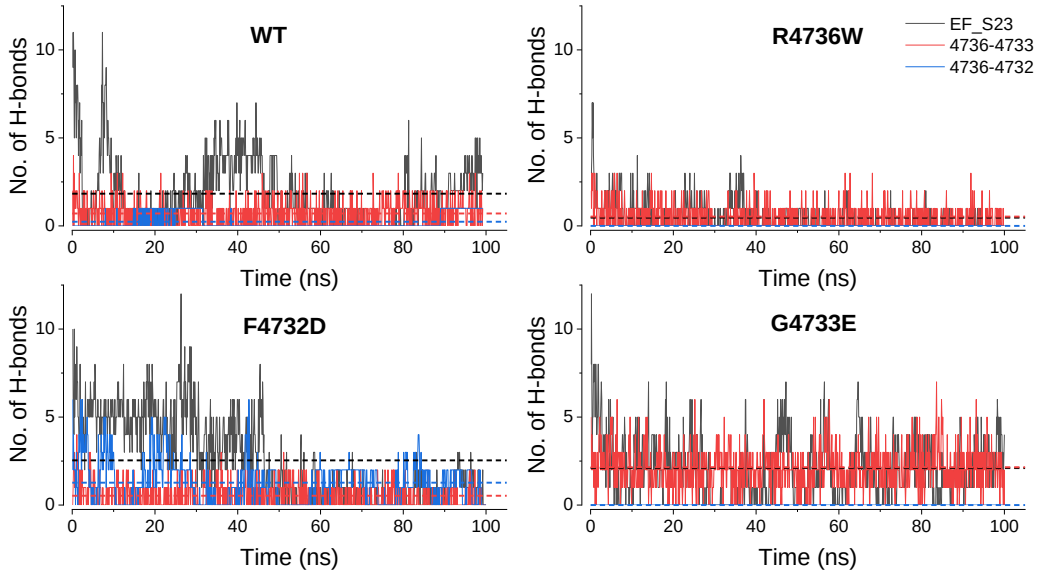


Fig. 2 Hydrogen bond formation between specific groups of residues. Black – H-bonds between the EF-hand region and S23 loop; red – H-bonds between residues 4736 and 4733; blue – H-bonds between residues 4736 and 4732. Trajectory averages are plotted as dashed lines of the corresponding color.

This result allows us to predict that the changes in hydrogen bonding will affect the strength and dynamics of the allosteric pathway leading to inactivation of RyR1 and thus to the appearance of the MH phenotype.

Acknowledgment. This work was supported by research grants APVV-21-0443 and APVV-21-0473. Research results were obtained in part using the computational resources procured in the national project National competence centre for high performance computing (project code: 311070AKF2), funded by European Regional Development Fund, EU Structural Funds Informatization of society, Operational Program Integrated Infrastructure.

References

1. Zahradnikova, A., Pavelkova, J., Sabo, M., Baday, S., & Zahradnik, I. (2025). Structure-based mechanism of RyR channel operation by calcium and magnesium ions. PLoS Computational Biology, 21(4), e1012950.
2. Gomez, A. C., Holford, T. W., & Yamaguchi, N. (2016). Malignant hyperthermia-associated mutations in the S2-S3 cytoplasmic loop of type 1 ryanodine receptor calcium channel impair calcium-dependent inactivation. Am. J. Physiol. Cell Physiol., 311(5), C749-C757.
3. Nayak, A. R., & Samso, M. (2022). Ca(2+) inactivation of the mammalian ryanodine receptor type 1 in a lipidic environment revealed by cryo-EM. Elife, 11, e75568.
4. Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., & Ferrin, T. E. (2023). Protein Sci, 32(11):e4792.
5. Jo, S., Kim, T., Iyer, V. G., & Im, W. (2008). CHARMM-GUI: a web-based graphical user interface for CHARMM. J. Comput. Chem., 29(11), 1859-65.
6. Hess, B., Kutzner, C., van der Spoel, D., & Lindahl, E. (2008). GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. J. Chem. Theory. Comput., 4(3), 435-47.

Magnetic nanoparticles– usefull platform for advanced biomedical applications

A. ZELENAKOVA

Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

In the recent past, magnetic nanoparticles (MNPs) emerged as the most promising building blocks to realize multifunctional devices to be used in the biomedical field. The greatest interest for MNPs resides in the possibility of exploiting their unique properties for both diagnostic and therapeutic purposes (i.e., theranostics). For example, the interactions between the particle's magnetization and the 1H nuclear magnetization strongly affect the nuclear relaxation times, making MNPs powerful contrast agents for magnetic resonance imaging (MRI) applications. Furthermore, the interaction of the MNP's magnetization with an alternating magnetic field of appropriate frequency and amplitude can cause a strong heating of the magnetic cores subsequently released to the surrounding tissues. This effect is known as magnetic fluid hyperthermia (MFH), and it is already applied in clinics for the therapeutic treatment of glioblastoma, prostate cancer, and some other tumors. Moreover, it has been extensively demonstrated in the literature that theMNP surface can be easily functionalized with ligands or biomolecules for the selective accumulation in target tissues (chemical targeting). Although simple in principle, the many barriers posed by biological processes make the application of this approach in clinics still far from being realized. MNPs could offer a unique possibility to by-pass this hurdle, as they can be driven to a desired site by the application of an external magnetic field gradient (magnetic targeting). In presented lecture the various biomedical applications of magnetic nanoparticles from fundamental and practical point of view will be discussed.

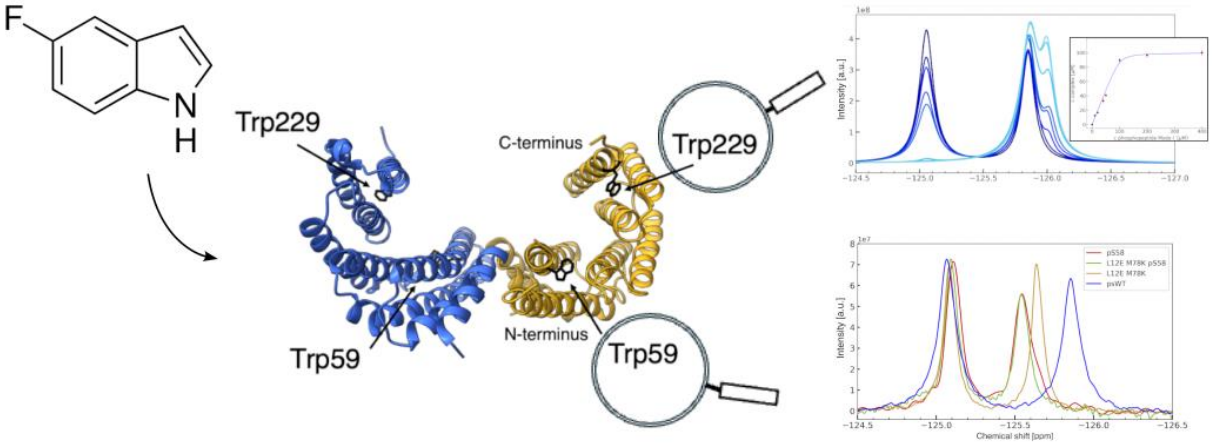
Acknowledgment. This work was supported by research grants APVV-20-0512, and by the VEGA No. 1/0470/25.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

^{19}F Tryptophan: an efficient NMR probe for protein complexes

[a, b]A. NAPLAVOVA, [a, b]A. KOZELEKOVA, [a, b]N. GASPARIK, [a, b]R. CRHA, [c]A. M. GRONENBORN, [a, b, d]J. HRITZ

[a]*National Center for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic*
[b]*Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
[c]*Department of Structural Biology, University of Pittsburgh, School of Medicine, Pittsburgh, USA*
[d]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*



The self-association of proteins is a fundamental mechanism in cell. The oligomerization offers a way of protein regulation, often broadening their functionality. One of the rarest amino acids tryptophan (Trp) has a unique role in protein self-association. It has been previously described that Trp is commonly present in so called dimerization hot spots partaking in dimer formation. A method that could selectively focus on Trp could therefore offer a unique probe into protein dimerization. Nuclear magnetic resonance is a powerful tool in structural biology. Traditional double ^{13}C, ^{15}N labelling can be complemented by the ^{19}F labelling which is a simple and straight-forward method. The biggest advantage of ^{19}F labelling is high signal intensity and a selective labelling of only chosen amino acid (usually Trp, Tyr or Phe), leading to less convoluted spectra without background noise. For these reasons, the ^{19}F labelling may present a technique especially useful for determination of parameters connected to protein dimerization.

Here, we employed solution 1D ^{19}F-Trp NMR spectroscopy to characterize substrate binding and dimerization of 14-3-3 proteins, focusing on 14-3-3ζ - an abundant human isoform as an example. Upon study of post-translational modifications, it has been discovered that phosphorylation of 14-3-3ζ at Ser58 located at dimeric interface leads to monomerization. Both conserved Trp residues within 14-3-3 proteins are located in distinct functionally important sites - the dimeric interface and the ligand-binding groove. We substituted them by 5F-Trp, thereby introducing a convenient NMR probe. Fluorination of the two Trp did not impact the stability and interaction properties of 14-3-3ζ in a substantive manner, permitting to carry out ^{19}F NMR experiments to assess 14-3-3's structure and behavior. Importantly, 5F-Trp228 reports on binding of substrates in the amphipathic binding groove of 14-3-3ζ and permitted to distinguish distinct recognition modes. Thus, we established that ^{19}F NMR

is a powerful approach to evaluate the binding of partner proteins to 14-3-3 and to characterize the properties of the resulting complexes.

Acknowledgement. This project was funded by the European Union's Horizon Europe program under grant agreement No 101087124 (ADDIT-CE), the European Union's Horizon 2020 - Research and Innovation Framework Programme under the H2020 Marie Skłodowska-Curie Actions grant agreement No 873127 (InterTau) and by the Czech Science Foundation (No. GF20-05789L). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor REA can be held responsible for them.

We acknowledge the CEITEC Proteomics Core Facility, the CF Biomolecular Interactions and Crystallography of CIISB, the Instruct-CZ Centre and Josef Dadok National NMR Centre of Instruct-ERIC EU consortium, funded by the MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project "Innovation of Czech Infrastructure for Integrative Structural Biology" (No. CZ.02.01.01/00/23_015/0008175).

AN acknowledges the Grant Agency of Masaryk University (MU) for the support of an excellent diploma thesis within the rector's program (no. MUNI/C/0091/2022).

References

1. Naplavova, A., Kozelekova, A., Crha, R., Gronenborn, A., Hritz, J. (2025). Harnessing the power of ^{19}F NMR for characterizing dimerization and ligand binding of 14-3-3 proteins. Int. J. Biol. Macromol., 305, 141253.
2. Crha, R., Kozelekova, A., Hofrova, A., Ilkovicova, L., Gasparik, N., Kaderavek, P., Hritz, J. (2024). Hiding in plain sight: Complex interaction patterns between Tau and 14-3-3ζ protein variants. Int. J. Biol. Macromol., 266, 130802.
3. Kozelekova, A., Naplavova, A., Brom, T., Gasparik, N., Simek, J., Houser, J., Hritz, J. (2022). Phosphorylated and phosphomimicking variants may differ - a case study of 14-3-3 protein. Front. Chem., 10:835733.
4. Trosanova, Z., Lousa, P., Kozelekova, A., Brom, T., Gasparik, N., Tungli, J., Weisova, V., Zupa, E., Zoldak, G., Hritz, J. (2022). Quantitation of human 14-3-3ζ dimerization and the effect of phosphorylation on dimer-monomer ekvilibria. J. Mol. Biol., 434, 167479.
5. Sharaf, N. G., & Gronenborn, A. M. (2015). ^{19}F -Modified Proteins and ^{19}F -Containing Ligands as Tools in Solution NMR Studies of Protein Interactions. Methods in enzymology, 565:67-95.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Cytochrome c oxidase - a key component of oxidative phosphorylation: Regulation and modulation of its catalytic activity

[a]D. JANCURA, [b]M.FABIAN

[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[b]*Center for Interdisciplinary Bioscences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*

Mammalian cytochrome c oxidase (CcO) is an integral membrane protein which catalyses the reduction of oxygen to water and additionally pumps protons across the mitochondrial inner membrane. The net reaction can be written as follows:

$$4\ c^{+2} + 8H^{+}_{i} + O_2 \rightarrow 4\ c^{+3} + 2\ H_2O + 4\ H^{+}_{o}$$

where c^{+2} and c^{+3} represents reduced and oxidized form of cytochrome c, respectively, H^{+}_{i} represents protons taken up from the matrix phase and H^{+}_{o} referring to protons released into cytosolic phase of mitochondria.

CcO is composed of 13 subunits and four redox centers (Cu_A, heme *a*, and binuclear catalytic site heme a_3 + Cu_B), involved in electron transfer (ET) and in the reduction of O_2 to water. Cu_A is close to the cytosolic surface of the protein and serves as the initial acceptor of electrons from cytochrome *c*. Electrons received by the oxidase are rapidly distributed between Cu_A and heme *a* (Fe_a). ET continues further to the catalytic center composed of heme a_3 (Fe_{a3}) and Cu_B, where oxygen reduction takes place.

Full reduction of CcO requires four electrons and the transfer of two electrons to the oxidized catalytic center is accompanied with uptake of two protons, ultimately from surrounding of the enzyme. The rate of heme a_3 reduction appears to be simultaneous with proton uptake and it has been concluded that the rate limiting process for this internal ET is the stabilization of the electrons by protons.

In our study we have found that a single group with a pK_a of ~6.8 is critical for the regulation of the electron flow to the oxidized catalytic site of the CcO. Our data further indicate that proton entry, coupled with electron transfer to the catalytic site during anaerobic reduction, is gated by this residue and that proton access to the catalytic site is determined by the rate of proton diffusion via the proton conducting K-channel [1]. Further, we have shown that the reduction of Cu_B stimulates, rather than inhibits, the rate of the electron transfer to heme a_3. The rate-limiting step of the reduction of catalytic site appears to be the initial electron transfer to this site that proceeds with identical rates to either heme a_3 or Cu_B. As a consequence of changes at the catalytic site induced by the first electron reduction, the second electron transfer can be realized more rapidly [2]. Our findings also suggest that the relaxation of the catalytic center during the transition of the “high-energy” metastable state of the oxidized CcO (O_H) to the “resting” oxidized state (O) is either shorter than 100 ms or there is no difference in the protonation and ligation state of the catalytic sites of O and O_H [3, 4].

Acknowledgement: This work was supported by the project of the Slovak Grant Agency VEGA 1/ 0028/22.

References

1. Antalik, M., Jancura, D., G. Palmer, G., & Fabian, M. (2005). A role for the protein in internal electron transfer to the catalytic center of cytochrome c oxidase. Biochemistry, 44, 14881-14889.
2. Jancura, D., Antalik, M., Berka, V., Palmer, G., & Fabian, M. (2006). Filling the catalytic site of cytochrome c oxidase with electrons : Reduced Cu_B facilitates internal electron transfer to heme a_3. J. Biol. Chem. 281, 20003-20010.
3. Jancura, D., Berka, V., Antalik, M., Bagelova, J., Gennis, R. B., Palmer G., & Fabian. M. (2006). Spectral and kinetic equivalence of oxidized cytochrome c oxidase as isolated and „activated“ by reoxidation. J. Biol. Chem., 281, 30319-30325.
4. Jancura, D., Tomkova, A., Sztachova, T., Berka, V., & Fabian, M. (2023). Examination of „high-energy“ metastable state of the oxidized (O_H) bovine cytochrome c oxidase: proton uptaktke and reaction with H_2O_2. Archives Biochem. Biophys., 747, art. numb. 109758.

Tiny motifs doing huge things: Side-chain Propelled Rings (SPuRs) in the aggregation domain of tau protein

[a]R. SKRABANA, [a]K. MARTONOVA, [a]A. POLAK, [a]S. NJEMOGA, [a]R. DVORSKY, [b]E. BARRERA, [a]O. CEHLAR

[a]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
[b]Institute of Histology and Embryology (IHEM), National University of Cuyo (UNCuyo), CONICET, Mendoza, Argentina

Secondary structure elements in proteins, such as sheets or helices are familiar to the protein science community. Equally well-recognized are locally stabilized backbone turns with the most frequent beta turn and related alpha, gamma, delta and pi turns. A common denominator of all those structures is the hydrogen pairing of backbone nitrogen and carbonyl oxygen atoms.

Less apparent albeit also frequently encountered in PDB structures are small motifs stabilized by hydrogen bonds between backbone and side chain atoms [1]. For these motifs we coined an acronym SPuRs (Side-chain Propelled Rings) to distinguish their peculiar feature [2]. Looking for SPuRs in available structures, we found a surprisingly large panel of them, with variable lengths and amino acids involved. In globular proteins, SPuRs are frequently on the margins of sheets or helices, but they are also in loops connecting protein domains. Considering intrinsically disordered proteins (IDPs), we expect much larger significance for SPuRs due to their independence on a globular character of molecule.

IDP tau, microtubule associated protein involved in the pathology of various neurodegenerative tauopathies, underlies oligomerization and aggregation during disease progression. We have studied in more details the aggregation domain of tau protein and its surroundings with respect of possible SPuR motifs and validated them by experimental and *in silico* methods. Targeting of tau SPuRs may represent a way to regulate its aggregation.

Acknowledgment. This work was supported by research grants APVV-21-0479, VEGA 2/0141/23, 2/0125/23 and EU Grants ADDIT-CE, InterTau.

References

1. Leader, D. P., & Milner-White, E. J. (2009). Motivated Proteins: A web application for studying small three-dimensional protein motifs. BMC Bioinformatics, 10(1), 60.
2. Martonova, K. (2021). Structure Motifs Regulating Disordered Proteins. Literature overview for PhD thesis.

Insights into the early oligomerization stages of truncated tau proteins

[a]S. NJEMOGA, [b]Z. BEDNARIKOVA, [c]E. E. BARRERA, [b]Z. GAZOVA, [a, d]O. CEHLAR

[a]*Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*
[b]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[c]*Institute of Histology and Embryology (IHEM), National University of Cuyo (UNCuyo), CONICET, Mendoza, Argentina*
[d]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia*

Numerous structures of tau fibrils from different tauopathies suggest a sequence-specific mechanisms of amyloid aggregation and polymorphism. Previously validated short-sequence amyloid motifs are regarded as carriers of tau amyloidogenicity because of their intrinsic beta propensity and their ability to assemble into stable amyloid fibrils core [1-2]. Truncation of tau polypeptide sequence has been identified as the trigger of amyloid aggregation by exposing amyloid-prone regions and disrupting the protective full-length tau conformation previously proposed [3].

Our aim was to identify important structural changes that could cause significantly different aggregation profiles in Tau321-391 and Tau326-391 variants found by AFM and ThT assay. In particular, the importance of tau residues 321-325 was found to be essential to the self-assembly of monomers, as opposed to tau 326-391, which was incapable of aggregation, tau 321-391 showed a strong aggregation tendency. To reveal the structural mechanisms underlying tau aggregation, we performed simulations of tau 321-391 and 326-391 monomers and dimers, as well as their truncated variants. MD simulations revealed increased propensity of N-terminus of 321 tau variants towards beta-structures in all atom simulations, but rather helical propensity in coarse-grained simulations. Moreover, simulations successfully replicated sequence-specific beta-sheet propensity in agreement with computationally predicted and experimentally established amyloid-nucleating motifs, confirming the sequence-dependent amyloid propensity. Our study demonstrated sequence-dependent mechanism of aggregation, where only five amino acid difference can lead to completely different in vitro aggregation profiles. In silico, both variants, starting with 326 or 321 residues, formed short-lived dimers, however the main differences were observed in the structural contents, which could influence the further oligomerization and aggregation process.

Acknowledgment. Part of the research results was obtained using the computational resources procured in the national project National competence centre for high performance computing (project code: 311070AKF2) funded by European Regional Development Fund, EU Structural Funds Informatization of society, Operational Program Integrated Infrastructure. This research was funded by the European Union's Horizon Europe program under the grant agreement No. 101087124. This work was also funded by the grants APVV 21-0479, APVV-22-0598 and Vega 2/0125/23 and 2/0141/23. This research has received funding from the European Union's Horizon 2020 research and Innovation programme under the Marie Skłodowska-Curie grant agreement No 873127. This research was further funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00623.

References

1. von Bergen, M., Friedhoff, P., Biernat, J., Heberle, J, Mandelkow, E. M., & Mandelkow E. (2000). Assembly of tau protein into Alzheimer paired helical filaments depends on a local sequence motif ((306)VQIVYK(311)) forming beta structure. Proc. Natl. Acad. Sci. U S A., 97(10), 5129-34.
2. Louros, N., Wilkinson, M., Tsaka, G., Ramakers, M., Chiara Morelli, C., Garcia, T., Gallardo, R., D'Haeyer, S., Goossens, V., Audenaert, D., Thal, D. R., Mackenzie, I. R., Rademakers, R., Ranson, N. A., Radford, S. E., Rousseau, F., & Schymkowitz, J. (2024). Local structural preferences in shaping tau amyloid polymorphism. Nature Communications, 15:1028.
3. Jeganathan, S., von Bergen, M., Brutlach, H., Steinhoff, H. J., & Mandelkow, E. (2006). Global hairpin folding of tau in solution. Biochemistry, 45(7), 2283-93.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Bioinformatic identification, expression, purification and structural characterization of domains in multidomain proteins

V. BAUEROVA-HLINKOVA & J. A. BAUER

Department of Biochemistry and Structural Biology, Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia

The structural and functional characterization of large multidomain proteins can be quite challenging. One common approach is to “divide and conquer”, that is, to partition the protein into separate parts, each of which can be characterized individually. One such protein, which is essential in animalia is the ryanodine receptor (RyR). It is an ion channel, whose main function is to control Ca2+ release in muscle cells and thereby to promote functional muscular contraction. The ryanodine receptor is a homotetramer formed by four monomeric subunits (Fig. 1A) in which each subunit consists of 10 domains. Isoform 2 of this protein (RyR2) is predominantly expressed in cardiac tissue and its malfunction is associated with various cardiac arrhythmias [1].

In our laboratory we originally identified the domains of RyR2 using bioinformatics tools [2, 3] and further solved the structure of the N-terminal domain (NTD; Fig. 1B) by X-ray crystallography and SAXS analysis under near-physiological conditions [4]. The structure confirmed the presence of three subdomains and revealed the role of the central helix, which connects the three subdomains through a hydrogen bond network. A combination of biophysical characterization and molecular dynamics studies [5] revealed the role of residues L414, I419 and R420, located in the central helix, whose mutations are associated with cardiac arrhythmias.

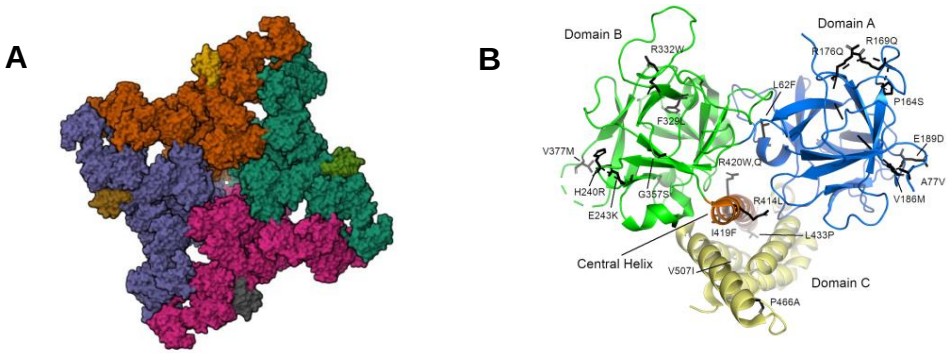


Fig. 1 (A) Quarternary structure of the human ryanodine receptor 2 (7u9z). **(B)** Crystal structure of the N-terminal domain of human Ryanodine receptor 2 (4jkq).

Acknowledgment. This work was financially supported by VEGA grant No. 2/0081/24 and Slovak Research and Development Agency, Contract no. APVV-23-0448.

References

1. Waddel, H. M. M., Mereacre, V., Alvarado, F. J., Munro, M. L. (2023). Clustering properties of the cardiac ryanodine receptor in health and heart failure, J. Mol. Cell Cardiol., 185:38-49.
2. Bauerova-Hlinkova, V., Hostinova, E., Gasperik, J., Beck, K., Borko, L., Lai, F. A., Zahradnikova, A., Sevcik J. (2010). Bioinformatic mapping and production of recombinant N-terminal domains of human cardiac ryanodine receptor 2. Protein Expr. Purif., 71(1), 33-41.

3. Bauerova-Hlinkova, V., Bauer, J., Hostinova, E., Gasperik, J., Beck, K., Borko, L., Faltinova, A., Zahradnikova, A., Sevcik, J. (2011). In: Bioinformatics - Trends and Methodologies. Ed. M. A. Mahdavi. Intech. Printed in Croatia. ISBN: 978-953-307-282-1 pp. 325-352.
4. Borko, L., Bauerova-Hlinkova, V., Hostinova, E., Gasperik, J., Beck, K., Lai, F. A., Zahradnikova, A., Sevcik, J. (2014). Structural insights into the human RyR2 N-terminal region involved in cardiac arrhythmias. Acta Cryst., 70(11), 2897-2921.
5. Bauer, J. A., Borko, L., Pavlovic, J., Kutejova, E., Bauerova-Hlinkova, V. (2020). Disease-Associated Mutations Alter the Dynamic Motion of the N-terminal Domain of the Human Cardiac Ryanodine Receptor. J. Biomol. Struct. Dyn., 38(4), 1054-1070.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Effects of rotenone and photobiomodulation on alpha-synuclein aggregates in differentiated SH-SY5Y cells

[a]K. STROFFEKOVA & [b]Z. BEDNARIKOVA

[a]Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

The world population will age in next few decades significantly. According to the WHO, the number of people older than 60 years will nearly double from 12% to 23% by 2050. Increase in aging correlates with an increase of the neurodegenerations in population, including Parkinson's (PD), Alzheimer (AD) diseases and ALS (amyotrophic lateral sclerosis). PD is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNc) and accumulation of insoluble cytoplasmic protein inclusions, including α-synuclein (αSNC). Photobiomodulation (PBM) by low-level near infrared (NiR) radiation was shown to have positive effects in cell repair processes and proliferation, in wound healing, muscle repair, and angiogenesis. The present work compares the levels of α-synuclein (αSNC) monomers and aggregates in non-differentiated and differentiated SH-SY5Y cells. We have established the 2D model of PD by using human neuroblastoma cell line SH-SY5Y and rotenone (ROT) treatment. In addition, we explore PBM effect on ROT treated cells (200nM Rot for 48hr, NiR 1J/cm^2). We have investigated αSNC species with specifically designed antibodies to recognize monomer and aggregated form. In non-differentiated SH-SY5Y cells, control cells displayed a low level of αSNC monomer and appreciably higher level of αSNC aggregates. Treatment with ROT increased αSNC level of both species, monomers and aggregates, respectively. These findings were confirmed further by the AFM analysis and Thioflavin T (ThT) assay, which showed that ROT treated cells have higher load of amyloid species based on the significantly higher ThT fluorescence intensity compared to non-treated cells and media. PBM treatment increased αSNC monomer level in both, control and ROT treated cells. In contrast, PBM notably decreased αSNC aggregates level in both. In the next step, we have differentiated SH-SY5Y cells by incubation with 1% FBS media and 10mM retinoic acid (RA) for 10-14 days. Differentiation media was changed every 72 hr. The SH-SY5Y differentiation was confirmed by expression of neuronal markers microtubule-associated protein 2 (MAP2) and tyrosine hydroxylase (TH). In differentiated SH-SY5Y cells (D9–D17), we have found noticeably higher load of αSNC aggregates in comparison with non-differentiated cells. The load of αSNC aggregates increased with the length of cultivation. In addition, differentiated SH-SY5Y cells were substantially more resistant to ROT cytotoxicity. The PBM treatment decreased αSNC aggregates level in control and ROT treated differentiated SH-SY5Y cells. The present results lay the groundwork for a PD research in more complex systems such as 3D organoids, or in the animal model *in vivo*.

Acknowledgment. This work was supported by the EU ERDF grant OPENMED (ITMS2014+: 313011V455), by the Slovak Grant Agency VEGA 1/0421/18;1/0557/20 and 1/0187/23; and by the Slovak Research and Development Agency APVV-21-0333 and APVV-22-0598.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Ionic liquid to tune cell membrane's viscoelasticity & amyloid fibril's properties: A combined atomic force microscopy and neutron scattering study

A. BENEDETTO

School of Physics, University College Dublin, Dublin, Ireland
Conway Institute for Biomolecular & Biomedical Research, University College Dublin, Dublin, Ireland
Department of Science, University Roma Tre, Rome, Italy

Ionic liquids (ILs) are a vast class of organic electrolytes composed of an organic cation and an organic or inorganic anion. Their melting temperature falls around room temperature, and they possess a low vapour pressure. In the last 20 years, several studies have shown moderate-to-high toxicity towards live cells and microorganisms for several ILs, breaking the dream of their absolute green character. Toxicity, however, is synonymous with affinity, and this has motivated several investigations focused on the mechanisms of action of ILs, which understanding can underpin the development of IL-based approaches in drug delivery, biomedicine, pharmacology, material science, and bio-nanotechnology broadly. Atomic force microscopy and neutron scattering are the two major approaches in use in my Lab, which we combine with computer simulations and a variety of cell biology assays (Fig. 1). After giving an overview of ILs in biophysics [1,2], I will focus on two representative cases of study under investigation in my Lab. First, I will discuss the effect of ILs on cell membrane models [3-5], cell migration and cell mechanics [6]. I will then move into amyloid fibrils, showing the effect of ILs on their morphology, surface electric potential and assembly [7]. I will conclude my seminar by presenting some current work underway in my Lab, including our preliminary results on lipid raft domains and cell rheology.

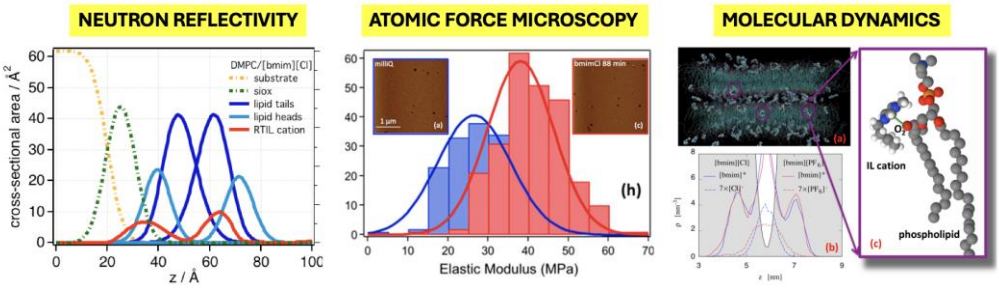


Fig. 1 Neutron reflectivity gives access to IL-partitioning between lipid and aqueous phases, atomic force microscopy to the effects of the absorbed IL on the biomembrane's morphology and mechano-elasticity, and computer simulations to the mechanism of insertion and several other observables.

References

1. Kumari, P., Pillai, V. V. S., Benedetto, A. (2020). Mechanisms of action of ionic liquids on living cells: the state of the art. Biophys. Rev., 12(5), 1187-1215.
2. Benedetto, A., Ballone, P. (2016). Room-Temperature Ionic Liquids Meet Biomolecules. ACS Sustain. Chem. Eng., 4(2), 392-412.
3. Benedetto, A. (2023). Ionic liquids meet lipid bilayers: a state-of-the-art review. Biophys. Rev., 15, 1909-1939.

4. Kumari, P., Faraone, A., Kelley, E. G., Benedetto, A. (2021). Stiffening effect of the [Bmim][Cl] ionic liquid on the bending dynamics of DMPC lipid vesicles. J. Phys. Chem. B, 125, 7241-7250.
5. Benedetto, A., Kelley, E. G. (2022). Absorption of the [bmim][Cl] Ionic Liquid in DMPC Lipid Bilayers across Their Gel, Ripple, and Fluid Phases. J. Phys. Chem. B, 126(17), 3309-3318.
6. Kumari P., Pillai, V. V. S., Rodriguez, B., Prencipe, M., Benedetto, A. (2020). Sub-Toxic Concentrations of Ionic Liquids Enhance Cell Migration by Reducing the Elasticity of the Cellular Lipid Membrane. J. Phys. Chem. Lett. 11, 7327.
7. Pillai, V., Kumari, P., Kolagatla, S., Garcia Sakai, V., Rudic, S., Rodriguez, B. J., Rubini, M., Tych, K. M., Benedetto, A. (2022). Controlling amyloid fibril properties via ionic liquids: the representative case of ethylammonium nitrate and tetramethylguanidinium acetate on the amyloidogenesis of lysozyme. J. Phys. Chem. Lett. 13, 7058-7064.

SHORT COMMUNICATIONS

RNA-Bender: A Novel Molecular Dynamics-Based Approach for Rapid Sequence-Based RNA Model Construction and Refinement

[a]G. WIECZOREK, [a,b]D. NIEDZIALEK

[a]Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[b]Ensemble[3] sp. z o.o., Centre of Excellence, Warsaw, Poland

We have developed a novel molecular dynamics-based approach, the RNA-Bender program, designed to facilitate the rapid, sequence-based construction of RNA models. Initiating the folding process with RNA-Bender requires detailed information regarding the shape of the RNA molecule (e.g., determined by Cryo-EM) and base pairing data (e.g., obtained from NMR measurements). This method allows for the controlled folding of RNA molecules by manipulating various fragments (such as bases, loops, hairpins, etc.) of the folded RNA molecule in a sequence defined by the user. To prevent unwanted energetic effects that could compromise the final RNA structure during manipulation, the structure is cooled in a controlled manner. We have successfully applied this method to resolve RNA structures obtained from Cryo-EM, refining them to angstrom-level resolution. We intend to present successful case studies and discuss the challenges our approach still faces.

Acknowledgment. We gratefully acknowledge Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and support within computational grant no. PLG/2024/017439.

References

1. Göç, Y. B., Poziemski, J., Smolińska, W., Suwała, D., Wieczorek, G., & Niedzialek, D. (2022). Tracking Topological and Electronic Effects on the Folding and Stability of Guanine-Deficient RNA G-Quadruplexes, Engineered with a New Computational Tool for De Novo Quadruplex Folding. International journal of molecular sciences, 23(19), 10990.

Immobilisation free in-solution kinetics using Flow Induced Dispersion Analysis (FIDA)

[a]K. RAY, [a]H. JENSEN, [a]A. C. HUNDAHL, presenting: [b]J. USKOBA

[a]*FIDA Biosystems ApS, Søborg, Denmark*
[b]*BioTech a.s., Prague, Czech Republic*

Flow induced dispersion analysis has been recently recognized as a powerful tool in drug discovery research due to its capabilities in providing many information from little amount of sample. It is possible to screen heterogeneity, stickiness, labelling quality and also affinity determination in equlibrium. When introducing FIDA Neo detectors in 2024 which provide extra Lambda Dynamics to the detection system, in-solution kinetics software had been also introduced. While affinity determination is a crucial information for many researchers, association constant and dissociation constant remained unravelled by FIDA. This no longer stands true.

Affinity determination is a crucial step in kinetics characterization. Premixing your samples and injecting the complex to the equimolar concentration of interaction partner prevents dissociation of interaction partner from the indicator, thus equilibrium state is established during affinity measurement. Changing the system from premixing your samples with each other and injecting the indicator itself to the interaction partner with varying concentrations leads to formation of the complex but not in equilibrium state. (Fig. 1) This change can be used for association constant determination based on the curve shift from equilibrium state. Since affinity is known from premix measurement and association constant is known from capmix measurement, it is possible to calculate dissocation constant which is extremely useful compared to immobilization technique like surface plasmon resonance or biolayer interferometry, where dissocation constant characterization is extremely important for correct affinity determination, but it can be very time consuming, especially for slow dissociating compounds.

Flow induced dispersion analysis is a truly unique technology. At the moment, this technology is the only one capable of characterizing complete in-solution kinetics in the world. The application portfolio is very versatile, from small molecules to large complexes.

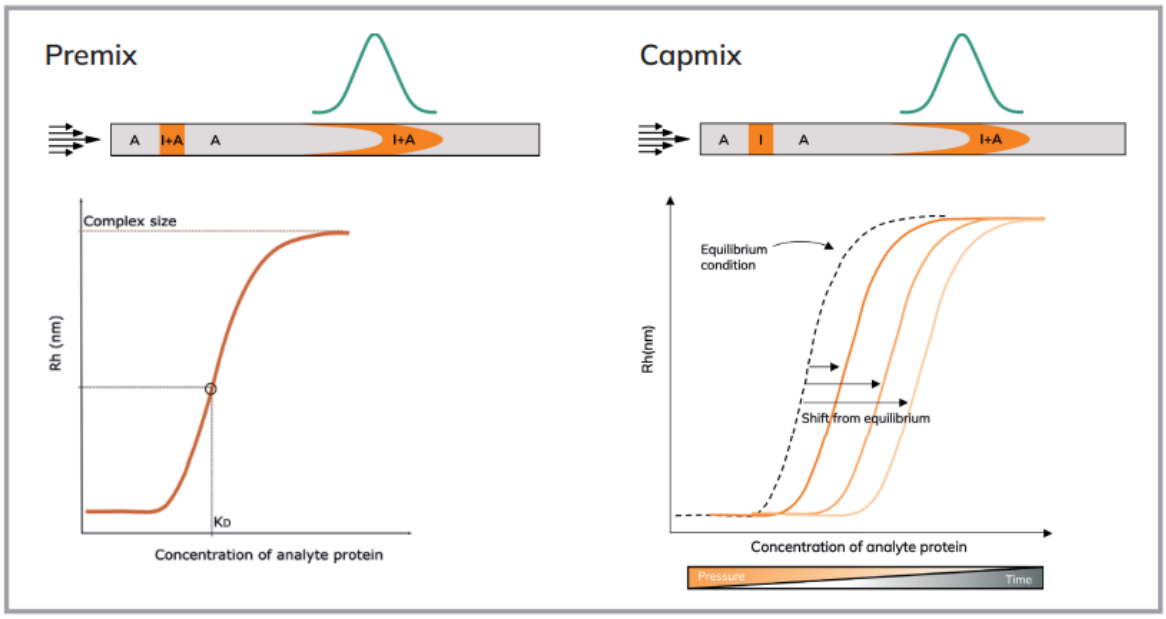


Fig. 1 The left image shows a typical binding curve under premix conditions (equilibrium) used to determine K_D. The right image shows the same typical binding curve as in the left image, but inder non-equilibrium conditions using capmix. The less amount of time allowed for interaction between binding partners the weaker apparent K_D is observed (Opaque orange) compared to the equilibrium K_D (dotted line).

References

1. Ray, K., Jensen, H., & Hundahl, A. C. (2024). IMMOBILISATION FREE IN-SOLUTION KINETICS USING FLOW INDUCED DISPERSION ANALYSIS (FIDA). www.fidabio.com

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Ultimate DSC, a new tool for protein characterization

[a]G. JOSSENS, [a]Y. RICCI, [a]J. - C. NEYT, [a]M. SIMOND, [b]J. L. GARDEN, [b]G. MOIROUX, presenting: [c]M. CERNIK

[a]Calneos SAS, Clermont-Ferrand, France
[b]Institut Neel CNRS, Grenoble, France
[c]Uni-Export Instruments, s.r.o., Prague, Czech Republic

In biochemical, biophysical or pharmaceutical research, proteins are an important subject in the development of new drugs or treatments.

The stability parameters of these proteins are necessary for all these developments. It is therefore necessary to know, for example, the denaturation temperatures of the proteins studied, as well as the energy involved in these denaturations (denaturation enthalpy and temperature).

The technology most commonly used to access these thermodynamic parameters is differential scanning calorimetry (DSC). One of the major problems with this technology is the large quantity of protein required to obtain usable results: up to 1 ml per experiment, for proteins that can sometimes be very expensive to produce.

In this context, Calneos has developed the Ultimate DSC, which allows the use of less than 100 µL of sample in extractable crucibles. One of the advantages is the drastic reduction in the amount of sample required to obtain usable thermograms. Another advantage is the elimination of tedious and sometimes unreliable cleaning procedures.

This presentation will focus on the main points to carried out measurements, explain the unique features as Joule effect calibration and real sample temperature measurement, and conclude with results obtained with Lysosyme or RNase in PSB buffer at low concentration and protein quantities.

Thermodynamics of the transition of ferryl (F) to the oxidized form of cytochrome *c* oxidase: implication for the proton pumping

[a]A. TOMKOVA, [a]T. SZTACHOVA, [a]D. JANCURA, [b]M. FABIAN

[a]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

[b]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*

In respiratory heme-copper oxygen reductases (HCOs), membrane bound complexes, the energy released during the reduction of dioxygen is utilized to build the transmembrane proton gradient by two mechanisms. One of these molecular mechanisms is the proton pumping, which remains the essential problem in the enzymology of HCOs. Large family of HCOs consists of cytochrome *c* oxidases (CcOs) found in all eukaryotes and some prokaryotes. In CcO electrons from cytochrome *c* are transferred through copper Cu_A to heme *a* and finally to the catalytic heme a_3-Cu_B center where O_2 is reduced to water. Most of the current pumping models rely on the energy released by the transfer of electrons from heme *a* to the two ferryl intermediates of heme a_3 [1-3]. One of these ferryl forms, denoted as F, is observed when three-electron reduced CcO reacts with O_2. One-electron reduction of this F state, producing the oxidized CcO (O), is assumed to release the main amount of energy for the pumping [4, 5]. Here, utilizing the purified bovine CcO the enthalpy changes of -13.6 kcal/mol *c* (pH 8.0, 25 ºC, I =0.145l) was determined by isothermal titration calorimetry for the F-to-O transition initiated by the reduction with ferrocytochrome *c*. The estimated ΔG of about - 24 kcal/mol for this reaction indicates that the reduction potential of F/O couple is close to +1.3 V. However, the structural changes, associated with the redox transition of heme *a*, are apparently the main driving force for the translocation of charges.

Acknowledgment. This work was supported by research grants of VEGA 0028/22.

References

1. Wikstrom, M., Krab, K., Sharma, V. (2018). Oxygen Activation and Energy Conservation by Cytochrome c Oxidase. Chem. Rev., 118, 2469-2490.
2. Popovic, D. M., Stuchebrukhov, A. A. (2004). Electrostatic study of the proton pumping mechanism in bovine heart cytochrome C oxidase. J. Am. Chem. Soc., 126, 1858-1871.
3. Blomberg, M. R. A., Siegbahn, P. E. M. (2015). How cytochrome c oxidase can pump four protons per oxygen molecule at high electrochemical gradient. Biochim. Biophys. Acta, 1847, 364-376.
4. Bloch, D., *et al.* (2004). The catalytic cycle of cytochrome c oxidase is not the sum of its two halves. Proc. Natl. Acad. Sci. U S A, 101, 529-533.
5. Verkhovsky, M. I., Jasaitis, A., Verkhovskaya, M. L., Morgan, J. E., Wikstrom, M. (1999). Proton translocation by cytochrome c oxidase. Nature, 400, 480-483.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

From Structure to Identification: Shimadzu Tools for Biomacromolecule Research

R. HODOSI

Shimadzu Slovakia, Bratislava, Slovakia

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Deciphering the role of small structural motifs in aggregation of tau protein

[a]A. POLAK, [a]K. MARTONOVA, [a]S. NJEMOGA, [b]E. BARRERA, [a]R. SKRABANA

[a]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
[b]Institute of Histology and Embryology (IHEM), National University of Cuyo (UNCuyo), CONICET, Mendoza, Argentina

Intrinsically disordered proteins (IDPs), among them disordered neuroproteins, are often prone to aggregation. IDPs lack a well-defined 3D structure, their hydrophobic and β-strand-promoting residues are more exposed to solution and post-translational modifications can also shift them to amyloidogenic state. However, we have also identified another potential regulators of amyloidogenesis – side-chain propelled rings (SPuRs) (1, 2). SPuRs are formed by intramolecular interactions between side-chain (SC) atoms and main-chain (MC) residues of a protein. An example of such SPuR is the hydrogen bond between SC carboxyl of D387 (i) and MC G389 (i+2) nitrogen, identified on solved structure of tau386-391 in complex with MN423Fab (2V17) (Fig. 1) (3). Originally, such interactions were identified on crystal structures of globular proteins. Their function is related to their localization at turns or loops and involves stabilization of secondary and terciary structures of proteins, protein folding and shaping of active sites of proteins. However, when translated to disordered proteins and, in our case specificaly tau protein, these motifs represent potential structure regulation sites in otherwise disordered protein. We suspect they play a role in either stabilization of aggregated forms or, on the other hand, in preventing aggregation by stabilizing tau in physiologic state. Our aim is to verify and asses these hypotheses by site-directed mutagenesis of residues creating the motifs and their subsequent functional characterisation including dynamic ligt scattering, surface plasmon resonance, in-cell fluorescence recovery after photobleaching and electron microscopy.

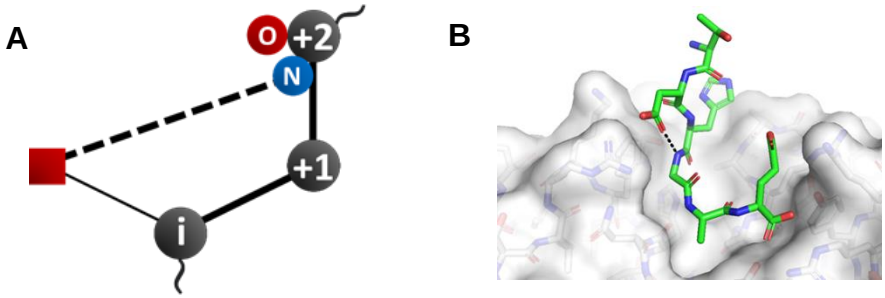


Fig. 1 (A) schematic representation of Asx-turn formed by SC-MC intramolecular hydrogen bond. **(B)** Structure of $tau_{387-391}$ in complex with MN423Fab with highlighted Asx-turn (structure PDB ID 2V17).

Acknowledgment. This work was supported by European Union's Horizon 2020 grant No 873127 (InterTau), APVV 21-0479, Vega 2/0125/23, 2/0141/23. The results were obtained under National competence centre for high performance computing (project:311070AKF2) funded by European Regional Development Fund, EU Structural Funds Informatization of society, Operational Program Integrated Infrastructure.

References

1. Martonova, K. (2021). Structure motifs regulating disordered proteins. Literature overview for PhD thesis.
2. Polak, A. (2025). Regulation of protein amyloid formation by small ordered motifs. Literature overview for PhD thesis.
3. Sevcik, J., Skrabana, R., Dvorsky, R., Csokova, N., Iqbal, K., Novak, M. C. (2007). X-ray structure of the PHF core C-terminus: insight into the folding of the intrinsically disordered protein tau in Alzheimer's disease. FEBS letters, 581(30), 5872-5878.

Design and Characterization of Circular DNA Minicircles as a Tool for Studying Non-B DNA Structures

L.TRIZNA, D. PITKOVA, A. VARHA, V. VIGLASKY

Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

Due to its conformational flexibility, DNA can form multiple non-canonical secondary structures, commonly termed non-B DNA motifs including G-quadruplexes, i-motifs, hairpins, triplexes, among others. The formation of such secondary structures is influenced not only by the intrinsic DNA sequence but also by various environmental factors such as temperature, pressure, pH, ionic strength, and/or the presence of molecular crowding agents [1]. Accumulating evidence indicates that the formation of non-canonical DNA structures contributes to multiple cellular processes, such as the regulation of gene expression at the levels of transcription and translation, epigenetic modulation of chromatin architecture, and DNA recombination. A growing body of research further suggests that the transient formation of these structures may modulate the expression of genes implicated in neurodegenerative disorders [2].

We have designed and characterized an artificial nanosystem based on circular DNA molecules. To determine the structural properties of the resulting DNA minicircles, we employed techniques such as circular dichroism spectroscopy, gel electrophoresis, and atomic force microscopy. The minicircle architecture is assembled from strategically designed single-stranded DNA sequences that serve as building blocks for the formation of double-stranded circular DNA. Additionally, the system allows for the optional formation of non-canonical DNA structures within the circular construct, enabled by the intentional introduction of partial non-complementarity in a specific region of the building sequence. A comprehensive approach to the preparation of artificial minicircles has been described [3]. Recently, our focus has been primarily on the incorporation of structural building blocks into plasmid DNA using restriction endonucleases. This approach could allow us to analyze the formation capacity and characteristics of non-B DNA motifs directly within the native cellular context.

Acknowledgment. This work was funded by the EU NextGenerationEU through the Recovery and Resilience Plan of the Slovak Republic under the project no. 09I03-03-V05-00008 – VVGS-2023-2958.

References

1. Wang, G., Vasquez, K. M. (2023). Dynamic alternative DNA structures in biology and disease. Nature Reviews Genetics, 24(4), 211-234.
2. Tateishi-Karimata, H., Sugimoto, N. (2021). Roles of non-canonical structures of nucleic acids in cancer and neurodegenerative diseases. Nucleic Acids Research, 49(14), 7839-7855.
3. Trizna, L., Olajos, J., Viglasky, V. (2024). DNA minicircles capable of forming a variety of non- canonical structural motifs. Frontiers in Chemistry, 26(12), 1384201.

Possibilities of measurement particle size, zeta potential and rheologic behavior with Anton Paar instruments

R. LANDL

Anton Paar Slovakia s.r.o., Bratislava, Slovakia

Anton Paar is a leading manufacturer of high-precision instrumentation for the physical and chemical characterization of materials. In the field of macromolecules and proteins, the company provides innovative solutions for determining particle size, zeta potential, and rheological properties—key parameters for understanding the stability, structure, and functionality of both biological and synthetic systems. The Litesizer series employs Dynamic Light Scattering (DLS) to precisely and reproducibly measure hydrodynamic diameter and size distribution of protein aggregates or polymeric structures, even at low concentrations. In addition, it enables zeta potential determination, which is crucial for assessing colloidal stability and predicting aggregation tendencies in sensitive protein formulations.

Complementary insights into functional behavior are provided by rheological measurements, which reveal viscoelastic properties and molecular interactions within macromolecular networks. Anton Paar's advanced rheometers allow researchers to study the behavior of proteins and biopolymers under various environmental and formulation conditions, delivering valuable information for pharmaceutical research, biomaterials development, and the food industry. By combining DLS, zeta potential, and rheology, Anton Paar offers a comprehensive toolkit for protein and macromolecule characterization—from particle size and electrostatic stability to functional performance—facilitating efficient translation of laboratory research into practical applications.

Fig. 1 Dynamic light scattering instrument

14-3-3ζ Variants as Modulators of Amyloid β Fibril Formation

[a]Z. BEDNARIKOVA, [b, c]A. KOZELEKOVA, [b, c]J. NOVOTNA, [b]K. KRALOVA, [b, c, d]J. HRITZ, [a]Z. GAZOVA

[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
[c]*National Center for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic*
[d]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*

Aggregation of the amyloid-β (Aβ) peptide into fibrillar structures is a hallmark of Alzheimer's disease, and proteins that interfere with this process may provide valuable mechanistic insights. Here, we examined the influence of dimeric and monomeric variants of 14-3-3 proteins on Aβ40 fibril formation. The 14-3-3ζ protein, a multifunctional regulator of diverse cellular processes, can exist in different oligomeric states (monomeric, homodimeric, heterodimeric), which may influence its functional role. Recently, 14-3-3ζ has been implicated as a potential modulator of amyloid fibrillation [1,2].

In this study, we examined the effects of dimeric and monomeric 14-3-3ζ variants on Aβ40 fibril formation. Fluorescence-based aggregation assays demonstrated that both proteins attenuate fibril formation in a concentration-dependent manner, though the monomeric protein was consistently more effective. Kinetic analyses suggested that the two variants interfere with distinct microscopic steps of the aggregation process. Atomic force microscopy confirmed these findings, revealing marked differences in fibril morphology and abundance depending on the protein variant and concentration. In addition, binding experiments indicated weak and dynamic interactions between Aβ40 and 14-3-3ζ variants, supporting a direct modulatory role.

Together, these findings indicate that the oligomeric state of 14-3-3ζ proteins plays a key role in their capacity to modulate Aβ40 fibrillation, with monomeric variants showing greater inhibitory potential than dimeric ones.

Acknowledgment. This work was supported by research grants the Slovak Research and Development Agency under Contract no. APVV-22-0598, APVV-18-0284, Slovak Research Agency VEGA 2/0141/25 and MVTS COST ML4NGP. This project was also funded by the European Union's Horizon Europe program under grant agreement No 101087124 (ADDIT-CE). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency (REA). Neither the European Union nor REA can be held responsible for them.

We acknowledge the CEITEC Proteomics Core Facility, the CF Biomolecular Interactions and Crystallography of CIISB, the Instruct-CZ Centre and Josef Dadok National NMR Centre of Instruct-ERIC EU consortium, funded by the MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project "Innovation of Czech Infrastructure for Integrative Structural Biology" (No. CZ.02.01.01/00/23_015/0008175).

References

1. Trosanova, Z., Lousa, P., Kozelekova, A., Brom, T., Gasparik, N., Tungli, J., Weisova, V., Zupa, E., Zoldak, G., Hritz, J. (2022). Quantitation of human 14-3-3ζ dimerization and the effect of phosphorylation on dimer-monomer equilibria. Journal of Molecular Biology, 434(7), 167479.

2. Heesink, G., van den Oetelaar, M. C. M., Semerdzhiev, S. A., Ottmann, C., Brunsveld, L., Blum, C., & Claessens, M. M. A. E. (2024). 14-3-3τ as a modulator of early α-synuclein multimerization and amyloid formation. ACS Chemical Neuroscience, 15(9), 1926-1936.

Ribosome display selection of non-immunogenic staphylokinase with improved fibrinolytic activity

M. TOMKOVA, M. NEMERGUT, I. PILIPCINCOVA, E. SEDLAK

Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

Thrombotic events, such as stroke, myocardial infarction, and pulmonary embolism, are common causes of mortality and disability worldwide. Recombinant staphylokinase (SAK) has been identified as a potential alternative plasminogen activator for thrombolytic therapy. However, the clinical use of wild-type SAK is restricted due to immunogenic reactions triggered by its bacterial origin. To reduce immunogenicity, non-immunogenic SAK variants have been engineered and evaluated. Recent clinical trials have shown that the non-immunogenic SAK variant is not inferior to tPA in the treatment of acute ischemic stroke [1]. However, non-immunogenic mutations in the SAK molecule may reduce its functional activity and stability.

To address these limitations, we used protein engineering approaches, specifically directed evolution, to improve the properties of SAK. Ribosome display was used to evolve a staphylokinase variant with enhanced function, using the non-immunogenic SAK 42D 3A as a template. Our primary aim was to increase plasmin binding affinity, assuming this would enhance activity. After five rounds of ribosome display, we discovered a mutant with higher fibrinolytic activity *in vitro* than the template. This mutant exhibited a clot lysis half-time of 38.3 ± 0.6 minutes, which is significantly faster than the template molecule (49.2 ± 3.3 min). However, the mutant showed reduced stability.

These results indicate that directed evolution can be used to enhance the activity of non-immunogenic SAK variants. However, further strategy optimization is required to achieve improvements in both activity and stability.

This work was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under projects No. 09I01-03-V04-00041, 09-I02-03-V01-00021 and by the Slovak Research and Development Agency (grant No. APVV-23-0013). The authors gratefully acknowledge the excellent collaboration with the members of the Loschmidt Laboratories at Masaryk University.

References

1. Gusev, E. I., Martynov, M. Y., Nikonov, A. A., Shamalov, N. A., Semenov, M. P., Gerasimets, E. A., Yarovaya, E. B., Semenov, A. M., Archakov, A. I., Markin, S. S., & FRIDA Study Group (2021). Non-immunogenic recombinant staphylokinase versus alteplase for patients with acute ischaemic stroke 4·5 h after symptom onset in Russia (FRIDA): a randomised, open label, multicentre, parallel-group, non-inferiority trial. Lancet Neurol., 20(9):721-728.

Programmability of protein-DNA bioconjugates self-assembly through functional ligand

[a]V. FEDOROVA, [b]V. HUNTOSOVA, [a]K. SIPOSOVA, [c]M. HUMENIK

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[b]Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia
[c]Department of Biomaterials, Faculty of Engineering Science, University Bayreuth, Germany

Nowadays, there is a growing demand for bio-based, safe, and effective drug delivery or healing-supporting mechanisms [1]. In this context, especially the amyloid protein-based nanomaterials are emerging as promising candidates for biotechnological and biomedical applications [2]. In addition, fibrillar morphologies of proteins with cross-β secondary structure associated with pathological aggregation in human body (e. g. amyloid β or tau) as well as proteins playing crucial roles in lower organisms, such as spider protein forming webs or bacterial proteins essential for forming biofilms [3, 4].

Presented work provides insight into the synthesis of bio-nanomaterials capable of binding biologically active ligands and act as biosensors or drug delivery systems. Recombinant spider protein eADF4(16) was chemically modified and subcequently conjugated with DNA aptamers, leading to the formation of protein-DNA bioconjugates that retain the key physico-chemical properties of both individual components.

Additionaly, this approach further enables the formation of highly organized fibril-based nanomaterials with programmable self-assembly through targeted ligand acting as self-assembling agent. When appropriate conditions are applied, the protein moiety of the conjugate undergoes fibrillization process, while DNA aptamers selectively bind ligands in solution, thereby linking individual fibrils.

Two types of DNA aptamers were used for the synthesis of bioconjugates: i) DNA oligomers targeting different exosites of human α-thrombin and ii) split aptamers forming ternary complex with ATP. Experimental results demonstrate that protein-DNA bioconjugates assemble into diverse higher-ordered morphologies in the presence of their targeted functional ligand, following reproducible pattern. Optimization of the reaction components concentrations and environmental factors, such as temperature, self-assembly time, or ion concentrations, may enable to design of specific, desired morphologies suitable for use as drug delivery systems.

Furthermore, DNA aptamers binding thrombin molecule are characterized by a G-quadruplex secondary structure, which can be destabilized under proper conditions, leading to ligand release. Such conditions include significant alterations on ion concentration essential for stabilizing G-quadruplex or rapid temperature shift of the system. Identifying optimal conditions that resemble the *in vivo* environment could lead to the origin of drug transporting system able to controllably release of desired ligand.

On the other hand, split aptamers are a great example for the use as the biosensors of specific molecules in the living organisms.

In summary, the establishment of a programmable platform based on bioconjugates synthesized in this study offers safe and effective drug transport/biosensoring foundation. This approach is based the inherent properties of both, protein and DNA components, including biocompatibility, biodegradability, and minimal immunogenicity.

Acknowledgment. This work was supported by research grants APVV 23-0013, VEGA 2/0034/22, SK-DAAD (2024-2025) and Doktogrant APP0643. V.H. acknowledges the support funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00007.

References

1. Ezike, T. C., Okpala, U. S., Onoja, U. L., Nwike, C. P., Ezeako, E. C., Okpara, O. J., Okoroafor, C. C., Eze, S. C., Kalu, O. L., Odoh, E. C., Nwadike, U. G., Ogbodo, J. O., Umeh, B. U., Ossai, E. C. & Nwanguma, B. C. (2023). Advances in drug delivery systems, challenges and future directions. Heliyon, 9(6).
2. Ling, S., Kaplan, D. L., & Buehler, M. J. (2018). Nanofibrils in nature and materials engineering. Nature Reviews Materials, 3(4), 1-15.
3. Bleem, A., & Daggett, V. (2017). Structural and functional diversity among amyloid proteins: agents of disease, building blocks of biology, and implications for molecular engineering. Biotechnology and Bioengineering, 114(1), 7-20.
4. Chiti, F., & Dobson, C. M. (2017). Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. Annual review of biochemistry, 86(1), 27-68.

Computational study of the tau protein aggregation

[a, b]L. ILKOVICOVA, [a, c]J. HRITZ

[a]*Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
[b]*National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic*
[c]*Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic*

The hallmark of neurodegenerative diseases is the abnormal accumulation of tau protein, transforming it from a soluble state into insoluble aggregates with unique structures depending on the disease [1]. Understanding the exact molecular mechanisms behind this aggregation and how post-translational modifications influence it still remains a significant challenge for experimental research. To address this, we employ computational approaches, specifically utilizing three variations of molecular dynamics simulations [2, 3], to thoroughly investigate the fibrillization process of paired helical filaments observed in Alzheimer's disease [4] and chronic traumatic encephalopathy type II [5]. Our results demonstrate that tau aggregation into these paired helical filaments predominantly occurs at one end of the fibril, with considerable energetic differences for dissociation from the two ends. Furthermore, phosphorylation (pSer324/pSer324 and pSer356) decreased the free energy minimum of the fibril, showing cooperation of these phosphorylations in the fibril stabilization. While histidine 329 protonation also contributes to fibril stabilization in both diseases, the impact of histidine 374 protonation differs between the two conditions.

Acknowledgment. Funded by the European Union's Horizon Europe program under the grant agreement No. 101087124 – ADDIT-CE. This publication reflects only the author's view, and the European Commission is not responsible for any use that may be made of the information it contains. The computational resources in IT4Innovations were granted by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90254).

References

1. Lövestam, S., Li, D., Wagstaff, J. L., Kotecha, A., Kimanius, D., McLaughlin, S. H., Murzin, A. G., Freund, S. M. V., Goedert, M., & Scheres, S. H. (2024). Disease-specific tau filaments assemble via polymorphic intermediates. Nature, 625(7993), 119-125.
2. Leonard, C., Phillips, C., & McCarty, J. (2021). Insight into seeded tau fibril growth from Molecular Dynamics simulation of the Alzheimer's disease protofibril core. Frontiers in molecular biosciences, 8, 624302.
3. Liu, H., Liu, X., Zhou, S., An, X., Liu, H., & Yao, X. (2019). Disclosing the template-induced misfolding mechanism of tau protein by studying the dissociation of the boundary chain from the formed tau fibril based on a steered molecular dynamics simulation. ACS Chemical Neuroscience, 10(3), 1854-1865.
4. Fitzpatrick, A. W. P., Falcon, B., He, S., Murzin, A. G., Murshudov, G., Garringer, H. J., Crowther, R. A., Ghetti, B., Goedert, M., & Scheres, S. H. W. (2017). Cryo-EM structures of tau filaments from Alzheimer's disease. Nature, 547(7662), 185-190.
5. Falcon, B., Zivanov, J., Zhang, W., Murzin, A. G., Garringer, H. J., Vidal, R., Crowther, R. A., Newell, K. L., Ghetti, B., Goedert, M., & Scheres, S. H. (2019). Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. Nature, 568(7752), 420-423.

POSTER PRESENTATIONS

Enhanced efficiency of Fe_3O_4@SiO_2 with specific ligand layer in magnetic separation RNA

[a]M. BARUTIAK, [a]A. ZELENAKOVA, [a]P. HRUBOVCAK, [a]L. NAGY, [a]J. SZUCSOVA, [a]Z. FABRICIOVA, [b]E. BENOVA, [b]N. KIRALY, [b]V. ZELENAK, [c]A. ANTOSOVA

[a]Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[c]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

We have designed core-shell nanoparticles that enhance the separation efficiency of nucleic acids and genetic material during RT-PCR assays. The improvement has been achieved by using a specific ligand modification of the nanoparticles' surface. Fe_3O_4@SiO_2 nanoparticles with a magnetic core size of around 10 nm and a silica shell were modified with an outer layer of organic ligand. In this way, four different samples were prepared, consisting of the same core-shell Fe_3O_4@SiO_2 system with different ligands attached to the surface. The specific ligands were chosen to enhance the RNA binding efficiency and differed from each other by functional groups. We characterised the prepared nanoparticle systems and studied their structural, morphological, and magnetic properties and their potential to bind nucleic acids by the extraction protocol and ethidium bromide fluorescence displacement assay. Our study shows that the modification of the nanoparticle surface with a suitable ligand results in a significant increase in the separation efficiency of RNA molecules.

Acknowledgment. This work was supported by research grants APVV-20-0512, and by the VEGA No. 1/0470/25.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Spider silk eADF4(C16) nanofibrillar protein hydrogels for biomedical application

[a]T. BIRO, [b]N. BEST, [c]V. HUNTOSOVA, [d]K. PAULOVICOVA, [a]K. SIPOSOVA, [b]M. HUMENIK

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[b]Department of Biomaterials, Faculty of Engineering Science, University Bayreuth, Germany
[c]Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia
[d]Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

Proteins exhibiting exceptional structural and functional properties are highly suitable precursors for the development of hydrogels. Such hydrogels retain characteristics typical of polymeric materials, yet their uniqueness arises from the diversity of structures and biological functions of the proteins themselves. Characterized by excellent physicochemical and biomimetic properties, biodegradability, and low immunogenicity, protein hydrogels can be used in a variety of applications, such as tissue engineering, drug delivery, or biotechnology in the form of biosensors. From a structural point of view, they are considered to be three-dimensional networks composed of hydrophilic polymer protein structures that have the ability to absorb water without being dissolved. Especially, amyloid-based hydrogels, in particular, exhibit tunable biochemical and mechanical properties [1-3], as amyloid proteins self-assemble into β-sheet-rich structures with remarkable stability and nanomechanical properties [4, 5]. These hydrogels can be chemically modified to incorporate functional ligands and bind macromolecules, polymers or small molecules, enabling diverse applications in biomedicine and beyond [6, 7]. Their fibrous shape also facilitates the cross-linking of chains with other polymers and the bridging of individual nanostructures, making them versatile building blocks for composite materials [8]. For example, the modified surface of nanofibrillar hydrogels provides numerous active sites, thereby increasing the efficiency of functional materials, such as enhancing adsorption capacity or supporting cell adhesion and growth. One such material is the recombinant spider silk protein eADF4(C16), derived from the silk of the orb-weaver spider *Araneus diadematus*.

Based on the ability of proteins to self-assemble into amyloid fibrils under specific conditions, the present work aims to fabricate single-protein hydrogels using lysozyme and the recombinant spider silk protein eADF4(C16). Additionally, we the second task was to develop hybrid hydrogels by conjugating proteins/fibrils with functional DNAs via chemical coupling. Understanding the organization of DNA hybrids within the hydrogel scaffold and their role in binding and releasing functional ligands is crucial for the controlled fabrication of nanostructured materials. We demonstrated the formation of nanohydrogels, consisting of fibrillar networks stabilized by β-sheets, which can be assembled on top of simple protein films. This technique can be applied on glass slides, enabling fluorescence assays and cell culture experiments.

Acknowledgment. This work was supported by research grants APVV 23-0013, APVV SK-TW-24-0005 and VEGA 2/0034/22, SK-DAAD (2024-2025) and MVTS SK-TW Supra-Sight. V.H. acknowledge the support funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00007.

References

1. Wang, X. (2024). Protein hydrogels for biomedical applications. Biosurface and Biotribology, 10(3), 106-131.
2. Sanchez-Cid, P. (2022). Novel Trends in Hydrogel Development for Biomedical Applications: A Review. Polymers, 14(15), 3023.
3. Tang, Y., *et al.* (2021). A review on recent advances of Protein-Polymer hydrogels. European Polymer Journal, 162, 110881.
4. Knowles, T. P. J., *et al.* (2016). Amyloid Fibrils as Building Blocks for Natural and Artificial Functional Materials. Advanced Materials, 28, 6546.
5. Hovanova, V., *et al.* (2023). Global analysis of kinetics revelas the role of secondary nucleation in recombinant spider silk self-assembly. Protein Science, 32(8), e4722.
6. Nelson, R., *et al.* (2006). Recent atomic models of amyloid fibril structure. Current Opinion in Structural Biology, 16, 260-265.
7. Maji, S. K., *et al.* (2009). Structure–activity relationship of amyloid fibrils. FEBS Letters, 583, 2610-2617.
8. Mao, X., (2025). Nano-fibrous biopolymers as building blocks for gel networks: Interactions, characterization and applications. Advances in Colloid and Interfaces Science, 338, 103398.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

When Structure Matters: How Linker Length and Coumarin Dimerization Shape Anti-Amyloid Activity

[a]B. BOROVSKA, [b]P. KRUPA, [a]Z. BEDNARIKOVA, [c]S. HAMULAKOVA, [b, d]M. S. LI, [a]Z. GAZOVA

[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Institute of Physics, Polish Academy of Sciences, Warsaw, Poland*
[c]*Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[d]*Life Science Lab, Institute for Computational Science and Technology, Quang Trung Software City, Ho Chi Minh City, Vietnam*

Protein aggregation into amyloid fibrils is a central feature of numerous human amyloid-related diseases, such as Alzheimer's disease ($A\beta_{42}$ peptide) and systemic amyloidosis. Although proteins differ in their amino acid sequence and native folds, amyloid fibrils share a common cross-β-sheet architecture, making them attractive targets for small molecule modulators [1]. Coumarins are bioactive aromatic compounds with reported antioxidant and anti-amyloid activity [2], yet how specific structural modifications translate into anti-amyloid efficacy is still an open question.

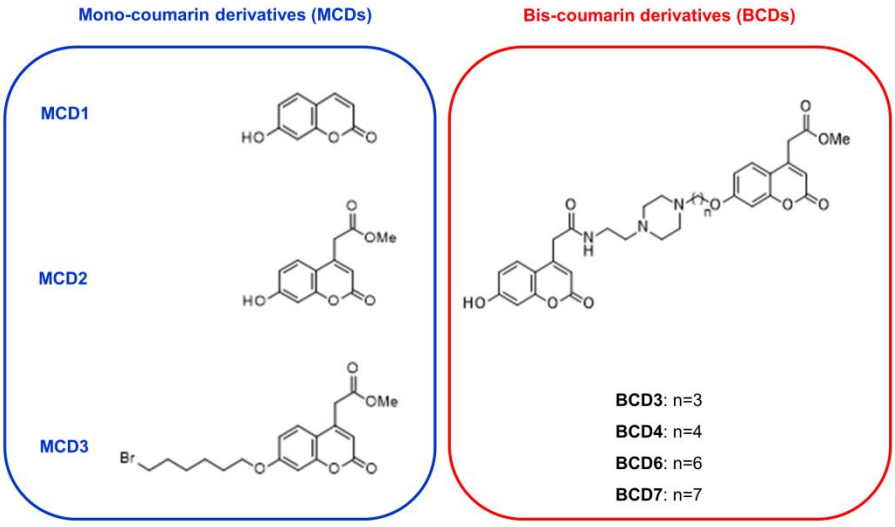


Fig. 1 The chemical structure of the studied mono-coumarin derivatives (MCDs) and the bis-coumarin derivatives (BCDs).

In this study, we investigated the ability of mono- (MCDs) and bis- (BCDs) coumarin derivatives (Fig. 1) to modulate fibrillation of two structurally distinct proteins: the globular protein - hen egg-white lysozyme (HEWL) and the intrinsically disordered $A\beta_{42}$ peptide, and explored their structure-activity relationship. The MCD1-MCD3 differed in the substituents on the coumarin core, whereas the BCDs consisted of two coumarin units connected by a flexible linker of varying length (3 (BCD3), 4(BCD4), 6(BCD6), or 7 (BCD7) methylene groups) (Fig. 1).

The anti-amyloid potential of the studied compounds was determined using Thioflavin-T (ThT) fluorescence assay and atomic force microscopy. Interestingly, the anti-amyloid activity of the studied compounds was different. Neither the presence of MCDs during fibrillation nor the incubation with mature fibrils of both proteins altered fibril morphology or abundance. In contrast, BCDs exerted substantial effects, highlighting a strong dependence on both linker length and concentration. Among the tested derivatives, BCD7 was the most potent, markedly inhibiting $A\beta_{42}$ aggregation at micromolar concentrations and effectively disassembling mature fibrils, while HEWL fibrils exhibited little susceptibility.

Kinetic analysis confirmed that BCD7 suppressed the characteristic sigmoidal aggregation profile of $A\beta_{42}$ peptide, whereas BCD3 exerted only modest effects. Computational modeling further supported these observations: molecular docking and molecular dynamics (MD) simulations indicated that the extended linker in BCD7 enables stable interactions with key residues in both the N-terminal region and hydrophobic core of $A\beta_{42}$ fibrils. Binding free energy calculations reinforced these findings, demonstrating BCD7's superior affinity in line with experimentally observed potency.

Together, these findings highlight that dimerization of coumarin moieties combined with an optimal linker length is the key structural feature for enhancing anti-amyloid activity. Our results provide mechanistic insight into how small-molecule architecture governs amyloid aggregation and offer valuable guidance for the rational design of next-generation amyloid inhibitors.

Acknowledgements. This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0598 and APVV-18-0284; Slovak Grant Agency VEGA 02/0141/25, 1/0037/22. The computational part of the work was supported by the National Science Center (Poland) Sonata 2019/35/D/ST4/03156.

References

1. Soto C. (2003). Unfolding the role of protein misfolding in neurodegenerative diseases. Nature reviews. Neuroscience, 4(1), 49-60. https://doi.org/10.1038/nrn1007
2. Frydman-Marom, A., Levin, A., Farfara, D., Benromano, T., Scherzer-Attali, R., Peled, S., Vassar, R., Segal, D., Gazit, E., Frenkel, D., & Ovadia, M. (2011). Orally administrated cinnamon extract reduces β-amyloid oligomerization and corrects cognitive impairment in Alzheimer's disease animal models. PloS one, 6(1), e16564. https://doi.org/10.1371/journal.pone.0016564

Analysing possible interaction of mitochondrial translocator protein with chloride intracellular channel CLIC5

[a]R. DEKHTIARENKO, [b]K. POLCICOVA, [a]Z. SEVCIKOVA TOMASKOVA

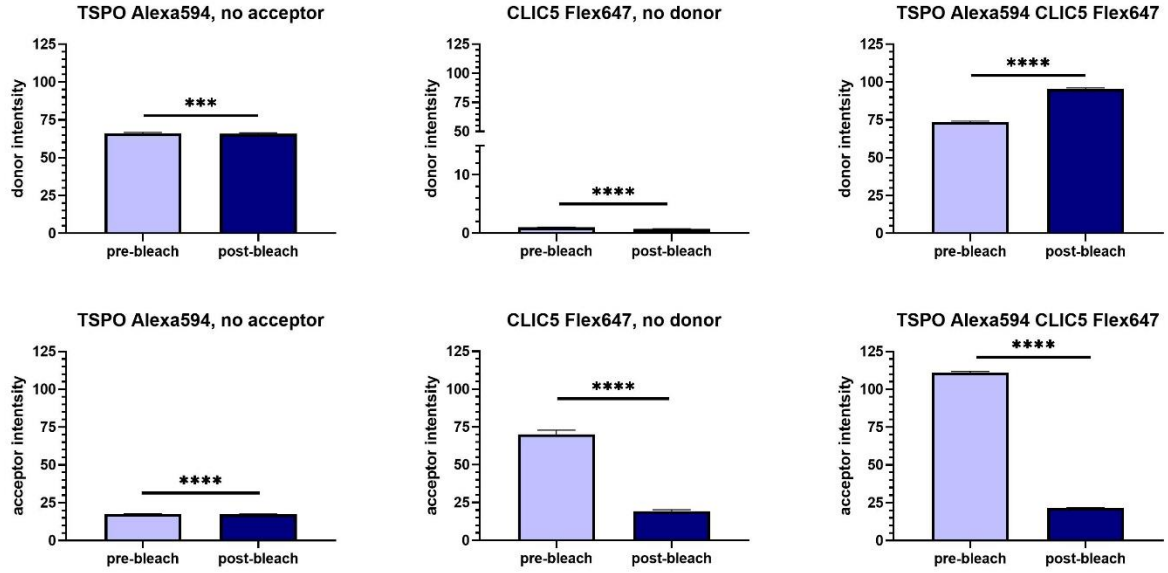
[a]Center of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia
[b]Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia

Cardiac arrhythmias are among the leading causes of death worldwide. These irregular heart rhythms can also result from ischemia/reperfusion, a process in which fluctuations in nutrient and oxygen supply occur. During reperfusion, oxygen radicals increase and at critical concentration, they flow out of the mi [1] influencing mitochondrial membrane potential throughout the whole cell. The route for superoxide anions was suggested by inner membrane anion channel, IMAC. Cardioprotective effects have been observed at both the cellular and whole-heart levels following the application of specific mitochondrial translocator protein (TSPO) ligands 4-chlorodiazepam (4Cl-DZP), as well as non-specific anion channel inhibitor 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS). While 4Cl-DZP is believed to modulate chloride channel activity indirectly via TSPO, the precise nature of this interaction remains unknown. Ponnalagu *et al.* (2006) showed that isoform 5 of chloride intracellular channel (CLIC5) is localized in the inner mitochondrial membrane of cardiomyocytes and cardiomyoblasts and, therefore, is the most probable candidate for the genetically unknown IMAC channel. We aimed to show whether the TSPO protein is localized in the vicinity of CLIC5 protein, sufficient to enable any potential physical contact.

We determined the colocalization of TSPO with CLIC5 protein. Both proteins were visualized by immunofluorescence labeling (FlexAble Coralite kit for rabbit IgG, 488nm). The images were acquired sequentially using Leica Sp8 STED 3X with 63X oil immersion objective. Both proteins colocalized with Mitotracker Deep Red (Pearson coefficient for TSPO was 0.8723 ± 0.0441 (mean, SD, N=83) and for CLIC5 was 0.8936 ± 0.0424 (mean, SD, N=53). For the mutual colocalization of these two proteins, CLIC5 was stained using Alexa 594 nm and TSPO was stained using FlexAble Coralite 488 nm. We quantified their colocalization with Pearson coefficient (r = 0.8188 ± 0.0523, mean, SD, N=85) and with Mander's coefficients that express the proportion of one signal colocalizing with the second signal. The proportion of TSPO signal that colocalized with CLIC5 signal was 0.8135 ± 0.0611 (mean, SD, N=86). On the hand, the proportion of CLIC5 signal that colocalized with TSPO was smaller, 0.7766 ± 0.0728 (mean, SD, N=86).

We used Forster resonance energy transfer (FRET) to analyze the protein-protein interaction that has been suggested by Aon *et al.* (2009). FRET is observable when two fluorophores are up to 10 nm apart, the efficiency of the energy transfer is sensitive not only to the distance but also to the mutual position of the fluorophores and to their excitation and emission spectral properties. For FRET measurement, we used Alexa 594 as donor fluorophore due to its more appropriate spectral properties in comparison to FlexAble Coralite 594. FlexAble Coralite 647 was used as acceptor. We used acceptor photobleaching mode, the intensity of acceptor was bleached down to the background level

within small regions of interest (ROIs) encompassing single mitochondrion or a small group of mitochondria. We observed an increase in the donor intensity to 130% of its pre-bleach value, with median efficiency 24% (median, Q1=17%, Q3=29%, N=1913 ROIs/90 cells).



TSPO protein is a protein localized at the contact sites of the inner and the outer mitochondrial membrane [3], sometimes it is referred to also as a protein of the outer membrane only [2]. On the other hand, CLIC5 is localized in the inner mitochondrial membrane, according to the study of Ponnalagu *et al.* (2016). Interestingly, when we performed the FRET experiments with another protein from the outer mitochondrial membrane, TOM20, we detected even higher efficiency of energy transfer (30%, Q1=26%, Q3=33%, N=1456 ROIs/90 cells). The controls containing only donor or only acceptor indicate that there is no other interference between the two fluorophores and that the observed increase in the donor intensity should be the result of the resonance energy transfer.

Our data imply that CLIC5 protein is localized in close apposition with TSPO protein, near the outer mitochondrial membrane. Further experiments (like crosslinked co-immunoprecipitation) are needed to show a possible functional connection between these two proteins that could explain the sensitivity of the mitochondrial chloride channel to TSPO ligands.

Acknowledgment. This work was supported by VEGA 2-0051-23 and APVV-0084-22.

References

1. Aon, M. A., Cortassa, S., Akar, F. G., Brown, D. A., Zhou, L., & O'Rourke, B. (2009). From mitochondrial dynamics to arrhythmias. Int. J. Biochem. Cell Biol., 40, 1940-1948.
2. Bader, S., Wolf, L., Milenkovic, V. M., Gruber, M., Nothdurfter, C., Rupprecht, R., & Wetzel, C. H. (2019). Differential effects of TSPO ligands on mitochondrial function in mouse microglia cells. Psychoneuroendocrinology, 106, 65-76.
3. Baglini, E., Baglini, E., Poggetti, V., Cavallini, C., Petroni, D., Forini, F., Nicolini, G., Barresi, E., Salerno, S., Costa, B., Iozzo, P., Neglia, D., Menichetti, L., Taliani, S., & Da Settimo, F. (2024). Targeting the Translocator Protein (18 kDa) in Cardiac Diseases: State of the Art and Future Opportunities. J. Med. Chem., 67(1), 17-37.

4. Kinnally, K. W., Zorov, D. B., Antonenko, Y. N., Snyder, S. H., McEnery, M. W., & Tedeschi, H. (1993). Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligands. Proc. Natl. Acad. Sci. USA, 90(4), 1374-1378.
5. Ponnalagu, D., Gururaja Rao, S., Farber, J., Xin, W., Hussain, A. T., Shah, K., Tanda, S., Berryman, M., Edwards, J. C., & Singh, H. (2016). Molecular identity of cardiac mitochondrial chloride intracellular channel proteins. Mitochondrion, 27, 6-14.

Cancer therapy by magnetic hyperthermia: impact of cobalt ferrite nanoparticles

[a, b]Z. FABRICIOVA, [a]A. ZELENAKOVA, [a]L. NAGY, [a]P. HRUBOVCAK, [a]M. BARUTIAK, [a]D. VOLAVKA, [a]M. LISNICHUK

[a]Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Department of Chemistry and Industrial Chemistry & INSTM RU, nM2-Lab, University of Genoa, Italy

In recent years, magnetic nanoparticles (MNPs) have gained increasing attention as promising agents for biomedical applications, owing to their ability to combine diagnostic and therapeutic functionalities within a single platform. One of the most promising therapeutic strategies is magnetic hyperthermia, in which MNPs exposed to an alternating magnetic field are capable of converting electromagnetic energy into heat. This effect enables the localised increase of temperature in tumour tissues, ultimately promoting cancer cell death while minimising side effects on surrounding healthy tissues.

Within this context, cobalt ferrite ($CoFe_2O_4$) nanoparticles represent a particularly attractive material due to their high magnetocrystalline anisotropy, chemical stability, and well-defined magnetic behaviour. By tuning their size, morphology, and surface chemistry, it is possible to significantly influence their magnetic behaviour and consequently their efficiency as hyperthermia agents. Thermal decomposition is a suitable synthesis route for producing highly crystalline $CoFe_2O_4$ nanoparticles with controlled morphology, such as spherical, cubic, or octapod-like shapes, enabling systematic studies on the impact of nanoparticle design.

This contribution will address the potential of cobalt ferrite nanoparticles as magnetothermal agents, with emphasis on the role of particle morphology and magnetic properties in determining their suitability for cancer therapy.

Acknowledgment. This work was supported by research grants APVV-20-0512, and by the VEGA No. 1/0470/25.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Dual effect of ionic liquids on lysozyme stability and activity

D. FEDUNOVA, V. VANIK, Z. GAZOVA

Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

Over the past twenty years, ionic liquids (ILs) have become an important class of solvents in biochemistry, biophysics, and biotechnology. They are organic salts composed of an organic cation combined with an organic or inorganic anion. The nearly unlimited number of possible combinations gives rise to their unique properties, which is why they are often referred to as *"designer solvents."* By carefully selecting the cation–anion pair, their physicochemical properties - such as melting temperature, polarity, hydrophobicity, density, viscosity, or solubility - can be precisely tuned [1]. Imidazolium-based ILs are among the most widely studied ILs because their structure allows systematic tuning of side-chain length, hydrophobicity, and functional groups. These variations strongly influence protein stability, folding, and activity, making them valuable for probing solvent–protein interactions. Their well-defined chemistry and broad availability also make them attractive candidates for biotechnological applications involving proteins [2].

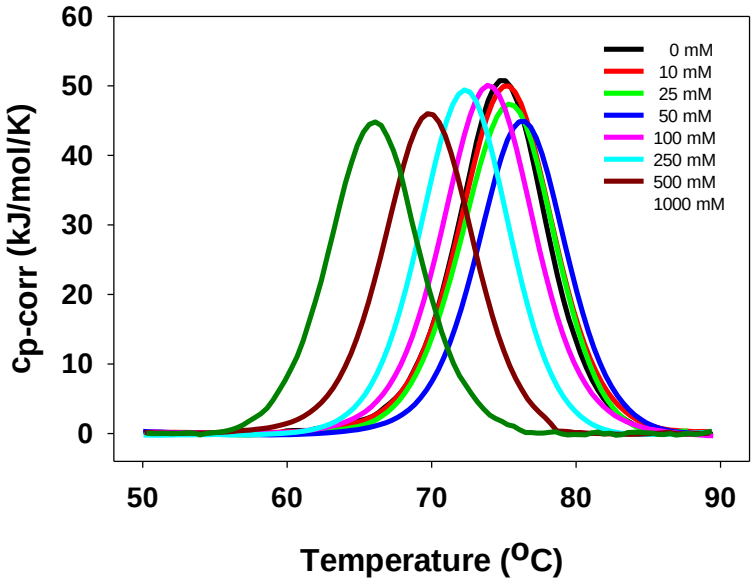


Fig. 1 DSC thermogram of lysozyme in ILs with allyl-imidazolium cation at increasing concentrations in 2 mM PP, pH 6.0. Lysozyme concentration is 140 µM.

We have studied the effect of imidazolium-based ionic liquids (ILs) with allyl, propyl, and benzyl side chains, combined with chloride anion, across a wide concentration range on the thermal stability, activity, and structure of lysozyme. The influence of cations differing in hydrophobicity and geometry on lysozyme stability and activity was characterized to better understand the mechanisms of protein stabilization. In addition to being widely used as a model protein, lysozyme also serves as an antibacterial and anti-inflammatory agent in both medicine and the food industry. Therefore identification of solvents capable to maintain or enhance lysozyme stability and activity is of great importance for its biotechnological applications.

At pH 6, calorimetric measurements revealed that at lower ILs concentrations (10 mM, 25 mM, 50 mM), all tested ILs enhanced the thermal stability of lysozyme, as evidenced by higher enthalpy change and elevated transition temperature. The effect of ILs with allyl substituent was the most prominent (Fig.1). Complementary spectroscopic analyses (CD, FTIR) together with fluorescence quenching confirmed that the native structure was preserved under these conditions. Furthermore, the bacteriolytic turbidity assay using *Micrococcus lysodeikticus* demonstrated increased enzymatic activity at these ILs concentrations.

However, at higher IL concentrations (100 mM–1 M), both the thermal stability and enzymatic activity of lysozyme decreased. CD spectra indicated alterations in tertiary structure, while fluorescence data showed that ILs predominantly influenced the microenvironment around tryptophan residues. The interactions between the selected ILs and lysozyme are relatively weak, as follows from dynamic quenching. of lysozyme fluorescence. Among the tested ILs, the benzyl-substituted cation exhibited the strongest destabilizing effect. Since Trp62 and Trp108 are located in the active site, the results imply that hydrophobic and π–π interactions in this region contribute to modulating lysozyme stability and activity. At higher concentrations, additional non-specific ionic interactions are likely involved.

In conclusion, the selected ILs are capable of preserving the native state of lysozyme and enhancing its activity in a concentration-dependent manner. These findings highlight the potential of these ILs for further studies and applications in biotechnology.

Acknowledgment. This work was supported by research grants of the Slovak research agency VEGA 2/0164/22 and 2/0141/25 and the Slovak Research and Development Agency under Contract No. APVV-22-0598 and APVV-18-0284.

References

1. Hu, Y., Xing, Y., Yue, H., Chen, T., Diao, Y., Wei, W., Zhang, S. (2023). Ionic liquids revolutionizing biomedicine: recent advances and emerging opportunities. Chem. Soc. Rev., 52, 7262-7293.
2. Schindl, A., Hagen, M. L., Shafaq, M., Gunsekera, H. A., Croft, A. K. (2019). Proteins in Ionic liquids: Reaction, Applications, and Futures. Front. Chem., 7, 347.

Photopolymeric microchambers for investigating the selectivity of photodynamic therapy in individual breast cancer cells

[a]M. GALIS, [b]S. TOMKOVA, [c]A. MIGASOVA, [d]J. KUBACKOVA, [c]M. ALMASI, [a]G. BANO, [b]V. HUNTOSOVA

[a]Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia
[c]Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[d]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

Breast cancer is one of the most common types of cancer worldwide. This invasive disease has a poor prognosis for patients due to its aggressiveness. Therefore, it is important to understand the mechanisms of the biological processes behind the disease development and to propose an appropriate treatment.

In the present work, SKBR3 cells (ductal breast carcinoma) were subjected to a selective treatment in which a metal-organic framework (MOF) as a transport system and methylene blue (MB) as a photosensitising agent enabled photodynamic treatment (PDT). Despite the modification of the particles by amination, the selectivity of the particles was not uniform and PDT led to a heterogeneous response of the cells. To better understand this effect, we proposed a single cell approach using photopolymeric chambers prepared by two-photon polymerization direct laser writing that can confine a certain number of cells. Using optical tweezers, SKBR3 cells treated with MOF-MB were transported into microchambers and exposed to PDT. The reaction of the cells was detected in the following hours. For multimodal detection, the samples were fixed in paraformaldehyde and immunostatined against HER2 receptors and α-tubuline, which are significantly altered by the PDT process.

In summary, the observation of single cells in photopolymer chambers revealed the correlations between the uptake of nanoparticles (MOF) and photosensitiser (MB) by SKBR3 cells and PDT efficacy in these cells. This approach can be improved by the delivery/inclusion of different phenotypes of cells in the microchamber, which could further enable the monitoring of the microbehaviour of cancer cells compared to non-cancer cells.

Acknowledgment. This work was supported by the Slovak Research and Development Agency, grant APVV-21-0333, and by the grant agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic, grant VEGA 2/0055/25. Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00007.

Cholesteryl-anchor mediated non-covalent aptamer functionalization of β-Casein self-assemblies towards therapeutic potential

[a]Z. GARAIOVA, [a]V. SUBJAKOVA, [a]A. A. MACIAS, [b]M. GARAIOVA, [b]R. HOLIC, [a]T. HIANIK

[a]Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia
[b]Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia

β-Casein is a naturally amphiphilic milk protein that spontaneously forms nanoscale micelles, making it a promising drug delivery platform [1, 2]. Nucleic acid aptamers are short single-stranded DNA or RNA oligonucleotides that fold into three-dimensional structures, enabling specific binding to molecular targets, such as cell-surface biomarkers [3]. The sgc8c DNA aptamer binds PTK7 receptor overexpressed on leukemic T cells and has been employed for targeted cancer detection and therapy [4]. The functionalization of β-casein micelles with aptamers remains largely unexplored.

We investigated the non-covalent anchoring of a cholesteryl-TEG modified sgc8c DNA aptamer to β-casein micelles. β-casein at 0.2, 2, and 5 mg/mL was prepared in 10 mM sodium phosphate buffer (pH 7) at 25 °C under stirring for 2 h, then filtered (0.2 μm) and incubated with sgc8c or cholesteryl-TEG-sgc8c aptamers at 0.5, 2, or 5 μM for 2 h.

Dynamic light scattering results showed that at 0.2 mg/mL, the β-casein based structures were heterogeneous (Z-average ~46 nm, PDI 0.70); increasing concentration of β-casein (2 mg/ml and 5 mg/ml) yielded more compact (31 nm and 28 nm) and uniform micelles (PDI 0.46 and 0.43), consistent with micellization behavior.

Agarose gel electrophoresis showed significant retardation of aptamer mobility at higher β-casein concentrations, indicating binding. At 2 mg/mL β-casein, 2 μM aptamer had clear binding, while at 5 μM some aptamers remained free. In contrast, at 5 mg/mL β-casein even 5 μM aptamer was retained. The cholesteryl-TEG significantly enhanced aptamer anchoring: binding and reduction in free aptamer were more pronounced than with unmodified sgc8c, likely due to stronger hydrophobic interactions between the cholesteryl moiety and the micellar core.

These findings suggest that cholesteryl-TEG tag enables efficient non-covalent insertion of sgc8c aptamer into β-casein micelles with potential applications for delivering therapeutics to leukemic cells.

Acknowledgment. This work was supported by the Science Agency VEGA research grant, project No. 1/0554/23.

References

1. Li, M., Fokkink, R., Ni, Y., Kleijn, J. M. (2019). Bovine beta-casein micelles as delivery systems for hydrophobic flavonoids. Food Hydrocolloids, 96, 653-662.
2. Cuggino, J. C., Picchio, L. M., Gugliotta, A., Burgi, M., Ronco, L. I., Calderon, M., Etcheverrigaray, M., Alvarez Igarzabal, C. I., Minari, R. J., Gugliotta, L. M. (2021). Crosslinked casein micelles bound paclitaxel

as enzyme activated intracellular drug delivery systems for cancer therapy. European Polymer Journal, 145, 110237.
3. Fu, Z., Xiang, J. (2020). Aptamer-Functionalized Nanoparticles in Targeted Delivery and Cancer Therapy. International Journal of Molecular Sciences, 21(23), 9123.
4. Poturnayova, A., Burikova, M., Bizik, J., Hianik, T. (2019). DNA Aptamers in the Detection of Leukemia Cells by the Thickness Shear Mode Acoustics Method. ChemPhysChem: a European journal of chemical physics and physical chemistry, 20(4), 545-554.

Multitarget *In Vitro* Evaluation of a Novel Quinacrine Analog: Interactions with DNA, RNA, HSA and Topoisomerase Inhibition

[a]A. GUCKY, [a]A. MIHOKOVA, [b]E. SCALZI, [c]J. KORABECNY, [a]M. KOZURKOVA

[a]Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Department of Pharmacy, University of Pisa, Italy
[c]Biomedical Research Center, University Hospital Hradec Kralove, Czech Republic

Low-molecular-weight ligands represent some of the most promising drug candidates in modern chemotherapy due to their broad spectrum of pharmacological effects. Studying their physicochemical properties and biological activity is essential to understanding their mechanisms of action and enabling further structural optimization. [1]. Acridine derivatives represent one class of such molecules and are known for their wide range of biologically significant activities, including anticancer, antiviral, antibacterial, antiprotozoal, antimalarial and numerous other effects. The pharmacological attractiveness of acridines stems mainly from the planar character of the azaheterocyclic chromophore that can be structurally modified in various positions and allows interaction with all sorts of biomolecular targets [2]. Differently substituted acridine derivatives have been proven to interact with DNA and RNA structural motifs, while also acting as potent topoisomerase inhibitors and cytotoxic agents, all of which emphasizes their importance in the search for novel anticancer therapeutics [3-5].

Our recent work was focused on studying the interactions between a novel quinacrine structural analog (QA1, Fig. 1) and various target biomacromolecules, including calf thymus DNA (ctDNA), single-stranded polyriboadenylic acid (polyA), double stranded polyriboadenylic-polyribouridylic acid (polyAU), human serum albumin (HSA) and topoisomerases (Topo) I and II. The results of absorption spectroscopy experiments disclosed the compound's ability to bind with all three tested polynucleotides, although a slight preference for single-stranded polyA structural motif was observed ($K_b = 2{,}29 \times 10^4$ $mol^{-1}.dm^3$) in comparison to double stranded motifs ($K_b = 0{,}56 \times 10^4$ $mol^{-1}.dm^3$). Binding of QA1 to the polynucleotides was further confirmed by shifts observed in emission and circular dichroism spectra, as well as thermal denaturation assays. In the latter, we observed the compound's ability to stabilize the double-stranded motifs by 5,5 °C (ctDNA) and 10,8 °C (polyAU), while also being able to induce the formation of a stable self-structured form of polyA (T_m = 73,0 °C). Additionally, QA1 was confirmed to spontaneously bind to HSA with high affinity ($K_b = 0{,}86 – 1{,}70 \times 10^6$ $mol^{-1}.dm^3$). This interaction is presumably mediated through hydrophobic interactions and occurs mainly in binding site I of the IIA subdomain, while a minor fraction of the molecules possibly binds to site III. Finally, the compound was also tested for its inhibition activity against Topo I and II. The results of these assays revealed that QA1 selectively inhibits Topo I over Topo II and that the inhibition is primarily mediated through its ability to intercalate between DNA base pairs. In summary, our findings offer valuable insights into the pharmacological profile of QA1 and provide basic foundations for further research of its biological activity.

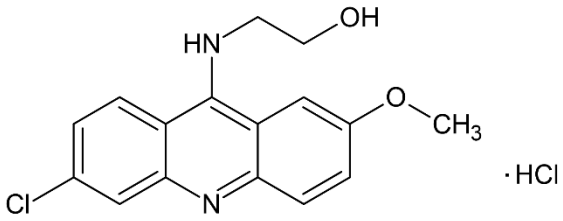


Fig. 1 Chemical structure of the studied compound QA1.

Acknowledgment: Financial support for this study was provided by VEGA Grant no. 1/0037/22.

References

1. Dobbelstein, M., Moll, Y. (2014). Targeting tumour-supportive cellular machineries in anticancer drug development. Nature Reviews Drug Discovery, 13(3), 179-196.
2. Kozurkova, M., Sabolova, D., Kristian, P. (2021). A new look at 9-substituted acridines with various biological activities. Journal of Applied Toxicology, 41(1), 175-189.
3. Krochtova, K., Haleckova, A., Janovec, L., Blizniakova, M., Kusnirova, K., Kozurkova, M. (2023). Novel 3,9-Disubstituted Acridines with Strong Inhibition Activity against Topoisomerase I: Synthesis, Biological Evaluation and Molecular Docking Study. Molecules, 28(3), 1308.
4. Janovec, L., Gucky, A., Krochtova, K., Michalkova, R., Kusnirova, K., Miskufova, V., Jager, D., Mojzis, J., Kozurkova, M. (2025). Development of 2,9-Disubstituted Acridines as Topoisomerase IIα Inhibitors with Strong Anticancer Activity: Synthesis, Biological Evaluation, and In Silico Study. ChemMedChem, e2500267.
5. Krochtova, K., Janovec, L., Bogarova, V., Haleckova, A., Kozurkova, M. (2024). Interaction of 3,9-disubstituted acridine with single stranded poly(rA), double stranded poly(rAU) and triple stranded poly(rUAU): molecular docking – A spectroscopic tandem study. Chemico-Biological Interactions, 394, 110965.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Novel betulinic acid-based ionic liquids: synthesis and anti-breast cancer activity

[a]M. GUNCHEVA, [b]P. OSSOWICZ-RUPNIEWSKA, [a]Y. RAYNOVA

[a]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[b]Department of Organic Chemical Technology and Polymer Materials, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology, Szczecin, Poland

Among its many biological activities observed in vitro, betulinic acid has shown strong potential as an anti-breast cancer agent by inhibiting multiple key enzymes in signaling pathways and cellular receptors [1]. It also exhibits synergistic effects with conventional chemotherapeutics and increases the chemosensitivity of chemoresistant tumor cells [2]. Additionally, it may reduce inflammation, thereby altering the tumor microenvironment, which contributes to tumor growth inhibition and activation of cell death mechanisms [3].

Despite the significant potential of betulinic acid (BA) in anticancer therapy, no pharmacological applications have been approved due to its poor water solubility and, consequently, low bioavailability. Therefore, BA formulations with improved physicochemical properties and preserved or enhanced biological activity are in demand. Recently, we discovered that converting BA into organic salts based on amino acid ester cations may improve its solubility, cytotoxicity, and selectivity in hormone-dependent and triple-negative breast cancer cells (TNBC) [4, 5].

Here, we report the synthesis of three new betulinic acid (BA) salts with arginine ethyl ester, glutamic acid ethyl ester, and glutamic acid isopropyl ester cations. ^{1}H NMR, ^{13}C NMR, and FTIR spectroscopy confirmed the structures of the compounds. The melting points and thermal stability of the BA derivatives were also assessed.

The cytotoxic effects of the three novel compounds on triple-negative breast cancer (TNBC) cells were evaluated by measuring mitochondrial activity using the colorimetric MTT assay. The estimated half maximum inhibition concentration (IC50) is within the micromolar range. We observed a slight increase in the cytotoxicity for the salt containing glutamic acid isopropyl ester compared to the acid form of BA and its sodium salt. Additionally, we found that all compounds were able to induce apoptosis through a caspase-dependent mechanism. A decrease in metastatic potential and suppression of colony formation in TNBC cells were also observed.

Acknowledgment. This work was supported by the Bulgarian National Science Fund, research grant № KP-06-H69/2.

References

1. Luo, R., Fang, D., Chu, P., Wu, H., Zhang, Z., Tang, Z. (2016). Multiple molecular targets in breast cancer therapy by betulinic acid. Biomedicine and Pharmacotherapy, 84, 1321-1330.
2. Cai, Y., Zheng, Y., Gu, J., Wang, S., Wang, N., Yang, B., Zhang, F., Fu, W., Wang, Z. (2018). Betulinic acid chemosensitizes breast cancer by triggering ER stress-mediated apoptosis by directly targeting GRP78. Cell Death and Disease. 9, 636.

3. Zheng, Y., Liu, P., Wang, N., Wang, S., Yang, B., Li, M., Chen, J., Situ, H., Xie, M., Lin, Y., Wang, Z. (2019) Betulinic acid suppresses breast cancer metastasis by targeting GRP78-mediated glycolysis and ER Stress Apoptotic Pathway. Oxididative Medicine and Cell Longelivity, 8781690.
4. Ossowicz-Rupniewska, P., Klebeko, J., Georgieva, I., Apostolova, S., Struk, Ł., Todinova, S., Tzoneva, R. D., Guncheva, M. (2024). Tuning of the anti-breast cancer activity of betulinic acid via its conversion to ionic liquids. Pharmaceutics, 16, 496.
5. Apostolova, S., Georgieva, I., Ossowicz-Rupniewska, P., Klebeko, J., Todinova, S., Tzoneva, R., Guncheva, M. (2025). In vitro cytotoxicity assessment of betulinic acid organic salts on triple-negative breast cancer cells. Sci 2025, 7(1), 2.

Raman characterization of the biocompatible photoresist Ormocomp

[a]Z. JURASEKOVA, [a]M. GALIS, [b]J. KUBACKOVA, [b]C. SLABY, [b]Z. TOMORI, [c]G. VIZSNYICZAI, [c]L. KELEMEN, [c]G. T. IVANYI, [a]G. BANO

[a]Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[c]HUN-REN Biological Research Centre Szeged, Institute of Biophysics, Szeged, Hungary

Photopolymer microstructures fabricated through two-photon polymerization direct laser writing (TPP-DLW) are extensively utilized in live cell experiments as scaffolds or containers that precisely localize cells in specific locations. Ormocomp is a biocompatible photoresist material appropriate for TPP-DLW applications. When polymerized under near-threshold conditions, Ormocomp exhibits high elasticity. The flexibility of Ormocomp microstructures is influenced by the degree of conversion, which is significantly affected by the polymerization laser power and writing speed.

Recently, flexible Ormocomp microrobots have been developed to be captured by optical tweezers for applications involving indirect single-cell manipulation [1]. The fabrication process of these microstructures must be optimized to achieve the necessary flexibility. This study employs Raman spectroscopy to quantitatively assess the degree of conversion of the photopolymer. The ratio of selected Raman lines significantly alters with an increase in polymerization laser power. Furthermore, non-trivial behavior is observed when the writing speed is varied. Our findings will facilitate the further development of flexible Ormocomp microstructures.

Acknowledgment. This work was supported by the Slovak Research and Development Agency, grants APVV-21-0333 and SK-HU-24-0009, and by the grant agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic, grant VEGA 2/0055/25.

References

1. Ivanyi, G. T., Nemes, B., Grof, I., Fekete, T., Kubackova, J., Tomori, Z., Bano, G., Vizsnyiczai, G., Kelemen, L. (2024). Optically Actuated Soft Microrobot Family for Single-Cell Manipulation. Advanced Materials, 36(32), 2401115.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Application of modular anticancer nanoparticles in Raman microscopy analysis of cells and 3D culture models

[a]Z. JURASEKOVA, [a]B. G. VARCHOL, [b]A. MIGASOVA, [c]X. LI, [c]M. ANDREANA, [c]A. UNTERHUBER, [b]M. ALMASI, [d]V. HUNTOSOVA

[a]Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[c]Center for Medical Physics and Biomedical Engineering, Medical University, Vienna, Austria
[d]Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

Raman spectroscopy (RS) is a non-invasive, label-free spectroscopy technique that identifies molecular vibrations (each spectrum acts as a unique fingerprint of the substance) and is well-suited for biomedical use due to weak signal of water. When combined with microscopy, it enables high-resolution spectral analysis and chemical imaging of a variety of samples, including biological specimens [1-3]. The integration of RS with other techniques like fluorescence microscopy or optical coherence tomography (OCT) holds significant potential for multimodal bioimaging since it combines molecular specificity (from RS) with structural or functional information from other modalities, offering thus a more comprehensive view of biological samples [4, 5]. This methodology is currently gaining importance in medicine, especially for the diagnosis, treatment, and targeting of tumors with enhanced safety and efficacy. In this context, an important related task involves designing, preparing, and investigating effective functional modular nanoparticles intended for use in both bioimaging and cancer treatments. Particularly, an attention for targeted drug delivery is focused on micro- and mesoporous nanoparticles, especially organometallic frameworks (MOF), due to their high drug-loading capacity, kinetic and thermodynamic stability, high biocompatibility, low cytotoxicity, and the potential for surface functionalization [6-8].

In this study, we employ UiO-66 and its derivative UiO-66-NH_2 nanoparticles [9, 10] as potential drug delivery carriers, taking advantage of their porous structure that enables the entrapment of a large number of hydrophilic drug molecules. We focus on 5-fluorouracil (5FU), a chemotherapeutic agent widely recognized as one of the most effective and commonly used drugs for treating various types of cancer [11, 12]. The favorable Raman properties of the selected MOF nanoparticles were investigated to evaluate their potential for modular functionality. Their reference vibrational spectra were recorded to optimize the methodology and identify specific spectral markers. Subsequently, UiO-66-NH_2 nanoparticles were detected within different cells to examine cellular responses to the 5FU biological activity. Finally, Raman microscopy in combination with OCT and multiphoton microscopy was used to examine interactions between UiO-66-NH_2 nanoparticles and patient-derived organoids of colorectal cancer. Our approach demonstrates the multimodal potential of MOF for bioimaging and their modularity in drug delivery. Improving this system through targeted surface modification would lead to controlled

delivery and simultaneous therapy, which could significantly improve the success of cancer treatment.

Acknowledgment. This work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic bilateral project SK-AT-23-0001; and by the grand of the Faculty of Science, University of P. J. Safarik in Kosice VVGS-2024-3101. This study was also funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project BCOrgFluorIDA No. 09I03-03-V04-00007.

References

1. Malsch, E. A., & Gangadeen, S. (2013). Non-contact, label-free monitoring of cells and extracellular matrix using Raman spectroscopy. Journal of Biophotonics, 6(10), 874-889.
2. Zhang, C., & Aldana-Mendoza, J. A. (2021). Coherent Raman scattering microscopy for chemical imaging of biological systems. Journal of Physics Photonics, 3(3), 032002.
3. Khadem, H., Mangini, M., Farazpour, S., & De Luca, A. C. (2024). Correlative Raman Imaging: Development and Cancer Applications. Biosensors, 14(7), 324.
4. Patil, C. A., Kalkman, J., Faber, D. J., Nyman, J. S., van Leeuwen, T. G., & Mahadevan-Jansen, A. (2011). Integrated system for combined Raman spectroscopy–spectral domain optical coherence tomography. Journal of Biomedical Optics, 16(10), 1011007.
5. Krafft, C., Popp, J., & Northrup, J. (2022). Looking for a perfect match: multimodal combinations of Raman spectroscopy for biomedical applications. Analytical and Bioanalytical Chemistry, 414, 6427-6444.
6. Khafaga, D. S. R., El-Morsy, M. T., Faried, H., Diab, A. H., Shehab, S., Saleh, A. M., & Ali, G. A. M. (2024). Metal–organic frameworks in drug delivery: engineering versatile platforms for therapeutic applications. RSC Advances, 14, 30201-30229.
7. Belsova, E., Hornebecq, V., Zelenak, V., Huntosova, V., Almasi, M., Macajova, M., & Berge-Lefranc, D. (2021). pH-responsive mesoporous silica drug delivery system, its biocompatibility and co-adsorption/co-release of 5-Fluorouracil and Naproxen. Applied Surface Science, 561, 150011.
8. Huntosova, V., Benziane, A., Zauska, L., Ambro, L., Olejarova, S., Joniova, J., Hlavkova, N., Wagnieres, G., Zelenkova, G., Zakany, F., Kovacs, T., Sedlak, E., Vamosi, G., & Almasi, M. (2025). The potential of metal–organic framework MIL-101(Al)–NH_2 in the forefront of antiviral protection of cells via interaction with SARS-CoV-2 spike RBD protein and their antibacterial action mediated with hypericin and photodynamic treatment. https://doi.org/10.2139/ssrn.5120631
9. Pourmadadi, M., Omrani, Z., Forootan, Z., Ebadi, M. S., & Yazdian, F. (2023). UiO-66 nanoparticles as a drug delivery system: A comprehensive review. Journal of Drug Delivery Science and Technology, 86, 104690.
10. Migasova, A., Zauska, L., Pevna, V., Kucharova, V., Huntosova, V., & Almasi, M. (2023). pH-responsive microporous material UiO-66(Zr)-NH_2 as a drug carrier. In Novel Trends in Chemistry, Research and Education: Book of Abstracts (p. 54). Kosice: University of P. J. Safarik in Kosice.
11. Longley, D. B., Harkin, D. P., & Johnston, P. G. (2003). 5-Fluorouracil: mechanisms of action and clinical strategies. Nature Reviews Cancer, 3(5), 330-338.
12. Alzahrani, S. M., Al Doghaither, H. A., Al-Ghafari, A. B., & Pushparaj, P. N. (2023). 5-Fluorouracil and capecitabine therapies for the treatment of colorectal cancer (Review). Oncology Reports, 50, 175.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Targeting Insulin Fibrils: Evaluating the Destruction Potential of Phytoalexins

[a]H. A. KAREEM, [a]A. ANTOSOVA, [b]M. BUDOVSKA, [a]Z. BEDNARIKOVA, [a]D. FEDUNOVA, [a]Z. GAZOVA

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[b]Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

Insulin, a vital peptide hormone for glucose regulation and diabetes management, is prone to forming insoluble amyloid fibrils under certain physicochemical conditions, posing significant challenges in both therapeutic administration and industrial production. Phytoalexins, a class of low-molecular-weight secondary metabolites produced by plants in response to pathogenic stress, are known for their broad pharmacological activities, including antibacterial, antifungal, anti-inflammatory, antidiabetic, and anticancer effects.

Our study aimed to examine the destructive effect of indole phytoalexins and their synthetic derivatives (Phy 7 to Phy 16) on insulin amyloid fibrils using ThT and Nile Red fluorescence assays and atomic force microscopy (AFM). Based on the results of fluorescence assays, three phytoalexins - Phy 7 (Cyclobrassinin), Phy 9 (2-phenylamino-4,9-dihydro-1,3-thiazino[6,5-*b*] indole) and Phy 11 *(N,N'*-bis(4,9-dihydro-1,3-thiazino[6,5-*b*]indol-2-yl) benzene-1,4-diamine) exhibited strong ability to destroy insulin amyloid fibrils, suggesting a notable capacity to interfere with the hydrophobic and β-sheet-rich region stabilizing insulin fibrils. These the most active phytoalexins and one less active compound (Phy 14), included for comparison, were selected for detailed concentration-dependent studies in the range of 70 pM to 700 µM. Among them, Phy 7 exhibited the highest destructive effect on insulin amyloid fibrils with a DC_{50} value of 21.9 µM (ThT assay) and 20.5 µM (Nile red assay), followed by Phy 11 and Phy 9. In comparison, Phy 14 showed significantly lower activity with a DC_{50} of 802.0 µM (ThT assay) and 1084.5 µM (Nile red assay). As presented in Fig. 1, AFM imaging showed reduced insulin fibrillar structures after treatment with the most potent phytoalexins Phy 7 and Phy 11, which is consistent with their high destructive activity observed in the ThT and Nile Red assays. The obtain results indicate the structural- and concentration-dependence of phytoalexin's derivatives to destroy insulin fibrillar aggregates.

The cytotoxicity of the studied Phy compounds on HEK-293 cells was assessed using the lactate dehydrogenase (LDH) assay. Analysis of the results revealed that compounds Phy 7 and Phy 11 did not exhibit any cytotoxic effects on HEK-293 cells.

This study identifies promising phytoalexin candidates, particularly Phy 7 and its derivative Phy 11 (bis-indolyl derivative substitution) as novel non-cytotoxic amyloid fibril-destroying agents. It highlights the potential of phytoalexins in combating insulin amyloidosis.

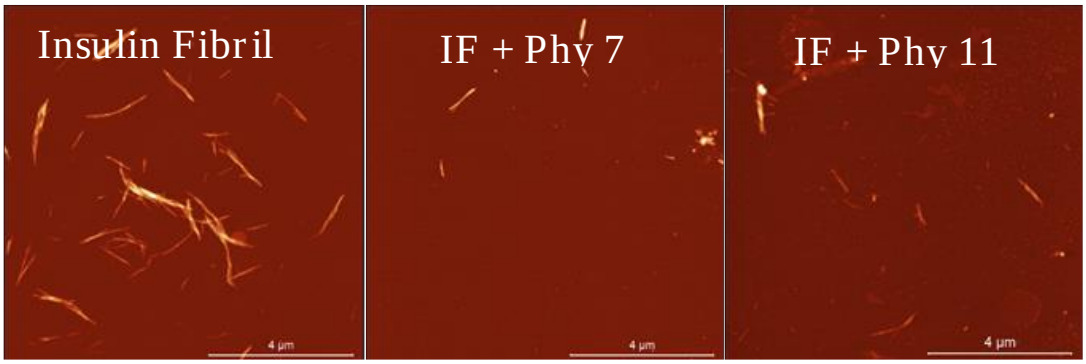


Fig. 1 AFM images of 10 µM insulin fibrils alone and in the presence of studied Phy 7 and Phy 11 after 16 hrs incubation at 37 °C with 300 rpm shaking. All images are 10 × 10 µm with a scale bar representing 4 µm.

Acknowledgement. This work was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00039 and by research grants: Slovak Research and Development Agency under the Contract no. APVV-22-0598 and APVV-18-0284, the Grant Agency VEGA 02/0164/22, 02/0141/25, 01/0347/23, 1/0037/22.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Red Blood Cell Stiffness and Deformability as Biophysical Indicators of Chronic Lymphocytic Leukemia and Therapy Response

[a]A. LANGARI, [b]V. STRIJKOVA, [c]A. ALEXANDROVA-WATANABE, [a]M. IVANOVA, [b]V. KATROVA, [d]M. GUENOVA, [d]L. GARTCHEVA, [c]E. ABADJIEVA, [c]T. TIANKOV, [a]S. KRUMOVA, [a]S. TODINOVA

[a]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria
[b]Institute of Optical Materials and Technologies "Acad. Yordan Malinovski", Bulgarian Academy of Sciences, Sofia, Bulgaria
[c]Institute of Mechanics, Bulgarian Academy of Sciences, Sofia, Bulgaria
[d]National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

Chronic lymphocytic leukemia (CLL) is primarily characterized by the accumulation of abnormal B lymphocytes. However, growing evidence suggests that the disease also affects non-malignant blood cells, including red blood cells (RBCs). Alterations in RBC morphology, deformability, and mechanical properties may contribute to microcirculatory disturbances and tissue hypoxia in CLL, yet they remain underexplored.

In this study, we combine atomic force microscopy (AFM) and microfluidic flow analysis to assess changes in the biophysical properties of RBCs in CLL.

RBCs are isolated from healthy donors, untreated CLL patients, and CLL patients undergoing therapy with either Obinutuzumab/Venetoclax or Ibrutinib. AFM is used to assess cell morphology, membrane roughness (Rrms), and stiffness (elastic modulus, Ea). Microfluidic image flow analysis is employed to quantify RBC deformability based on the elongation index (EI) across a range of shear rates (89–625 s^{-1}).

AFM analysis reveals that RBCs from untreated CLL patients have significantly reduced membrane roughness and increased stiffness compared to healthy controls, along with a shift from the typical biconcave discocyte morphology to pathological forms (echinocytes and spherocytes). Obinutuzumab/Venetoclax therapy restores membrane roughness to levels approaching those of the controls, but the membrane stiffness remains unchanged. In contrast, Ibrutinib treatment normalizes both the roughness and the stiffness.

Consistent with the nanomechanical analysis, microfluidic measurements confirm that RBCs from untreated CLL patients exhibit significantly lower EI values at all shear rates, indicating poor deformability. RBCs from patients treated with Ibrutinib- show substantial recovery of deformability, with EI values approaching those of healthy controls at lower and intermediate shear rates. However, at high shear rates (535–625 s^{-1}), EI remains significantly reduced, suggesting incomplete recovery of membrane flexibility. Obinutuzumab/Venetoclax-treated patients are to be characterized in the future.

This study demonstrates that CLL is associated with profound biophysical alterations in RBCs, which are differently affected by Obinutuzumab/Venetoclax and Ibrutinib therapy. Thus, our findings highlight the importance of evaluating RBC stiffness and deformability as potential markers of disease severity and treatment efficacy.

Acknowledgment. This work was supported by Grant KP-06-H73/3, competition for financial support for basic research projects—2023, Bulgarian National Science Fund.
Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation, and by the Center for Competence in Mechatronics and Clean Technologies–MIRACle (No. BG16RFPR002-1.014-0019-C0)1, financed by the Program "Research, Innovation, and Digitalization for Smart Transformation" (PRIDST) 2021–2027.

Fluorescence study of the interaction between Silver (I), Zinc (II), and Gallium (III) thiophene-2-carboxylates and human serum albumin

[a]H. MATAJOVA, [b]M. RENDOSOVA, [b]Z. VARGOVA, [a]M. KOZURKOVA

[a]Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia
[b]Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

Human serum albumin (HSA) is the most abundant protein of blood plasma and plays an important role in the regulation of colloidal osmotic pressure. Due to its ligand-binding capacities, it is also responsible for being a transport protein for both endogenous (such as fatty acids, hormones, toxic metabolites (e.g. bilirubin), bile acids, amino acids, and metals) and exogenous substances (a wide variety of drug molecules) [1-5]. The binding property of albumin reduces the toxicity of many drugs and prolongs their half-life, as drugs compete for binding at specific sites. So albumin has significant role in the pharmacokinetics and pharmacodynamics of many drugs [6].

In this study, we investigated the binding properties of three 2-thiophenecarboxylate (Tio2c) complexes with different central metal atoms Ag(I), Zn(II) and Ga(III), $[Ag(Tio2c)]_2$ (AgTio2c), $\{[Zn_2(Tio2c)_4]_2\}_n$ (ZnTio2c) and $[Ga(Tio2c)_3]\cdot H_2O$ (GaTio2c) to HSA using fluorescence spectroscopy. Fluorescence quenching experiments were conducted at four different temperatures, and the binding constant (K_b), Stern–Volmer quenching constant (K_{SV}), and bimolecular quenching constant (K_q) were calculated for each complex. Based on the result, all complexes exhibited spontaneous binding with moderate affinity (K_b ~10^4 M^{-1}) to a single binding site on HSA. The K_{SV} values of AgTio2c, ZnTio2c, GaTio2c were recorded also in the order of 10^4 M^{-1}. AgTio2c exhibited dynamic quenching, while ZnTio2c showed static quenching behavior associated with ground-state complex formation. Thermodynamic parameters (ΔH, ΔS and ΔG) were also calculated and indicated that the binding process is endothermic and primarily driven by hydrophobic interactions. These findings suggest that these complexes can effectively bind to HSA, affecting their biological transport and activity. Among them, the AgTio2c complex appears to be the most suitable for biological applications. However, further testing is needed to determine its complete bioavailability.

Acknowledgment. This work was financially supported by the Scientific Grant Agency of Ministry of Education, Research, Development and Youth of the Slovak Republic (VEGA) under grant numbers 1/0268/24 and 1/0037/22. This support is gratefully acknowledged.

References

1. Yamasaki, K., Chuang, V. T. G., Maruyama, T., & Otagiri, M. (2013). Albumin–drug interaction and its clinical implication. Biochimica et Biophysica Acta (BBA)-General Subjects, 1830(12), 5435-5443.
2. Peters Jr, T. (1995). All about albumin: biochemistry, genetics, and medical applications. Academic press.
3. Fanali, G., Di Masi, A., Trezza, V., Marino, M., Fasano, M., & Ascenzi, P. (2012). Human serum albumin: from bench to bedside. Molecular aspects of medicine, 33(3), 209-290.
4. Kragh-Hansen, U., Chuang, V. T. G., & Otagiri, M. (2002). Practical aspects of the ligand-binding and enzymatic properties of human serum albumin. Biological and Pharmaceutical Bulletin, 25(6), 695-704.

5. Vallner, J. J. (1977). Binding of drugs by albumin plasma protein. Journal of pharmaceutical sciences, 66(4), 447-465.
6. Abbas, A., & Humma, Z. (2021). Clinical significance of albumin: structure, function and role in different pathophysiological states. International Journal of Applied Chemical and Biological Sciences, 2(1), 13-23.

Hsp70-SBD as a Therapeutic Modulator: Biophysical and Structural Perspectives in Light Chain Amyloidosis

M. NEMERGUT, M. TOMKOVA

Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

Light chain amyloidosis is a protein misfolding disorder characterized by the deposition of monoclonal immunoglobulin light chains as insoluble amyloid fibrils in tissues and organs, leading to progressive dysfunction. Current treatments mainly focus on reducing amyloidogenic light chain production through chemotherapy or monoclonal antibody therapies, yet effective methods to directly prevent or reverse amyloid fibril formation remain limited. The Hsp70 chaperone system plays a crucial role in protein homeostasis by assisting protein folding, preventing aggregation, and facilitating the degradation of misfolded proteins. This study aims to explore Hsp70 chaperone-based strategies to mitigate light chain aggregation and their potential for future therapeutic development. In the first step, we developed an effective method for producing soluble Hsp70-SBD in sufficient quantity and quality, allowing us to perform basic biophysical characterization, including anti-aggregation properties against the JTO light chain involved in multiple myeloma. We are now developing new variants with higher specificity and improved anti-aggregation properties using evolutionary techniques like yeast display.

Acknowledgment. This work is funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00116.

References

1. Sternke-Hoffmann, R., Pauly, T., Norrild, R. K., *et al.* (2023). Widespread amyloidogenicity potential of multiple myeloma patient-derived immunoglobulin light chains. BMC Biol., 21, 21.
2. Saibil, H. (2013). Chaperone machines for protein folding, unfolding and disaggregation. Nat. Rev. Mol. Cell Biol., 14, 630-642.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Role of proline-, cysteine-, and poly-L-lysine coated magnetic nanoparticles in amyloid aggregation

[a]O. PARMAR, [a]A. ANTOSOVA, [c,d]P. VERDUCCI, [c]E. BYSTRENOVA, [a]M. GANCAR, [b]I. ANTAL, [b]M. KUBOVCIKOVA, [a]Z. BEDNARIKOVA, [b]M. KONERACNA, [b]V. ZAVISOVA, [a]Z. GAZOVA

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[b]Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[c]Consiglio Nazionale Delle Ricerche, Istituto Per Lo Studio Dei Materiali Nanostrutturati (CNR-ISMN), Bologna, Italy
[d]Facolta Chimica e Tecnologia Farmaceutiche, Alma Mater Studiorum-Universita di Bologna, Italy

Proteins and peptides that aggregate into amyloid fibrils with a characteristic cross-β-sheet structure are the hallmarks of a group of diseases known as amyloidoses [1]. Magnetic nanoparticles (MNPs) could variously interfere with the amyloid aggregation of proteins depending on their physico-chemical properties, such as surface area, size, shape, charge, and concentration. Amino acid functionalization of MNPs surface provides a more extensive surface area to interact with macromolecules allowing them to be used as potential anti-amyloid agents [2].

In this study, we investigated the effect of magnetic nanoparticles (MNPs) functionalized with proline (Pro-MNPs), cysteine (Cys-MNPs), and poly-L-lysine (PLL-MNPs) on the fibrillization of α-lactalbumin (α-LA). α-LA, a small globular, acidic and Ca^{2+}-binding protein present in mammalian milk, can be used as a model protein for amyloid aggregation *in vitro*. Amino acid-functionalized nanoparticles (aa-MNPs) were synthesized using a precipitation method [3]. Their hydrodynamic diameter, zeta potential, and isoelectric point were characterized by dynamic light scattering and laser Doppler velocimetry. The effect of aa-MNPs on α-LA fibril formation and preformed fibrils was analyzed using Thioflavin T fluorescence assay and atomic force microscopy (AFM). Lactate dehydrogenase (LDH) assay was used to determine cytotoxicity of studied nanoparticles. The results demonstrated that aa-MNPs disaggregate preformed α-LA fibrils and inhibit fibril formation, depending on their concentration, size, surface charge, and specific surface area. Kinetic parameters of α-LA fibril formation - including lag time, half-time of aggregation, and aggregation rate constant - were determined from growth curves (Fig.1). All aa-MNPs caused a significant decrease in steady-state fluorescence intensities. The presence of Cys-MNPs and Pro-MNPs led to the shortening of the lag phase, on the other hand, PLL-MNPs prolonged the lag phase. Determined DC_{50} and IC_{50} values (concentration of aa-MNPs causing a 50 % decline in fluorescence intensities corresponding to 50 % fibrils destruction or inhibition of fibrils formation) suggest that Pro-MNPs has the strongest inhibitory effect on fibril formation (lowest IC_{50} value), while Cys-MNPs exhibited the highest disaggregation activity (lowest DC_{50} value). These findings were confirmed by AFM imaging. Samples from disaggregation experiments using Cys-MNPs showed small aggregates and free aa-MNPs, whereas fibrillization in presence of Pro-MNPs revealed significantly shorter fibrils compare the fibrils formed alone. Cytotoxicity, assessed by LDH assay and optical microscopy of

human kidney cells (HEK293) treated with aa-MNPs, indicated relatively high toxicity of PLL-MNPs and low toxicity of Pro-MNPs.

In summary, our results suggest that the coating of nanoparticles with studied amino-acids play a crucial role in influencing amyloid aggregation. These findings suggest diverse mechanisms of MNPs' anti-amyloid effect and help understand the impact of different surface modifications on protein amyloid aggregation. Our results indicate that Pro-MNPs effectively inhibit α-LA fibril formation while exhibiting low cytotoxicity, making them promising candidates for anti-amyloid therapeutics.

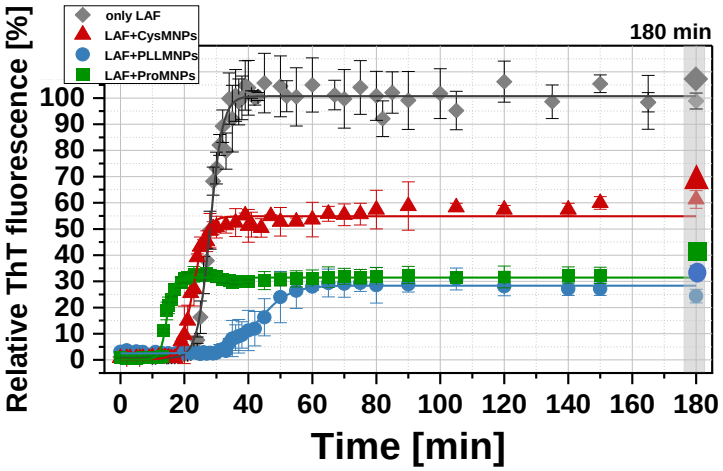


Fig. 1 Kinetic profiles of α-lactalbumin (α-LA) fibril formation alone (grey diamonds) and in the presence of aa-MNPs: Cys-MNPs (red triangles), PLL-MNPs (blue circles), and Pro-MNPs (green squares) (w/w ratio - α-LA: aa-MNPs = 1:1) measured using ThT assay. The error bars represent the average deviation of multiple measurements (n = 5). The curves were obtained by the Boltzmann sigmoidal function fit of the average experimental values.

Acknowledgement. This work was supported by the Slovak Science Grant Agency VEGA-Project No. VEGA-2/0141/25, and No. VEGA-02/0049/23. the Slovak Research and Development Agency under the Contract no. APVV-22–0598, the Operational Programme Integrated Infrastructure funded by the ERDF ITMS2014+ 313011T553 DIAGNAD.

References

1. Iadanza, M. G., Jackson, M. P., Hewitt, E. W., Ranson, N. A., & Radford, S. E. (2018). A new era for understanding amyloid structures and disease. Nature Reviews Molecular Cell Biology, 19(12), 755-773.
2. Antosova, A., *et al*. (2019). Destroying activity of glycine coated magnetic nanoparticles on lysozyme, α-lactalbumin, insulin and α-crystallin amyloid fibrils. Journal of Magnetism and Magnetic Materials, 471, 169-176.
3. Antosova, A., *et al*., (2024). Anti-amyloid activity of aminoacid functionalized magnetic nanoparticles on αLactalbumin aggregation. Nano-Structures & Nano-Objects, 40, 101413.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Non canonical 3WJ DNA motif – helical ligand interaction

D. PITKOVA, L. TRIZNA, V. VIGLASKY

Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

Non-canonical DNA motifs are interesting molecular targets for the scientific community due to their ability to regulate various physiological processes at the gene level and may serve as building blocks in the development of biodegradable nucleic acid-based nanostructures [1]. There are some specific DNA sequences that have the potential to accept so-called three-way junctions (3WJs). These branched structures consist of three double-stranded chains forming a central cavity, have a threefold symmetry with an almost flat pyramidal arrangement. The specific binding of helical ligands to the central cavity of the 3WJ has the potential to dynamically regulate the physical and chemical properties of such nanoparticles. The $[M_2L_3]^{4+}$ type ligands used in our work bind non-covalently to the hydrophobic DNA cavity of 3WJ [2]. We analyzed the effect of helical ligands on the topology and stability of DNA 3WJ motifs using electrophoretic and spectral methods, and in addition we also monitored their overall denaturation profile using the TGGE method.

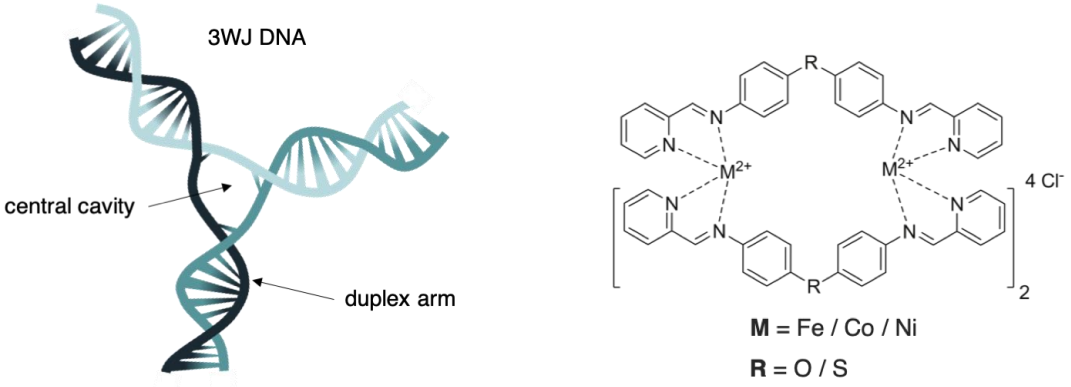


Fig. 1 Structure of helical ligands used in our work.

Acknowledgment: This work was supported by Grant Agency of The Ministry of Education, Research, Development and Youth of the Slovak Republic VEGA 1/0347/23.

References

1. Kaushik, M., Kaushik, S., Roy, K., Singh, A., Mahendru, S., Kumar, M., Chaudhary, S., Ahmed, S., & Kukreti, S. (2016). A bouquet of DNA structures: Emerging diversity. Biochemistry and Biophysics Reports, 5, 388-395.
2. Malina, J., Kostrhunova, H., Scott, P., & Brabec, V. (2023). Metallohelices stabilize DNA three-way junctions and induce DNA damage in cancer cells. Nucleic Acids Research, 51(14), 7174-7183.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Structural characterization of the amyloid-forming protein RIPK1

[a, b]P. POLONIO, [c, d]J. P. LOPEZ-ALONSO, [c]H. JIANG, [a]S. ANDRES-CAMPOS, [a]F. ESCOBEDO, [a]G. TITAUX, [c, e]I. UBARRETXENA-BELANDIA, [a]M. MOMPEAN

[a]*Institute of Physical Chemistry "Blas Cabrera" (IQF-CSIC), Madrid, Spain*
[b]*Autonomous University of Madrid, Doctoral School, Madrid, Spain*
[c]*Institute of Biophysics (UPV/EHU, CSIC), Leioa, Spain*
[d] *Basque Resource for Electron Microscopy, Leioa, Spain*
[e] *Ikerbasque Foundation for Science, Bilbao, Spain*

In nature, various functional amyloids have evolved to carry out essential biological processes. Their assembly conformations result from selective evolutionary pressure to optimize structure for function.

An example of functional amyloid involvement can be found in necroptotic signaling pathways, where distinct homomeric and heteromeric assemblies mediate signal transduction and execution. Upon activation of canonical necroptosis, receptor-interacting protein kinase 1 (RIPK1) self-associates and becomes activated, subsequently recruiting RIPK3 monomers to form a heteromeric complex known as the "necrosome". This complex serves as a nucleation seed for further homomeric RIPK3 amyloid assembly. The resulting RIPK3 fibrils then act as a scaffold for the oligomerization of the pseudokinase MLKL, which ultimately executes necroptosis through membrane disruption. A similar mechanism operates in non-canonical necroptotic pathways, where RIPK1 engages in alternative heteromeric complexes—such as with Z-DNA binding protein 1 (ZBP1) or TIR-domain-containing adaptor-inducing interferon-β (TRIF)—that also lead to RIPK3 amyloid formation and MLKL-mediated membrane permeabilization [1].

All amyloidogenic interactions within these pathways rely on a short amyloid-forming sequence known as the RIP homotypic interaction motif (RHIM). While the structural features of the RHIM have been elucidated for RIPK3 in both human and mouse, the corresponding region in human RIPK1 remains uncharacterized. To address this gap, we aimed to determine the structure of the human RIPK1 RHIM in order to better understand the molecular basis of RHIM-mediated specificity in signaling complexes.

To this end, we employed an integrative approach combining solid-state nuclear magnetic resonance (SSNMR) and cryo-electron microscopy (cryo-EM). SSNMR measurements on isotopically diluted fibrils—fibrils made of U-^{13}C,^{15}N RIPK1 mixed with an excess of non-labeled RIPK1—revealed the local fold of monomeric layers within the fibril identifing side-chain conformations at atomic resolution (Fig. 1A). In parallel, cryo-EM imaging of multiple fibril orientations enabled the resolution of the macromolecular architecture, including the symmetry, helical twist, and axial rise of the stacked protomers within the fibril (Fig. 1B) [2].

As a result, we resolved a high-resolution structure of the assembled RIPK1 RHIM, revealing interprotomer interactions that appear critical for monomer incorporation and fibril stabilization. To validate these findings, we introduced single-amino-acid mutations and demonstrated that individual residues can modulate both homomeric and heteromeric RHIM assemblies, providing insight into the specificity of these interactions. Based on our structural and mutational data, we propose a mechanism by which the ordered assembly of RIPK1 enables RIPK3 nucleation during canonical necroptosis [2].

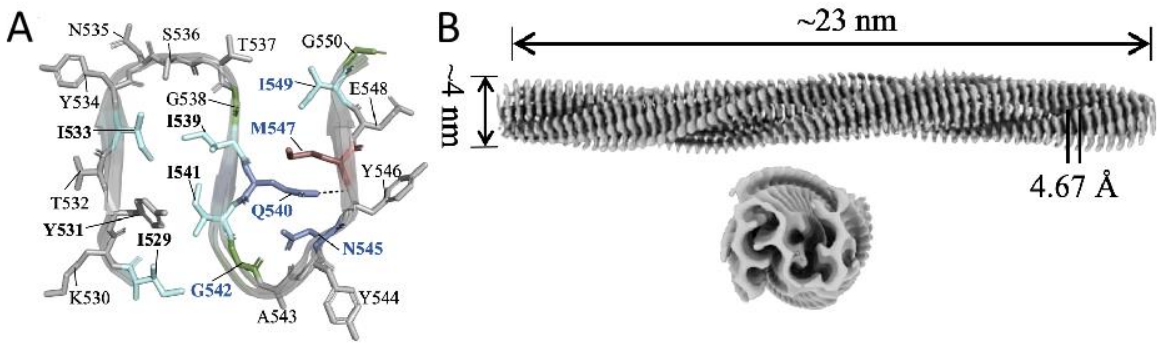


Fig. 1 Resolved structures of human RIPK1 RHIM fibrils. **(A)** SSNMR obtained model of hRIPK1 protomer showing a core of three beta-strands, residues labeled. **(B)** Maps resulted from cryo-EM characterization of hRIPK1 fibrils showing pitch, rise and widht parameters. Adapted from ref 2.

Acknowledgment. This work is funded by the European Union (ERC, 101042403 – BiFOLDOME, to M.M). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them. NMR experiments were performed in the "Manuel Rico" NMR Laboratory (LMR) of the Spanish National Research Council (CSIC), a node of the Spanish Large-Scale National Facility (ICTS R-LRB). G.T.A.-D. and P.P. acknowledge FJC2021-047976-I fellowships funded by MCIN/AEI/ 10.13039/501100011033 and European Union Next Generation EU/PRTR, and PIPF-2022/BIO-25611 funded by the Dept. of Education, Science and Universities from the Community of Madrid, respectively. This work has been supported by grant PID2022-143177NB-I00 to I. U.-B from the Spanish Ministry of Science, Innovation and Universities. Cryo-EM was performed at the Basque Resource for Electron Microscopy located at Instituto Biofisika (UPV/EHU, CSIC), supported by the Department of Science, Universities and Innovation and the Innovation Fund of the Basque Government, with additional support from MCIN (Recovery, Transformation and Resilience Plan) and the Basque Government "Biotechnology Complementary Plan Applied to Health" with funding from European Union NextGenerationEU (PRTR-C17.I1; PRTR-C17.I01.P01.S13) (AAAA_ACG_AY_2539/22_05).

References

1. Clucas, J., & Meier, P. (2023). Roles of RIPK1 as a stress sentinel coordinating cell survival and immunogenic cell death. Nature Reviews Molecular Cell Biology, 24(11), 835-852.
2. Polonio, P., Lopez-Alonso, J. P., Jiang, H., Escobedo, F., Titaux, G., Ubarretxena-Belandia, I., & Mompean, M. (2025). Structural Basis for the Assembly of Amyloid Fibrils by the Master Cell-Signaling Regulator Human Receptor-Interacting Protein Kinase 1. Biophysics.

Exciton and electron transfer in photosynthetic complex

M. PUDLAK

Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

Purple phototropic bacteria's photosynthetic complex transports light and electrons with ultrafast speed, and transforms light into chemical energy in reaction centers with high efficiency. We show that electrons are directed by the vibration modes into the needed site where they are localised [1]. We describe the exciton transport between the light-harvesting complex LH1 and the reaction center dimer. We also describe the hot electron transfer (ET). This type of ET can be used in the bacterial reaction centers. The electron-hole separation is more efficient in this case. The result is high productivity of chemical energy by solar energy in biological systems [2].

Acknowledgment. This work was supported by MVTS project CA21146.

References

1. Pudlak, M., & Pincak, R. (2023). Photosynthetic Complex: Exciton transfer and Electron-Hole Separation Quantum Yields. J. Phys. Chem. A, 127, 5795-5804.
2. Pudlak, M. (2023). Impact of the unrelaxated vibrational modes on hot-electron transfer. J. Chem. Phys., 159, 244105.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Biogenic Magnetosome-mediated disassembly of protein amyloid fibrils triggered by ultrasound and magnetic field

[a]M. ROMANOVOVA, [b]M. MOLCAN, [c]R. BIELAS, [c]F. RATAJCZAK, [a]V. FEDOROVA, [c]A. JOZEFCZAK, [a]K. SIPOSOVA

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[b]Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[c]Department of Acoustics, Faculty of Physics and Astronomy, Adam Mickiewicz University, Poznan, Poland

Amyloid fibrils, highly ordered protein aggregates formed by insulin and lysozyme, are implicated in neurodegenerative and metabolic diseases due to their exceptional stability and resistance to enzymatic degradation. Although considerable advances have been made in deciphering the molecular underpinnings of amyloidogenesis and in developing novel anti-amyloid therapeutics, a definitive and universally effective cure remains elusive. A diverse array of strategies has been investigated to inhibit fibril formation, induce fibril disassembly, or enhance their clearance, among which nanotechnology-driven platforms employing nanoparticles have garnered significant attention.

In this study, we investigated the potential of naturally occurring biogenic magnetosomes, Fe_3O_4 nanocrystals enveloped by a phospholipid membrane and organized in chain-like structures to promote the disassembly of amyloid fibrils. Using Thioflavin T fluorescence assays and atomic force microscopy, we assessed fibril degradation under various conditions, including passive incubation with magnetosomes and stimulation by ultrasound (US) and alternating magnetic fields (AMF). Magnetosomes alone induced concentration-dependent disruption of fibrils, but combined US and AMF treatment markedly enhanced disassembly, even at low nanoparticle doses (Fig.1). This synergistic effect likely arises from localized heating via magnetic hyperthermia, mechanical stress from magneto-mechanical motion, and ultrasound-induced acoustic streaming and cavitation, which together destabilize the stable cross-β sheet network. Notably, combined treatment reduced the magnetosome concentration required for 50% disassembly of fibrils more than a six-fold enhancement.

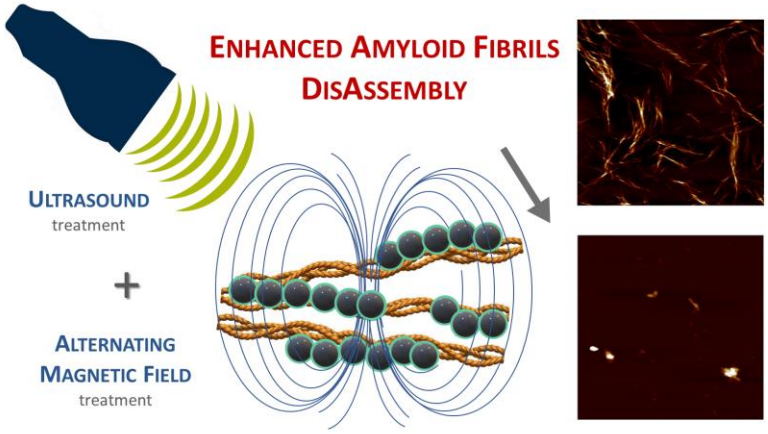


Fig. 1 Schematic presentation of Stimuli-Responsive Disassembly of Amyloid Fibrils by Biogenic Magnetosomes under Ultrasound and Magnetic Fields

These findings demonstrate that biogenic magnetosomes, when combined with physical stimuli, provide a powerful, non-pharmacological approach for amyloid clearance. This strategy may offer new opportunities for therapeutic intervention in amyloid-related disorders, particularly where conventional treatments are limited, and highlights the potential of nanoparticle-assisted physical methods for future biomedical applications.

Acknowledgment. This work was supported by research grants VEGA 2/0034/22, 2/0028/25, MVTS SK-TW Supra-Sight and by the "PhytoAPP" EU framework (2021-2026). The PhytoAPP Project has received funding from the European Union's Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie Grant Agreement No.101007642.

Beyond Oxidative Stress: Cytochrome c in Alzheimer's Pathogenesis

K. SIPOSOVA, E. VALUSOVA, V. FEDOROVA, A. MUSATOV

Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia

The etiology of Alzheimer's disease (AD) is highly complex, and the molecular determinants driving its onset and progression remain only partially elucidated. Among the proposed mechanisms, oxidative stress is considered a central contributor, not only through induction of lipid peroxidation and membrane destabilization, but also by promoting amyloid-β (Aβ) production via β-secretase activation. These events may establish a self-perpetuating cycle of Aβ accumulation and oxidative injury, driven by reciprocal interactions between Aβ peptides and cellular membranes. Within this pathogenic framework, cytochrome *c* (Cyt *c*) emerges as a protein of particular interest due to its diverse biological functions, including a critical role in redox homeostasis. In this study, we investigated the impact of Cyt *c* on $A\beta_{(1-40)}$ aggregation dynamics, fibril stability, and morphological features. Our findings reveal a pronounced anti-amyloidogenic activity of Cyt *c*, with lysine residues identified as key mediators of this effect. To substantiate these observations, we extended our analyses to apo-Cyt *c*, free lysine, and poly-lysine derivatives. In summary, these results highlight Cyt *c* as a potential modulator of Aβ-associated pathology and underscore its broader relevance in the molecular landscape of AD pathogenesis.

Acknowledgment: This work was supported by research grants VEGA 2/0034/22, APVV23-0013 and MVTS SK-TW Supra-Sight and by the "PhytoAPP" EU framework (2021-2026). The PhytoAPP Project has received funding from the European Union's Horizon 2020 Research and Innovation programme under the Marie Skłodowska-Curie Grant Agreement No.101007642.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Development of protocol for covalent functionalization of β-casein micelles with DNA aptamers for potential targeted delivery

[a]V. SUBJAKOVA, [b]M. GARAIOVA, [b]R. HOLIC, [a]T. HIANIK, [a]Z. GARAIOVA

[a]*Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia*
[b]*Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia*

Casein micelles, naturally occurring nanostructures derived from milk proteins, have gained significant interest in biomedical applications due to their biodegradability, biocompatibility and ability to encapsulate therapeutics as potential nanocarriers in drug delivery. They possess amphiphilic properties that are suitable for encapsulation of hydrophobic as well for hydrophilic therapeutics [1]. Caseins with their carboxyl groups) (from aspartate/glutamate residues) provides reactive sites for modification with targeting ligands, such as nucleic acid aptamers. DNA/RNA aptamers are single-stranded oligonucleotides with high specificity and affinity for certain molecules, making them attractive candidates in biosensing and targeted cancer therapy [2]. Their covalent crosslinking onto casein nanostructures could enhance the selective delivery of therapeutic agents to cancer cells, thereby improving treatment efficacy and reducing side effects.

In this study, we focused on the sgc8c aptamer, which specifically binds to the protein tyrosine kinase-7 (PTK7), a biomarker overexpressed on the surface of certain cancer cells [3]. The conjugation of this aptamer (10 mM) to β-casein (2 mg/ml) was performed through EDC/NHS-mediated coupling reaction with concentrations 2 mM/5 mM and 20 mM/50 mM, respectively at the condition: 4h at 25°C or 24h at 4°C (Fig. 1). EDC/NHS conjugation is a widely used method for covalently linking molecules, through the formation of amide bonds between carboxyl groups and amino groups.

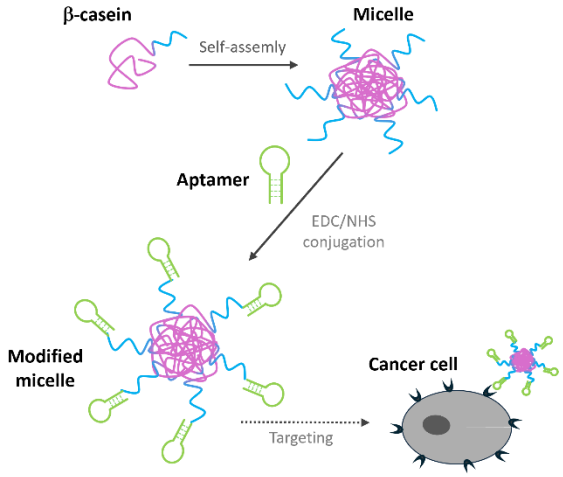


Fig. 2 Schematic illustration self-assembly of b-casein micelles and covalent conjugation with DNA aptamer via carbodiimide chemistry using EDC/NHS coupling agents for as potential drug delivery cancer targeting system.

The reaction time, temperature, and ratio of EDC/NHS to reactants need to be optimized for different applications. Samples were examined by dynamic light scattering (DLS) and agarose gel electrophoresis. DLS measurements revealed an increased hydrodynamic diameter Z-average (nm) following EDC/NHS treatment, while the polydispersity index (PDI) decreased, particularly at higher concentrations for both reaction conditions (4 h at 25°C and 24 h at 4°C). This is due to mutual crosslinking within the micelles and between the micelles. Individual micelles may get covalently bonded into larger units which are more rigid [4]. Crosslinking creates a more uniform size distribution resulting in lower PDI. The presence of DNA aptamers did not significantly affect the hydrodynamic size. However, some β-casein–aptamer conjugates exhibit retarded migration in agarose gel electrophoresis, while others migrate freely, indicate successful conjugation alongside the presence of unbound aptamers. This highlights the need for optimization and purification to enhance the specificity and efficacy of these conjugates for further applications in targeted cancer therapy.

Acknowledgment. This work was supported by research grant of VEGA agency (Project No. 1/0554/23).

References

1. Sadiq, U., Gill, H., & Chandrapala, J. (2021). Casein micelles as an emerging delivery system for bioactive food components. Foods, 10(8).
2. Ning, Y., Hu, J., & Lu, F. (2020). Aptamers used for biosensors and targeted therapy. Biomedicine & pharmacotherapy, 132, 110902.
3. Sicco, E., Baez, J., Ibarra, M., Fernandez, M., Cabral, P., Moreno, M., Cerecetto, H., & Calzada, V. (2020). Sgc8-c Aptamer as a Potential Theranostic Agent for Hemato-Oncological Malignancies. Cancer biotherapy & radiopharmaceuticals, 35(4), 262-270.
4. Huang, G. P., Shanmugasundaram, S., Masih, P., Pandya, D., Amara, S., Collins, G., & Arinzeh, T. L. (2015). An investigation of common crosslinking agents on the stability of electrospun collagen scaffolds. Journal of biomedical materials research. Part A, 103(2), 762-771.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Directed Evolution in Action: Engineering Haloalkane Dehalogenase Variants for Enhanced Activity

I. TIMKOVA, M. NEMERGUT, E. SEDLAK

Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia

Halogenated aliphatic compounds, with chlorinated derivatives being particularly prevalent, constitute a structurally diverse class of organic pollutants characterized by their notable environmental persistence and pronounced toxicity. Haloalkane dehalogenases (HLDs) represent a highly promising class of enzymes capable of catalyzing the dehalogenation of haloalkanes, thereby facilitating their enzymatic detoxification. Consequently, HLDs are integral to bioremediation strategies aimed at mitigating the ecological impact of these recalcitrant contaminants [1]. Haloalkane dehalogenases are hydrolytic enzymes, which catalyze the cleavage of the halogenated compounds. HLDs belong to the α/β-hydrolase superfamily and are composed of two domains – α/β-hydrolase domain and the cap domain (Fig.1). The active site is in the cavity at the interface of the domains and is composed of the catalytic pentad of the amino acids and two access tunnels [2].

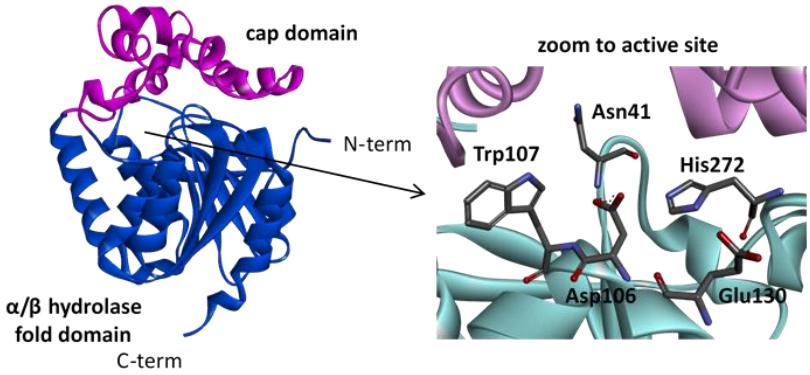


Fig. 1 Tertiary structure of HLD DhaA and topology of catalytic pentad inside the active site of DhaA [PDB: 4HZG].

We exploit Halo Tag technology in selection of HLDs, which are represented by enrichment of the genes encoding HLDs using ribosome display (RD). After the 4th round of the RD, we obtained variants of input DhaA115 protein, RD4-32 and RD4-37. Circular dichroism and differential scanning calorimetry showed that evolved variants are properly folded and stable, even the thermal stability of both DhaA variants is decreased. On the other hand, analysis of their catalytic activities showed that RD4-37 variant possesses increased catalytic activity in comparison with DhaA115 protein. Preliminary structural analysis suggests that the observed changes in activities of evolved variants are caused by the mutations in the access tunnels.

Acknowledgment. This work was supported by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00112 and 09-I02-03-V01-00021 and by the grant agency of the Ministry of Education, Science, Research, and Sport of the Slovak Republic (grant no. VEGA 1/0074/22).

References

1. Janssen, D. B. (2004). Evolving haloalkane dehalogenases. Curr. Opin. Chem. Biol., 8(2), 150-159.
2. Hasan, K. (2018). A mini review of haloalkane dehalogenase: From molecular characterization to applications. Communications in Science and Technology, 3(1), 15-18.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

In Vitro Characterization of the Interaction between Conventional and Novel Bruton's Tyrosine Kinase Inhibitors and Human Red Blood Cells via Atomic Force Microscopy and Biofluidic Analysis: A Pilot Study

[a]S. TODINOVA, [b]A. ALEXANDROVA-WATANABE, [c]V. STRIJKOVA, [a]A. LANGARI, [d]N. ANASTASSOVA, [d]M. ARGIROVA, [d]D. YANCHEVA, [a]M. IVANOVA, [c]V. KATROVA, [b]E. ABADJIEVA, [b]T. TIANKOV, [e]E. STOYANOVA, [a]S. KRUMOVA, [d]M. GUNCHEVA

[a]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria
[b]Institute of Mechanics, Bulgarian Academy of Sciences, Sofia, Bulgaria
[c]Institute of Optical Materials and Technologies "Acad. Yordan Malinovski", Bulgarian Academy of Sciences, Sofia, Bulgaria
[d]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[e]Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria

Chronic lymphocytic leukemia (CLL) remains an incurable disease despite advances in conventional chemotherapy. Recently, novel targeted therapies, particularly those inhibiting Bruton's tyrosine kinase (BTK), have shown promise in improving patient outcomes. While these agents have been widely studied for their antitumor efficacy, their effects on red blood cells (RBCs) remain poorly understood.

This study investigates the *in vitro* effects of the conventional BTK inhibitor - Ibrutinib (IBR) and two newly synthesized BTK inhibitors, namely 2-(2-hydroxy-3-methoxybenzylidene)-1-(1H-benzimidazol-2-yl)hydrazine (**compound 1**) and 3-(2-amino-1H-benzo[d]imidazol-1-yl)-N'-(2-hydroxy-4-methoxybenzylidene)propanehydrazide (**compound 2**) on the nanomechanical and rheological properties of RBCs from healthy donors via atomic force microscopy (AFM) and a microfluidic system (BioFlux). Among a series of N-substituted 1H-benzimidazole-2-yl hydrazone derivatives, compounds **1** and **2** demonstrated strong antiproliferative activity against HG-3 cells, a model for chronic lymphocytic leukemia. Their respective half-maximal inhibitory concentrations (IC_{50}) were 17.6 µM for IBR, 3.42 µM for **1**, and 6.67 µM for **2**. Notably, at a concentration of 20 nM, compound **1** exhibited twice stronger the *in vitro* inhibitory activity against BTK compared to IBR.

Atomic force microscopy revealed that untreated RBCs exhibited an average membrane roughness (Rrms) of 4.09 ± 1.2 nm and Young's modulus value (Ea) of 441 ± 46 kPa. Treatment with IBR resulted in dose-dependent decreases in membrane roughness and increased membrane stiffness (Young's modulus), suggesting cytoskeletal disruption. In contrast, the newly synthesized compounds **2** and **1** caused less pronounced effects on membrane roughness and elasticity compared to IBR. Compound 2 showed a reduction of Rrms at higher concentrations, while **1** exerted a significant effect only at 12.5 µM, indicating a dose-specific interaction. Young's modulus measurements revealed that **2** and **1** significantly reduced membrane stiffness at concentrations of 12.5 and 25 µM, suggesting a softening effect on the RBC membrane, likely due to alterations in the underlying cytoskeletal structure.

Microfluidic analysis revealed that Ibrutinib caused dose-dependent changes in RBC aggregation behavior. Initial disaggregation occurred at a low concentration of 6.25 µM, followed by partial recovery at 12.5 µM. At higher doses (25 µM), IBR promoted the formation of fewer, but denser aggregates and clusters. Compound **2** induced more stable but fewer aggregates, while **1** showed mild effects without clear dose dependency, indicating a minimal impact on membrane adhesive interactions.

This pilot study demonstrates that the observed biophysical alterations reflect differential impacts on cytoskeletal organization and membrane–protein interactions among the tested compounds. Our data suggest that **2** and **1** exert a lower hemotoxic effect than Ibrutinib, supporting their potential as safer therapeutic alternatives in targeting BTK while minimizing erythrocyte-related side effects. RBC biophysical properties - membrane roughness, stiffness, and aggregation degree emerge as sensitive indicators of drug-induced changes and can serve as effective biomarkers of cytoskeletal integrity and membrane remodeling.

Acknowledgment. This work was supported by Grant KP-06-H73/3, competition for financial support for basic research projects—2023, Bulgarian National Science Fund. Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science was used in this investigation and by the Center for Competence in Mechatronics and Clean Technologies–MIRACle (No. BG16RFPR002-1.014-0019-C0)1, financed by the Program "Research, Innovation, and Digitalization for Smart Transformation" (PRIDST) 2021–2027.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Motion Analysis of Shrimps and Microrobots: Insights into Active Matter Behavior

[a]Z. TOMORI, [b]A. STREJCKOVA, [c]R. GALAJDA, [a]C. SLABY, [d]D. HORVATH, [e]G. BANO

[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*Department of Chemistry, Biochemistry & Biophysics, University of Veterinary Medicine & Pharmacy, Kosice, Slovakia*
[c]*Department of Biology &Physiology, University of Veterinary Medicine &Pharmacy, Kosice, Slovakia*
[d]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Kosice, Slovakia*
[e]*Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia*

Active-matter systems consist of “active particles,” with self-propulsion. Their grouping is subject of many studies (colloidal systems, animals, human crowds etc.) where similar behavioral rules were found. Our research interest is focused in study of models valid for both – living animals and microrobots [1, 2]. The model input is the position and orientation of active particles during experiment captured by a camera and recorded as video file. In this contribution we describe the experimental setup and the video analysis software related to both – microrobots (hexbugs) and living animals (shrimps).

Video analysis of microrobots. We exploited small (4.4 cm length) toy robot “Hexbug nano” supplied with pair of 6 rubbery legs that move the robot forward by vibrating electric motor. Due to this simple and low-cost construction is hexbug popular also among physicists as a model of active matter. Experimental setup consists of measuring area encircled by permanent magnets that push away the magnet glued on to top of hexbug. The excentricity of loading on the top of hexbug has influence to its trajectory [3] and can be exploited for its correction.

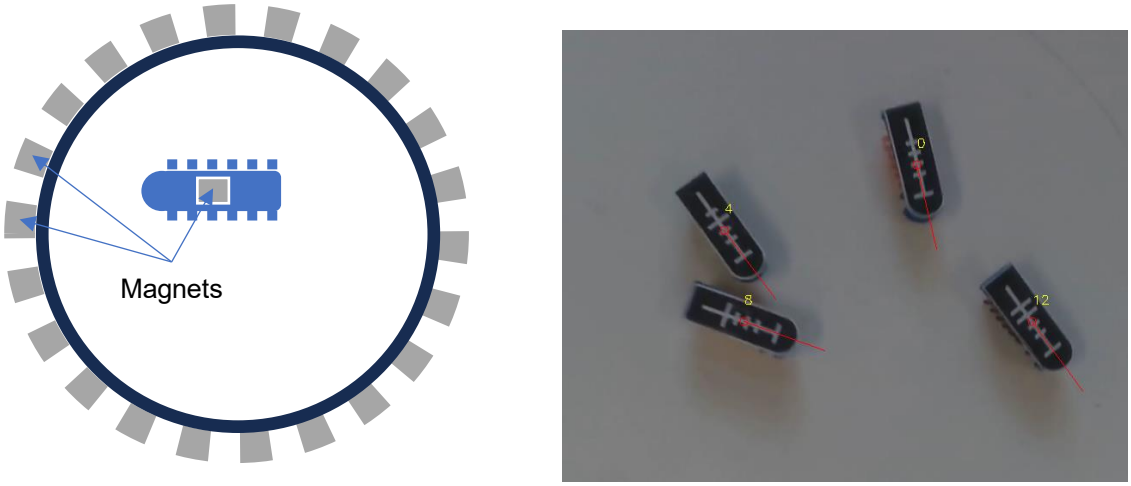


Fig. 1 Experimental setup (left), detected positions, orientation and ID (right).

In video analysis software, each frame from video file is initialy transformed into binary image by comparing pixel value with given threshold. Subsequently a hierarchical contours detection is applied where only inner contours are detected (white drawings on the black hexbug body). From such contours we compute the positions and main axis orientations of all hexbugs. The bars perpendicular to the main axis can serve as a "bar code" to get unique ID for each hexbug (see Fig. 1 right). Software is based on C++ version of OpenCV library using Visual Studio 2022 environment.

Video analysis of shrimps' movement. Experimental animals (1-4 shrimps) are placed into a circular Petri dish illuminated by a homogeneous light from the bottom. Camera with resolution 1920x1080 records 10 min. video from each experiment. The sophisticated software „idtracker.ai" [4] performs analysis based on the machine learning. Initially, the selected video images where segmented to create a training set for a model. After training, the model is able to find a position of shrimps on the whole video including crossing ones (see Fig. 2). We extended existing software by adding modules for computing of orientation (angle 0-360 degrees) and interpolation of angles in positions where angle is not possible to find. Interactive enter of missing or manual correction of wrongly detected values is possible. We have also developed modules for video stabilization and replay showing resulting (x, y, angle) values along with video. Our modules were created in C++/winrt using Visual Studio development environment.

Fig. 2 Crossing shrimps

Acknowledgment. This work was supported by research grants APVV-21-0333, VEGA 2/0055/25.

References

1. Horvath, D., Slaby, C., Tomori, Z., Hovan, A., Miskovsky, P., & Bano, G. (2023). Bouncing dynamics of inertial self-propelled particles reveals directional asymmetry. Phys. Rev. E, 107(2-1), 024603.
2. Bano, G., Slaby, C., Strejckova, A., Tomori, Z., Hovan, A., Miskovsky, P., & Horvath, D. (2024). Controlled stigmergy in quasi-one-dimensional active particle systems. Phys. Rev. E, 110(2-1), 024605.
3. Barona Balda, A., Argun, A., Callegari, A., & Volpe, G. (2024). Playing with active matter. American Journal of Physics, 92(11), 847-858.
4. Romero-Ferrero, F., Bergomi, M. G., Hinz, R. C., *et al.* (2019). Idtracker.ai: tracking all individuals in small or large collectives of unmarked animals. Nature Methods, 16, 179-182.

Small Au25 -based probe for enhanced infrared sensing of cytochrome c

[a]E. VALUSOVA, [a]V. FEDOROVA, [a]K. SIPOSOVA, [b]M. ANTALIK

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[b]Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

Infrared spectroscopy is an important and widely used analytical tool in the fields of biology, chemistry, pharmacy and medicine. Dipole-active modes can offer key information on the structural and conformational properties of small molecules but also larger structures such as proteins. Since protein absorption in the infrared range is relatively low, large quantities of protein are typically required for analysis. One way to overcome this issue is the use of surface enhanced infrared absorption spectroscopy (SEIRA). As a rule, local electrical fields are strongly enhanced by plasmonic effects on rough or patterned metal surfaces. Conventional processes of manufacturing sensors with different spot areas are extremely costly [1] and cause difficulties with SEIRA [2]. Some nanostructures are currenly designed as gold nanoparticles with a protecting layer.

Gold nanoparticles of the extremely small size regime (≤ 3 nm) undergo remarkable changes in the atom packing structure and in their optical properties. These atomically precise nanoclusters induce a breakup of the continuous energy levels into discrete energy levels [3], but these unique molecular-like properties are highly dependent on the size and structure of the clusters [4]. Due to their enhanced optical and biocompatibility properties and the ease with which the structures can be produced, these ultrasmall nanoparticles (nanoclusters) are attracting growing interest in research and seeing use in a variety of bio-applications [4]. Among the great superiorities of nanoclusters, the family of atomically precise thiolate-protected gold $Au_{25}(SR)_{18}$ nanoclusters has been studied for potential applications in the fields of sensing, cancer therapy and catalysis [5].

In this study, we describe a simple approach for the preparation of complexes consisting of $Au_{25}(SG)_{18}$ and iron-containing proteins through variable degrees of non-covalent interactions. The cytochrome *c* (cyt *c*) was found to be the most attractive iron-containing protein due to its widely reported multiple functions and catalytic activities [6]. When is adsorbed on glutathione-coated Au_{25} nanoclusters, the broadband enhanced detection of infrared vibrational signatures of cytochrome *c* was performed within a matter of minutes. The structure of the Au_{25} nanosensor also eliminates the need for complicated immobilization procedures or lengthy processes with aptamers. The Au_{25} sensor was also found to possess the capacity to selectively distinguish iron-containing proteins from non-iron containing models. The infrared SEIRA technique therefore constitutes a viable alternative to existing approaches. On this basis, the $Au_{25}(SG)_{18}$ sensor offers considerable promise as a plattform for monitoring and assessing cytochrome *c* in future applications.

Acknowledgment. This work was supported by research grants VEGA 2/0034/22, APVV23-0013 and MVTS SK-TW Supra-Sight.

References

1. Enders, D., & Pucci, A., (2006). Surface enhanced infrared absorption of octadecanethiiol on wet-chemically prepared Au nanoparticle films. Applied Physics Letters, 88, 184104.
2. Hoffmann, J. M., Xin, X., Richter, J., Hartung, A., Ma, T. W. W., & Taubner, T. (2013). Low-cost infrared resonant structures for surface-enhanced infrared absorption spectroscopy in the fingerprint region from 3 to 13 μm. Journal of Physical Chemistry C, 117, 11311-11316.
3. Maity, S., Bain, D., & Patra, A. (2019). An overview on the current understanding of the photophysical properties of metal nanoclusters and their potential applications. Nanoscale 11, 22685-22723.
4. Hossain, S., Hirayama, D., Ikeda, A., Ishimi, M., Funaki, S., Samanta, A., Kawawaki, T., & Negishi, Y. (2023). Atomically precise thiolate-protected gold nanoclusters. Current status of designabiility of the structure and physicochemical properties. Aggregate 4, e255.
5. Luo, D., Wang, X., Zeng, S., Ramamurthy, G., Burda, C., & Basilion, J. P. (2019). Targeted gold nanocluster-enhanced radiotherapy of prostate cancer. Small 15, 1900968.
6. Kalpage, H. A., Bazylianska, V., Recanali, M. A., Fite, A., Liu, J., Wan, J., Mantena, N., Malek, M. H., Podgorski, I., Heath, E. I., Vaishnay, A., Edwards, B. F., Grossman, L. I., Sandarson, T. H., Lee, J., & Hüttemann, M. (2019). Tissue-specific regulation of cytochrome c by post-translational modifications: respiration, the mitochondrial membrane potential, ROS, and apoptosis. FASEB Jpurnal, 33, 1540-1553.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Detection of changes in DNA secondary structure caused by exposure to the conazole fungicide prothioconazole

[a]V. VEREBOVA, [a, b]J. STANICOVA

[a]Department of Chemistry, Biochemistry & Biophysics, University of Veterinary Medicine & Pharmacy, Kosice, Slovakia
[b]Institute of Biophysics & Informatics, First Faculty of Medicine, Charles University, Prague, Czech Republic

Prothioconazole (PTC) is a broad-spectrum fungicide used to protect canola, mustard, poppy, rice, and cereals [1]. Together with tebuconazole, it is the main active substance of the new fungicide formulation Tilmor®. It provides plants with excellent protection against fungal diseases. It blocks ergosterol biosynthesis, thereby preventing the formation of cell membranes of the pathogen. It differs from other azole active substances in that it affects the pathogen at multiple sites, the onset of action is very rapid and it is distributed acropetally into the plant, thus providing plants with long-term fungicidal protection [2]. Despite the above positive effects on target organisms, PTC is also characterized by adverse effects on non-target organisms [3]. These include nausea, endocrine disruption [4], and elevated levels of reactive oxygen species, which can result in lipid peroxidation, tissue destruction, DNA damage [5], some adverse cardiovascular effects, and other physiological disorders [6]. Consequently, analysis of the impact of PTC on the structure and stability of the DNA molecule can provide valuable insights for a comprehensive understanding of the potential risks posed by the fungicide PTC.

The interaction of PTC with calf thymus DNA was investigated by spectroscopic methods (absorption and fluorescence spectroscopy) supplemented by viscosity measurements and by studying the changes in thermodynamic parameters in the DNA denaturation process. By adding DNA to the PTC solution, a slight decrease in absorbance intensity was observed in the region of the triazole heterocycle of PTC (at 204 nm). On the other hand, there is an increase in absorbance in the region of 240 nm in the presence of DNA, due to which we assumed that PTC interacts with the DNA macromolecule. Fluorescence measurements were accompanied by quenching of PTC fluorescence by increasing DNA concentration. In addition to a significant decrease in emission intensity, a bathochromic shift of the maximum emission wavelength by 3 nm was also observed. We also detected the appearance of a new peak at 370 nm when PTC interacted with DNA, which is likely due to the existence of unbound DNA, and this phenomenon is the result of nonradiative energy transfer between PTC and DNA [7]. The binding constant between DNA and the pesticide PTC determined by fluorescence spectroscopy was 4.8×10^4 L/mol, excluding the intercalation mode of interaction. Similarly, viscosity measurements did not confirm the possibility of PTC intercalation into the DNA structure. The calculated thermodynamic parameters suggest the incorporation of PTC into the groove of the DNA macromolecule by hydrogen and/or hydrophobic bonds as well as destabilization of the DNA secondary structure.

Acknowledgment. This work was supported by research grant VEGA 1/0240/25 - Combined exposure to selected pesticide formulations containing neonicotinoids and triazoles and its effect on non-target organisms.

References

1. Dong, X., Tong, Z., Chu, Y., Sun, M., Wang, M., Gao, T., & Duan, J. (2019). Dissipation of prothioconazole-desthio in rice fields and risk assessment of its dietary intake. Journal of Agricultural and Food Chemistry, 67(23), 6458-6465.
2. Bayer Crop Science Slovenko, (2020). Tilmor 240EC Fungicide. Available online: TilMOR 240 EC Label - EN.
3. Joy, V. C., Pramanik, R., & Sarkar, K. (2005). Biomonitoring insecticide pollution using non-target soil microarthropodas. Journal of Environmental Biology, 26(3), 571-577.
4. Berrada, H., Fernandez, M., Ruiz, M. J., Moltó, J. C., Mañes, J., & Font, G. (2010). Surveillance of pesticide residues in fruits from Valencia during twenty months (2004/05). Food Control, 21(1), 36-44.
5. Li, M., Ma, X., Wang, Y., Saleem, M., Yang, Y., & Zhang, Q. (2022). Ecotoxicity of herbicide carfentrazone-ethyl towards Eisenia fetida in soil. Comparative Biochemistry and Physiology. Toxicology & Pharmacy, 253, 109250.
6. Shen, J., Liu, P., Sun, Y., Xu, X., Guo, L., Rao, Q., Chen, M., & Liu, X. (2021). Embryonic exposure to prothioconazole induces oxidative stress and apoptosis in zebrafish (Danio rerio) early life stage. The Science of the Total Environment, 756, 143859.
7. Zhang, Y., Zhang, G., Zhou, X., Li, Y. (2013). Determination of acetamiprid partial-intercalative binding to DNA by use of spectroscopic, chemometrics, and molecular docking techniques. Analytical and bioanalytical chemistry, 405(27), 8871-8883.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Conformation of The Ryanodine Receptor Isoform Models Created Using the AlphaFold 3 Server

[a]A. WAHEED, [b]A. ZAHRADNIKOVA

[a]Bioinformatics Laboratory, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia
[b]Department of Cell Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia

Ryanodine receptors (RyRs) are calcium channels responsible for calcium release from the endoplasmic/sarcoplasmic reticulum. The three mammalian isoforms, namely RyR1, RyR2, and RyR3, have ~70% sequence identity and similar overall structure [1]. RyR mutations are associated with genetic disorders such as malignant hyperthermia (RyR1), catecholaminergic polymorphic ventricular tachycardia (CPVT), and neurological disorders (RyR3) [1]. The RyR core (the C-terminal quarter), where the binding sites for the regulators Ca^{2+}, Mg^{2+}, ATP and caffeine are located [2], has a high density of mutations [3].

Here we aimed to evaluate the reliability of structures of the core of human RyR isoforms, predicted by the AlphaFold 3 server [4], from the viewpoint of structural validity and similarity to experimentally determined structures.

Fig. 1 shows the top AlphaFold 3 predictions. In all isoforms, the flexible DR1 region, which is not resolved in the experimental structures, was replaced by a chain of 11 glycine residues. While the overall shapes of all three isoforms are similar, the structures differ in the length and charge of the luminal loop, and RyR2 additionally displays a positively charged (blue) region above the cytoplasmic side of the membrane.

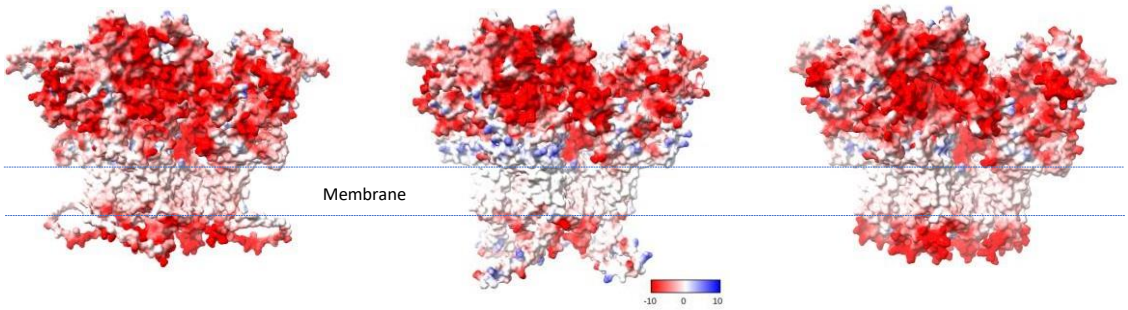


Fig. 1 AlphaFold-predicted structures of human RyR1(A), RyR2(B), and RyR3(C) isoforms. The surface is colored by the electrostatic potential.

Structural validation was performed using MolProbity [5], focusing on the distribution of Ramachandran outliers (Rama Z score), number of clashes per 1000 residues (ClashScore) and overall structure quality (MolProbity score). The predicted models demonstrated acceptable structural quality (Tab. 1). Comparison of the AlphaFold 3 models to the experimental structures with the best resolution showed a good overall Alignment Score. The models most closely resembled the experimentally observed closed-state conformation (RMSD < 2 Å). Interestingly, there were no differences between the deviations of individual isoforms from the respective closed state structures, although only RyR1 and RyR2 structures are present in the AlphaFold 3 template database.

Tab. 1 MolProbity Validation and structure comparison of RyR1, RyR2, and RyR3 isoforms.

		RyR1	**RyR2**	**RyR3**
	Rama Z Score	0.84 ± 0.06	0.79 ± 0.44	1.10 ± 0.09
	Clash Score	11.66 ± 1.22	9.19 ± 0.35	10.32 ± 0.51
	MolProbity Score	2.13 ± 0.10	1.94 ± 0.03	1.90 ± 0.09
Reference state		**RyR1**	**RyR2**	**RyR3**
Closed	Reference PDB ID	8VJJ	7UA5	9CTE
	Alignment Score	5171.8	5192.9	5005.5
	n /RMSD (Å)	1034 / 1.964	1031 / 1.193	990 / 1.299
Open	Reference PDB ID	5T9V	7UA9	9C1F
	Alignment Score	5125.6	5114.9	4899.5
	n /RMSD (Å)	1052 / 7.244	1031 / 2.098	1001 / 2.366
Inactivated	Reference PDB ID	7TDG	-	-
	Alignment Score	4930.3	-	-
	n /RMSD (Å)	1040 / 2.489	-	-

These data show that the structural quality of AlphaFold models is satisfactory and comparable to the quality of the experimental structures [3]. The ability of AlphaFold 3 to predict distinct conformational states and the conformational variability of a large dataset of AlphaFold models will be analyzed in the poster and the potential of AlphaFold 3 for investigating allosteric regulation and the effect of pathogenic mutations on RyR function will be evaluated.

Acknowledgment. This work was supported by research grants APVV-21-044 and APVV-21- 0473.

References

1. Hadiatullah, H., He, Z., & Yuchi, Z. (2022). Structural insight into ryanodine receptor channelopathies. Frontiers in Pharmacology, 13, 897494.
2. des Georges, A., Clarke, O. B., Zalk, R., Yuan, Q., Condon, K. J., Grassucci, R. A., Hendrickson, W. A., Marks, A. R., & Frank, J. (2016). Structural basis for gating and activation of RyR1. Cell, 167(1), 145-157.e17.
3. Zahradnikova, A., Pavelkova, J., Sabo, M., Baday, S., & Zahradnik, I. (2025). Structure-based mechanism of RyR channel operation by calcium and magnesium ions. PLoS Computational Biology, 21(4), e1012950.
4. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A. … Jumper, J. M. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature 630, 493-500.
5. Williams, C. J., Headd, J. J., Moriarty, N. W., Prisant, M. G., Videau, L. L., Deis, L. N., Verma, V., Keedy, D. A., Hintze, B. J., Chen, V. B., Jain, S., Lewis, S. M., Arendall 3rd, W. B., Snoeyink, J., Adams, P. D., Lovell, S. C., Richardson, J. S., & Richardson, D. C. (2018). MolProbity: More and better reference data for improved all-atom structure validation. Protein Science, 27(1), 293-315.

13th International Conference Structure & Stability of Biomacromolecules, Kosice, September 9 – 12, 2025

Structural Comparison of SERCA Conformations

E. WETTER, M. MAJEKOVA

Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Recent research has emphasized the importance of disrupted calcium signaling in various pathological processes. A key role in maintaining calcium homeostasis is ensured by a calcium ATPase SERCA, which pumps calcium ions from the cytoplasm into the lumen of the reticulum. The activity of SERCA, which can be decreased under pathological conditions, can be enhanced by activators, usable in the treatment of chronic diseases. Inhibition of SERCA activity can lead to apoptosis, which can be used in the treatment of oncological diseases.

While the mechanism of SERCA inhibition is mostly known (due to the abundance of 3D structures), the mechanism of allosteric activation has not been elucidated to date. Therefore, we decided to collect and systematically compare the available SERCA conformations corresponding to individual catalytic steps (Fig. 1).

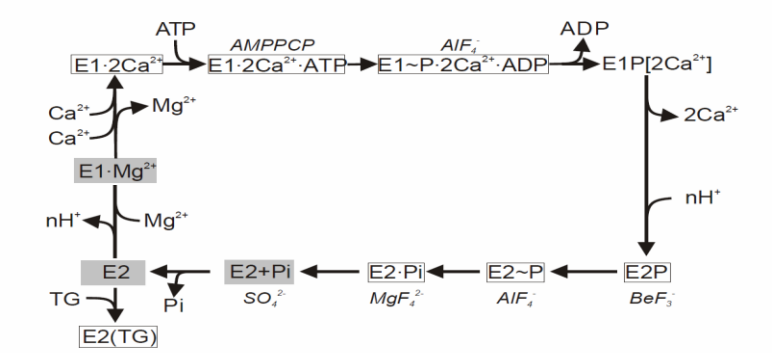


Fig. 1 Scheme of catalytic steps for active transport of calcium ions by SERCA.

The structures have been aligned using MUSTANG structure alignment in YASARA to study structural differences among the structures and compared concerning RMSD values and ligands present.

Acknowledgment. This work was supported by research grants the projects APVV-20-0543, VEGA 2/0103/22, and Devana-p1238-25-t.

References

1. Clausen, J. D., McIntosh, D. B., Woolley, D. G., Andersen, J. P. (2011). Modulatory ATP Binding Affinity in Intermediate States of E2P Dephosphorylation of Sarcoplasmic Reticulum Ca2+-ATPase. Journal of Biological Chemistry, 286, 11792-11802.
2. Aguayo-Ortiz, R., Espinoza-Fonseca, L. M. (2020). Linking Biochemical and Structural States of SERCA: Achievements, Challenges, and New Opportunities. IJMS, 21, 4146.
3. Rodriguez, Y., Majekova, M. (2020). Structural Changes of Sarco/Endoplasmic Reticulum Ca2+-ATPase Induced by Rutin Arachidonate: A Molecular Dynamics Study. Biomolecules, 10, 214.

ProteoForce Group: Laser Tweezers Laboratory for In-Depth Analysis of Molecular Forces in Macromolecules

M. ZOLDAKOVA, J. PAVLINSKA, L. HOMOLOVA, O. RAGAC, N. CHOMOVA, P. RAGAN, S. VAVRAKOVA, K. PAPAYOVA, S. DATTA, S. SOVOVA, P. VARGA, J. G. JOHANNESSON, G. ZOLDAK

Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Kosice, Slovakia

This contribution presents the ProteoForce project aimed at establishing Slovakia's first advanced laser optical tweezers laboratory dedicated to single-molecule force spectroscopy. The initiative builds on Nobel Prize-winning technology developed by Arthur Ashkin and is focused on the in-depth analysis of mechanical properties of clinically relevant proteins, particularly clients of the Hsp70 chaperone family, including membrane proteins.

The laboratory will be equipped with state-of-the-art laser optical tweezers and confocal fluorescence allows for measurement molecular forces with high subnanometer resolution. The project investigates force propagation within proteins during, for example, chaperone-assisted folding. Using engineered protein variants and optical tweezers force-clamp techniques, we are planning to analyze site-specific mechanical responses and conformational transitions in real time.

Further objectives include establishing national and international research collaborations, promoting open science principles via FAIR-compliant data sharing, and a training early-stage researchers. The laboratory infrastructure will also serve as a platform for high-risk/high-gain exploratory research on membrane protein mechanics and chaperone–client interactions under load, leveraging advanced biochemical modifications and fluorescence labeling.

This effort should make Slovakia visible on the map of European biophysical research infrastructures and contribute directly to strategic goals in fundamental science.

Acknowledgment. This work was Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V03-00008.

References

1. Ashkin, A. (1997). Optical trapping and manipulation of neutral particles using lasers. Proceedings of the National Academy of Sciences, 94(10), 4853-4860.
2. Zoldak, G., & Rief, M. (2013). Force as a single molecule probe of multidimensional protein energy landscapes. Current Opinion in Structural Biology, 23(1), 48-57.

AI-Identified Compounds Influence $A\beta_{42}$ Fibril Formation and Stability

[a]M. GANCAR, [a]Z. BEDNARIKOVA, [b]C. YUE, [b]Y. TANG, [b]L. MA, [b]B. - B. ZENG, [b]R. WANG, [a]Z. GAZOVA

[a]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*
[b]*School of Pharmacy, East China University of Science and Technology, Shanghai, China*

The aggregation of amyloid-β ($A\beta_{42}$) peptides into fibrils is a hallmark of Alzheimer's disease (AD). We developed a knowledge graph-enhanced drug-target interaction (DTI) prediction framework to identify potential inhibitors of $A\beta_{42}$ fibril formation and disruptors of pre-formed fibrils. A key innovation of our approach lies in protein representation learning: we enriched protein embeddings by integrating structural features from Node2Vec applied to a biomedical knowledge graph with contextual sequence representations from ProteinBERT. This dual-embedding strategy enabled more comprehensive protein characterization. The model demonstrated strong predictive performance across multiple metrics (AUC = 0.972, Precision = 0.914, Recall = 0.931, Accuracy = 0.921, AUPR = 0.970). Using this framework, we screened compounds from the TCMSP, ETCM, and TCMID databases, leading to the selection of nine candidate molecules (T1–T9) with predicted anti-AD activity. Specifically, their anti-amyloid activities were tested in this study through Thioflavin T (ThT) fluorescence assay at a 1:5 protein-to-compound ratio (20 μM $A\beta_{42}$, 100 μM compound, 50 mM sodium phosphate buffer, pH 7.4). Among the tested compounds, only T1 significantly reduced fibril formation (by approximately 45%, Fig. 1A) and disrupted pre-formed fibrils (decreasing the ThT signal by roughly 75%, Fig. 1B). T2 showed similar but less pronounced effects. Interestingly, T3 did not inhibit fibril formation but appeared to destabilize pre-formed fibrils. In contrast, T4, T5, and T6 facilitated fibril formation and had only moderate disrupting activity, while T7, T8, and T9 showed weak inhibitory potential without notable disruption of fibrils.

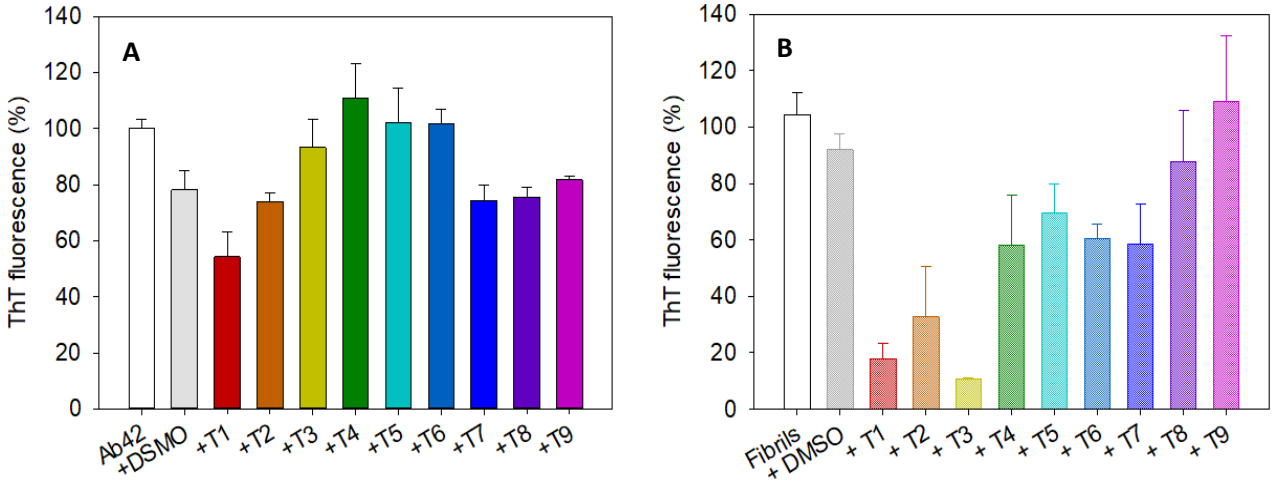


Fig. 1 ThT fluorescence intensities normalized to control ($A\beta_{42}$ fibrils formed alone) showing the effects of compounds T1–T9 on $A\beta_{42}$ fibril formation **(A)** and pre-formed $A\beta_{42}$ fibrils **(B)** at a 1:5 protein-to-compound ratio. The data represent averages from three independent wells with standard deviation.

Atomic force microscopy imaging confirmed T1's effectiveness in both inhibiting fibril formation and disrupting pre-formed fibrils, whereas T2 and T3 yielded inconclusive results, requiring further investigation. These findings highlight T1 as a promising candidate for AD treatments and underscore the value of AI-driven compound screening, with ongoing studies to clarify the effects of T2 and T3.

Acknowledgements. This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0598, APVV- and APVV-SK-CN-23-0025; and the National Natural Science Foundation of China (Grant 82173746).

AUTHOR INDEX

LIST OF CONTRIBUTORS

1. Abadjieva Emilia — Institute of Mechanics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 4, 1113 Sofia, Bulgaria.
2. Abdul Kareem Hanan — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
3. Alexandrova-Watanabe Anika — Institute of Mechanics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 4, 1113 Sofia, Bulgaria.
4. Almasi Miroslav — Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.
5. Anastassova Neda — Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria.
6. Andreana Marco — Center for Medical Physics and Biomedical Engineering, Medical University, AKH Vienna, Leitsstelle 4L, Währinger Gürtel 18-20, 1090 Vienna, Austria.
7. Andres-Campos Sara — Institute of Physical Chemistry “Blas Cabrera” (IQF-CSIC), Calle Serrano 119, 28006 Madrid, Spain.
8. Antal Iryna — Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
9. Antalik Marian — Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54, Kosice, Slovakia.
10. Antosova Andrea — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
11. Argirova Maria — Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria.
12. Baglaeva Iuliia — Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.

13.	Bano Gregor	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
14.	Barrera Exequiel Ernesto	Institute of Histology and Embryology of Mendoza "Dr. Mario H. Burgos" (IHEM), National University of Cuyo (UNCuyo), CONICET, Av. Del Libertador 80, Mendoza, Argentina.
15.	Barutiak Michael	Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.
16.	Bauer Jacob A.	Department of Biochemistry and Structural Biology, Institute of Molecular Biology, Slovak Academy of Sciences, Dubravska cesta 21, 845 51 Bratislava, Slovakia.
17.	Bauerova-Hlinkova Vladena	Department of Biochemistry and Structural Biology, Institute of Molecular Biology, Slovak Academy of Sciences, Dubravska cesta 21, 845 51 Bratislava, Slovakia.
18.	Bednarikova Zuzana	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
19.	Benedetto Antonio	School of Physics, University College Dublin, Belfield, Dublin 4, Ireland. Conway Institute for Biomolecular & Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland. Department of Science, University Roma Tre, Via della Vasca Navale 79, 00146 Rome, Italy.
20.	Benova Eva	Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.
21.	Best Nadine	Department of Biomaterials, Faculty of Engineering Science, University of Bayreuth, Prof.-Rüdiger-Bormann-Str. 1, D-95447 Bayreuth, Germany.
22.	Bielas Rafal	Department of Acoustics, Faculty of Physics and Astronomy, Adam Mickiewicz University in Poznan, ul. Uniwersytetu, Poznanskiego 2, 61-614 Poznan, Poland.

23.	Biro Tomas	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
24.	Borovska Barbora	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
25.	Budovska Mariana	Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54, Kosice, Slovakia.
26.	Bystrenova Eva	Consiglio Nazionale Delle Ricerche, Istituto Per Lo Studio Dei Materiali Nanostrutturati (CNR-ISMN), Area della Ricerca di Bologna, Via Piero Gobetti, 101, 40129 Bologna, Italy.
27.	Cehlar Ondrej	Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Odbojarov 10, 832 32 Bratislava, Slovakia.
28.	Cernik Marek	Uni-Export Instruments, s.r.o., Sultysova 642/15, 169 00 Prague 6, Czech Republic.
29.	Crha Radek	National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic.
30.	Datta Shubhashis	Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
31.	Dekhtiarenko Roman	Centre of Biosciences, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.
32.	Dvorsky Radovan	Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia.
33.	Escobedo Fatima	Institute of Physical Chemistry “Blas Cabrera” (IQF-CSIC), Calle Serrano 119, 28006 Madrid, Spain.

34. Fabian Marian — Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

35. Fabriciova Zaneta — Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.

Department of Chemistry and Industrial Chemistry & INSTM RU, nM2-Lab, University of Genoa, Via Dodecaneso 31, 16146 Genova, Italy.

36. Fedorova Viktoria — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

37. Fedunova Diana — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

38. Galajda Richard — Department of Biology & Physiology, University of Veterinary Medicine and Pharmacy, Komenskeho 73, 041 81 Kosice, Slovakia.

39. Galis Matus — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

40. Gancar Miroslav — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

41. Garaiova Martina — Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Dubravska cesta 9, P.O.BOX 63, 840 05 Bratislava, Slovakia.

42. Garaiova Zuzana — Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia.

43. Garden Jean Luc — Institut Neel CNRS Grenoble, 25 Av. des Martyrs, 38042 Grenoble, France.

44. Gartcheva Lidia — National Specialized Hospital for Active Treatment of Hematological Diseases, Zdrave Str. 2, 1756 Sofia, Bulgaria.

45.	Gasparik Norbert	National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic.
46.	Gazova Zuzana	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
47.	Gronenborn Angela Maria	Department of Structural Biology, University of Pittsburgh, School of Medicine, Biomedical Science Tower 3, 3501 Fifth Avenue, 15260 Pittsburgh, USA.
48.	Gucky Adrian	Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.
49.	Guenova Margarita	National Specialized Hospital for Active Treatment of Hematological Diseases, Zdrave Str. 2, 1756 Sofia, Bulgaria.
50.	Guncheva Maya	Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria.
51.	Hamulakova Slavka	Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54, Kosice, Slovakia.
52.	Hianik Tibor	Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia.
53.	Hodosi Richard	Shimadzu Slovakia, Rontgenova 28, 851 01 Bratislava, Slovakia.
54.	Holic Roman	Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Dubravska cesta 9, P.O.BOX 63, 840 05 Bratislava, Slovakia.
55.	Homolova Lucia	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

56.	Horvath Denis	Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
57.	Hritz Jozef	National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic. Department of Chemistry, Faculty of Science, Masaryk University, Kotlarska 267/2, 611 37 Brno, Czech Republic.
58.	Hrubovcak Pavol	Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.
59.	Humenik Martin	Department of Biomaterials, Faculty of Engineering Science, University of Bayreuth, Prof.-Rüdiger-Bormann-Str. 1, D-95447 Bayreuth, Germany.
60.	Hundahl Adam Coln	FIDA Biosystems ApS, Generatorvej 6, 2860 Søborg, Denmark.
61.	Huntosova Veronika	Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
62.	Chomova Natalia	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
63.	Ilkovicova Lucia	Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic. National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic.
64.	Ivanova Miroslava	Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria.
65.	Ivanyi Gergely Tamas	HUN-REN Biological Research Centre Szeged, Institute of Biophysics, Temesvari krt. 62., H-6726 Szeged, Hungary.

66.	Jagielska Elzbieta	Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Adolfa Pawinskiego 5, 02-106 Warsaw, Poland.
67.	Jancura Daniel	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
68.	Jensen Henrik	FIDA Biosystems ApS, Generatorvej 6, 2860 Søborg, Denmark.
69.	Jiang Hanxing	Institute of Biophysics CSIC, Science Park of the UPV/EHU, Barrio Sarriena s/n, 48940 Leioa, Bizkaia, Spain.
70.	Johannesson Jonatan Göran	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
71.	Jossens Guillaume	Calneos SAS, 15 Rue Jean Claret, 63000 Clermont-Ferrand, France.
72.	Jozefczak Arkadiusz	Department of Acoustics, Faculty of Physics and Astronomy, Adam Mickiewicz University in Poznan, ul. Uniwersytetu, Poznanskiego 2, 61-614 Poznan, Poland.
73.	Jurasekova Zuzana	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
74.	Katrova Vesela	Institute of Optical Materials and Technologies "Acad. Yordan Malinovski", Bulgarian Academy of Sciences, "Acad. G. Bontchev" Str., Bl. 109, 1113 Sofia, Bulgaria.
75.	Kelemen Lorand	HUN-REN Biological Research Centre Szeged, Institute of Biophysics, Temesvari krt. 62., H-6726 Szeged, Hungary.
76.	Kiraly Nikolas	Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.
77.	Koneracna Martina	Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
78.	Korabecny Jan	Biomedical Research Center, University Hospital Hradec Kralove, Sokolovska 581, 500 05 Hradec Kralove, Czech Republic.

79. Kozelekova Aneta — National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic.

Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic.

80. Kozurkova Maria — Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.

81. Kralova Katerina — Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic.

82. Krumova Sashka — Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria.

83. Krupa Pawel — Institute of Physics, Polish Academy of Sciences, aleja Lotników 32/46, 02-668 Warsaw, Poland.

84. Kubackova Jana — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

85. Kubovcikova Martina — Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

86. Landl Richard — Anton Paar Slovakia s.r.o., Hattalova 12/A, 831 03 Bratislava, Slovakia.

87. Langari Ariana — Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria.

88. Li Mai Suan — Institute of Physics, Polish Academy of Sciences, aleja Lotników 32/46, 02-668 Warsaw, Poland.

Life Science Lab, Institute for Computational Science and Technology, Quang Trung Software City, Tan Chanh Hiep Ward, District 12, Ho Chi Minh City, Vietnam.

89. Li Xu — Center for Medical Physics and Biomedical Engineering, Medical University, AKH Vienna, Leitsstelle 4L, Währinger Gürtel 18-20, 1090 Vienna, Austria.

90. Lisnichuk Maksym — Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.

91. Lopez-Alonzo Jorge Pedro — Biofisika Institute CSIC, Science Park of the UPV/EHU, Barrio Sarriena s/n, 48940 Leioa, Bizkaia, Spain.

Basque Resource for Electron Microscopy (BREM), Instituto Biofisika CSIC, Science Park of the UPV/EHU, Barrio Sarriena s/n, 48940 Leioa, Bizkaia, Spain.

92. Ma Lei — School of Pharmacy, East China University of Science and Technology (ECUST), No. 130 Meilong Road, Xuhui District, 200237 Shanghai, China.

93. Macias Ashley Antonella — Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia.

94. Majekova Magdalena — Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Dubravska cesta 9, 841 04 Bratislava, Slovakia.

95. Martonova Katarina — Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia.

96. Matajova Henrieta — Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.

97. Migasova Alexandra — Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.

98. Mihokova Anna — Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.

99. Moiroux Gaêl — Institut Neel CNRS Grenoble, 25 Av. des Martyrs, 38042 Grenoble, France.

100. Molcan Matus — Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

101. Mompean Miguel — Institute of Physical Chemistry “Blas Cabrera” (IQF-CSIC), Calle Serrano 119, 28006 Madrid, Spain.

102.	Musatov Andrej	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
103.	Nagy Lubos	Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.
104.	Naplavova Alexandra	National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic.
105.	Nemergut Michal	Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
106.	Neyt Jean-Claude	Calneos SAS, 15 Rue Jean Claret, 63000 Clermont-Ferrand, France.
107.	Niedzialek Dorota	Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Adolfa Pawinskiego 5, 02-106 Warsaw, Poland. Ensemble^3 sp. z o.o., Centre of Excellence, Wolczynska St. 133, 01-919 Warsaw, Poland.
108.	Njemoga Stefana	Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia.
109.	Novotna Jana	National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic. Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic.
110.	Nowacka Martyna	Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Adolfa Pawinskiego 5, 02-106 Warsaw, Poland.
111.	Ossowicz-Rupniewska Paula	Department of Organic Chemical Technology and Polymer Materials, Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology, Piastow 42, 71-065 Szczecin, Poland.

112.	Papayova Kristina	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
113.	Parmar Olga	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
114.	Paulovicova Katarina	Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
115.	Pavlinska Julia	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
116.	Pilipcincova Ivana	Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
117.	Pitkova Diana	Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54, Kosice, Slovakia.
118.	Polak Adam	Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia.
119.	Polcicova Katarina	Biomedical Research Center, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.
120.	Polonio Paula	Institute of Physical Chemistry “Blas Cabrera” (IQF-CSIC), Calle Serrano 119, 28006 Madrid, Spain. Autonomous University of Madrid, Doctoral School, Calle Francisco Tomas y Valiente, nº 2 Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain.
121.	Pudlak Michael	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
122.	Ragac Ondrej	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

123.	Ragan Peter	Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
124.	Ratajczak Filip	Department of Acoustics, Faculty of Physics and Astronomy, Adam Mickiewicz University in Poznan, ul. Uniwersytetu, Poznanskiego 2, 61-614 Poznan, Poland.
125.	Ray Kritika	FIDA Biosystems ApS, Generatorvej 6, 2860 Søborg, Denmark.
126.	Raynova Yuliana	Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria.
127.	Rendosova Michaela	Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.
128.	Ricci Yannice	Calneos SAS, 15 Rue Jean Claret, 63000 Clermont-Ferrand, France.
129.	Romanovova Michaela	Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.
130.	Sabala Izabela	Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Adolfa Pawinskiego 5, 02-106 Warsaw, Poland.
131.	Sabo Miroslav	Bioinformatics Laboratory, Biomedical Research Center, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.
132.	Scalzi Eduardo	Department of Pharmacy, University of Pisa, Via Bonanno Pisano 12, 561 26 Pisa PI, Italy.
133.	Sedlak Erik	Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.
134.	Sevcikova Tomaskova Zuzana	Centre of Biosciences, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.
135.	Simond Mickael	Calneos SAS, 15 Rue Jean Claret, 63000 Clermont-Ferrand, France.

136. Siposova Katarina — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

137. Skrabana Rostislav — Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia.

138. Slaby Cyril — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

139. Sovova Simona — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

140. Stanicova Jana — Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy, Komenskeho 73, 041 81 Kosice, Slovakia.

Institute of Biophysics and Informatics, First Faculty of Medicine, Charles University, Salmovska 1, 120 00 Prague, Czech Republic.

141. Stoyanova Elena — Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Boulevard "Tsarigradsko shose" 73A, 1113 Sofia, Bulgaria.

142. Strejckova Alena — Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy, Komenskeho 73, 041 81 Kosice, Slovakia.

143. Strijkova Velichka — Institute of Optical Materials and Technologies “Acad. Yordan Malinovski”, Bulgarian Academy of Sciences, “Acad. G. Bontchev” Str., Bl. 109, 1113 Sofia, Bulgaria.

144. Stroffekova Katarina — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

145. Subjakova Veronika — Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia.

146. Sugimoto Naoki — Frontier Institute for Biomolecular Engineering Research, Konan University 7-1-20, Minatojima-minamimachi, Chuo-ku, 650-0047 Kobe, Japan.

147. Susko Tomas — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

148. Sztachova Tereza — Department of Biophysics, Institute of Experimental Physics Slovak Academy of Sciences, Watsonova 47, 040 01, Kosice, Slovakia.

149. Szucsova Jaroslava — Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.

150. Tang Yun — School of Pharmacy, East China University of Science and Technology (ECUST), No. 130 Meilong Road, Xuhui District, 200237 Shanghai, China.

151. Tiankov Tihomir — Institute of Mechanics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 4, 1113 Sofia, Bulgaria.

152. Timkova Ivana — Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

153. Titaux Gustavo — Institute of Physical Chemistry “Blas Cabrera” (IQF-CSIC), Calle Serrano 119, 28006 Madrid, Spain.

154. Todinova Svetla — Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria.

155. Tomkova Adriana — Department of Biophysics, Institute of Experimental Physics Slovak Academy of Sciences, Watsonova 47, 040 01, Kosice, Slovakia.

156. Tomkova Maria — Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

157. Tomkova Silvia — Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

158. Tomori Zoltan — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

159. Trizna Lukas — Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54, Kosice, Slovakia.

160. Ubarretxena-Belandia Iban — Institute of Biophysics CSIC, Science Park of the UPV/EHU, Barrio Sarriena s/n, 48940 Leioa, Bizkaia, Spain.

Ikerbasque – Basque Foundation for Science, Plaza Euskadi 5, 48009 Bilbao, Bizkaia, Spain.

161. Unterhuber Angelika — Center for Medical Physics and Biomedical Engineering, Medical University, AKH Vienna, Leitsstelle 4L, Währinger Gürtel 18-20, 1090 Vienna, Austria.

162. Uskoba Jozef — BioTech a.s., Sluzeb 4, 108 00 Prague 10, Czech Republic.

163. Valusova Eva — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

164. Vanik Vladimir — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

165. Varga Peter — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

166. Vargova Zuzana — Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.

167. Varha Adriana — Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54, Kosice, Slovakia.

168. Varchol Bianka Gizela — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

169. Vavrakova Sandra — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

170. Verebova Valeria — Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy, Komenskeho 73, 041 81 Kosice, Slovakia.

171. Verducci Patrizia — Consiglio Nazionale Delle Ricerche, Istituto Per Lo Studio Dei Materiali Nanostrutturati (CNR-ISMN), Area della Ricerca di Bologna, Via Piero Gobetti, 101, 40129 Bologna, Italy.

Facolta Chimica e Tecnologia Farmaceutiche, Alma Mater Studiorum-Universita di Bologna, Campus Navile, Complesso Battiferro, Via della Beverara, 123/1, 40131 Bologna, Italy.

172. Viglasky Viktor — Department of Biochemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54, Kosice, Slovakia.

173. Vizsnyiczai Gaszton — HUN-REN Biological Research Centre Szeged, Institute of Biophysics, Temesvari krt. 62., H-6726 Szeged, Hungary.

174. Volavka Dominik — Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.

175. Waheed Amna — Bioinformatics Laboratory, Biomedical Research Center, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.

176. Wang Rui — School of Pharmacy, East China University of Science and Technology (ECUST), No. 130 Meilong Road, Xuhui District, 200237 Shanghai, China.

177. Wetter Erik — Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Dubravska cesta 9, 841 04 Bratislava, Slovakia.

178. Wieczorek Grzegorz — Laboratory of Protein Engineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Adolfa Pawinskiego 5, 02-106 Warsaw, Poland.

179. Yancheva Denitsa — Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 9, 1113 Sofia, Bulgaria.

180. Yue Chengyuan — School of Pharmacy, East China University of Science and Technology (ECUST), No. 130 Meilong Road, Xuhui District, 200237 Shanghai, China.

181. Zahradnikova Alexandra — Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Dubravska cesta 9, 845 05 Bratislava, Slovakia.

182. Zavisova Vlasta — Department of Magnetism, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

183. Zelenak Vladimir — Department of Inorganic Chemistry, Institute of Chemistry, Faculty of Science, University of P. J. Safarik in Kosice, Moyzesova 11, 041 54 Kosice, Slovakia.

184. Zelenakova Adriana — Department of Condensed Matter Physics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Park Angelinum 9, 041 54 Kosice, Slovakia.

185. Zeng Bu-Bing — School of Pharmacy, East China University of Science and Technology (ECUST), No. 130 Meilong Road, Xuhui District, 200237 Shanghai, China.

186. Zimovcak Peter — Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia.

187. Zoldak Gabriel — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

188. Zoldakova Miroslava — Department of Biophysics, Institute of Physics, Faculty of Science, University of P. J. Safarik in Kosice, Jesenna 5, 040 01 Kosice, Slovakia.

Book of Contributions, 13th International Conference Structure & Stability of Biomacromolecules, September 9 – 12, 2025, Kosice, Slovakia

Editors: Z. Gazova, A. Antosova, J. Kubackova
Reviewers: G. Fabriciova, R. Varhac
Number of pages: 139

ISBN: 978-80-89656-27-1
EAN: 9788089656271