RBC2026

Regional Biophysics Conference 2026

BOOK OF ABSTRACTS

1 – 4 June 2026

Belgrade, Serbia

Organized by the Biophysical Society of Serbia (BSS)

11th Regional Biophysics Conference 2026
1-4th June, Belgrade, Serbia

RBC2026

Regional Biophysics Conference 2026

BOOK OF ABSTRACTS

1 – 4 June 2026

Belgrade, Serbia

Organized by the Biophysical Society of Serbia (BSS)

RBC2026

Regional Biophysics Conference 2026

BOOK OF ABSTRACTS

Izdavač

Srpska akademija nauka i umetnosti, Beograd

Urednici

Ksenija Radotić Hadži-Manić, Miloš Mojović

Štampa

Srpska akademija nauka i umetnosti, Beograd

Tiraž

170

ISBN 978-86-6184-125-5

Beograd, 2026.

Organizing Committee

- Ksenija Radotić
- Pavle Andjus
- Miloš Mojović
- Dejan Žikić
- Branislav Jelenković
- Miljko Satarić
- Đura Nakarada
- Jasna Simonović Radosavljević
- Dragana Bartolić
- Mihailo Rabasović
- Nemanja Rajković
- Aleksandar Krmpot
- Milena Milošević

Scientific advisory board

- Velia Minicozzi (Italy)
- Miklos Kellermayer (Hungary)
- Nadica Ivošević DeNardis (Croatia)
- Matej Kanduc (Slovenia)
- Ksenija Radotić (Serbia)
- Pavle Andjus (Serbia)
- Miloš Mojović (Serbia)
- Nebojša Milošević (Serbia)
- Dejan Žikić (Serbia)
- Anja Sadžak (Croatia)
- Alberto Diaspro (Italy)
- Andras Lukacs (Hungary)
- Tanja Dučić (Spain/Serbia)
- Srđan Antić (USA)
- Lars Klimaschewski (Switzerland)
- Vladimir Parpura (China)
- Zuzana Gažová (Slovakia)

Supported by:

- Biophysical Society of Serbia (BSS)
- Ministry of Science, Technological Development and Innovations, Serbia
- Serbian Academy of Sciences and Arts
- European Biophysical Societies' Association (EBSA)
- International Union for Pure and Applied Biophysics (IUPAB)
- Nanion Technologies GmbH
- KEFO d.o.o. Beograd

TABLE OF CONTENTS

SCIENTIFIC SESSION I

MOLECULAR AND STRUCTURAL BIOPHYSICS

Disordered LEA Proteins as Regulators of $A\beta_{40}$ Amyloid Aggregation: Targeting Secondary Nucleation in Fibril Formation

Zuzana Bednarikova[1], Ana Pantelic[2], Tatiana Ilina[2], Vladimir Perovic[3], Zuzana Gazova[1], Marija Vidovic[2]

[1]*Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01, Kosice, Slovakia*
[2]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11042 Belgrade, Serbia*
[3]*Institute of Nuclear Sciences "VINČA ", University of Belgrade, 11001 Belgrade, Serbia*

Aggregation of the amyloid β (Aβ) peptide is a key molecular process in Alzheimer's disease. Aβ self-assembles into β-sheet–rich fibrils via a nucleation-dependent pathway involving primary nucleation, elongation, and secondary nucleation. Targeting these microscopic steps offers a strategy to control amyloid formation and toxicity[1].

Here, we investigated late embryogenesis abundant (LEA) proteins as modulators of $A\beta_{40}$ aggregation. LEAPs are mostly intrinsically disordered proteins associated with desiccation tolerance in *Ramonda serbica*. Twelve recombinant LEA proteins were selected by *in silico* screening and evaluated their ability to affect the amyloid aggregation of $A\beta_{40}$ using the thioflavin T (ThT) fluorescence assay. Circular dichroism spectroscopy and atomic force microscopy were applied to assess structural and morphological changes of $A\beta_{40}$ peptide and amyloid fibrils.

Several LEA proteins delayed $A\beta_{40}$ fibril growth and altered aggregate morphology, indicating their interference with the assembly pathway. Kinetic data of $A\beta_{40}$ fibrilization were analyzed using AmyloFit[2], revealing that the most effective proteins, LEA17 and LEA28, primarily suppress secondary nucleation.

These findings highlight specific LEA proteins as promising regulators of $A\beta_{40}$ amyloid aggregation and provide insight into their inhibitory mechanisms.

1. Xu, Y., *et al.* 2022, Essays Biochem. 66(7), 959-975.
2. Meisl, G., *et al*., 2016, Nat. Protoc. 11, 252–272.

Acknowledgment: *This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0598, APVV-SK-SRB-23-0062 and APVV-DS-FR-24-0060, VEGA 2/0141/25, a multilateral project "NEPTUNE" with the Republic of Serbia and the Czech Republic; and by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-136/2026-03/200042) and by the MVTS COST ML4NGP project.*

Multi-domain Proteins and the Allosteric Puzzle: Lessons from SHP2

Paolo Calligari[1]

[1]*Department of Chemical Science and Technologies, Tor Vergata University of Rome, Rome 00133 Italy, paolo.calligari@uniroma2.it*

The phosphatase protein SHP2 plays a crucial role in regulating key cellular signaling pathways. Activating mutations in SHP2 have been linked to developmental disorders such as Noonan syndrome and are associated with multiple cancer types[1,2]. SHP2 has a multi-domain structure comprising two SH2 domains (N-SH2 and C-SH2) followed by a catalytic PTP domain. SH2 domains recognize and bind polypeptide sequences containing phosphotyrosine residues.

In the absence of external stimuli, SHP2 remains in a closed, autoinhibited state, where the N-SH2 domain blocks the catalytic site of the PTP domain. Activation occurs when SH2 domains interact with phosphorylated partners, leading to structural rearrangements. The great part of the pathological mutations alters this finely tuned mechanism, ultimately promoting the active state even in the absence of external stimuli. However, the exact role and interplay of SH2 domains in partner recruitment and binding remain only partially understood. Upon SHP2 activation, N-SH2 undergoes an allosteric change, which corresponds to greater accessibility of its binding site to phosphorylated sequences. We recently proposed that phosphopeptide binding to N-SH2 triggers SHP2 activation via a conformational selection mechanism[3].

Although structural data exist for both inactive and active states, the overall activation mechanism of SHP2 remains elusive. Traditional simulations face challenges due to slow interconversion rates (on the order of seconds). To address this, we employed enhanced sampling techniques, combined with coarse-grained molecular dynamics (Martini force field). These approaches allowed us to identify bona fide activation pathways and explore the topological constraints imposed by bis-phosphorylated peptide binding to the dynamics of both SH2 domains.

1. R. Frankson et al., Cancer Res. 77 (2017) 5701–5705.
2. M. Tartaglia et al., Am. J. Hum. Genet. 78 (2006) 279–290.
3. P. Calligari et al., Comp Struct Biotech J 19 (2021) 6125-6139.

Validation of Acoustic Detection of Myelin Basic Protein (MBP)

Sára Mikuličová, Sandro Spagnolo, Tibor Hianik

Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Department of Nuclear Physics and Biophysics, 84248 Bratislava, Slovakia, sara.mikulicova@fmph.uniba.sk

Myelin Basic Protein (MBP) is an integral component of the myelin sheath in central nervous system. For appropriate conduction of nerve impulses, the integrity of this multilayered proteolipid membrane covering the axon is essential. During neurodegenerative diseases such as multiple sclerosis (MS), demyelination occurs. It is commonly accompanied by the increased levels of MBP in cerebrospinal fluid (CSF)[1]. Previous detection focused on the use of electrochemical methods[2]. In our work we focused on the use of acoustic methods. Using antifouling surface molecules with DNA aptamers immobilized on gold electrode, we developed a biosensor for the detection of MBP using Quartz Crystal Microbalance with Dissipation monitoring (QCM-D). The surface was functionalized with a mixed self-assembled monolayer of 0.5 mM SH-MEG-MIX (mixture of 0.25 mM 2-(2-mercaptoethoxy)-ethanol (SH-MEG-OH) and 0.25 mM 3-(2-mercaptoethanoxy) propanoic acid (SH-MEG-COOH), followed by covalent immobilization of DNA aptamers. A recombinant MBP with size 22 kDa has been detected in buffer with limit of detection (LOD) of 7.8 ng/ml. The sensor has been validated also in a MBP spiked artificial cerebrospinal fluid.

1. Martinsen, V. and Kursula, P., 2022, Multiple sclerosis and myelin basic protein: insights into protein disorder and disease, Amino Acids, 54, 1, 99-109.
2. Serin, M. and Kara, P., 2025, Demyelination detection in CSF based on electrochemical monitoring of myelin basic protein in comparison between Apta vs. Immuno sensing strategies, Bioelectrochemistry, 161, 108834.

Acknowledgment: *This work was funded by the Grant Agency for Science VEGA 1/0445/23 and by NextGenertion EU Recovery Plan, project No. 09I03-03-V04-00145.*

From Flexibility to Crystals: Structural and Biophysical Characterization of a Thermostable Metagenomic Esterase

Ivana Kekez[1], Lucija Brtan[1], Aleksandra Maršavelski[1]

[1]*Faculty of Science, University of Zagreb, Department of Chemistry, Zagreb, Croatia, ikekez@chem.pmf.hr*

The accumulation of plastic waste calls for innovative biotechnological strategies enabling efficient and sustainable polymer degradation.[1,2] Our work focuses on the development and structural optimization of novel biocatalysts for bioplastic processing. We previously identified a novel esterase, MGY,[3] and reconstructed ancestral variants (118, 119, and 121) active toward polylactic acid (PLA). Among them, variants 118 and 119 exhibited superior solubility and yielded the highest amounts of purified protein, and were therefore selected for in-depth structural and biophysical characterization. Variant 119 displayed the highest thermal stability (T_m = 84 °C) reported for this enzyme family to date. To obtain diffraction-quality crystals and enhance structural homogeneity, molecular dynamics (MD) simulations were performed to assess conformational flexibility. B-factors derived from RMSF analysis identified a highly flexible loop around residue 200 in both variants, as well as pronounced N-terminal mobility in variant 119. Guided by these insights, an N-terminally truncated construct (ΔN17_119) was engineered, and limited proteolysis was applied to reduce loop flexibility. This structure-guided stabilization strategy proved critical for successful crystallization. The resolved crystal structure validates the design approach and establishes a framework for rational engineering of robust polyester-degrading enzymes, while preserving catalytic activity toward PLA.

1. Han, Y., Wang, R., Wang, D., Luan, Y., 2024, Int. Biodeter. Biodegr. 189, 105746.
2. Hajighasemi, M. et al., 2018, Environ. Sci. Technol. 52, 12388–12401.
3. Kekez, I., Josić, E., Močibob, M., Mendeš, D., Maršavelski, A., 2024, Acta Cryst. A. 80(a1), e122–e122.

Acknowledgment: *This work was funded through the National Recovery and Resilience Programme (NextGenerationEU), project Enzyme engineering for sustainable recycling of bioplastics (NPOO.C3.2.R2-I1.06.0041), and supported by the COST Action CA21162 Short-Term Scientific Missions grant.*

Perylene Bisimide Derivatives in Water Solutions, Effects of NaCl on Aggregation Kinetics and Spectroscopic Properties

Miles Martinati[1], Giovanni Batignani[1], Gaia De Maria[1], Giancarlo Fabrizi[2], Yuri Gazzilli[2], Valeria Pennacchietti[3], Fang Yang[4], Alberto Boffi[3], Tullio Scopigno[1], Roberta Piacentini[3,5]

[1]*Department of Physics, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy*
[2]*Department of Chemistry and Pharmaceutical Technologies, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy*
[3]*Department of Biochemical Sciences "Alessandro Rossi Fanelli", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy, roberta.piacentini@uniroma1.it*
[4]*Ningbo Key Laboratory of Biomedical Imaging Probe Materials and Technology, Laboratory of Advanced Theranostic Materials and Technology, Chinese Academy of Sciences (CAS) Key Laboratory of Magnetic Materials and Devices, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, Ningbo 315201, China*
[5]*D-Tails s.r.l. S.B., Via Agrigento, 4A/4B, 00161, Rome, Italy*

In fluorescence-based biochemical and cell biology applications, probes must be stable, biocompatible, and strongly emissive in water. Perylene bisimide derivatives (PBIs) are attractive candidates as they satisfy many of these requirements, such as favorable photophysical properties and high stability[1]. However, their use is limited by their strong tendency to aggregate in aqueous saline environments. This behavior arises from the rigid aromatic core of PBIs that promotes π-π stacking and drives the formation of supramolecular structures with distinctive spectroscopic and thermodynamic properties. Aggregation is further influenced by solvent effects, which regulate solubility, hydrogen bonding, intermolecular interactions, and aggregate morphology. Although the structural and spectroscopic features of PBI aggregates have been widely investigated, their self-assembly kinetics in solution remain poorly understood, despite their importance for mechanistic understanding and biological applications[2]. In this work, we addressed this gap by studying the salt-induced self-assembly kinetics of two representative PBI derivatives, chosen as model systems with different charge properties. To better interpret the spectroscopic changes, we used stimulated Raman scattering under resonance conditions, a technique that enhances vibrational signals coupled to electronic transitions while suppressing fluorescence background and providing deeper structural insight into the aggregation process[3].

1. Rehm, S.; Stepanenko, V.; Zhang, X.; Rehm, T. H.; Würthner, F. Spermine Functionalized Perylene Bisimide Dyes—Highly Fluorescent Bola Amphiphiles in Water. Chemistry – A European Journal 2010, 16 (11), 3372–3382. https://doi.org/10.1002/chem.200902839.
2. Slavnova, T. D.; Chibisov, A. K.; Görner, H. Kinetics of Salt-Induced J-Aggregation of Cyanine Dyes. J. Phys. Chem. A 2005, 109 (21), 4758–4765. https://doi.org/10.1021/jp058014k.
3. Batignani, G.; Ferrante, C.; Fumero, G.; Martinati, M.; Scopigno, T. Femtosecond Stimu-lated Raman Spectroscopy. Nature Reviews Methods Primers 2024, 4 (1), 34. https://doi.org/10.1038/s43586-024-00314-6.

Acknowledgment: *The national project from MinSal "Traiettoria 4 Biotecnologie, Bioinformatica e Sviluppo Farmaceutico, Linea di azione 4.1" and Italian Fund for Applied Sciences (FISA), project "ROAD - Retina Observation for Alzheimer Diagnostic", CUP J53C25000530001, funded by the Italian Ministry of University and Research (MUR), under Call No. 1233 of 01/08/2023, to Alberto Boffi are gratefully acknowledged. Fang Yang discloses support for the research of this work from funder Key Program of the Natural Science Foundation of Zhejiang province (Z25C100007), Special Exchange Program of Chinese Academy of Sciences (China-Romania), "Innovation Yongjiang 2035" Key R&D Program of Ningbo (2024Z217) and International Science & Technology Cooperation Program (2024H006).*

Effects of Choline Chloride-Based Deep Eutectic Solvents on the Structure and Thermal Stability of Lysozyme

Peter Zimovcak[1], Zuzana Gazova[1], Diana Fedunova[1]

[1]*Institute of Experimental Physics, Slovak Academy of Sciences, Department of Biophysics, Košice, Slovakia, zimovcak@saske.sk*

Deep eutectic solvents (DESs) are non-toxic alternatives to organic solvents, formed by combining hydrogen bond donors and acceptors in defined molar ratios [1]. Their tunable polarity, hydrogen-bonding capacity, and water content enable controlled modulation of protein conformation, making them useful for studying protein stability and aggregation [2].

We studied the effects of two choline chloride-based DESs, with glutaric (GA) and malic acids (MA) as hydrogen bond donors, on the structure and stability of lysozyme (LZ). Understanding the effects of these conditions on LZ properties is relevant for their applications in food and pharmaceutical formulations, as well as for controlled aggregation studies. LZ properties were studied in aqueous solution and in 60–80% (w/w) DES at pH 2.7. At room temperature, near-UV CD and fluorescence measurements show that the tertiary LZ structure remains compact across all conditions. In contrast, calorimetry revealed reduced thermal stability in all DES systems, with lower transition temperatures than in aqueous solution. This behavior is characteristic of co-solvent systems strongly interacting with protein surfaces. DESs appear to facilitate unfolding at elevated temperatures, indicating the preferential interaction with the unfolded state. The stronger destabilizing effect of GA compared to MA likely arises from its flexible linear chain, which enables more effective interactions with LZ backbone than the more constrained MA.

1. Fatima, U., Yadav, N., Venkatesu, P., 2024, International Journal of Biological Macromolecules. 268:131997.
2. Sanchez-Fernandez, A., Basic, M., Xiang, J., Prevost, S., Jackson, A. J., Dicko, C., 2022, Journal of the American Chemical Society. 144:23657-23667.

Acknowledgment: *This work was funded by the Slovak grant Agency VEGA 2/0161/26 and 2/0141/25, the Slovak Research and Development Agency under Contract no. APVV-22-0598 and the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09-I02-03-V01-0002.*

Structural Investigation of Protein Assemblies With Small-angle X-ray Scattering

Bence Fehér[1,2]

[1]*HUN-REN-Semmelweis University Nanobiophysics Research Group, Tűzoltó street 37-47, Budapest, H-1094, Hungary*
[2]*Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary, feher.bence1@semmelweis.hu*

Small-angle neutron and X-ray scattering (SANS and SAXS) are powerful techniques widely used to probe the structure of colloids, polymers, and self-assembled systems in soft matter physics. In recent decades, these methods have found growing application in biophysics, particularly for the low-resolution structural characterization of globular and membrane proteins in artificial membrane environments.

Our research focuses on casein proteins, the primary milk proteins, which are intrinsically disordered and therefore lack a defined crystal structure. In milk, caseins naturally form self-assembled structures known as casein micelles. Notably, certain casein subtypes—such as α-casein and κ-casein—have been shown to form amyloid fibrils under physiological conditions, suggesting a potential role in pathological processes including breast cancer and mastitis.

Here we show an exact mathematical approach of the detailed structural description of re-assembled casein micelles. Furthemore, to elucidate the structural and kinetic aspects of casein fibrillogenesis, we combine small-angle X-ray scattering (SAXS), molecular dynamics (MD) simulations, and atomic force microscopy (AFM). Our approach enables us to investigate how varying experimental conditions influence fibril formation and to correlate macroscopic scattering data with molecular-level insights.

The Effect of Peptide Diversity on Protein Amyloid Aggregation

Anja Dimić[1], Natalija Polović[2], Jelica Milošević[2]

[1]*Innovative Centre of the Faculty of Chemistry, University of Belgrade, Belgrade, Serbia*
[2]*Faculty of Chemistry, University of Belgrade, Department of Biochemistry, Belgrade, Serbia, jelica@chem.bg.ac.rs*

Amyloid aggregation is believed to represent the ultimate stabilization state of protein solutions. Increasing evidence that natively folded proteins can undergo amyloid aggregation under specific conditions supports this hypothesis. Here, we investigated amyloid aggregation pathways in novel model systems – plant cysteine proteases. Papain and ficin share a similar tertiary structure composed of two domains with distinct α-helix and β-sheet folds, but differ in the expression of single versus multiple isoforms in papaya and fig, respectively. This inspired us to examine the effect of peptide diversity on fibril formation propensity. Preliminary results showed that the formed structures depend on sequence diversity within the protein solution. To further test this hypothesis, ficin isoforms were purified, revealing a higher propensity for ordered aggregation in single-isoform solutions. Amyloid aggregation was analyzed by monitoring aggregation kinetics using Thioflavin T fluorescence, secondary structure changes by FTIR spectroscopy, and aggregate morphology by AFM. Regardless of isoform composition, the studied cysteine proteases formed aggregates with positive ThT fluorescence following complete unfolding in the presence of a chaotrope and subsequent incubation under reducing conditions in 90% ethanol. Differences between the ficin mixture and single isoforms were more evident in the infrared spectra, particularly through the greater retention of native-like secondary structures in the case of higher peptide diversity.

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract 451-03-136/2025-03/ 200168.*

Liquid-Liquid Phase Separation by the Intrinsically Disordered PEVK Domain of the Giant Muscle Protein Titin

Miklós Kellermayer[1], Hedvig Tordai[1], Bence Fehér[1], Anikó Szabó[2], Péter Vilmos[2]

[1]*Semmelweis University, Department of Biophysics and Radiation Biology, Budapest, Hungary, kellermayer.miklos@semmelweis.hu*
[2]*HUN-REN Biological Research Center, Institute of Genetics, Szeged, Hungary*

Titin is a giant filamentous protein that spans the half muscle sarcomere between the Z- and M-lines and provides elasticity, structural template and mechanosensor function. Titin's I-band section contains a heavily spliced domain called PEVK, named so due to its propensity of prolines, glutamates, valines and lysines. Even though PEVK has been shown to contain structural motifs such as poly-proline helices, by large it is an intrinsically disordered protein (IDP). IDPs display a tendency for liquid-liquid phase separation (LLPS), a reversible demixing process characterized by the formation of liquid droplets. Here we hypothesized that PEVK might also display LLPS. To test our hypothesis, we carried out *in vivo* experiments and molecular dynamics (MD) simulations. The 733-residue-long middle part of the full-length PEVK was inserted into a construct containing, C-terminally from the PEVK, the genes encoding mCherry and the photodimerizing protein Cry2. Cultured Drosophila S2R+ cells were transfected with the construct, and protein expression was induced by the addition of 1 mM copper-sulfate. Photoactivation and fluorescence imaging were carried out by using confocal and TIRF microscopy. We found that, upon a few-second illumination with 488-nm laser light, mCherry positive droplets formed rapidly in the cytoplasm. The droplets often fused together and evaded the mechanical probing by an AFM cantilever tip, thereby revealing their liquid nature. MD simulations suggested that PEVK indeed displays self-interaction, and that calcium enhances the effect. Altogether, our results demonstrate that titin's PEVK domain is a protein capable of LLPS, which may have yet-to-be-revealed implications for muscle function.

Acknowledgment: *This work was funded by grants from the Hungarian National Research, Development and Innovation Office (K143321 and 150826 to M.K.) and the Thematic Excellence Programme (2020-4.1.1.-TKP2020) of the Ministry for Innovation and Technology in Hungary, within the framework of the Therapeutic Development and Bioimaging thematic programs of the Semmelweis University.*

Tuning the Structure and Nanomechanics of κ-Casein Amyloid Fibrils with Food-Grade Osmolytes

Negar Rahimi[1,2], Barnabás-Bőcskei Antal[1,2], Amiesam Manshazadeh[1], Neshat Moslehi[3,4], Bence Fehér[1,2]

[1]*HUN-REN-Semmelweis University Nanobiophysics Research Group, Tűzoltó street 37-47, Budapest, H-1094, Hungary, e-mail of the presenting author: n.rahimi.99@gmail.com*
[2]*Institute of Biophysics and Radiation Biology, Semmelweis University, Tűzoltó street 37-47, Budapest, H-1094, Hungary*
[3]*Institute for Complex Molecular Systems (ICMS), Eindhoven University of Technology, 5600 MB Eindhoven, The Netherlands*
[4]*Laboratory of Self-Organizing Soft Matter, Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, 5600 MB Eindhoven, The Netherlands*

Amyloid fibrils are highly ordered protein aggregates that, beyond their pathological roles, are increasingly recognized as functional structures in food systems. κ-casein is an interesting model due to its dual role: it stabilizes casein micelles in milk yet can form amyloid fibrils under destabilizing conditions. Understanding how food components influence fibril formation and properties is therefore important for both food science and the broader study of protein self-assembly.

In this work, we investigate how food-grade sugars acting as osmolytes modulate the fibrillation behavior, structure, and nanomechanical properties of κ-casein amyloid fibrils. Aggregation kinetics are monitored using Thioflavin T fluorescence assays to assess the influence of sugars on fibril nucleation and growth. The morphology and mechanical properties of the resulting fibrils are characterized using atomic force microscopy, enabling nanoscale measurements of fibril dimensions and stiffness. Complementary small-angle X-ray scattering experiments provide insight into fibril organization in solution, while all-atom molecular dynamics simulations probe how osmolytes influence κ-casein conformational dynamics and intermolecular interactions.

Preliminary observations suggest that sugars can influence fibril formation and nanomechanical properties without significantly altering the core fibrillar architecture. By combining experimental and computational approaches, this study aims to elucidate how osmolytes regulate κ-casein aggregation and how common food additives can tune the structural and mechanical properties of amyloid fibrils formed by dietary proteins.

Aknowledgement: *This research project was supported by HUN-REN Welcome Home and Foreign Researcher Recruitment Programme 2023. This project was supported by the National Research, Development and Innovation Office of Hungary under STARTING grant (150679) and under grant FK146081. We acknowledge [KIFÜ] for awarding us access to resources based in Hungary at Komondor for CPU and GPU time.*

Bioactive Phenolics in *Picea omorika* Knot Extracts

Nevena Preradović[1], Đura Nakarada[2], Ana Đurić[3], Uroš Gašić[4], Ksenija Radotić[1], Miloš Mojović[2], Jasna Simonović Radosavljević[1]

[1]*University of Belgrade – Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, Belgrade, 11030, Serbia, nevena@imsi.bg.ac.rs*
[2]*Center for Physical Chemistry of Biological Systems, BioScope Labs, Faculty of Physical Chemistry, University of Belgrade, 11158 Belgrade, Serbia*
[3]*Department of Experimental Oncology, Institute for Oncology and Radiology of Serbia, 11000 Belgrade, Serbia*
[4]*Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, 11108 Belgrade, Serbia*

Phenolic compounds from conifer tissues exhibit diverse biological activities, including antioxidant and cytotoxic effects. Knots of *Picea omorika* remain underexplored despite evidence of enrichment in specialized phenolics, particularly lignans.

Ethyl acetate extracts from *P. omorika* knots were subjected to qualitative HPLC-MS/MS analysis to profile phenolic compounds. Antioxidant activity was assessed by DPPH and hydroxyl radical scavenging using electron paramagnetic resonance (EPR) spectroscopy. Cytotoxicity was evaluated by MTT assay on MRC5, A549, MDA-MB-231, and HeLa human cell lines.

A total of 55 phenolic compounds were identified, including a rich set of lignans. The extract exhibited significant hydroxyl radical scavenging (57.83%), as detected by EPR and moderate DPPH activity (12.06%). In contrast, moderate cytotoxic effects were observed with IC_{50} values ranging from 184 to 204 μg/mL across cell lines. The EPR-based approach enables direct detection of short-lived radical species, providing a specific assessment of antioxidant activity. These findings demonstrate the usefulness of EPR spectroscopy for evaluating radical scavenging activity in complex plant extracts and indicate *P. omorika* knot tissues as a source of bioactive phenolics.

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contracts 451-03-33/2026-03/200053, 451-03-33/2026-03/200146, 451-03-34/2026-03/200146, 451-03-33/2026-03/200043, 451-03-33/2026-03/200007.*

Structural Insights Into the *Ramonda serbica* LEA4 Protein Family

Ana Pantelić[1], Tatiana Ilina[1], Dejana Milić[1], Miroslav Gančár[2], Zuzana Bednarikova[2], Marija Vidović[1]

[1]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11042 Belgrade, Serbia ana.pantelic@imgge.bg.ac.rs*
[2]*Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01, Kosice, Slovakia*

Defined by a relative water content of less than 5%, desiccation triggers cellular stress that leads to irreversible protein denaturation and aggregation, compromised membrane integrity, and destabilisation of nucleic acids [1]. *Ramonda serbica*, an ancient resurrection plant, exhibits a remarkable ability to survive desiccation and fully recover metabolic functions upon rehydration. The hallmarks of desiccation tolerance are Late Embryogenesis Abundant Proteins (LEAPs). As a heterogeneous group of small intrinsically disordered proteins, LEAPs typically maintain a random coil conformation when hydrated but transition into α-helices during drying.

In this study, we expressed and purified three members of the LEA4 *R. serbica* protein group. Structural characterization using circular dichroism (CD) and Fourier Transform Infrared (FTIR) spectroscopy revealed that all three LEAPs remain predominantly disordered. However, in the presence of 2,2,2-trifluoroethanol and lipid-mimetic detergents, these proteins undergo a disorder-to-order transition, folding primarily into α-helical structures. This structural plasticity suggests that the protective role of LEAPs is closely linked to their ability to interact with desiccation-sensitive membranes and proteins. Our findings provide new insights into the relationship between the structural duality of LEAPs and the molecular mechanisms underlying desiccation tolerance in resurrection plants.

1. Farrant, JM., Brandt, W., Lindsey, GG., 2007, Plant Stress 1:72–84.

Acknowledgment: *This research was funded by the Science Fund of the Republic of Serbia (PROMIS project LEAPSyn-SCI, grant no. 6039663), the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-33/2026-03/200042), a bilateral project with the Republic of Slovakia (337-00-3/2024-05/17) and supported by the Slovak Research and Development Agency (Contract no. APVV-SK-SRB-23-0062). T.I. wishes to acknowledge the support of COST Action CA21160 (ML4NGP) for approving Short-Term Scientific Missions in Košice.*

Cytochrome *c* as a Modulator of Amyloid-β Aggregation and Fibril Stability

Viktoria Relovska[1], Eva Valusova[1], Nikitas Georgiou[2], V. Huntosova[2], Katarina Siposova[1]

[1]*Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia, fedorova@saske.sk*
[2]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, P. J. Safarik University, Kosice, Slovakia*

Alzheimer's disease (AD) is a major neurodegenerative disorder whose molecular determinants remain incompletely understood. Oxidative stress plays a key role by promoting lipid peroxidation, membrane destabilization, and increased amyloid β (Aβ) production via β-secretase activation, thereby enhancing peptide-membrane interactions and accumulation. Cytochrome *c* (Cyt *c)*, due to its role in redox homeostasis, has emerged as a potential modulator of these processes. Here, we investigated its effect on $A\beta_{1–40}$ aggregation, fibril stability, and morphology, revealing a pronounced anti-amyloidogenic activity mediated primarily by lysine residues. To probe this effect, lysine-rich Cyt *c*-derived fragments were examined; however, their activity was weaker compared to the full-length protein, underscoring the importance of the native structural context. Additional experiments with apo-Cyt *c*, free lysine, poly-lysine, and polyarginine further highlighted the role of cationic residues in Aβ modulation. Complementary, *in silico* docking (ClusPro) between an amyloid β peptide and Cyt *c* yielded a top−ranked cluster with a score of −886.5, indicating a favorable and potentially biologically relevant interaction. Overall, Cyt *c* is identified as a potential modulator of Aβ aggregation, with both lysine residues and protein structural integrity playing key roles, contributing to a more comprehensive understanding of AD pathogenesis.

Acknowledgment: *This work was funded by the APVV 23-0013, VEGA 2/0053/26, and MVTS SK-TW Supra-Sight. The work was also funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09-I02-03-V01-00021.*

Structural and Dynamical Changes of the *Streptococcus Pyogenes* Transcriptional Regulator Mtsr Induced by Mn^{2+} Binding

Silvia – Maria Franov[1], Ivana Lešić Ašler[2], Dijana Žilić[2], Ivo Crnolatac[2], Branimir Bertoša[1]

[1]University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia, sfranov@chem.pmf.hr
[2]Ruđer Bošković Institute, Zagreb, Croatia

The ability of *Streptococcus pyogenes* to adapt to changes in manganese availability relies on the DtxR-family regulator MtsR, which controls metal-dependent gene expression.[1,2] Despite its known regulatory role[1], the molecular mechanism by which binding of Mn^{2+} ions increases affinity of MtsR to DNA remains unresolved. To address this question, computational (molecular dynamics (MD) simulations) and experimental methods (EPR spectroscopy, circular dichroism (CD), and differential scanning calorimetry (DSC)) were applied to characterize manganese-dependent conformational changes of MtsR. Biophysical characterisation of apo, partially metallated, and fully metallated (holo) states of MtsR showed structural stabilization upon Mn^{2+} binding. Only fully metallated MtsR adopts compact conformation with the arrangement of the DNA-binding domains that is capable for binding to the two neighbouring major grooves of the DNA. In the holo state, the DNA binding domains (DBDs) are separated by ~34 Å, consistent with the spacing of adjacent major grooves in B-form DNA (Fig.1). Whereas the apo form of the protein samples expanded conformations with DBD separations of ~60 Å, incompatible with productive DNA binding. At the molecular level, allosteric mechanism induced by Mn^{2+} binding and based on Arg157-centered interdomain interactions was identified. This agrees with metal-induced rearrangements in related DtxR-family regulators[3] and defines the basis of manganese-dependent MtsR activation.

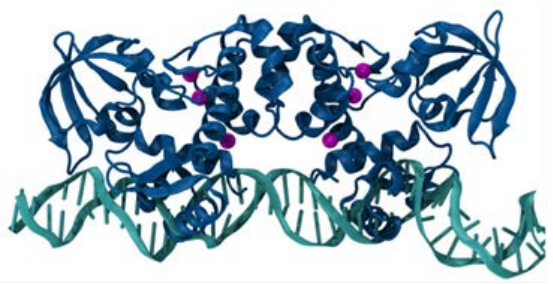


Figure 1. Mn^{2+} - bound MtsR in complex with operator DNA.

1. Bates, C.S. *et al*., 2005, Infect. Immun. 73 6832–6841.
2. Bates, C.S. et al., 2003, Infect. Immun. 71 1042–1055.
3. Radman, K. *et al*., 2023, Int. J. Biol. Macromol. 253 127572.

Acknowledgments: *This work was supported by the Croatian Science Foundation under project IP-2020-02-3446 – "Manganese metallosensors"*

Fullerene-Phytocompound Composites as Anti-Amyloid Agents: Insights From Structural and Dynamical Studies

Katarina Siposova[1], Viktor Petrenko[2,3], Mona Sarter[4], Oleksandr Tomchuk[4], Svitlana Lyubchyk[5], Sergiy Lyubchyk[5]

[1]*Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia, siposova@saske.sk*
[2]*BCMaterials, Basque Center for Materials, Applications and Nanostructures, UPV/EHU Science Park, Leioa, Spain*
[3]*IKERBASQUE, Basque Foundation for Science, Bilbao, Spain*
[4]*ISIS Neutron and Muon Source, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Didcot, United Kingdom*
[5]*Department of Engineering, Centre of Industrial Engineering Management and Sustainability, Universidade Lusófona, Lisbon, Portugal*

Protein misfolding and aggregation into amyloid fibrils are hallmarks of neurodegenerative diseases, leading to cellular toxicity and neuronal dysfunction. Identifying compounds capable of inhibiting or destabilizing amyloid aggregates remains a major challenge. Fullerene-based nanomaterials are promising modulators of protein aggregation, while phytochemicals exhibit anti-amyloid activity but suffer from poor solubility and bioavailability. Fullerene-phytochemical complexes offer a strategy to overcome these limitations and enhance anti-amyloid performance. In this study, lysozyme was used as a model system to investigate amyloid formation and inhibition in the presence of these complexes. The analysis combined ThT fluorescence, AFM, SAXS, and QENS to probe structural and dynamic properties of amyloid systems. ThT and AFM confirmed that fullerene-phytochemical derivatives effectively inhibit fibril formation and disrupt preformed aggregates. SAXS revealed nanoscale structural changes and differences in aggregation kinetics upon fullerene addition. QENS experiments, performed at the IRIS spectrometer (ISIS Neutron and Muon Source, UK), provided key insight into protein dynamics on the pico- to nanosecond timescale. Differences in spectral broadening and elastic peak intensity between amyloids, fullerenes, and their mixtures indicate significant changes in protein mobility and flexibility, highlighting the impact of fullerenes on amyloid dynamics. Overall, this study demonstrates that fullerene–phytochemical complexes modulate both structure and dynamics of amyloid systems, emphasizing the importance of protein dynamics in aggregation and inhibition processes.

Acknowledgment: *This work was funded by the APVV 23-0013, VEGA 2/0053/26, MVTS SK-TW Supra-Sight and 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.*

Disordered Oligomers Formed by Truncated Tau Proteins

Ondrej Cehlar[1,2], Stefana Njemoga[1], Andrej Kovac[1], Petra Majerova[1], Tomas Sadecky[2], Vladimir Garaj[2], Exequel E. Barrera[3]

[1]*Institute of Neuroimmunology, Laboratory of Structural Biology of Neurodegeneration, Slovak Academy of Sciences, Bratislava, Slovakia, ondrej.cehlar@savba.sk*

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

[3]*Instituto de Histología y Embriología (IHEM), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CC56, Universidad Nacional de Cuyo, Mendoza M5502JMA, Argentina*

Soluble oligomers are thought to be the toxic agents of several neurodegenerative diseases and not the relatively inert mature filaments. Oligomers can propagate between neurons and could be isolated by in vivo microdialysis from brain interstitial fluid. Tau oligomers can be induced in vitro by seeding with amyloid-β (Aβ) oligomers or using tau with modified cysteines[1]. However, intermediate filaments with shorter amyloid interface compared to mature filaments were observed early in the course of tau aggregation[2].

Previously we have summarized structural data about oligomers formed by amyloid-β, α-synuclein, tau a prion protein, that show substantial lack of structural information about tau protein oligomers[3].

Isolated in vitro tau oligomers prepared from truncated tau were characterized by ion mobility mass spectrometry, size exclusion chromatography, dot blot and western blot using oligomer specific antibodies T22, A11 and TOMA-1. The structures of tau oligomers will be further characterized by SAXS, solid-state and solution state NMR and hydrogen-deuterium exchange techniques.

Moreover, representative structures of tau dimers and trimers were obtained after clustering of coarse-grained MD simulations. Set of previously reported small molecules with potential to inhibit tau aggregation was docked in the presumed binding pockets identified in these structures. The inhibition was probed by measuring the ThT signal of the aggregation reaction in the presence of small molecules.

1. Lasagna-Reeves, C. L., Castillo-Carranza, D., Sengupta, U. et al. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. Sci Rep 2, 700 (2012).
2. Lövestam, S., Li, D., Wagstaff, J.L. et al. Disease-specific tau filaments assemble via polymorphic intermediates. Nature 625, 119–125 (2024).
3. Cehlar, O.; Njemoga, S.; Horvath, M.; Cizmazia, E.; Bednarikova, Z.; Barrera, E.E. Structures of Oligomeric States of Tau Protein, Amyloid-β, α-Synuclein and Prion Protein Implicated in Alzheimer's Disease, Parkinson's Disease and Prionopathies. Int. J. Mol. Sci. 2024, 25, 13049.

Acknowledgment: *This research was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00623. This work was supported by research grants VEGA 2/0125/23 and 1/0825/25. This research was further funded by the European Union's Horizon Europe program under the grant agreement No. 101087124.*

Disorder-to-Order Transition of LEA4 Family Proteins in *Ramonda serbica*

Tatiana Ilina[1], Ana Pantelić[1], Zuzana Bednáriková[2], Marija Vidović[1]

[1]*Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11042 Belgrade, Serbia*
[2]*Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01, Kosice, Slovakia*

Ramonda serbica Panč. is an ancient resurrection plant characterized by exceptional tolerance to extended desiccation and the capacity to rapidly restore metabolic function upon rehydration. A key mechanism underlying this tolerance involves the accumulation of protective late embryogenesis abundant proteins (LEAPs). These intrinsically disordered proteins are believed to serve a critical function during dehydration by maintaining the native conformations of cellular proteins, nucleic acids, and membranes. Comparative transcriptomic and proteomic analyses have revealed that LEA4 family proteins represent the dominant class of desiccation-responsive LEAPs.

In the present study, we used circular dichroism (CD) spectroscopy to characterize secondary structure of 10 members of LEA4 protein family, which were recombinantly produced and purified. To simulate low-water-content conditions, increasing concentrations of trifluoroethanol (TFE) were tested, while critical micelle concentration of sodium dodecyl sulfate (SDS) was used as a membrane-mimicking environment. All tested LEA proteins underwent a gradual structural transition from an intrinsically disordered state to an α-helical conformation upon increasing concentrations of TFE and in the presence of SDS. We propose this disorder-to-order transition of LEA4 family members plays a crucial protective role during desiccation enabling rapid recovery of cell components upon rehydration. Further studies on LEA protein functions in living plants would help to better understand the mechanisms of desiccation tolerance in resurrection plants.

Acknowledgement: *This research was funded by the Science Fund of the Republic of Serbia–RS (PROMIS project LEAPSyn-SCI, grant no.6039663) and by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-33/2026-03/200042). TI thanks COST Action ML4NGP (CA21160) for providing funding for the Short-Term Scientific Mission in Košice, which supported our research and collaboration efforts.*

Studying Bacterial Ecosystems Using Engineered Microfluidic Habitats

Peter Galajda[1]

[1]*Institute of Biophysics, HIN-REN Biological Research Centre, Szeged, Hungary, galajda.peter@brc.hu*

Ecological and physiological interactions between cells are strongly determined by their adjacency relationships. The spatial structure of an environment determines the geometry of cell–cell interactions; therefore, community dynamics depend critically on it.

We present engineered synthetic microbial ecosystems where spatially and temporally structured habitats were created using microfluidic technologies. In these systems, the geometry of the habitat, the (dynamic) chemical landscape, and the spatial distribution of cells were controlled. We explored how the geometric structure of the environment, the presence of chemical gradients, and the topology of cell-cell interactions affect population dynamics, competition, and coexistence. For example, we showed that a patchy habitat structure can lead to the formation of metapopulations (a population of coupled populations) and support the coexistence of bacterial strains that outcompete each other in shaken flasks. We demonstrated that antibiotic gradients could lead to rapid evolution of resistance, in which the emergent spatial structure of the population plays a key role. We also explored how pulses of signal molecules shape bacterial communication (quorum sensing). While our previous results help us understand the significance of spatial and temporal structures in microbial ecosystems, we are developing a platform to achieve complete control over the structure of spatiotemporal landscapes in future experiments.

SCIENTIFIC SESSION II

MEMBRANE AND CELL BIOPHYSICS

Molecular Pathology of Ion Channels in Diseases And Their Pharmacological Targeting

Gyorgy Panyi[1]

[1]*Faculty of Medicine, University of Debrecen, Department of Biophysics and Cell Biology, Debrecen, Hungary, panyi@med.unideb.hu*

Ion channels allow selective and regulated transport of ions across biological membranes. Ion channels have versatile biological functions including the regulation of the resting membrane potential and electrical excitability of the cells. In general, loss-of-function (LOF) and gain-of-function (GOF) of ion channels can lead to pathological conditions that can be managed by molecules targeting and modulating ion channel functions. Of these, the talk will focus on two voltage-gated K^+ channels.

The Kv1.3 channel primarily expresses in immune cells and in the CNS. Kv1.3 inhibition is a promising therapeutic approach in certain autoimmune diseases (e.g. multiple sclerosis) mediated by effector memory T cells. To address this, we have identified and characterized several high affinity and selective Kv1.3 inhibitor peptides derived from scorpion toxins. We have also generated fluorescence-conjugated peptides that can serve as a tool to identify Kv1.3 channel expressing cells.

LOF mutations of the KCNQ2 gene encoding the Kv7.2 K^+ channel leads to hyperexcitability syndromes, such as benign familial neonatal seizures. We have characterized a novel LOF mutation of Kv7.2, S113F in the extracellular S1-S2 loop. We found that the peak current density is substantially reduced in the S113F mutant. Linking S113F with the wild-type subunits in concatamers showed that the presence of a single mutant subunit confers the LOF phenotype to the tetramers.

Support: *2024-1.2.3-HU-RIZONT-2024-00099*

Characterization of a Novel Hyperpolarization-Activated Delayed Anion Current (Hadac) in Plasma Membrane of the Filamentous Fungus *Phycomyces blakesleeanus*

Aleksandar Milovanović[1], Katarina S. Stevanović[1], Anđela Trkulja[1], Nataša V. Todorović[2], Miroslav Živić[1]

[1]*Faculty of Biology, University of Belgrade, Belgrade, Serbia, m3029_2024@stud.bio.bg.ac.rs*
[2]*Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*

Ion movement across the plasma membrane is essential for homeostasis and responses to external stimuli. In fungi, ion distribution and fluxes across the cell membrane are largely determined by H^+-ATPase, which maintains the resting potential. While carrier-mediated mechanisms, relying on the proton gradient, are well described in fungi, ion channel activity remains largely unexplored, particularly ion channels that may transport large organic acids. Here, we present a biophysical profile of a novel anion current, in the membrane of cytoplasmic droplets derived from sporangiophores of *Phycomyces blakesleeanus*, mediated by a channel permeable to chloride (Cl^-), bicarbonate (HCO_3^-), gluconate and citrate ions. The patch-clamp whole-cell recorded currents were evoked by voltage steps from -150 to +90 mV. The current is apparently active at hyperpolarizing potentials, mildly rectified, with a pronounced delay in current activation (211.8±19.6 ms), most prominent at -150 mV. Current density (pA/µF) at -150 mV was significantly different between Cl^- (-34±7) and both bicarbonate (-22±3) and citrate (-18±2). The current is also active with intracellular gluconate at pH 5.5, indicating lack of inactivation by high [H^+]. Current densities with gluconate and Cl^- were indistinguishable, demonstrating that the novel hyperpolarization-activated delayed anion current (HADAC) nearly equally carries large organic acid anions as Cl^-. We propose its role in efflux of organic acids.

Scanning Electron Microscopy Study of Multilamellar Vesicles Produced by the Rapid Solvent Exchange Method

Marija Raguž[1], Krešimir Kuliš[2], Ante Bilušić[3], Mateo Šerić[1]

[1]*University of Split School of Medicine, Department of Medical Physics and Biophysics, Split, Croatia, marija.raguz@mefst.hr*
[2]*Institute of Emergency Medicine of Split-Dalmatia County, Split, Croatia*
[3]*Faculty of Natural Sciences, University of Split, Department of Physics, Split, Croatia*

In this study, we investigated the properties of multilamellar vesicles prepared from a mixture of 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) and cholesterol (Chol). The aim was to determine how increasing Chol concentration affects vesicle size, morphology, lipid-domain formation, and the appearance of Chol crystals. The liposomes were prepared using the rapid solvent exchange method, deposited onto an indium tin oxide (ITO) conductive surface, dried, and then monitored by scanning electron microscopy (SEM). We evaluated whether SEM is a suitable method for this type of research.

We successfully observed consistent MLV morphology for Chol/POPS mixing ratios ranging from 0 to 1.5, both in buffer and in Milli-Q water. In buffer, vesicle size decreased by approximately a factor of three as the Chol concentration increased, whereas this parameter remained essentially unchanged in Milli-Q water. We also detected the formation of Chol crystals at a Chol/POPS mixing ratio as low as 0.25, which was an unexpected result. Furthermore, Chol crystals were always formed outside the MLV lipid bilayer what was in agreement with differential scanning calorimetry data reported in the literature.

Acknowledgment: *This work was funded by the Croatian Science Foundation under Grant HRZZ IP-2024-05-3923.*

Synaptic Force Shielding in T-Cell Receptor–Ligand Interactions

Lukas Schrangl[1,2], Florian Kellner[3], René Platzer[3], Vanessa Mühlgrabner[3], Paul Hubinger[3], Josephine Wieland[4], Reinhard Obst[4], José L. Toca-Herrera[1], Johannes B. Huppa[3], Gerhard J. Schütz[2], Janett Göhring[3,5]

[1]*BOKU University, Institute of Biophysics, Department of Natural Sciences and Sustainable Resources, Vienna, Austria, lukas.schrangl@boku.ac.at*
[2]*TU Wien, Institute of Applied Physics, Vienna, Austria*
[3]*Medical University of Vienna, Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria*
[4]*Ludwig-Maximilians-Universität München, Institute for Immunology, Biomedical Center, München, Germany*
[5]*BOKU University, Institute of Molecular Biotechnology, Department of Biotechnology and Food Science, Vienna, Austria*

As a key player in immunity, T-cells use their T-cell receptors (TCRs) to identify antigenic peptides on major histocompatibility complexes (pMHCs) at the membrane of antigen-presenting cells (APCs). While the exact process remains elusive, theories suggest mechanical forces as major factors.

To quantify forces exerted by single TCRs, we built a force sensor based on a flexible peptide acting as a nanoscopic spring. Force-induced length changes were monitored via single-molecule (sm) FRET. Primary CD4+ T-cells interacted with sensors bound to supported lipid bilayers (SLBs) that mimic APCs. Forces of 2–7pN were registered by a small fraction of the sensors at low ligand densities, which did not activate the T-cells, but emulated conditions in which the cells scan for antigen. In contrast, high ligand doses lead to T-cell activation and immune synapse formation. Here, up to 7pN could be observed only with gel-phase SLBs, while forces were mostly absent on fluid SLBs. This indicates that the immune synapse shields TCR–pMHC bonds from forces perpendicular to the cellular interface.

Mechanical forces are thought to modulate T-cell activation by affecting TCR–pMHC bond lifetimes (catch- or slip bonds). We quantified the bond lifetime between receptor and ligand via smFRET on (high force) gel-phase and (low force) fluid SLBs. We found no evidence of catch- nor slip bonds within the immune synapse, raising further doubt about the role of forces in T-cell antigen recognition.

Energy Profile of Proton Pumping Mechanism in Mammalian Cytochrome c Oxidase under the Influence of Membrane Electrochemical Gradient

Dragan M. Popović[1], Ivana S. Đorđević[1]

[1]*Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, University of Belgrade, Department of Chemistry, Belgrade, Serbia, dragan.popovic@ihtm.bg.ac.rs*

Cytochrome *c* oxidase (CcO) is an energy-transducing membrane-bound enzyme in the respiratory electron transport chain. It couples the reduction of O_2 to H_2O with proton pumping across the mitochondrial membrane, generating an electrochemical gradient essential for energy storage and use in aerobic cells.[1] The precise coordination between protonation and redox reactions is key to its function.[2] Recent time-resolved electrometric studies have shed light on the step-by-step process of charge translocation in CcO. In this work, combined DFT/electrostatic calculations[2-4] are employed to obtain the energetics of the proton- and electron-transfer reaction steps during the O→E transition. The energy profile of the reaction mechanism is examined in relation to the redox states of metal centers, dielectric solvation effects, membrane potential, and the risk of proton leakage through the Glu242 gating site of the enzyme pump. Special attention is given to side reactions that may compromise pump efficiency, as well as the natural strategies biological systems use to mitigate or prevent their effects.

1. Popović, D.M., Đorđević, I.S., 2020, J. Serb. Chem. Soc. 85:1429-1444.
2. Popović, D.M., Stuchebrukhov, A.A., 2012, BBA-Bioenerg. 1817:506-517.
3. Popović, D.M., 2022, Combined DFT/electrostatics approach: Exploring the coupling of biological electron and proton transfer reactions in cytochrome c oxidase, in: C.D. Grant (ed.) Advances in Biology, vol. 2, pp. 1-66, Nova Science, New York.
4. Popović, D.M., 2013, Amino Acids 45:1073-1087.

Acknowledgment: *This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Contract No. 451-03-33/2026-03/200026. The authors also thank COST Actions CA21101 (COSY) and CA22143 (EuMINe) for their partnership through the European Cooperation in Science and Technology.*

Oxidative Degradation of Polyunsaturated Lipid Membranes: Structural Changes, Mechanistic Insights and Flavonoid Protection

Anja Sadžak[1], Mihael Eraković[1], Manfred Kriechbaum[2], Janez Mravljak[3], Jan Přibyl[4], Nadica Maltar-Strmečki[1], Suzana Šegota[1]

[1]*Division of Physical Chemistry, Ruđer Bošković Institute, 10000 Zagreb, Croatia, asadzak@irb.hr*
[2]*Institute of Inorganic Chemistry, Graz University of Technology, 8010 Graz, Austria*
[3]*University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia*
[4]*CEITEC MU, Masaryk University, 62500 Brno, Czech Republic*

Peroxidation of polyunsaturated fatty acids (PUFAs) in biological membranes is a central mechanism through which oxidative stress compromises membrane stability and initiates radical damage.[1] Despite extensive research on lipid peroxidation, the mechanistic pathways and antioxidant protection of PUFAs remain poorly characterized. In this study, we investigate peroxidation in liposomal bilayers composed of docosahexaenoic acid, one of the constitutional PUFAs in various cellular membranes using controlled in vitro conditions to simulate oxidative stress.[2] By initiating oxidative stress under controlled conditions, the distinct reactivity profiles and propagation kinetics of PUFA peroxidation were elucidated. Simultaneously, membrane structural changes were monitored by atomic force microscopy (AFM), electron paramagnetic resonance (EPR), and small-angle X-ray scattering (SAXS).

Since this radical-driven chain reaction can escalate rapidly within PUFA-rich membranes, it is crucial to determine how antioxidant molecules intervene to interrupt or modulate these pathways and provide effective protection. Therefore, we evaluated the antioxidant role of three structurally-different flavonoids, assessing both their capacity to suppress oxidative product formation and their influence on bilayer stability. Together, these findings point toward promising directions for future research into antioxidant–membrane interactions and their potential applications in managing oxidative damage.

1. O.I. Aruoma, Free radicals, oxidative stress, and antioxidants in human health and disease, J. Am. Oil Chem. Soc. 75 (1998) 199–212
2. A. Sadžak*, M. Eraković, M. Kriechbaum, J. Mravljak, J. Přibyl, N. Maltar-Strmečki, S. Šegota*, "Oxidative degradation of polyunsaturated lipid membranes: Structural changes, mechanistic insights and flavonoid protection", Journal of Colloid and Interface Science 5 709 (2026) 139894

Acknowledgment: *The authors acknowledge Croatian Science Foundation (IP-2016-06- 8415) and CF Nanobiotechnology of CIISB, Instruct-CZ Centre, supported by MEYS CR (projects no. LM2018127 and LUC24105) and CEITEC of A4L_ACTIONS, supported by European Union's Horizon 2020 under grant agreement No. 964997. They also thank the "CERIC-ERIC" for the generous support in the use of the Somapp Lab (SAXS equipment), a core facility supported by the Austrian Federal Ministry of Education, Science and Research, the Graz University of Technology, the University of Graz and Anton Paar GmbH.*

Effect of Dipole Potential Modulators Phloretin and 6-Ketocholestanol on Membrane Potential

Shahrukh Husain[1], Levente Szolyka[1], Gyorgy Panyi[1], Zoltan Varga[1], Tamas Kovacs[1], Gyorgy Vamosi[1] and Ferenc Papp[1]

[1]Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, shahrukh.husain@med.unideb.hu

Membrane potential (MP) is the voltage difference across biological membranes generated by unequal ion distribution and typically ranges between −20 and −80 mV in many cell types. It plays a central role in electrical excitability, muscle contraction and intracellular signaling. In contrast, dipole potential (DP) originates from the orientation of lipid and interfacial water dipoles within the membrane bilayer, producing a localized electric field of approximately 100–300 mV. Although DP does not directly arise from ion gradients, it can influence membrane protein function and ion permeation.

Phloretin and 6-ketocholestanol (6Keto) are well-known modulators of DP, decreasing and increasing it, respectively. The aim of this study was to investigate whether pharmacological modulation of DP affects MP. Membrane potential was measured using the patch-clamp technique in current-clamp mode following 30 minutes of pre-incubation with either phloretin or 6Keto.

These two compounds alter MP in a cell-dependent manner (i.e., dependent on ion channel expression) compared to control conditions. These findings suggest that the relationship between DP and MP is more substantial than previously assumed. Furthermore, the results indicate that DP-modifying molecules may influence membrane permeability, potentially through direct or indirect effects on ion channel activity. Understanding this interplay may provide new insights into membrane biophysics and ion channel regulation.

Acknowledgment: *This research was funded by the University of Debrecen Scientific Research Bridging Fund (DETKA) (FP) and also supported by the University of Debrecen Program for Scientific Publication (DETKA) (ZV). This work was also supported by the Ministry of Culture and Innovation of Hungary from the National Research, Development, and Innovation Fund, grant 2024-1.2.3-HU-RIZONT-2024-00099. This research was also funded by the Hungarian National Research, Development and Innovation Office (OTKA K143071, OTKA 132906).*

Exploring Liposome-Encapsulated Schiff Bases as Antioxidants for Honey Bee Health

Đura Nakarada[1], Miljan Bigović[2], Marija Kaluđerović[2], Nevena Prlainović[3], Miloš Mojović[1]

[1]*Center for Physical Chemistry of Biological Systems, BioScope Labs, Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia, djura@ffh.bg.ac.rs*
[2]*Faculty of Natural Science and Mathematics, University of Montenegro, Podgorica, Montenegro*
[3]*Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

Schiff bases derived from thiocarbohydrazide are widely studied for their diverse biological activities, including strong antioxidant potential. In this work, the antioxidative properties of (*E*)-*N*′-((*E*)-1-(2-hydroxyphenyl)ethylidene)-2-(1-(2-hydroxyphenyl)ethylidene)hydrazine-1-carbothiohydrazide (SB), a novel Schiff base, were evaluated by electron paramagnetic resonance (EPR) spectroscopy through scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (˙OH) radicals. To examine the influence of a lipid environment, SB was incorporated into dipalmitoylphosphatidylcholine (DPPC) liposomes using a modified thin-film hydration method followed by extrusion. EPR results showed strong radical-scavenging activity in solution, reducing DPPH and ˙OH signals by 69.53% and 37.31%, respectively. After incorporation into DPPC liposomes, DPPH scavenging decreased to 46.12%, while ˙OH scavenging remained similar (34.38%). The reduced DPPH activity likely reflects partial shielding of SB within the lipid bilayer, limiting access to radicals, whereas reactivity toward ˙OH is less affected. The SB retains notable antioxidative activity in lipid vesicles, highlighting liposomes as suitable carriers that modulate interactions with reactive species. Given the role of oxidative stress in honey bee decline, these results support further exploration in apicultural research, including studies using the AmE-711 honey bee cell line, to assess potential benefits for bee health.

Acknowledgment: *This research was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, contract numbers 451-03-33/2026-03/200146 and 451-03-34/2026-03/200146 and by the Science Fund of the Republic of Serbia, project Waste2ProtectBees No. 5455.*

Toward High-Throughput Characterization of Giant Unilamellar Vesicles by Real-Time Deformability Cytometry

Megi Tinev[1], Bojan Božič[1]

[1]*Faculty of Medicine, University of Ljubljana, Institute of Biophysics, Ljubljana, Slovenia, megi.tinev@mf.uni-lj.si*

Existing approaches for extracting membrane properties of giant unilamellar vesicles (GUVs), including fluctuation spectroscopy, micropipette aspiration, and electrodeformation, provide rich physical information but are typically applied to relatively small vesicle numbers per experiment[1]. Statistically relevant characterization, however, requires the analysis of several hundreds of vesicles[2]. These methods therefore remain challenging in practice, especially if performed manually, as they may be time-consuming and prone to subjective bias.

In our work, we explore real-time deformability cytometry (RT-DC) as a potential high-throughput method for GUV characterization. RT-DC enables rapid measurement of vesicle cross-sectional area and deformability for large vesicle populations under controlled microfluidic flow conditions. Preliminary experiments reveal the potential of RT-DC to resolve differences in measured deformability-area distributions due to different membrane compositions.

Based on experimentally relevant channel geometry, flow rates, carrier-fluid conditions, and measured vesicle contours, we used OpenFOAM simulations to resolve normal and tangential hydrodynamic stress distributions on the vesicle surface, and its steady velocity through the channel. Together, these quantities define the hydrodynamic stress state acting on the deformed vesicle membrane and establish the physical basis for future extraction of membrane mechanical parameters from RT-DC measurements.

1. Dimova, R., 2019, Annu. Rev. Biophys. 48:93-119.
2. Hermann, E., Bleicken, S., Subburaj, Y., *et al.*, 2014, Bioinformatics. 30:1747-1754.

Acknowledgment: *This work was supported by the Slovenian Research and Innovation Agency (ARIS, contract P1-0055).*

Reshaping RNA: Bulges Guide High-Affinity PNA Triplex Formation

Maja Marušič[1], Sara Farshineh Saei[2], Md Motiar Rahman[2], Christopher A. Ryan[2], Janez Plavec[1,3,4] and Eriks Rozners[2]

[1]*Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia, marusic.maja@ki.si*

[2]*Department of Chemistry, Binghamton University, The State University of New York, Binghamton, New York 13902, United States*

[3]*Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia*

[4]*EN-FIST Center of Excellence, SI-1000 Ljubljana, Slovenia*

Controlling RNA structural dynamics is central to RNA function and remains a major challenge for molecular targeting. Peptide nucleic acids (PNAs) enable sequence-specific recognition of RNAs through triplex formation,[1] although the physical basis of PNA recognition in context of structural motifs remains unclear. Among these, bulges represent common and highly dynamic RNA features that disrupt local geometry and influence ligand binding. Using NMR spectroscopy, SHAPE-MaP and thermodynamic analysis, we show that PNA actively remodels the bulged RNA into a binding-competent state. Triplex stability emerges from a cooperative interplay between bulge identity, stacking interactions, and backbone-mediated contacts. Adenine-containing bulges promote enhanced stacking and binding, while a subset of amide–phosphate hydrogen bonds exhibits exceptional stability, persisting even after base-triple interactions are lost.

Our results reveal a previously unrecognized role of backbone interactions and suggest a partial decoupling between base pairing and overall dsRNA-PNA triplex stability. The persistence of backbone contacts raises the possibility of PNA redistribution along RNA, while the strong preference for bulged motifs indicates that structural features of RNA and not just sequence dominate target selection. The structural adaptability is therefore established as a key determinant of PNA–RNA recognition, with implications for targeting dynamic RNA elements in biological systems.

1. C. A. Ryan, M. M. Rahman, V. Kumar, E. Rozners, 2023, J. Am. Chem. Soc., 10497–10504.

Acknowledgment: *This work has been supported by Slovenian Research Agency [P1-0242] and CERIC-ERIC consortium (projects no. 20232149 and 20242193).*

Calcium: A Life and Death Messenger

Miljko V. Satarić[1], Tomas Nemeš[2]

[1]*Serbian Academy of Sciences and Arts, Belgrade, Serbia*
[2]*Faculty of Technical Sciences, University of Novi Sad, Serbia*

Calcium is recognized as an essential messenger that accompanies cells troughout their entire lifespan, from their origin at fertilization till the programmed apoptosis.
Here we elaborated the roles of cellular microtubules in calcium signaling which tunes the wavelike beating of cilia and flagella. The nine microtubule doublets (MTDs) in axoneme represent a kind of electrical conduit. They enable the formation and propagation of "calcium ionic clouds", which are the signals for initiating and controlling cilia and flagella beats. In that respect we have considered MTDs as nonlinear electric transmission lines obeying basic Kirchoff's laws. It leads to nonlinear differential equation with travelling-wave localized solution described by squared secans-hyperbolic function of space and time named calcium ionic cloud". Then we subjected this localized signal to the Shanonn's information theory. In final segment we analysed the diffusion of tagged and untagged calcium ions in flaggelar signaling and we have proven that "calcium ionic cloud" messages propagate faster than calcium messengers.

1. M. V. Satarić, T. Nemeš and S. Zdravković, Calcium messages in flagella are faster than messenger particles, BioSystems 232, 105003(2023).
2. M. V. Satarić and T. Nemeš, On the role of calcium diffusion and its rapid buffering in intraflagellar signaling, Eur. Biophys. J. 52(8)705(2023).

Infrared Vibrational Signatures of Disease-Induced Perturbations in Membrane Hydration Water

Elisa Bonaccorso[1], Giovanna D'Angelo[1,2]

[1]*Department of Mathematical and Computational Sciences, Physical Science and Earth Science (MIFT), University of Messina, V.le F. Stagno d'Alcontres 31, 98166 Messina, Italy, elisa.bonaccorso@studenti.unime.it*
[2]*IMM-CNRsedediMessina, Viale Ferdinando Stagno d'Alcontres, 31, 98166 Messina, Italy*

The water layers in proximity of biological membranes are essential for cellular architecture, biochemical reactions, and intermolecular interactions. The interfacial water network, highly sensitive to the membrane chemical composition and structure, undergoes strong perturbation and reorganization under pathological alterations, e.g. cholesterol dyshomeostasis[1] in neuronal membranes[2]. We used Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy to investigate model neuronal membranes under controlled humidity and varying cholesterol content, designed to mimic physiological and pathological states. Pathological alterations in membrane lateral organization leave distinct signatures in the OH stretching (ν – OH) band, serving as markers of water-lipid coupling and anomalous hydrogen-bond geometry in the first hydration layers (~10 Å). Shape and frequency shifts in the ν-OH band reflect distinct water populations (structured network vs multimeric forms). Complementary analysis of the carbonyl stretching band gives information on water-lipid interactions, revealing a structurally and dynamically heterogeneous H-bond network, strongly coupled to lipid phase state and composition. These results show that disease-induced membrane anomalies imprint detectable signatures on the vibrational pattern of the H-bonding network, rendering it a sensitive "molecular mirror" for early diagnosis of pathological membrane perturbations.

1. Oh, Myong In, Chang In Oh, and Donald F. Weaver, 2020, The Journal of Physical Chemistry B 124.18:3686-3694
2. Fernández-Pérez, Eduardo J., et al., 2018, Frontiers in aging neuroscience 10: 226.

Time-Controlled Immune Cell Interactions Studied With Optical Tweezers

Éva Hegedüs[1], Tamás Fekete[2], Gergely Iványi[2, 3], György Vámosi[1], Lóránd Kelemen[2]

[1] *Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary*
[2] *Institute of Biophysics, HUN-REN BRC, Szeged, Hungary*
[3] *Doctoral School of Multidisciplinary Medical Sciences, University of Szeged, Szeged, Hungary*

The formation of the immunological synapse between B and T immune cells lies in the center of their activation. On the molecular level, it involves the binding of interleukins (such as IL-15) and activating antigens by their respective receptors. Actin is one of the main factors in the organization of the receptors involved. A novel biophysical tool using optical tweezer-actuated polymer microtools was applied to study the rate of the physical attachment during synapse formation in a highly controlled manner. The T cell, maneuvered with the trapped microtool, was brought into contact with the B cell for pre-defined durations, and the probability of the attachment was determined. The effect of IL-15, the antigen *Staphylococcus* enterotoxin E (SEE) and the actin polymerization-blocking agent latrunculin on the process was studied. We found that the synapse formation is considerably delayed when actin formation is blocked.

Acknowledgment: *This work was supported by the Hungarian Scientific Research Fund (K146028 and 150958).*

SCIENTIFIC SESSION III

COMPUTATIONAL BIOPHYSICS

A Dance Story of Two Anisotropicaly Charged Colloids in an Asymmetric Ionic Solution

Nataša Adžić[1], Emanuela Bianchi[2]

[1]*Institute of Physics Belgrade, University of Belgrade, Scientific Computing Laboratory, Belgrade, Serbia, natasa.adzic@ipb.ac.rs*
[2]*Institute of Theoretical Physics, Technical University of Vienna, Vienna, Austria*

Biomolecules in polar solutions acquire charge through the dissociation of ionizable groups or the adsorption of ions from the solvent. Their interactions are strongly influenced by the distribution of surrounding ions. Electrostatic interactions between charged colloids in ionic environments have been extensively studied, and most theoretical approaches rely on two key approximations: (i) the assumption of a homogeneous charge distribution on the colloids, and (ii) weak correlations between the colloidal charges and the surrounding ionic cloud. These approximations are typically valid in highly concentrated monovalent salt solutions, where mean-field theories provide an accurate description. Recently, mean-field theories have also been extended to inhomogeneously charged particles. However, a common limitation of these approaches is their inability to accurately predict colloidal interactions when even small amounts of polyvalent salt are added to the solution.

In this work, we address two key complexities that arise in charged biological systems: the anisotropy of colloidal charge and the asymmetry of the ionic environment. We develop a theoretical description based on the path-integral approach for the interaction between two Janus colloids immersed in a mixed ionic solution containing both monovalent and polyvalent salts. The obtained results provide insights beyond the mean-field approximation into the full orientational interaction landscape of two charged Janus particles.

Physically Informed Machine Learning for DNA Sequence–Current Relationships

Stefan Milenkovic[1], Dan Potempkin[2], Hemani Chhabra[2], Matteo Ceccarelli[1] and Aleksei Aksimentiev[2]

[1]*University of Cagliari, Department of Physics, Cagliari, Italy, smilenkovic@dsf.unica.it*

[2]*University of Illinois, Department of Physics, Urbana, IL, United States of America*

Nanopore sequencing links DNA sequence to ionic current through complex steric and electrostatic interactions inside the pore. While deep learning models can learn this mapping, they often lack interpretability. In this talk, I present a hierarchy of models showing that carefully engineered physicochemical descriptors—augmented with molecular dynamics (MD)-derived statistical features—enable simple linear regression models to outperform neural networks while retaining mechanistic transparency. The framework generalizes across k-mer lengths and pore types, demonstrating that physically informed features capture the essential determinants of nanopore current.

Prediction of Ryanodine Receptor States by the AlphaFold3 Server

A. Waheed[1], A. Zahradníková[2]

[1]*Biomedical Research Center, Slovak Academy of Sciences, Bioinformatics Laboratory, Bratislava, Slovakia (ueenamna@savba.sk)*
[2]*Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Department of Cell Cardiology, Bratislava, Slovakia*

Ab initio structural prediction with AlphaFold3 has shown high accuracy[1], but its performance in predicting multiple structural states of a single protein has not been explicitly tested. In this work, we compared the predictions of the AlphaFold3 server for three ryanodine receptor paralogs, differing in the number of experimental structures available for use in the prediction (48 in 3 states, 17 in 3 states, and 0 for RyR1, RyR2, and RyR3, respectively).

The conformations of experimental structures were compared with those of 300 AlphaFold3 models of the C-terminal quarter of human RyRs (100 per paralog) with ~5000 residues per the tetrameric model. Experimental and simulated structures were aligned to the central domain, and domain positions relative to the closed and inactivated rRyR1 reference states were calculated[2]. Geometry of the triangle W4716 - F3753 - I4996, important for RyR function[3], was also evaluated.

Experimental structures showed three conformational variants corresponding to the closed, open, and primed/inactivated states, as previously shown[2]. Predicted RyR1 models corresponded solely to the primed state, while RyR2 models corresponded solely to the closed state. RyR3 models formed a continuum between the closed and primed conformations, with ~16% in the closed state, none in the primed state, and ~84% of the models had no experimental counterpart. Thus, the AlphaFold3 server does not reproduce the known conformational variability of RyR.

1. Abramson, J., Adler, J., Dunger, J., *et al*., 2024, Nature 630:493–500.
2. Zahradníková, A, Pavelková, J., Sabo, M., *et al*., 2025, PLoS Comput. Biol. 21:1–40.
3. Murayama, T., Ogawa, H., Kurebayashi, N., *et al*., 2018, Commun. Biol. 1:98.

Acknowledgment: *This work was supported by the Grant Programme for SAS PhD Students, project no. APP0843, and by projects APVV-21-0443 and APVV-21-0473 from the Slovak Research and Development Agency*

A Molecular Dynamics Workflow for Modelling the *E. coli* Cell Wall

Boris Gomaz[1], Matej Kožić[1], Branimir Bertoša[1]

[1]*Faculty of Science, University of Zagreb, Department of Chemistry, Zagreb, Croatia, bgomaz@chem.pmf.hr*

The Gram-negative bacterial cell wall is a multi-layered assembly composed of the inner membrane (IM), a cross-linked peptidoglycan (PG) mesh, and an asymmetric outer membrane (OM).[1,2] Despite its biological importance, fully atomistic simulations of the complete cell wall are challenging and uncommon, although recent studies have begun to address these limitations.[3]

Here, we present an atomistic molecular dynamics (MD) workflow for the construction and simulation of a realistic *Escherichia coli* cell wall model. The system is built starting from a biologically relevant inner membrane composed of multiple phospholipids species. The asymmetric outer membrane is derived from the inner membrane architecture, with lipopolysaccharides incorporated into the outer leaflet. A chemically accurate peptidoglycan network is assembled between the membranes from repeating disaccharide units (GlcNAc and MurNAc) with attached peptide stems. This modular buildup results in a physically connected envelope that captures the structural organization of the *E. coli* cell wall.

The constructed models provide a platform for molecular dynamics (MD) simulations aimed at probing interlayer coupling, membrane fluidity and stability, and membrane permeability. Using all-atom MD simulation, membrane perturbations, including electroporation phenomena and the possibility of DNA translocation across the bacterial envelope, are studied.

1. Pluhackova, K., Horner, A., 2021, BMC Biol. 19(4):1-22.
2. Gumbart, J.C., Beeby, M., Jensen, G.J., Roux, B., 2014, PLoS Comput. Biol. 10(2): e1003475.
3. Hwang, H., Paracini, N., Parks, J.M., Lakey, J.H., Gumbert, J.C., 2018, Biochim. Biophys. Acta, Biomembr. 1860(12):2566-2575.

Acknowledgment: *This work is conducted under the MI-DNA Disc Project that was funded by the EU Commission in the framework of the Horizon Europe - EIC Transition Open programme under grant agreement number 101115215.*

A Multi-Scale View of Multicomponent IDP Condensates From Simulation and Experiment

Miloš T. Ivanović[1], Nicola Galvanetto[1], Robert B. Best[2] and Benjamin Schuler[1]

[1]*Department of Biochemistry, University of Zurich, Zurich, Switzerland; m.ivanovic@bioc.uzh.ch*
[2]*National Institutes of Health, Bethesda, MD, USA*

Biomolecular condensates assembled from IDPs govern myriad cellular processes, yet the principles linking molecular motions to emergent material properties are yet to be fully understood. Combining single-molecule FRET and multi-million–atom explicit-solvent simulations, we show that condensates of oppositely charged IDPs preserve sub-microsecond chain reconfiguration and pico- to nanosecond side-chain exchange despite very high bulk viscosity [1]. Extending this experiment–simulation approach [2] to multicomponent condensates spanning diverse sequences and salt concentrations, we identify striking correlations between viscosity, translational diffusion, and chain dynamics [3]. Multi-million–atom simulations recapitulate these trends and reveal that sequence- and salt-dependent inter-residue contact lifetimes govern the effective friction linking molecular and macroscopic scales, in line with both polymer-physics models and independent measurements [3]. Rapid exchange of charged interaction partners at high residue densities emerges as a general mechanism by which highly charged nuclear compartments avoid dynamic arrest. Finally, we developed and validated a tailored strategy to refine force-field parameters for condensates and benchmark complementary routes, providing a toolkit for predictive simulations.

1. Galvanetto, N.[*], Ivanović[*], M., *et al.*, 2023, Nature 619, 876-883 ([*]: equal contribution)
2. Ivanović, M., and Best, R., 2025, Curr. Opin. Struct. Biol. 93, 103101
3. Galvanetto, N.[*], Ivanović[*], M., *et al.*, 2025, Proc. Natl. Acad. Sci. U.S.A. 122, e2424135122

Probing the Interaction of Ghrelin With the Human L-Type Calcium Channel Cav1.2 by Molecular Docking

Milica Popović[1,2], Đura Nakarada[2], David Lutz[3], Milena Milošević[4], Miloš Mojović[2]

[1]*Vinča Institute of Nuclear Sciences, University of Belgrade, Department for Bioinformatics and Computational Chemistry, Belgrade, Serbia, milica.popovic@vin.bg.ac.rs*
[2]*Faculty of Physical Chemistry, University of Belgrade, Center for Physical Chemistry of Biological Systems – BioScope Labs, Belgrade, Serbia*
[3]*Faculty of Medicine, Medical University of Varna, Department of Anatomy and Cell Biology, Varna, Bulgaria*
[4]*Faculty of Biology, University of Belgrade, Center for Laser Microscopy, Belgrade, Serbia*

Ghrelin is a 28-residue acylated peptide hormone regulating appetite, growth hormone release, and energy homeostasis. Beyond endocrine roles, it exhibits neuroprotective and cardioprotective effects and is increasingly considered relevant in age-related disorders, where it modulates neuronal activity and synaptic plasticity[1]. In cardiomyocytes, the ghrelin receptor agonist hexarelin reduces inward L-type Ca^{2+} currents (I_{CaL})[2], mediated by Cav1.2, a predominant voltage-gated Ca^{2+} channel in the brain, heart, and smooth muscle[3]. Whether ghrelin can directly interact with Cav1.2 or modulate its activity through indirect signaling or weak, transient binding interactions, remains unknown.

Molecular docking was applied to probe interactions between ghrelin, in its receptor-bound conformation (PDB: 7F9Y), and human Cav1.2 (PDB: 8WE8). The aim was to identify plausible contact surfaces and key interacting residues, and to assess their correspondence with known ligand and toxin binding sites.

Docking identifies a plausible binding region within an extracellular cleft of Cav1.2, where ghrelin adopts an extended conformation along the channel surface. The site combines hydrophobic and polar contacts, consistent with engagement of ghrelin's *n*-octanoyl group with a hydrophobic patch on the channel, providing a structural starting point for experimental tests of direct ghrelin–Cav1.2 interaction.

1. Stoyanova, I., 2014, Neurobiol. Dis. 72:72-83.
2. Bresciani, E., Rizzi, L., Coco, S., et al., 2019, Int. J. Mol. Sci. 20:4361- 4374.
3. Gao, S., Yao, X., Chen, J., et al., 2023, Cell 186:5363-5374.

Acknowledgment: *This research was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, contract numbers 451-03-33/2026-03/200146, 451-03-34/2026-03/200146, and 451-03-34/2026-03/200178.*

DFT Conformational Analyses and Magnetic Properties of the Copper(II) Compounds With *N*-Alkylated And *N*,*N*-Dialkylated Amino Acids in Solutions

Jasmina Sabolović[1]

[1]*Institute for Medical Research and Occupational Health, Division of Occupational and Environmental Health, Zagreb, Croatia, e-mail: jasmina.sabolovic@imi.hr*

Paramagnetic neutral bis(aminoacidato)copper(II) [$Cu(aa)_2$] coordination compounds represent the physiological form of copper(II) amino acid compounds in blood plasma, comprising about 0.2% of an exchangeable Cu(II) pool for transport, distribution, and storage.[1] They also serve as valuable model systems for exploring noncovalent interactions between amino-acid side chains and copper binding sites in metalloproteins. Adding an N-methyl group to a molecule can significantly alter its properties and biological activity, affecting lipophilicity, solubility, metabolic stability, and enhancing pharmacological effects.[2,3]

Generally, experimental studies did not determine the complete structures of $Cu(aa)_2$ in solutions. This computational study aims to fill that gap by predicting the structural properties for the copper(II) compounds with N-methylated and N,N-dimethylated L-valine and L-alanine, and L-N,N-dipropylalanine in solutions. Using density functional theory (DFT) with the B3LYP-D3 functional and a polarizable continuum model for solutions, low-energy conformers are identified. These structures are validated through DFT calculations of the hyperfine coupling constant tensor of the ^{63}Cu center, allowing comparison with experimental electron paramagnetic resonance data. The goal is to uncover the effects of intra- and intermolecular interactions on the stability of these compounds as part of their physicochemical characterization for potential pharmacological applications.

1. Kirsipuum, T., Zadorožnaja, A., Smirnova, J., et al., 2020, Sci. Rep. 20:5686.
2. Barreiro, E. J., Kummerle A. E., Fraga, C. A. M., 2011, Chem. Rev. 111:5215-5246.
3. Chatterjee, J., Rechenmacher, F., Kessler, H., 2013, Angew. Chem. Int. Ed. 52:254-269.

Acknowledgment: *This work was funded by the European Union – Next Generation EU Programme (contract of 8 December 2023, Class: 643-02/23-01/00016, Reg. no. 533-03-23-0006).*

Molecular Dynamics study of DNA interactions with inner *E. coli* membrane

Matej Kožić[1], Marta Vukić[1], Boris Gomaz[1], Branimir Bertoša[1]

[1]*University of Zagreb, Faculty of Science, Department of Chemistry, Zagreb, Croatia, mkozic@chem.pmf.hr*

Despite being a routine procedure done daily in labs all around the world, the process of molecules of DNA entering into a cell during *in vitro* transformation is not fully mechanistically understood. Two main transformation approaches are: 1. Electroporation and 2. Heat-Shock. Electroporation uses pulses of electrical field to create pores in a membrane through which even large molecules of DNA can enter into the cell, while heat-shock transformation uses temperature changes and calcium chloride to get DNA to pass through a membrane into a cell.

Electroporation has been successfully studied using Molecular Dynamics (MD) as far back as 2005[1,2]. Recently, an *in silico* model of the inner membrane of *E. Coli* has been developed by Pluhackova *et al* (*Avanti* membrane)[3]. This new and improved membrane coupled with improved modern force-fields presents a great foundation to study mechanisms behind DNA-membrane interactions in atomistic detail. Simulating the *Avanti* membrane with different concentrations of different ions including Ca^{2+} showed the differences of ion membrane penetration depth and frequency (Figure 1.). Thus, the goal of the presented study is to shed light onto the atomistic mechanisms of DNA passing through a membrane using all-atom MD simulations.

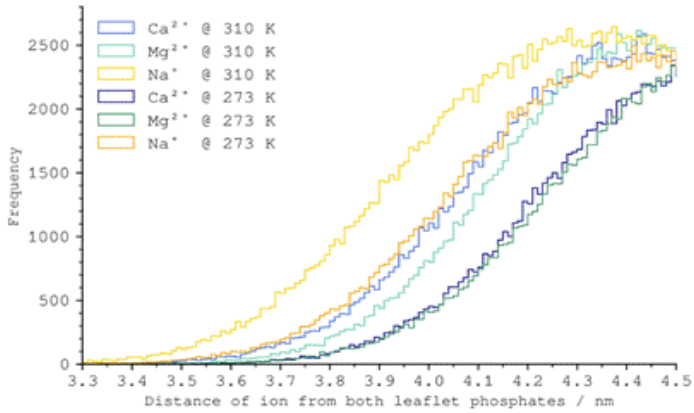


Figure 1. Histogram of ion penetration into the membrane at different temperatures. The lower the distance from both leaflets (x axis), the deeper the ion penetrated.

1. Tarek M., 2005, *Biophysical Journal* 4045-4053, vol 88 issue 6.
2. Delmotte L., Tarek M., 2012, *J Membr Biol.* 531-543, 9.
3. Pluhackova K., Horner A., 2021, *BMC Biology* vol 19 article 4.

Acknowledgment: *This work is conducted under the MI-DNA Disc Project that was funded by the EU Commission in the framework of the Horizon Europe - EIC Transition Open programme under grant agreement number 101115215.*

The Synergy of Computational and Experimental Research in Biophysics

Branimir Bertoša[1]

[1]*Faculty of Science, University of Zagreb, Department of Chemistry, Zagreb, Croatia*

Combination of computational and experimental methods presents the core of modern biophysical studies. Through this approach both sets of methods, experimental and computational, gain additional value. Computational methods provide insight into microscopic level of studied system and enable understanding of biophysical events at molecular level. Despite significant advances in computational biophysical methods over the last few decades, computational results still need, or at least it is desirable to have, experimental validation. On the other hand, experimental methods provide credible results obtained from examining the system at the macroscopic level. Thus, computational results enable deeper interpretation of those experimental findings. Therefore, combining computational and experimental methods produces synergy that adds additional value to biophysical studies. Several examples of synergistic effects between computational and experimental methods in various biophysical studies will be presented. Allostery of transcription factors that control manganese homeostasis in various bacteria, as well as their interactions with DNA, were studied computationally and experimentally.[1,2] Usage of horseradish peroxidase for construction of pathogen detection test in water samples was investigated by combination of computational and experimental methods.[3] Finally, DNA interaction with the *E. coli* membrane was studied.

1. Radman, K., Crnolatac, I., *et al.*, 2025, Int. J. Biol. Macromol. 290:138828.
2. Manenica, M. & Bertoša, B. 2025, Int. J. Biol. Macromol. 311:142937.
3. Škulj, S., Kožić, M., *et al.*, 2024 Comput. Struct. Biotechnol. J. 23:742-751.

Acknowledgment: *This work was funded by: (i) the European Union's Horizon 2020 research and innovation programme under grant agreement No 952110, project Marilia, (ii) the Croatian Science Foundation, grant number IP-2020-02-3446, project Manganese metallosensors, (iii) Horizon Europe – EIC Pathfinder Challenges Program (Grant 101115215), project MI-DNA DISC.*

SCIENTIFIC SESSION IV

BIOINFORMATICS

Engineering Thermostable Ancestral Polyesterase for Efficient Bioplastic Degradation

Aleksandra Maršavelski[1], Ivana Kekez[1], Lucija Brtan[1], Anđela Pustak[2], Margaux Aubel[3], Erich Bornberg-Bauer[3]

[1]*Faculty of Science, University of Zagreb, Department of Chemistry, Zagreb, Croatia, amarsavelski.chem@pmf.hr*
[2]*Ruđer Bošković Institute, Division of Materials Chemistry, Zagreb, Croatia*
[3]*Institute for Evolution & Biodiversity, University of Münster, Münster, Germany*

Plastic pollution represents a growing environmental challenge, driving the need for efficient and sustainable biocatalysts capable of degrading synthetic polymers, particularly polyesters.[1] In this work, we describe the engineering of a novel metagenome-derived polyesterase, MGY, exhibiting poly(lactic acid) (PLA)–degrading activity. Although MGY showed promising catalytic potential, its low solubility, poor expression yield, and limited thermostability restricted its applicability. To address these limitations, ancestral sequence reconstruction[2] was employed to generate thermostable ancestral variants of MGY. All reconstructed enzymes retained substantial PLA-degrading activity, and one variant additionally displayed activity toward polycaprolactone (PCL). The most thermostable variant exhibited a melting temperature of 84 °C, representing the highest value reported to date for a PLA-degrading enzyme. Thermal characterization of PLA and PCL substrates enabled identification of optimal temperature windows for enzymatic degradation. Structural and computational analyses revealed that enhanced thermostability primarily arises from loop rigidification,[3] achieved through direct stabilization of a short loop and remote core mutations that stabilize a longer loop–helix motif near the active site. Despite an unchanged primary sequence of this flexible region, remote mutations increased the number of simultaneous hydrophobic contacts, significantly reducing structural mobility. These findings highlight ancestral sequence reconstruction as an effective strategy for engineering robust polyester-degrading enzymes and provide mechanistic insight into enzyme thermostability relevant for sustainable bioplastic recycling.

1. Wang, Y., van Putten, R. J., Tietema, A., Parsons, J. R., Gruter, G. M., 2024, *Green Chem.* 26:3698–3716.
2. Spence, M. A., Kaczmarski, J. A., Saunders, J. W., Jackson, C. J., 2021, *Curr. Opin. Struct. Biol.* 69:131–141.
3. Yu, H., Huang, H., 2014, *Biotechnol. Adv.* 32:308–315.

Acknowledgment: *This work was funded through the National Recovery and Resilience Programme (NextGenerationEU), project Enzyme engineering for sustainable recycling of bioplastics (NPOO.C3.2.R2-I1.06.0041), and supported by the COST Action CA21162 Short-Term Scientific Missions grant.*

PML Nuclear Bodies as Cellular Aging Markers: Morphological and Proteomic Analysis

Eugene Y. Smirnov[1], Sergey A. Silonov[1], Konstantin K. Turoverov[1], Alexander V. Fonin[1]

[1]*Laboratory of Structural Dynamics, Stability and Folding of Proteins, Institute of Cytology, Russian Academy of Sciences, St. Petersburg 194064, Russia, e-mail: e.smirnov@incras.ru*

Aging represents a fundamental physiological process characterized by age-associated alterations across multiple levels of biological organization. One prominent manifestation of organismal aging is the acceleration of replicative senescence, also termed cellular senescence. The promyelocytic leukemia (PML) protein, a structural component of PML nuclear bodies, participates in these processes, though underlying mechanisms remain unclear.

We employed immunofluorescence to examine PML body morphology and protein localization during senescence in primary fibroblasts from healthy donors and patients with accelerated aging syndromes (ataxia-telangiectasia, Cockayne syndrome). Senescent cells showed increased PML body size and number, plus nuclear enlargement, suggesting these parameters as cellular aging markers.

Bioinformatic analysis and mass spectrometry-based proteomics revealed that 30% of PML interactome proteins and 45% of PML body proteome components directly link to senescence/aging processes (CellAge/GenAge databases). These proteins likely participate in post-translational modifications and client protein sequestration within PML bodies, modulating transcription factor activity, DNA damage responses, and apoptosis regulation.

Our findings highlight PML bodies' pivotal role in cellular aging and provide foundation for investigating senescence mechanisms and age-related pathologies.

Vitamin B Complex Suppresses Neuroinflammation in Activated Microglia: An Integrated *In Vitro* and *In Silico* Approach With Dynamical Modeling

Marija Rakić[1], Tanja Lunić[1], Marina Bekić[2], Sergej Tomić[2], Katarina Mitić[1], Stefan Graovac[3] , Bojan Božić[1*], Biljana Božić Nedeljković[1*]

[1]*University of Belgrade, Faculty of Biology, 11000 Belgrade, Serbia; *biljana@bio.bg.ac.rs*
[2]*University of Belgrade, Institute for the Application of Nuclear Energy, INEP, 11080 Belgrade, Serbia*
[3]*University of Belgrade, Faculty of Physics, 11000 Belgrade, Serbia*

Activated microglia play a critical role in regulating neuroinflammation and neurodegeneration. In this study, the anti-inflammatory effects of a vitamin B complex (VBC; B1, B2, B3, B5, B6, and B12) on the function and phenotype of lipopolysaccharide (LPS)-stimulated BV2 microglial cells were investigated *in vitro*. To assess microglia-neuron interactions, conditioned media from VBC-treated microglia were applied to SH-SY5Y neuronal cells, and their effects on neuronal viability were evaluated. To further elucidate the underlying mechanisms, molecular docking analyses were performed to examine the binding affinity of individual VBC components to key proteins of the Toll-like receptor 4 (TLR4) signalling pathway, as well as inducible nitric oxide synthase (iNOS). In addition, a dynamical model simulating VBC-mediated inhibition of LPS-activated TLR4 signalling was developed, showing strong agreement with experimental data ($adjR^2$ = 0.9715 for TNF-α and 0.9909 for IL-6). The results demonstrated that VBC treatment significantly reduced the secretion of pro-inflammatory mediators in activated microglia, attenuated their neurotoxic effects on neuronal cells, and promoted a phenotypic shift towards an anti-inflammatory (M2-like) profile. Furthermore, the developed dynamical model provides mechanistic insight into the contribution of individual VBC components to the overall inhibitory effect on TLR4 signalling and enables the rational optimization of VBC formulations, as well as the identification of novel inhibitors targeting this pathway.

Grant Support: *Supported by the Ministry of Education and Science of the Republic of Serbia (Contract numbers: 451-03-68/2022-14/200178 and 451-03-68/2022-14/200019).*
Results previously published in: Rakić, M. et al. (2023). Vitamin B complex suppresses neuroinflammation in activated microglia: in vitro and in silico approach combined with dynamical modeling. *International Immunopharmacology*, *121*, 110525.

Reassessing the Predictive Value of the Global Health Security Index During the SARS-CoV-2 Omicron Wave

Anđela Rodić[1], Sofija Marković[1], Milica Pap[1], Marija Mijajlović[1], Marko Đorđević[1]

[1]*Faculty of Biology, University of Belgrade, Department of General Physiology and Biophysics, Belgrade, Serbia, sofija.markovic@bio.bg.ac.rs*

The Global Health Security Index (GHSI) was introduced as a tool for evaluating countries' capacity to respond to infectious disease threats. Nevertheless, during the first phase of the COVID-19 pandemic, countries with higher GHSI scores frequently experienced poorer outcomes, generating debate regarding the index's practical relevance. The SARS-CoV-2 Omicron wave, distinguished by rapid spread and comparatively lower disease severity, offers a suitable context for reexamining the relationship between preparedness and epidemic dynamics. In this study, Random Forest machine learning models were used to investigate determinants of Omicron transmissibility, quantified by the average effective reproduction number, in 104 countries worldwide. The analysis incorporated the 2019 and 2021 GHSI editions together with demographic, socioeconomic, health-related, and Google mobility indicators. To improve model stability and interpretability, correlated variables were grouped and decorrelated within related domains. Across both GHSI versions, higher preparedness scores were consistently associated with lower transmissibility. Variables reflecting preexisting population immunity, demographic structure, and timing of epidemic onset had the greatest predictive importance overall. However, after excluding these covariates, the GHSI and mobility indicators emerged as the leading determinants of transmission intensity. The combination of limited preparedness and elevated population movement was associated with particularly rapid spread, a pattern observed in multiple African countries. Overall, the findings support the relevance of the GHSI framework for capturing preparedness factors associated with epidemic control during the Omicron period. The contradictory patterns observed during the early pandemic likely resulted from additional influences not accounted for by the index, including delayed responses and lack of prior experience with a novel global outbreak.

SCIENTIFIC SESSION V

NEUROBIOPHYSICS

Microscopic Imaging of Human Multipolar Neurons: Computational and Monofractal Morphological Characterization Across the Aging Process

Dejana Milošević[1], Mila Opačić[1], Nebojša Milošević[2]

[1]*Faculty of Medicine, University of Belgrade, Belgrade, Serbia, dejanamilosevic46@gmail.com*
[2]*Faculty of Medicine, University of Belgrade, Department of Biophysics, Belgrade, Serbia*

Introduction: The dentate nucleus is one of the principal cerebellar nuclei, playing a critical role in the planning, initiation, and regulation of voluntary movements. Previous studies have primarily examined neuronal number, classification, and structural features. However, few investigations have addressed age-related changes in neuronal morphology, particularly in multipolar neurons of the human dentate nucleus.

Aim: The primary objective of this study was to analyze age-related morphological changes in the dentate nucleus by quantifying three neuronal image parameters: size, shape, and dendritic arbor complexity. A supplementary objective was to compare morphological features between neurons during maturation and aging.

Results: Our findings demonstrate that only neuronal surface area and dendritic arbor complexity exhibit significant age-related changes, with both parameters decreasing progressively with age. For other morphological parameters, no consistent or reliable relationship with age could be established.

Conclusions: These results corroborate previous findings on age-related neuronal changes in the principal olivary nucleus and the human neostriatum. Furthermore, they align with studies on glial cell morphology in the principal olivary nucleus, reinforcing the broader pattern of structural simplification associated with aging in the human brain.

Noise-Induced Resonance in a Model of the Hypothalamic–Pituitary–Adrenal Axis

Miha Štorman[1], Barbara Arcet[1], Marko Gosak[1,2,3*]

[1]*Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia,*
[2] *Alma Mater Europaea, Maribor, Slovenia*
[3] *Institute of Physiology, Faculty of Medicine, University of Maribor, Maribor, Slovenia*
**marko.gosak@um.si*

The hypothalamic–pituitary–adrenal (HPA) axis is a complex neuroendocrine regulatory system governing stress responses and basal homeostasis through hormonal feedback loops. Its dynamics can be captured by nonlinear differential equation models describing the interactions between stress hormones [1]. As shown in previous analyses of a minimal three-dimensional submodel, the system exhibits rich dynamical behavior, including supercritical and subcritical Hopf bifurcations, degenerate (Bautin) bifurcations, and coexistence of multiple limit cycles [2]. However, the HPA axis operates in a fluctuating physiological environment. Hormone secretion is pulsatile, receptor binding stochastic, and external stress inputs noisy. Motivated by these intrinsic and extrinsic fluctuations, we investigate noise-induced dynamical phenomena in the vicinity of bifurcation points. After a systematic bifurcation analysis in biologically relevant parameter regimes, stochastic perturbations are introduced. We demonstrate the emergence of stochastic and coherence resonance, characterized by noise-induced amplification and temporal regularization of cortisol oscillations. Importantly, the character of the resonance critically depends on the underlying deterministic bifurcation type. These results provide a mechanistic link between bifurcation structure and fluctuation-driven endocrine variability, offering insight into how physiological noise shapes stress-axis dynamics and may contribute to dysregulation in stress-related disorders.

1. Stanojević, A., Marković, V.M., Čupić, Ž., Kolar-Anić, Lj., Vukojević, V., 2018, Curr. Opin. Chem. Eng. 21:34-42.
2. Arcet, B., et al., 2020, Commun. Math. Comput. Chem. 83:331-356.

Acknowledgment: *This work was funded by the Slovenian Research and Innovation Agency (research core funding no. P3-0396 and research projects J3-60062 and SN-ZRD/22-27/0552).*

A Brain-On-A-Chip System Integrating Bbb Cell Types and Brain Cortical Organoids From Stem Cells

Judit P. Vigh[1,2], Anna E. Kocsis[1,2], Nóra Kucsápszky[1,2], Ana R. Santa-Maria[1,3], Luíza Santa Brígida de Barros Góes[4], Emese Bélai[1], Lilla Kókity[1], Melinda Pirity[1], András Dér[1], Mária A. Deli[1,*], Fruzsina R. Walter[1,*]

[1]*HUN-REN Biological Research Centre, Szeged, Hungary*

[2]*Doctoral School of Biology, University of Szeged, Szeged, Hungary*

[3]*Wyss Institute, Harvard University, Boston, MA, USA*

[4]*Instituto de Química de São Carlos, Universidade de São Paulo, 13566-590 São Carlos, SP, Brazil * authors contributed equally*

Integrating stem cell-based in vitro BBB models and human brain cortical organoids into lab-on-a-chip (LOC) systems provides a complex environment to study BBB functions in health and disease. Here our goal was to advance our existing LOC model and optimize a new, dynamic microphysiological system by combining a human stem cell-derived BBB model with human cortical organoids. Our goal was to examine BBB properties and functionality in the presence of organoids, and also test the system under different stressors. Barrier integrity was evaluated by impedance measurement and permeability assay for fluorescent markers. Tight junction morphology was analyzed by immunostaining. Organoid viability and morphology were characterized by calcein AM and by immunolabeling for MAP 2, β-tubulin neuronal and GFAP astroglial markers. We found appropriate BBB integrity in the presence of cortical organoids; the system was set up for co-culture and was sustainable for further experiments.

Organoids expressed key neuronal and glial markers. Brain endothelial functions and organoid response was tested after the addition of tetrahydrocannabinol and iodinated contrast agents.

With the use of this brain-on-a-chip system, we could evaluate the effects of external stressors at the BBB and also the brain organoids. Here, we pave the way to a new era of research tools for toxicological, pharmacological, and pathological investigations.

Acknowledgement: *The project was supported by the National Research, Development and Innovation Office OTKA-K 143766 and ERA-NET-Neuron, B3Phrenia (to M.A.D.), NKKP Advanced Grant-153360 (to F.R.W.).*

Also by the Hungarian Academy of Sciences Lendület „Momentum" Program (to F.R.W., LP2025-22/2025).

Priming the Defense: Low-Dose Irradiation Rapidly Activates Antioxidant Systems in BV2 Cells

Stefana Dejković[1], Milica Milošević[2], Anđela Laudanović[2], Đura Nakarada[1], Marko Daković[1], Milena Milošević[2], Miloš Mojović[1]

[1]*Centre for Physical Chemistry of Biological Systems, Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia, stefana@ffh.bg.ac.rs*
[2]*Centre for Laser Microscopy, Faculty of Biology, University of Belgrade, Belgrade, Serbia*

Rationale: The detailed mechanism of response of brain tissue cells to exposure to low dose irradiation is still unrevealed. Although neurons are known to be extremely radioresistant[1], the impact of ionizing radiation on glial cells, especially microglia, should not be overlooked. In this study we evaluated the response of microglial cells up to 24h post-irradiation. **Methods:** BV2 cells were irradiated using a standard clinical X-ray diagnostic device with a total dose of 9 mGy. Antioxidant activity was monitored over 24h post-irradiation by measuring the reduction of the TEMPOL spin probe using a Bruker BioSpin Elexsys II E540 EPR spectrometer. Cell count and viability were examined using FDA/PI and MTT tests. **Results and Conclusion:** BV2 cells showed the lowest antioxidative activity 2h after irradiation, while the highest was 5h post-irradiation, as measured by TEMPOL reduction, that may be attributed to the clearance of the generated reactive oxygen species. We speculate that this specific increase 5h post-irradiation is possibly due to the time required for the recruitment of endogenous redox modulators. No cell death was detected 24h post-irradiation with viability assays. While FDA/PI test showed no statistically significant difference between control and irradiated cells, MTT test pinpointed to higher metabolic activity in the irradiated group, which could suggest an alteration in the metabolism of irradiated cells.

1. Menzel F, Kaiser N, Haehnel S, Rapp F, Patties I, Schöneberg N, Haimon Z, Immig K, Bechmann I. Impact of X-irradiation on microglia. Glia. 2018 Jan;66(1):15-33. doi: 10.1002/glia.23239. Epub 2017 Oct 10. PMID: 29024033.

Acknowledgment: *This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers 451-03-34/2026-03/200146, 451-03-33/2026-03/200146 and 451-03-34/2026-03/200178.*

Detection of Age-Related Morphological Changes in Astrocyte Projections Using Multifractal Spectra

Zorana Jelesijević[1], Dragana Radošević[2], Nemanja Rajković[3]

[1]*Faculty of Medicine, University of Belgrade, Department of Biophysics, Belgrade, Serbia, zorana.jelesijevic@med.bg.ac.rs*
[2]*Faculty of Medicine, University of Novi Sad, Department of Anatomy, Novi Sad, Serbia*
[3]*Faculty of Medicine, University of Belgrade, Department of Biophysics, Belgrade, Serbia*

Astrocytes, including fibrous and protoplasmic types, are a major class of glial cells whose morphology plays an important role in maintaining neuronal microenvironment and brain homeostasis. Quantitative characterization of their structural complexity can provide insights into age-related morphological changes. Multifractal analysis offers a detailed description of the spatial organization of complex two-dimensional biological patterns, such as cellular projections. This study investigates age-related morphological changes in two-dimensional projections of astrocyte cells using multifractal spectra and examines correlations between spectral parameters and age, as well as differences between age groups. A sample of 105 binary images of astrocyte projections was analyzed using three multifractal spectra: the generalized dimension spectrum $D_Q(Q)$, the Hölder exponent spectrum $\alpha(Q)$, and the singularity spectrum $f(\alpha)$ vs Q. The sample was divided into three age groups: Group 1 (36 - 60 years), Group 2 (61 - 75 years), and Group 3 (76 - 90 years). Among the analyzed spectra, only the singularity spectrum showed sufficient sensitivity to age-related morphological changes. Its parameters exhibited statistically significant correlations with age across much of the spectrum and enabled differentiation between age groups. The observed changes indicate a decrease in the complexity of both fine and coarse structures of monofractal sub-objects within the overall multifractal structure in the two older groups compared to the youngest group. The separation of the youngest group from the two older groups suggests that the most pronounced morphological changes occur between the first and second age intervals, after which the morphology remains statistically similar. Further studies on larger samples are needed to confirm these findings.

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-66/2024-03/200110.*

Photobiomodulation Effect on A-Synuclein Aggregates and Autophagy in Parkinson's Like Cells and Possible Implications for Treatment of Neurodegenerative Diseases

Stroffekova K.[1], Mitter Z.[1], and Bednarikova Z.[2]

[1]*Department of Biophysics, Institute of Physics, PJ Safarik University, Kosice, Slovakia;* katarina.stroffekova@upjs.sk
[2]*Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia*

According to the WHO, the population older than 60 years will nearly double from 12% to 23% by 2050. The aging correlates with an increase of the neurodegenerative diseases, 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 a-synuclein (aSNC). The present study focused on changes in the levels of a-synuclein (aSNC) monomers and aggregates in SH-SY5Y cells treated with rotenone (ROT) and photobiomodulation (PBM, low-level near infrared (NiR) radiation). We have established the 2D model of PD by using human neuroblastoma cell line SH-SY5Y and ROT treatment (200nM Rot for 48hr), followed by PBM (808nm NiR, 1J/cm^2). ROT increased aSNC level of both species, monomers and aggregates, respectively. PBM treatment notably decreased aSNC aggregates in both, control and ROT cells through improvement of the autophagy. We have compared our findings between non differentiated and differentiated SH-SY5Y cells. These results can be 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 Slovak VEGA 1/0421/18;1/0557/20 and 1/0187/23; and APVV-21-0333 and APVV-22-0598; and by the EU NextGenerationEU project No. 09-I02-03-V01-00021*

Collective β-Cell Dynamics under GABA Modulation: Integrating High-Resolution Confocal Imaging, Network Analysis, and Computational Modeling

Jan Zmazek[1,2], Lidija Križančić Bombek[3], Vladimir Grubelnik[4], Jasmina Kerčmar[3], Lana Kralj[3], Andraž Stožer[3], Marko Marhl[1,3], Marko Gosak[1,3,*]

[1]*Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia*
[2]*Community Health Centre Ljubljana, Metelkova ulica 9, 1000 Ljubljana, Slovenia*
[3]*Faculty of Medicine, University of Maribor, Maribor, Slovenia*
[4]*Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor, Slovenia*
[*]*marko.gosak@um.si*

Islets of Langerhans are multicellular microorgans that regulate glucose homeostasis through pulsatile hormone secretion. Their predominant cell type, the β-cell, responds to glucose with coupled metabolic and electrical oscillations that drive rhythmic Ca^{2+} signals and insulin release [1]. β-cells are functionally heterogeneous yet electrically and metabolically coupled, forming networks that generate complex spatiotemporal activity patterns increasingly analyzed using tools from complex network theory [2]. Recent evidence indicates that γ-aminobutyric acid (GABA), synthesized and released within islets, modulates electrical excitability, metabolic processes, collective cellular activity, and secretory responses [3]. However, its role in coordinating β-cell dynamics remains incompletely understood. In this study, we combine high-resolution multicellular Ca^{2+} imaging in acute tissue slices with network analysis and computational modeling to investigate how GABA modulation reshapes β-cell activity. By manipulating intracellular GABA levels and selectively targeting GABA_A and GABA_B receptors, we assess their effects on oscillatory dynamics and functional connectivity. Our results show that GABA signaling nontrivially influences both intracellular activity and network synchronization. Computational modeling further provides mechanistic insight into how GABA-dependent changes in excitability and coupling shape the emergent behavior of β-cell networks.

1. Rorsman, P., Ashcroft, F.M., 2018, Physiol. Rev. 117-214:98.
2. Šterk, M., Zhang, Y., Pohorec, V., Leitgeb, E. P., Dolenšek, J., Benninger, R. K. P., et al., 2024, PLoS Comput. Biol. 20(5):e1012130.
3. Ferreira, S.M., Hagan, D.W., Stis, E.S., et al., 2025, Mol. Metab. 102: 102268.

Acknowledgment: *This work was funded by the Slovenian Research and Innovation Agency (research core funding no. P3-0396 and research projects J3-60062 and SN-ZRD/22-27/0552).*

SCIENTIFIC SESSION VI

MEDICAL BIOPHYSICS

Protein-based HER2 Targeting Using DARPin–AsLOV2C450A Photosensitizing Conjugate

Alexandra Poliaková[1,2], Kristína Felčíková[1], Silvia Tomková[2] Ľuboš Ambro[2], Erik Sedlák[2,3], Veronika Huntošová[2,4]

[1]*Department of Biophysics, Institute of Physics, FS, P.J. Šafárik University, Jesenná 5, 04 011 Košice, Slovakia,*
[2]*Center for Interdisciplinary Biosciences, TIP, P.J. Šafárik University, Jesenná 5, 04 011 Košice, Slovakia,*
[3]*Pavol Jozef Šafárik University, Faculty of Science, Department of Biochemistry, Moyzesova 11, 040 01 Košice, Slovakia,*
[4]*Institute of Animal Biochemistry and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Dúbravská cesta 9, 840 05 Bratislava, Slovakia*

Targeted delivery of therapeutic agents to tumor cells is an important strategy for improving cancer treatment selectivity. The human epidermal growth factor receptor 2 (HER2), represents an attractive target for receptor-mediated therapies.[1] In this work, we developed a protein-based delivery platform combining a HER2-specific designed ankyrin repeat protein (DARPin) with the photosensitizing AsLOV2 C450A domain. The AsLOV2 binds flavin mononucleotide (FMN), which functions as a chromophore and enables light-activated generation of reactive oxygen species, making the system suitable for photodynamic applications. Furthermore, the C450A mutation binds FMN inside for prolonged time[2], that facilitates a decent delivery system of FMN to the cells via mentioned DARPin HER2-AsLOV2 C450A conjugate targeting cell´s HER2 receptors.
The fusion proteins were produced in *E. coli* and purified for physicochemical characterization. Spectroscopic analysis confirmed the presence of FMN, while thermal measurements indicated stability of the individual domains within the conjugate. Cellular experiments demonstrated low cytotoxicity in the absence of light and selective binding of the DARPin–AsLOV2 C450A conjugate to HER2-receptors of SKBR3 breast cancer cells. These findings demonstrate that the engineered fusion proteins retain both targeting capability and photosensitizing functionality, supporting their potential use as targeted delivery systems for directed photodynamic therapy.

1. Calce, E., Monfregola, L., Saviano, M. 2015, HER2-mediated anticancer drug delivery: strategies to prepare targeting ligands highly specific for the receptor. Curr Med Chem, pp. 2525-38.
2. Petrenčáková, Martina, *et al*. 2020, Conformational properties of LOV2 domain and its C450A variant within broad pH region, in: *Biophysical chemistry*, pp. 106339.

Acknowledgment: *This work was funded by the* project vvgs-2025-3481, 004UPJŠ-4/2024, APVV-20-0340 and by the EU NextGenerationEU through the Recovery and Resilience Plan of the Slovak Republic under project no. 09I03-03-V04-00007.

Biophysical Effects of Laser Wavelength and Pulse Duration on Skin: Comparative Analysis of Picosecond and Millisecond Lasers

Aleksandra Markoski Smiljković[1], Marko Pajić[2], Isidora Janićijević Grubišić[3], Ljubica Ilić[4], Katarina Žikić[5]

[1]*Rejuva Laser and Aesthetic, Belgrade, Serbia,* aleksandramarkoski@yahoo.com
[2]*Institute of Biophysics, Faculty of Medicine, University of Belgrade, Serbia*
[3]*Eye Clinic, Clinical Hospital Center Zvezdara, Belgrade, Serbia*
[4]*Institute of Radiology, Military Medical Academy, University of Defense, Belgrade, Serbia*
[5]*Institute of Applied Physics, University of Bern, Bern, Switzerland*

Skin aging represents a complex biophysical process driven by collagen degradation, loss of elasticity, and cumulative UV-induced damage. Modern therapeutic approaches rely on quantum optics and laser–tissue interaction, where biological effects depend on wavelength, energy density, and pulse duration in relation to the thermal relaxation time (TRT) of target chromophores.

This study analyzes and compares the biophysical effects of picosecond and millisecond laser systems on skin tissue. Treatments were performed using lasers operating at wavelengths such as 532 nm and 1064 nm, within the visible and near-infrared spectrum, with varying exposure times.

Comparative analysis shows that millisecond lasers predominantly induce photothermal effects, resulting in controlled thermal diffusion, collagen denaturation, and subsequent neocollagenesis and elastin remodeling. In contrast, picosecond lasers operate below the thermal relaxation time, producing a dominant photoacoustic effect characterized by stress confinement and mechanical disruption at the cellular level, with minimal thermal damage.

The results demonstrate that pulse duration is a critical parameter in defining the dominant biophysical mechanism. While millisecond lasers are effective for thermal remodeling, picosecond lasers enable precise, non-thermal tissue interaction and improved safety profiles.

Understanding these mechanisms allows optimization of laser-based therapies and individualized treatment protocols.

1. Feng J, Shen S, Song X, Xiang W. Efficacy and safety of picosecond laser for the treatment of melasma: a systematic review and meta-analysis.
2. Zhang X, Wang R, Lv J, Sun Q. Q-Switched 1064 nm Nd:YAG laser rejuvenates photoaging skin of rats by downregulating miR-196b-5p.
3. Dadkhahfar S, Fadakar K, Robati RM. Efficacy and safety of long pulse Nd:YAG laser versus fractional erbium:YAG laser in the treatment of facial skin wrinkles.

From Molecules to Meaning: Unlocking Cancer Across Scales with Synchrotron Light

Tanja Dučić

ALBA Synchrotron Light source, Experimental department, Barcelona, Spain, tducic@cells.es

Despite remarkable advances in oncology research, cancer remains a fundamentally multiscale disease, governed by processes that span from molecules to cells and tissues, and extending from molecular assemblies to whole-cell architecture. Addressing this complexity requires experimental approaches capable of seamlessly integrating structural and chemical information across length scales[1]. It will be presented how synchrotron radiation is redefining cancer biophysics by enabling comprehensive, high-resolution interrogation of tumor systems in near-native states.

This work leverages complementary synchrotron-based techniques, including X-ray fluorescence and diffraction imaging, X-ray spectroscopy and tomography, as well as Fourier-transform infrared (FTIR) spectromicroscopy[2,3], to elucidate the intricate relationship between cellular ultrastructure and biochemical composition. Soft X-ray tomography provides three-dimensional visualisation of intact cells at nanometer resolution, revealing organelle remodelling and spatial heterogeneity associated with malignancy. In parallel, micro- and nano-FTIR spectroscopy deliver label-free chemical mapping of biomolecular alterations in proteins, lipids, and nucleic acids during cancer progression at cellular and tissue levels.

By integrating structural and spectroscopic information, these approaches provide a powerful framework to decode tumor heterogeneity, identify robust biomarkers, and support the development of targeted therapeutic strategies. Overall, this perspective highlights how advanced synchrotron imaging and spectroscopy, combined with emerging data-driven methodologies, are bridging molecular mechanisms with clinically relevant insights and opening new pathways in precision oncology.

1. Salditt, T., Dučić, T., 2014, *Super-Resolution Microscopy Techniques in the Neurosciences*, Springer: 257–290.
2. Dučić, T., Pereiro, E., Ninkovic, M., Sperling, S., Rohde, V., Fernández-González, C., Algarra, M., González-Muñoz, E., 2025, *Journal of Nanobiotechnology*, 23(1):629. https://doi.org/10.1186/s12951-025-03687-2
3. Algarra, M., et al., 2024, *ACS Omega*, 9(12):13818–13830. https://doi.org/10.1021/acsomega.3c08459

Acknowledgment: *This work is supported by the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033) through projects PID2021-122613OB-I00 and PID2024-155480OB-100.*

Pressure as a Determinant of Pulse Wave Velocity: Experimental Evidence Supporting the Clinical Link Between Arterial Stiffness and Hypertension

Bojana Stojadinović[1], Zorica Nestorović[1]

[1]*Faculty of Medicine, University of Belgrade, Department of Biophysics, Belgrade, Serbia, bojana.stojadinovic@med.bg.ac.rs*

Introduction: Pulse wave velocity (PWV) is the gold standard for assessing arterial stiffness, improves prediction of cardiovascular events, and is strongly associated with the progression of hypertension. Despite its clinical significance, the direct biomechanical role of pressure in modulating PWV remains insufficiently defined.

Methods: The influence of pressure on PWV was investigated using an experimental biophysical model of the cardiovascular system consisting of elastic tubes filled with a viscous fluid. Pulse waves were generated under controlled laboratory conditions. PWV measurements were performed over a pressure range of 0 to 120 mmHg, with pressure systematically increased in increments of 20 mmHg.

Results: The obtained results demonstrate a progressive increase in PWV with rising transmural pressure across the entire investigated range. These findings demonstrate a direct biomechanical effect of pressure on pulse wave propagation, with increasing pressure producing higher PWV values.

Conclusion: These findings identify pressure as a fundamental determinant of PWV and provide a biophysical basis for interpreting results from large-scale clinical studies linking arterial stiffness with future hypertension and cardiovascular risk. Experimental validation of pressure-dependent changes in PWV may enhance the interpretation of vascular stiffness measurements in daily clinical practice.

From Erythrocyte-Based Cardiovascular Risk Assessment to the Development of a Synthetic Erythrocyte

Catarina S. Lopes[1,2], Ryan Gouveia e Melo[3], Luís Mendes Pedro[2,3], Filomena A. Carvalho[1,2], Nuno C. Santos[1,2]

[1]*GIMM – Gulbenkian Institute for Molecular Medicine, Lisbon, Portugal, nsantos@medicina.ulisboa.pt*
[2]*Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal*
[3]*Hospital de Santa Maria, Lisbon, Portugal*

Erythrocytes are deformable cells that undergo progressive changes affecting blood flow. We have previously demonstrated the biomedical relevance of the measurement of single-molecule fibrinogen-erythrocyte receptor binding and erythrocyte-erythrocyte adhesion, using atomic force microscopy (AFM)-based force spectroscopy, at the level of clinical prognosis in heart failure[1] and essential arterial hypertension[2] patients. The adhesion between human erythrocytes was further assessed by comparing AFM-based force spectroscopy measurements with micropipette aspiration. These data were used for the development of a physical/mathematical model of the biomedical relevant interaction between erythrocytes[3]. Recently, we evaluated changes in fibrinogen-erythrocyte and erythrocyte-erythrocyte interactions in carotid artery disease (CAD) patients, and characterized the biomechanical properties of atherosclerotic plaques from CAD patients. Blood collected from CAD patients, before and after endarterectomy surgery (3-year follow up) was analyzed and compared with healthy blood donors. This study comprising hemorheological parameters, erythrocyte interactions, and cell biomechanical properties, provided clinically relevant data on post-surgery improvement of patients and the evolution of the restenosis process. In parallel, we are participating in the development of a synthetic erythrocyte aimed at overcoming limitations on blood availability for transfusions, combining the lipid membrane with a cytoskeleton of self-assembled peptides or DNA origami.

1. Guedes, A.F., Carvalho, F.A., Malho, I., *et al.*, 2016, Nature Nanotechnol. 11:687-692.
2. Domingues, M.M., Carvalho, F.A., Santos, N.C., 2022, Annu. Rev. Biophys. 51:201-221.
3. Lopes, C.S., Curty, J., Carvalho, F.A., *et al.*, 2023, Commun. Biol. 6:192.

Acknowledgment: *This work was funded by Fundação para a Ciência e a Tecnologia project PTDC/EMD-TLM/7289/2020 and Horizon Europe project SynEry – HORIZON-EIC-2021-PATHFINDEROPEN-01.*

Multi-Target Alzheimer's Disease Compounds Identified by AI: Evaluation of Systems-Level Predictions and Direct Biochemical Activity

Miroslav Gancar[1], Zuzana Bednarikova[1], Chengyuan Yue[2], Barbora Borovská[1], Yun Tang[2], Lei Ma[2], Bu-Bing Zeng[2], Rui Wang[2], Zuzana Gazova[1]

[1]*Institute of Experimental Physics, Slovak Academy of Sciences, Department of Biophysics, Kosice, Slovakia, gancar@saske.sk*
[2]*School of Pharmacy, East China University of Science and Technology, Shanghai, China*

The aggregation of amyloid-β (Aβ) peptides and cholinergic dysfunction are central features of Alzheimer's disease (AD). We combined an AI-driven drug–target interaction framework with experimental validation to evaluate natural compounds predicted for AD therapy. Compound selection was based on a systems-level, multi-target strategy, where candidates are predicted to modulate combinations of disease-relevant proteins involved in signaling and neuroinflammation rather than directly targeting amyloid structures or classical enzymes. A knowledge graph-enhanced model integrating Node2Vec embeddings with ProteinBERT representations achieved high predictive performance (AUC = 0.972, AUPR = 0.970) and enabled prioritization of nine compound candidates (T1–T9), complemented by six additional compounds (M1–M6).

The T- and M-compounds were then evaluated for their biochemical activity, namely their ability to inhibit $A\beta_{40/42}$ aggregation and to disrupt amyloid fibrils, using Thioflavin T assays and supported by AFM imaging. Inhibition of BACE1 and AChE was also tested, alongside cytotoxicity (LDH assay) and antioxidant activity under oxidative stress. *In vitro* experiments show limited direct effects on aggregation and enzymatic targets but moderate cytoprotective and antioxidant activity in selected cases.

The obtained findings should be interpreted in the context of assay selection, as the AI-driven approach prioritizes systems-level targets, whereas the *in vitro* study focused on direct biochemical endpoints.

Acknowledgment: *This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0598, APVV-18-0284, and APVV-SK-CN-23-0025; DIAGNAD ITMS2014+ 313011T553 supported by the Operational Programme Integrated Infrastructure (OPII) funded by the ERDF, and the National Natural Science Foundation of China (Grant 82173746).*

Influence of Diastolic Pressure on Pulse Wave Reflection in a Biophysical Model of the Cardiovascular System

Ljubica Ilić[1], Isidora Janićijević[2], Aleksandra Markoski[3], Marko Pajić[4], Katarina Žikić[5]

[1]*Institute of Radiology, Military Medical Academy, University of Defense, Belgrade, Serbia*
[2]*Eye Clinic, Clinical Hospital Center Zvezdara, Belgrade, Serbia*
[3]*Rejuva Laser and Aesthetic, Belgrade, Serbia*
[4]*Institute of Biophysics, Faculty of Medicine, University of Belgrade, Serbia*
[5]*Institute of Applied Physics, University of Bern, Bern, Switzerland*

Wave propagation through the arterial system represents a complex biophysical process arising from the interaction between left ventricular dynamics, the mechanical properties of the arterial wall, and the physical characteristics of blood. A proper understanding of arterial hemodynamics is essential for both clinical diagnostics and research into cardiovascular pathophysiology. With aging, structural and functional changes occur in the arterial wall, including reduced elasticity and vascular compliance, which lead to an increase in pulse wave velocity (PWV) and alterations in wave reflection patterns. In this study, the influence of diastolic pressure and heart rate on pulse wave propagation and reflection was investigated using a biophysical model of the cardiovascular system. The results indicate that an increase in diastolic pressure leads to an increase in PWV due to the nonlinear elastic properties of the arterial wall. Consequently, the reflected wave returns earlier and overlaps with the incident wave during systole, resulting in augmentation of systolic arterial pressure and changes in pulse waveform morphology. An increase in cardiac pump frequency produced higher pressure wave amplitudes and a steeper systolic pressure upstroke, reflecting altered arterial hemodynamics. At lower pump frequencies, a stronger superposition of incident and reflected waves was observed, while at higher frequencies the separation between the two wave components became more pronounced. Additionally, increasing the distance to the reflection site shifted the reflected wave toward the diastolic phase.

These findings confirm the significant influence of diastolic pressure and heart rate on pulse wave dynamics and highlight their important role in the biophysical mechanisms associated with vascular aging and arterial stiffness.

1. Ilić L., Žikić K., Nestorović Z., Smiljković B., Žikić D. Development of novel experimental setup for hands-on cardiovascular biophysics education. European Biophysics Journal. 2025.
2. Laurent S., Cockcroft J., Van Bortel L., et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European Heart Journal. 2006.
3. Nichols W., O'Rourke M., Vlachopoulos C. McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. Hodder Arnold.
4. Mitchell G.F. Arterial stiffness and wave reflection: biomarkers of cardiovascular risk. Hypertension. 2010.

Anticancer, Antioxidant, and Insulin Anti-Amyloid Activities of Newly Synthesized Bis-Indoles

Andrea Antošová[1], Mariana Budovská[2], Aneta Salayová[3], Radka Michalková[4], Ladislav Janovec[2], Viktória Miškufová[4], Hanan Abdul Kareem[1], Ján Mojžiš[4], Zuzana Gažová[1]

[1]*Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Košice, Slovak Republic*
[2]*Department of Organic Chemistry, Institute of Chemistry, Faculty of Science, Pavol Jozef Šafárik University in Košice, Moyzesova 11, 040 01 Košice, Slovak Republic*
[3]*Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy in Košice, 041 81 Košice, Slovak Republic*
[4]*Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, SNP 1, 040 11 Košice, Slovak Republic*

Bis-indole derivatives have recently emerged as promising multifunctional bioactive molecules[1]. Their antioxidant properties and ability to modulate amyloid fibril formation highlight their potential for the development of therapeutics targeting amyloid-related diseases[2]. A series of novel nature-inspired bis-indole derivatives containing a phenyl linker was designed, synthesized, and evaluated as potential multitarget agents. The compounds were investigated for their cytotoxic, antioxidant, and anti-amyloid activities. In an *in vitro* MTT assay, bis-indole diurea showed selective inhibitory activity toward A549 lung cancer cells. Antioxidant evaluation using ABTS and DPPH assays identified bis-indole thiazino derivative as a strong free-radical scavenger. Nile red fluorescence, circular dichroism spectroscopy, atomic force microscopy, and molecular docking revealed that bis-indole dithiourea and thiazino bis-indole derivatives strongly inhibited insulin fibril formation, whereas bis-indole diurea was the most effective disruptor of preformed fibrils. Molecular docking revealed different binding affinities and orientations of bis-indoles toward amyloidogenic regions of insulin, which may explain their distinct effects on fibril inhibition and disaggregation. Overall, the synthesized bis-indoles act as multifunctional agents with anti-amyloid, antioxidant, and selective anticancer activities, highlighting their therapeutic potential.

1. Marinescu, M., 2024, Antibiotics, 13, 1212.
2. Ganegamage, S.K., Ramirez, E., *et al*., 2024, ACS Omega, 9, 1216–1229.

Acknowledgment: *This research was funded in part by the Grant Agency of the Ministry of the Education, Science, Research and Sport of the Slovak Republic VEGA 1/0347/23, VEGA 02/0164/22, VEGA 02/0141/25, and the Slovak Research and Development Agency under the contract No. APVV-22-0598 and by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00039.*

Biophysical and Mathematical Approaches to Cardiovascular Age Estimation

Biljana Smiljković[1]

[1]*Faculty of Medicine, University of Belgrade, Department of Medical Physiology, Belgrade, Serbia, bilja.djuric@ymail.com*

Cardiovascular aging is a complex process characterized by structural, hemodynamic and dynamical changes within the vascular system. Chronological age does not necessarily reflect vascular condition, emphasizing the need for quantitative approaches to assess biological cardiovascular age. This lecture presents a biophysical and mathematical framework based on signal analysis. The arterial system is considered as a wave propagation model, where increased arterial stiffness leads to higher pulse wave velocity, earlier wave reflection and changes in waveform morphology. Non-invasive photoplethysmography enables extraction of signal-derived biomarkers, including morphological, temporal and dynamical features. Classical approaches such as heart rate variability and entropy-based measures are discussed alongside nonlinear methods. Particular emphasis is placed on detrended fluctuation analysis, which quantifies long-range correlations and signal complexity. Selected results from photoplethysmographic recordings illustrate age-related changes in arterial stiffness, waveform characteristics and scaling exponents ($\alpha 1$, $\alpha 2$), reflecting reduced physiological adaptability. Cardiovascular aging can be interpreted as a progressive loss of dynamical complexity, and combined signal analysis provides a promising approach for non-invasive estimation of cardiovascular biological age.

1. Ilić L, Žikić K, Nestorović Z, Smiljković B, Žikić D. Development of novel experimental setup for hands-on cardiovascular biophysics education. Eur Biophys J. 2025 Oct;54(7):521-525. doi: 10.1007/s00249-025-01781-9.
2. Djurić B, Žikić K, Nestorović Z, Lepojević-Stefanović D, Milošević N, Žikić D. Using the photoplethysmography method to monitor age-related changes in the cardiovascular system. Front Physiol. 2023 Jul 19;14:1191272. doi: 10.3389/fphys.2023.1191272.
3. Djurić B, Suzić S, Stojadinović B, Nestorović Z, Ivanović M, Suzić-Lazić J, Nešić D, Mazić S, Tenne T, Zikich D, Žikić D. An improved design of optical sensor for long-term measurement of arterial blood flow waveform. Biomed Microdevices. 2017 Sep;19(3):48. doi: 10.1007/s10544-017-0196-x

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract 451-03-66/2024-03/200110*

Label-Free Electrochemical and QCM Aptasensors for Sensitive MicroRNA Detection as a Promising Tool for Cancer Diagnosis

Tomáš Holka[1], Jana Korčeková[3], Boris Lakatoš[1], Miroslav Gál[2], Alexandra Poturnayová[3,4]

[1]*Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia, tomasholka2000@gmail.com*
[2]*Institute of Inorganic Chemistry, Technology and Materials, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia*
[3]*Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia*
[4]*Department of Laboratory Medicine, Faculty of Health Care and Social Work, Trnava University in Trnava, Univerzitné námestie 1, 918 43 Trnava, Slovakia*

MicroRNAs (miRNAs) are short, non-coding RNA molecules that play an important role in regulating numerous developmental and physiological processes. Due to their stability in biological fluids and their specific expression patterns, miRNAs are considered promising biomarkers and have attracted considerable attention as potential non-invasive tools for the diagnosis and monitoring of different types of cancer [1].

However, early and accurate detection of cancer-related biomarkers remains a significant challenge. Therefore, novel analytical approaches such as aptasensors have attracted considerable attention. Aptasensors enable sensitive and selective detection of biomarkers, including miRNAs, even at very low concentrations, while offering advantages such as rapid analysis and cost- effectiveness [2].

In this work, we focus on the development of electrochemical and quartz crystal microbalance (QCM) aptasensors for the detection of microRNA as a potential cancer biomarker. In the experimental part, particular attention was first devoted to the optimization of individual steps involved in the construction of the aptasensor, as well as to the comparison of different types of aptamers for aptasensor fabrication. Subsequently, calibration curves were constructed for various concentrations of microRNA, and the limit of detection (LOD) and linear range obtained by the two employed techniques were compared. In the near future, the developed aptasensor will be applied to the analysis of real biological samples.

1. Wang, J., Chen, J., Sen, S., 2015, MicroRNA as biomarkers and diagnostics, Journal of Cellular Physiology, *231*(1), 25–30.
2. Negahdary, M., Moradi, A., Heli, H., 2019, Application of electrochemical aptasensors in detection of cancer biomarkers, Biomedical Research and Therapy, *6*(7), 3315-3324.

Acknowledgment: *This work was funded by the Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences VEGA 1/0157/24 and VEGA 2/0120/26 and by Slovak Research and Development Agency APVV-24-0491.*

Differential Effects Of cGMP on Ca^{2+} Signaling in Pulmonary and Mesenteric Artery Smooth Muscle Cells: A Theoretical Modeling Study

Katarina Meštrović[1,2], Aleš Fajmut[1,2]

[1]*Faculty of Natural Sciences and Mathematics, University of Maribor, Department of Physics, Maribor, Slovenia, katarina.mestrovic1@student.um.si*
[2]*Faculty of Health Sciences, University of Maribor, Maribor, Slovenia*

Cyclic 3',5'-guanosine monophosphate (cGMP) plays an important role in the regulation of vascular tone through mechanisms that affect the cytoplasmic $[Ca^{2+}]_i$ in vascular smooth muscle cells (SMCs). Based on existing biophysical models[1,2], we developed an extended model of Ca^{2+} signaling that incorporates the known direct and indirect effects of cGMP, as well as its additional influence on Ca^{2+}-activated chloride channels (ClCa). We applied the model to SMCs from the pulmonary and mesenteric arteries, which differ in the expression of ClCa channel subtypes: mesenteric artery contains both cGMP-independent ClCa and cGMP-dependent ClCacGMP channels, whereas pulmonary artery contains only cGMP-independent ClCa channels[3]. We then simulated the effect of cGMP on Ca^{2+} signaling in oscillatory and stationary regimes and calculated the average $[Ca^{2+}]_i$ as an indicator of SMC contractile state. In the pulmonary artery model, increasing cGMP decreased $[Ca^{2+}]_i$, consistent with its relaxing effect. In contrast, in the mesenteric artery model, cGMP above a certain threshold increased $[Ca^{2+}]_i$, although experimental data indicate relaxation[3]. These results suggest an important role for additional Ca^{2+}-independent regulatory mechanisms and identify the ClCacGMP channel as a key contributor to increased Ca^{2+} influx at high cGMP concentrations. Agreement with some experimental observations[3] highlights the importance of including different ion channel subtypes in models of vascular physiology.

1. Kapela, A., Bezerianos, A., Tsoukias, N. M., 2008, J. Theor. Biol. 253:238–260.
2. Jacobsen, J.C., Aalkjaer, C., et al., 2007, Am. J. Physiol. Heart Circ. Physiol. 293:H229–H237.
3. Matchkov, V.V., Aalkjaer, C., Nilsson, H.,2004, J. Gen. Physiol. 123:121–134.

Intrinsic Mechanical Control of Tumor Spheroid Morphology and Rheology in a 3D Vertex Model

Urban Železnik[1, 2], Matej Krajnc[2], Tanmoy Sarkar[3]

[1]*Faculty of Mathematics and Physics, University of Ljubljana, 1000 Ljubljana, Slovenia*
[2]*Institut Jožef Stefan, Department of Theoretical Physics, 1000 Ljubljana, Slovenia, urban.zeleznik@ijs.si*
[3]*School of Physics, Indian Institute of Science Education and Research Thiruvananthapuram, Maruthamala PO, Thiruvananthapuram, Kerala 695551, India*

One of the hallmarks of pre-migratory tumors is the progressive loss of compact morphology. To investigate how tumors intrinsically regulate their shape during growth, we employ a three-dimensional (3D) vertex model of multicellular aggregates that incorporates key structural features of tumor spheroids, including the surface, a proliferative rim, and a necrotic core. Focusing on tumor-intrinsic mechanical interactions, we examine how their collective effects guide morphological evolution. We show that spheroids acquire lobulated morphologies through an interplay between differential tensions at the spheroid surface and the living–necrotic interface, together with differential growth within the proliferative rim. Spheroid shapes are further modulated by fluctuations emerging from active, cell-scale forces. These simulations are enabled by a computational framework that overcomes a major limitation of 3D vertex models—the lack of cell division—by introducing a graph-based polyhedral division algorithm within the Graph Vertex Model. In ongoing work, we extend this framework to probe the rheological response of tumor spheroids through *in silico* compression, centrifugation, and fusion experiments, isolating intrinsic cell–cell mechanics. Results show that surface tension governs mechanical response, relaxation dynamics, and fusion behavior, providing a complementary perspective on the physical principles underlying tumor morphology.

Development of Effective Genetically Encoded Light-Responsive Systems for Biomedical Applications

Kristína Felčíková[1], Alexandra Poliaková[1,2], Michal Nemergut[2], Andrej Hovan[1], Gregor Bánó[1], Erik Sedlák[2,3]

[1]*Department of Biophysics, Faculty of Science, P.J. Šafárik University, Jesenná 5, 040 01 Košice, Slovakia*
[2]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, P.J. Šafárik University, Jesenná 5, 040 01 Košice, Slovakia*
[3]*Department of Biochemistry, Faculty of Science, P.J. Šafárik University, Moyzesova 11, 040 01 Košice, Slovakia*

The effectiveness of photodynamic therapy critically depends on engineered photosensitizers with well-defined light-activated cofactor interactions. Flavin mononucleotide (FMN) is a natural photosensitizer with a high quantum yield of singlet oxygen ($\Phi\Delta$ = 0.51–0.65)[1], but its binding in a protein, forming thus genetically encoded photosensitizers (GEPS), is accompanied by significantly decreased efficiency (>10-fold) due to protein matrix quenching[2]. Therefore, our design of GEPS is based on forced FMN dissociation induced by oxidation of amino acids at the FMN binding site. Inspired by strategies from luciferase engineering, we designed proteins with mutations that enhance cofactor dissociation while preserving protein structure. Based on structural analysis, several LOV2 variants were designed, expressed and purified. Phosphorescence and fluorescence measurements confirmed enhanced singlet oxygen production and efficient FMN dissociation[3]. To achieve specific targeting, the LOV2 variants were conjugated with Designed Ankyrin Repeat Protein (DARPin). DARPins are small, stable engineered binding proteins that recognize specific targets with high affinity. In this study, we employed a DARPin that specifically recognizes the HER2 receptor, which is frequently overexpressed in cancers such as breast cancer[4]. These results demonstrate a promising strategy for designing targeted, light-activated protein therapeutics, enabling more efficient and selective photodynamic therapy in cancer treatment.

1. Westberg M., Bregnhoj M., Etzerodt M., Ogilby P.R., J. Phys. Chem. B 121 (2017) 2561-2574.
2. Ruiz-González R., Cortajarena A.L., Mejias S.H., Agut M., Nonell S., Flors C. J. Am. Chem. Soc. (2013) 9564-9567.
3. Felčíková K., Hovan A., Polák M., et al., Prot. Sci. 33 (2024) 4921.
4. Jost C., Schilling J., Tamaskovic R., Schwill M., Honegger A., Plückthun A., Structure. (2013) 1979-1991.

Acknowledgment: *This work was funded by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA), grant No. 1/0216/25.*

Molecular and Dynamical Insights of Amylin Supramolecular Assembly and Aggregation: Implications for Pancreatic Amyloidosis and Diabetes Development

Aleksandar Jeremic

The George Washington University, Department of Biological Sciences, Washington, D.C., U.S.A. jerema@gwu.edu

Human islet amyloid polypeptide (hIAPP), or amylin, is a 37-aa peptide hormone produced and co-secreted with insulin by pancreatic islet β-cells. Human amylin is the principal constituent of amyloid deposits in the pancreatic human islets, a hallmark of diabetes. Although much progress has been made in understanding the actions of toxic amylin oligomers and aggregates, the cellular events and factors that contribute to amylin oligomerization, aggregation and toxicity in the pancreas remain largely unknown. Our recent biochemical and biophysical studies using atomic force microscopy, laser scanning confocal and transmission electron microscopy, CD/fluorescence/mass spectroscopy, as well as transcriptomics approaches revealed that membrane cholesterol, copper and the proteasome complex play pivotal roles in the prevention of amylin-induced protein stress and proteotoxicity in pancreatic β–cells, and consequently diabetes. In contrast to the stimulatory effect of negatively charged lipids like phosphatidyl serine on amylin aggregation, cholesterol slowed down amylin transition to β-sheet enriched structures, reduced amylin fibrillization rates, and consequently limited its deposition on the synthetic and native membranes by creating submicron-domains of inert amylin deposits. Conversely, cholesterol-depleting agents stimulated amylin aggregation and toxicity in human islet cells. The similar inhibitory effect on amylin self-assembly, oligomerization and amylin-induced redox stress was observed in the presence of essential micro-element, copper. These findings offer new insight into the etiology of islet amyloidosis and diabetes, thus providing a platform for rational design of new anti-diabetic and anti-amyloid drugs.

Acknowledgment: *This work was funded by the National Institute of Health (grants no. R01DK091845, R01EB027648, R03EB032464).*

An Educational Arterial Model for Demonstrating Pulse Wave Propagation in Cardiovascular Biophysics

Marko Pajić[1], Isidora Janićijević Grubišić[2], Aleksandra Markoski Smiljković[3], Ljubica Ilić[4], Katarina Žikić[5]

[1]*Institute of Biophysics, Faculty of Medicine, University of Belgrade, Serbia, marko@srbsect.org*
[2] *Eye Clinic, Clinical Hospital Center Zvezdara, Belgrade, Serbia*
[3]*Rejuva Laser and Aesthetic, Belgrade, Serbia*
[4]*Institute of Radiology, Military Medical Academy, University of Defense, Belgrade, Serbia*
[5]*Institute of Applied Physics, University of Bern, Bern, Switzerland*

A fundamental understanding of biophysics and fluid dynamics is essential for interpreting cardiovascular physiological phenomena. However, medical students often encounter difficulties in mastering hemodynamic concepts due to their mathematical complexity. To facilitate learning, an experimental biophysical model of the cardiovascular system was developed as an alternative to in vivo and in vitro studies.

The model simulates the vascular tree using transparent silicone tubes, one-way valves, reservoirs, and pressure sensors connected to a data acquisition system. Its central component is a silicone aorta immersed in water and connected to rubber and peristaltic pumps for generating pulsatile flow. A 40% ethyl alcohol solution was used to mimic blood viscosity.

Pressure waveforms were recorded at multiple locations along the system, enabling real-time visualization and quantitative analysis. The setup allowed investigation of pulse wave propagation, waveform morphology, pulse wave velocity, and the influence of resistance and constrictions on wave transmission.

The model successfully demonstrated wave reflection at bifurcations and valves, as well as the superposition of incident and reflected waves. Time delays between sensors enabled accurate pulse wave velocity calculations. Additional resistance and stenosis further emphasized reflection phenomena.

This experimental setup provides an effective educational tool for teaching cardiovascular biophysics, offering hands-on experience in hemodynamic analysis and supporting a deeper understanding of physiological wave phenomena.

1. Žikić, D., Žikić, K., 2022, Eur. Biophys. J. 51:365–374.
2. Ilić, L., Žikić, K., Nestorović, Z., Smiljković, B., Žikić, D., 2025, Development of novel experimental setup for hands-on cardiovascular biophysics education, Eur. Biophys. J. https://doi.org/10.1007/s00249-025-01781-9
3. Caiazzo, A., Vignon-Clementel, I.E., 2018, Mathematical modeling of blood flow in the cardiovascular system, Springer, Cham.

Biophysical Assesment of Vascular Ageing Through Continuous Pulse Waveform Analysis and Quantitative Retinal Microvascular Assessment

Isidora Janićijević-Grubišić[1], Ljubica Ilić[2], Marko Pajić[3], Aleksandra Markoski Smiljković[4], Katarina Žikić[5]

[1]*Eye Clinic, Clinical Hospital Center Zvezdara, Belgrade, Serbia, isidorajanicijevic17@gmail.com*
[2]*Institute of Radiology, Military Medical Academy, University of Defense, Belgrade, Serbia*
[3]*Institute of Biophysics, Faculty of Medicine, University of Belgrade, Serbia*
[4]*Rejuva Laser and Aesthetic, Belgrade, Serbia*
[5]*Institute of Applied Physics, University of Bern, Bern, Switzerland*

Vascular ageing is accompanied by progressive functional and structural changes including both large arteries and microcirculation. Increased arterial stiffening of large arteries and reduction in the structural complexity of the microvascular network are one of the most reliable markers of vascular ageing and target organ damage. Biophysical investigations provide a non-invasive way for assessing these age related functional and structural changes across large arteries, while the retinal vasculature represents a unique in vivo model for the quantitative evaluation of microvascular architecture.

This pilot study aimed to investigate age related changes in retinal microstructural and microvascular parameters across diffrent age groups and to examine their association with arterial stiffnesss assessed by continuous pulse waveform analysis. The biophysical component is of a particular relevance, as continuous signal acquisition enables not only conventinal evaluation of vascular dynamics, but also advances spectral and nonlinear analyses of pulse waveform behavior.

Carotid artery hemodynamics were assessed using non invasive optical sensors and continuous pulse waveform analysis and retinal fundus umages were analyzed to determine fractal dimension as a measure of retinal vascular network complexity. Correlation analysis was performed betwen carotid derived parameters , retinal fractal dimension and patient age.

A correlation was observed betwen ageing and reduction in retinal fractal dimension as well as with ageing and increased arterial stiffness. This pattern suggest parallel age related changes in microvascular and macrovascular function.

These findings suggest that biophysical investigations combining carotid pulse waveform analysis with retinal fractal assesment may provide an integrated non-invasive approach to the study of vascular ageing . This pilot study supports the concept of a close relationship betwen macrovascular ageing and microvascular remodeling and highlights the potential value of retinal fractal dimension as a biomarker of a systemic vascular ageing.

TRPV4 Modulates Mechanically Induced Calcium Waves in Human Lens Epithelium

Sofija Andjelic[1], Dasa Sarman[1], Bernarda Kinkela[1], Taja Vita Petrovic[1], Katarina Mestrovic[2], Marko Hawlina[1], Marko Gosak[2,3]

[1]*Eye Hospital, University Medical Centre Ljubljana, Slovenia, sofija.andjelic@kclj.si*
[2]*Faculty of Natural Sciences and Mathematics, University of Maribor, Slovenia*
[3]*Faculty of Medicine, University of Maribor, Slovenia*

Although TRPV4 (transient receptor potential vanilloid 4) channel expression has recently been demonstrated in human lens epithelium, its functional role in Ca^{2+} signaling in lens epithelial cells (LECs) remains unclear. LECs form a tightly coupled epithelial sheet supporting coordinated intercellular communication and lens function, making it important to determine how TRPV4 contributes to local Ca^{2+} entry and propagation of multicellular Ca^{2+} waves.

Human postoperative anterior lens capsule preparations were loaded with the Ca^{2+} indicator Fura-2. Mechanically induced Ca^{2+} waves were analyzed under control conditions and after pharmacological TRPV4 inhibition with HC using spatially resolved quantitative analysis to assess wave velocity, amplitude, and kinetics.

Mechanical stimulation of a single LEC triggered a radially propagating intercellular Ca^{2+} wave. TRPV4 inhibition reduced propagation velocity from ~12 to ~7 μm/s and Ca^{2+} signals showed a consistent 15–25% decrease in amplitude, rise rate, and decay rate. The reduction in wave velocity occurred across all cataract types. In contrast, alterations in intracellular Ca^{2+} signaling kinetics were statistically significant only in mild cataracts, whereas no significant differences were detected in more advanced cataract stages.

These findings indicate that TRPV4 facilitates efficient intercellular Ca^{2+} wave propagation in human lens epithelium and contributes to coordinated multicellular responses in lens tissue.

Acknowledgment: *This work was funded by the Slovenian Research and Innovation Agency (research core funding nos. P3-0333, P3-0396, , and research project nos. J3-60062 and J3-50107).*

SCIENTIFIC SESSION VII

BIOMIMETIC AND FUNCTIONAL INTERFACES

RNA-induced Conformational Changes of Stress Granules Scaffold Protein, G3BP1

S.A. Silonov[1], A.A. Gavrilova[1], E.Y. Smirnov[1], Y.A. Zagryadskaya[2], Y.V. Bershatsky[3], A.V. Fonin[1]

[1]*Group of Biocondensates and cell membrane-less organelles, Institute of Cytology of RAS, St,Petersburg, Russia*
[2]*Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, Moscow Institute of Physics and Technology, Dolgoprudny, Russia*
[3]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of Russian Academy of Sciences, Moscow, Russia.*

Stress granules are the most studied membrane-less organelles. These organelles play a significant adaptive role in cell life by regulating different cell processes and storage of biomolecules in stress conditions. The formation of membrane-less organelles, and, respectively, stress granules is based on liquid-liquid phase separation of RNA and intrinsically disordered proteins. One of the scaffold proteins of stress granules is the Ras GTPase-activating protein-binding protein 1 (G3BP1). Complex coacervation of this protein with RNA induces the formation of RNA-protein liquid droplets. We studied the RNA-induced LLPS of G3BP1 by a wide range of methods including NMR, SAXS, steady state and fluorescence spectroscopy. We showed that G3BP1 “unwinds” its structure and becomes more dynamic in LLPS conditions. The obtained results allowed us to suggest a new model of change of structural-dynamic properties of G3BP1 in its LLPS.

Diamond vs. Gold: Interfacial Stability and Performance of QCM-Aptasensors for Oncogenic miRNA Detection

Michaela Hornychová[1], Miroslav Gál[1], Alexandra Poturnayová[2]

[1]*Faculty of Chemical and Food Technology, Slovak University of Technology, Department of Inorganic Technology, Bratislava, Slovakia, michaela.hornychova@stuba.sk*

[2]*Institute of Molecular Physiology and Genetics SAS, Centre of Biosciences Slovak Academy of Sciences, Bratislava, Slovakia*

This study explores the design and fabrication of biosensor platforms for the sensitive detection of circulating miRNAs, which serve as promising biomarkers for oncological diseases. Diamond surfaces are of significant interest due to their unique biocompatibility, chemical resistance, and electro-optical properties. We address key analytical limitations associated with miRNA determination, including low abundance in body fluids, short sequence length, and high sequence homology among related targets, which necessitate stringent requirements for interfacial stability and assay selectivity. This work integrates surface chemistry, biosensor engineering, and molecular biology to construct functional bioreceptor layers on gold and diamond-coated Quartz Crystal Microbalance sensors. Surface-immobilized DNA aptamers served as biorecognition elements to systematically compare the functionalization strategies and interfacial behavior of both substrates.

Results indicate that while diamond-based sensors offer significant potential for biomedical applications, low repeatability remains a critical obstacle as successful detection was primarily achieved on uncleaned diamond surfaces. This limitation underscores the need for further optimization of surface conditioning to achieve the reliability required for clinical implementation and rapid diagnostic screening.

Acknowledgment: *This work was supported by the Science Grant Agency; project no. VEGA 1/0157/24, APVV-24-0491, EULiST 101124310.*

The Examination of Cinnamic Acid Binding Interactions Strength to Human Serum Albumine Using Biomimetic Chromatography

Vladimir Vlatković[1], Milica Radan[2], Saša Lazović[1], Darija Obradović[1]

[1]*Institute of Physics Belgrade, National Institute of the Republic of Serbia, University of Belgrade, Serbia, vlatkovic@ipb.ac.rs*
[2]*Institute for the Study of Medicinal Plants "Dr. Josif Pančić", Belgrade, Serbia*

Biomimetic high-performance liquid chromatography (HPLC) employs biologically relevant stationary phases and organic mobile phases at physiological pH values, simulating conditions in the human body [1]. In this system, compound–plasma protein interactions on a human serum albumin (HSA) stationary phase are governed by hydrogen bonding, hydrophobic, ionic, and electrostatic forces [2].

This study aimed to examine how different system parameters influence the HSA binding strength of insulin-mimetic-related compounds, represented by cinnamic acid (CA), a cinnamon bioactive constituent. The experiments were performed using an HPLC system with a biomimetic HSA column and UV detection. The mobile phase consisted of a phosphate buffer/isopropanol mixture.

The influence of three independent variables: phosphate buffer strength (x_1), isopropanol ratio (x_2), and temperature (x_3) was investigated. The response variable was expressed as $\log_{10}$(PPB). The interaction behavior can be adequately described by a quadratic model ($R^2_{adj} = 0.96$; $R^2_{pred} = 0.78$).

The most pronounced effect was observed for the quadratic term of x_1, consistent with the established dependence – an increase in buffer strength decreases mobile phase polarity, negatively affecting the stability of the HSA-ligand complex.

The results indicate that the biomimetic HSA chromatography system, together with the developed quadratic model, can reliably reflect the binding strength of CA and structurally related molecules.

1. Obradović, D., Radan, M., Đikić, T., *et al*., 2022, J Pharm Biomed Anal. 114593: 211.
2. Starek, M., Dąbrowska, M., Lazović, S., *et al*., 2025, Microchem. J. 114824: 216.

Acknowledgment: *This research was supported by the Science Fund of the Republic of Serbia, GRANT No 13534, Proof of Concept - DIAMICA. The authors also acknowledge funding provided by the Institute of Physics Belgrade, National Institute of the Republic of Serbia, through the grant from the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia.*

Lysozyme and Spider Silk Amyloid Fibrils as Building Blocks for Novel Hydrogels

Tomas Biro[1], Veronika Huntosova[2], Katarina Siposova[1], Martin Humenik[3]

[1]*Department of Biophysics, Institute of Experimental Physics SAS, Kosice, Slovakia, biro@saske.sk*
[2]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, P. J. Safarik University, Kosice, Slovakia*
[3]*Department of Biomaterials, Faculty of Engineering Science, University Bayreuth, Bayreuth, Germany*

Amyloid fibrils, once primarily associated with neurodegenerative diseases, are now recognized as fundamental building blocks of functional biomaterials. One such class of materials is hydrogels and nanohydrogels, which can be characterized as three-dimensional networks capable of absorbing large quantities of water or other biological fluids without dissolving. This ability to swell allows them to mimic living tissues in terms of viscoelasticity and biocompatibility, which is crucial for the preparation of extracellular matrices used in 3D cultures for cancer research and organ development[1,2,3]. Based on the ability of amyloid fibrils to form hydrogels, the present work aims to prepare and characterize hydrogels using the model protein lysozyme, the recombinant spider silk protein eADF4(C16), and their combination. Fibrils of these proteins have the potential to endow hydrogels with robust and tunable mechanical properties through modulation of the β-sheet structure. This level of adaptability provides a promising and versatile tool for tailored biomedical applications, cell.culture experiments, and tissue engineering.

1. Tyagi, G., Sengupta, S., 2024, Biophys Rev. 16(6):737-751.
2. Chai, Q., Jiao, Y., Yu, X., 2017, Gels. 3(1):6.
3. Kumari, A., Ahmad, B., 2019, RSC Adv. 9(64):37424–37435

Acknowledgment: *This work was funded by the APVV 23-0013, APVV SK-TW-24-0005, VEGA 2/0053/26, 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.*

Catalogue of Shapes for Prolate Lipid Vesicles With Relative Volumes Below That of the Twin Shape

Bojan Božič[1], Saša Svetina[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biophysics, Ljubljana, Slovenia, bojan.bozic@mf.uni-lj.si*

The shapes of the lipid vesicles are presented for the region that has not yet been well studied, namely for relative volumes slightly smaller than that of the two-sphere shape[1]. The shapes are determined by minimising the mechanical energy of the membrane under the constraints of membrane area and vesicle volume. According to the area difference elasticity model, the relevant mechanical energy terms are the local and non-local bending energies of the membrane[2,3]. The appropriate parameters for vesicle shapes are the difference between the areas of the outer and inner monolayers, which is reflected in the average mean curvature of the membrane, and the relative volume of the vesicle, defined with respect to the membrane area. Examples of the shapes of the individual classes are shown in the phase diagram with the lowest membrane mechanical energy.

1. Christ, S., Litschel, T., Schwille, P., *et al.*, 2021, Soft Matter 17:319-330.
2. Svetina, S., Žekš, B., 1989, Eur. Biophys. J. 17:101-111.
3. Božič, B., Svetina, S., Žekš, B., *et al.*, 1992, Biophys. J. 61:963-973.

Acknowledgment: *This work was funded by the Slovenian Research and Innovation Agency (ARIS), Contract P1-0055.*

Interaction Between Flavonoids and Membranes Under Oxidative Stress: Implications for Cell Membrane Integrity

Anja Sadžak[1], Suzana Šegota[1]

[1]*Ruđer Bošković Institute, Division of Physical Chemistry,* Zagreb, Croatia, ssegota@irb.hr

Oxidative stress is a major contributing mechanism of cellular damage in numerous diseases. It occurs when the production of reactive oxygen species (ROS) exceeds the capacity of endogenous antioxidant defense systems, disrupting cellular redox homeostasis. ROS react with nearby molecular targets (proteins, DNA, lipids), disrupting their structure and membranes and compromising their function.

Flavonoids, bioactive molecules, exhibit diverse biological activities, including antibacterial, anti-inflammatory, anticancer, and antioxidant effects, and can alter various cellular processes. Flavonoids can act by scavenging free radicals or modifying their propagation through the cell membrane. In addition to direct scavenging, flavonoids can inhibit lipid peroxidation by chelating metal ions, which typically catalyse the Fenton reaction. Depending on their hydrophilicity/ hydrophobicity, flavonoids can penetrate the hydrophobic regions of biological membranes or remain near the polar head groups, thereby affecting membrane fluidity and the extent of their biological activities. Furthermore, the location of flavonoids within the membrane can influence their efficiency in preventing and controlling lipid peroxidation.

An overview of the protective effect of three structurally different flavonoids on the molecular structure, integrity, and elasticity of lipid model and cell membranes under oxidative attack, using a combination of biophysical techniques, will be presented.

1. Sadžak, A.; Eraković, M.; Kriechbaum, M.; Mravljak, J.; Přibyl, J.; Maltar-Strmečki, N.; Šegota, S., 2026, J. Colloid Interf. Sci. 139894:709.
2. Sadžak, A., Brkljača, Z., Eraković, M., Kriechbaum, M., Maltar-Strmečki, N., Pribyl, J., Šegota, S. 2023, J. Lipid Res. 100430: 64.
3. Jazvinšćak Jembrek, M., Oršolić, N., Mandić, L., Sadžak, A. Šegota, S. 2021, Antioxidants 1628:10.
4. Sadžak, A., Vlašić, I., Kiralj, Z., Batarelo, M., Oršolić, N., Jazvinšćak Jembrek, M., Kušen, I.,
5. Šegota, S. 2021, Molecules 845: 26.
6. Sadžak, A., Brkljača, Z., Crnolatac, I., Baranović, G., Šegota, S., 2020, Colloids Surf. B. Biointerfaces 111147: 193.
7. Sadžak, A., Mravljak, J., Maltar-Strmečki, N., Arsov, Z., Baranović, G., Erceg, I., Kriechbaum, M., Strasser, V., Přibyl, J., Šegota, S., 2020, Antioxidants 430-460: 9
8. Jazvinšćak Jembrek, M., Vlainić, J., Čadež, V. Šegota, S., 2018, PLoS One 0200119. 13.

Acknowledgment: *This work was funded by the Croatian Science Foundation under the project IP-2016-06-8415 entitled Protective mechanisms and effects of flavonoids embedded in nanocarriers in model membranes and neurons.*

Electroformation of Giant Unilamellar Vesicles with High Cholesterol Contents from Damp Lipid Films under Physiological Conditions

Mateo Šerić[1,2], Lovro Čeliković[3], Marija Raguz[1]

[1]*University of Split School of Medicine, Department of Medical Physics and Biophysics, Split, Croatia, mateo.seric@mefst.hr*
[2]*Faculty of Science, University of Split, Doctoral Study of Biophysics, Split, Croatia*
[3]*Faculty of Science, University of Split, Graduate Study of Molecular biology, Split, Croatia*

Giant unilamellar vesicles (GUVs) are widely used as model systems for studying cellular membrane properties due to their cell-like size. Electroformation is the most commonly used method for GUV production. Traditionally, electroformation is performed by swelling a dry lipid film deposited on conductive electrodes under an applied alternating electric field.[1] A major limitation of dry-film electroformation is the incorporation of high cholesterol (Chol) concentrations in vesicle bilayers. In that case, during dry-film preparation, Chol tends to crystallize, leading to a Chol demixing artefact that results in reduced and uncontrolled Chol incorporation into the vesicle bilayers. To overcome this limitation, we developed a modified electroformation protocol based on damp lipid films that includes rapid solvent exchange, ultrasonication, plasma cleaning, and spin-coating.[2] Using this approach, we investigated lipid mixtures with Chol/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine molar ratios ranging from 0 to 2 by fluorescence microscopy. In addition to modifications addressing Chol demixing, we evaluated the influence of the ionic strength of the internal solution. Four different buffers at pH 7 were supplemented with approximately 150 mM concentrations of different salts to assess vesicle formation yield and size distribution under physiological conditions.

1. Estes, D. J., Mayer M., 2005, Biointerfaces 42(2), 115-123.
2. Mardešić, I.; Boban, Z.; Raguz, M., 2024, Membranes 14, 215.

Acknowledgment: *This work was funded by the Croatian Science Foundation (Croatia) under Grant IP-2024-05-3923*

Exploring Liposome-Encapsulated Schiff Bases as Antioxidants for Honey Bee Health

Đura Nakarada[1], Miljan Bigović[2], Marija Kaluđerović[2], Nevena Prlainović[3], Miloš Mojović[1]

[1]*Center for Physical Chemistry of Biological Systems, BioScope Labs, Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia, djura@ffh.bg.ac.rs*
[2]*Faculty of Natural Science and Mathematics, University of Montenegro, Podgorica, Montenegro*
[3]*Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

Schiff bases derived from thiocarbohydrazide are widely studied for their diverse biological activities, including strong antioxidant potential. In this work, the antioxidative properties of (*E*)-*N*′-((*E*)-1-(2-hydroxyphenyl)ethylidene)-2-(1-(2-hydroxyphenyl)ethylidene)hydrazine-1-carbothiohydrazide (SB), a novel Schiff base, were evaluated by electron paramagnetic resonance (EPR) spectroscopy through scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (•OH) radicals. To examine the influence of a lipid environment, SB was incorporated into dipalmitoylphosphatidylcholine (DPPC) liposomes using a modified thin-film hydration method followed by extrusion. EPR results showed strong radical-scavenging activity in solution, reducing DPPH and •OH signals by 69.53% and 37.31%, respectively. After incorporation into DPPC liposomes, DPPH scavenging decreased to 46.12%, while •OH scavenging remained similar (34.38%). The reduced DPPH activity likely reflects partial shielding of SB within the lipid bilayer, limiting access to radicals, whereas reactivity toward •OH is less affected. The SB retains notable antioxidative activity in lipid vesicles, highlighting liposomes as suitable carriers that modulate interactions with reactive species. Given the role of oxidative stress in honey bee decline, these results support further exploration in apicultural research, including studies using the AmE-711 honey bee cell line, to assess potential benefits for bee health.

Acknowledgment: *This research was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, contract numbers 451-03-33/2026-03/200146 and 451-03-34/2026-03/200146 and by the Science Fund of the Republic of Serbia, project Waste2ProtectBees No. 5455.*

EPR Evaluation of Antioxidant Liposomal Chaga Extracts from Different Origins With Potential Relevance for Honeybee Oxidative Stress

Nevena Preradović[1], Stefan Jelisić[2], Đura Nakarada[3], Vuk Maksimović[1], Miloš Mojović[3]

[1]*Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, University of Belgrade, Department of Life Sciences, Belgrade Serbia*
[2]*Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia, stefan.jelisic@vet.bg.ac.rs*
[3]*Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia*

Inonotus obliquus (chaga), a medicinal mushroom used in traditional folk medicine, is a rich source of bioactive compounds with strong antioxidant potential. In this study, 50% ethanol extracts of chaga from Bosnia and Herzegovina, Russia, and Bulgaria were encapsulated into DPPC liposomes. The systems were characterized by dynamic light scattering and zeta potential, while phytochemical composition was analyzed by HPLC–MS. Antioxidant activity was evaluated using electron paramagnetic resonance (EPR) via DPPH radical reduction and hydroxyl radical scavenging with the DEPMPO spin–trapping method. All liposomes had sizes below 200 nm (163.5 nm control; 153.6 nm Bosnian; 122 nm Russian; 106 nm Bulgarian) and zeta potentials from −34 to −44 mV, indicating good stability. Liposomes with Bosnian extract showed the highest radical scavenging activity (49.24% DPPH; 50.96% hydroxyl), followed by Russian (46.1%; 29.71%) and Bulgarian samples (42.36%; 22%), while empty liposomes showed minimal activity. These differences likely reflect apparent variations in phytochemical composition and encapsulation efficiency. Overall, chaga–loaded liposomes demonstrated stability and significant antioxidant capacity, suggesting potential biomedical applications. Considering the role of oxidative stress in honeybee health, these systems may also be promising for future evaluation in apicultural models, including the AmE–711 honey bee embryonic cell line, for cytoprotective effects.

1. Ern, P.T.Y., Quan, T.Y., Yee, F.S., Yin, A.C.Y., 2024, Therapeutic properties of *Inonotus obliquus* (Chaga mushroom): A review, Mycology 15(2):144-161.
2. Shashkina, M.Y., Shashkin, P.N., Sergeev, A.V., 2006, Chemical and medicobiological properties of chaga (review), Pharm Chem J 40:560-568.
3. Szychowski, K.A., Skóra, B., Pomianek, T., Gmiński, J., 2021, *Inonotus obliquus* – from folk medicine to clinical use, J. Tradit. Complement. Med. 11(4):293-302.

Acknowledgment: *This work was funded by the Ministry of Education, Science, Technological Development and Innovations of the Republic of Serbia, contract numbers 451-03-33/2026-03/200053, 451-03-33/2026-03/200146, 451-03-34/2026-03/200146 and 451-03-34/2026-03/200143, and by the Science Fund of the Republic of Serbia, project Waste2ProtectBees No. 5455.*

Tuning the Size of Protein-Based Physical Nanogels: The Role of pH and Ionic Strength

Ana Vesković, Anđela Tasić, Anica Smiljanić, Ana Popović Bijelić

Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia, ana@ffh.bg.ac.rs

Nanogels are 3D nanoscale polymeric matrices capable of absorbing large amounts of water while maintaining stability in aqueous environments. By integrating the properties of both nanoparticles and hydrogels, these platforms offer high loading capacity and favorable biological response. While synthetic polymers afford considerable tunability, naturally derived protein-based materials are increasingly prioritized owing to their superior biocompatibility. Bovine and human serum albumins (BSA and HSA) have been extensively investigated for drug delivery due to their exceptional binding capacity, while ovalbumin (OVA) has demonstrated suitability for encapsulating bioactive compounds in functional food design. The aim of this study was to investigate the effects of ionic strength and pH on the nanogel size, with particular focus on physically crosslinked BSA- and OVA-based nanogels. Namely, the size and deformability of nanogels must correspond to their intended function and route of administration, and therefore it is essential to understand how the noncovalent interactions stabilizing the polymeric network govern these properties and, consequently, diffusion behavior and biological clearance. The nanogels were prepared by thermal synthesis across a range of protein concentrations, and were characterized by dynamic light scattering (DLS). The results indicate that pH and ionic strength strongly modulate nanogel size and dispersity, with effects that are specific to the protein.

Acknowledgment: *This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Contract 451-03-34/2026-03/200146.*

Stability and Reversibility of Organic Molecule Modifications of Monolayer MoS_2

Antun Lovro Brkić[1], Antonio Supina[1], Davor Čapeta[1], Lucija Ptiček[2], Livio Racané[2], Marina Šekutor[3], Christian Teichert[4], Ida Delač[1]

[1]*Institute of Physics, Centre for Advanced Laser Techniques, Zagreb, Croatia, idelac@ifs.hr*
[2]*Faculty of Textile Technology, University of Zagreb, Zagreb, Croatia*
[3]*Ruđer Bošković Institute, Department of Organic Chemistry and Biochemistry, Zagreb, Croatia*
[4]*Chair of Physics, Montanuniversität Leoben, Leoben, Austria*

Our research is focused on the modification of the two-dimensional materials' (2DM) properties with organic molecules. Starting point was the synthesis and characterization of 2DMs, and studying their stability under various conditions. In the research presented here, we modified 2D MoS_2 by deposition of the L63MS (6–(4,5-dihydro-1H-imidazol-3-ium2-yl)2(naphthalene-2-yl)benzothiazole methanesulfonate) molecule solution under ambient conditions and investigated the stability of our samples and the reversibility of this modification. By analyzing the optical signatures of the samples using photoluminescence spectroscopy, Raman spectroscopy, and surface quality using atomic force microscopy, we demonstrated that the surface modification is stable, while heating of the modified samples restores the optical properties of the pristine MoS_2[1]. Furthermore, we were interested in the evolution of optical properties of the modified 2D MoS_2 during thermal treatment. During these experiments, the interaction of the samples with laser light at varying temperature regimes revealed unexpected changes in the material's physical and electronic properties, namely, formation of organic molecule agglomerates on 2DM[2]. Thus, we conducted a series of experiments aimed at exploring the effects of laser parameters and exposure duration on the modified MoS_2 during thermal treatment. In this presentation I will show our results from different stages of this research and the current status of our work.

1. Brkić, A. L., Supina, A., Čapeta, D., *et al*., 2025, Nanotechnology 36, 065702
2. Brkić, A. L., Supina, A., Čapeta, D., *et al*., 2025, Nano Express 6, 035013

Acknowledgment: *This work was funded by the Croatian Science Foundation under the project number HRZZ-UIP-2020-02-8891 and European Regional Development Fund (ERDF) project 'Materials for clean energy, advanced sensors and quantum technologies' (Grant No. PK.1.1.10.0002).*

SCIENTIFIC SESSION VIII

INNOVATIONS IN IMAGING AND SPECTROSCOPY

The Interaction of SARS-CoV-2 Antivirals With Lipid Systems: Structural Study

Mária Klacsová[1], Juan Carlos Martínez Guil[2], Daniela Uhríková[1]

[1]*Faculty of Pharmacy, Comenius University Bratislava, Department of Physical Chemistry of Drugs, Bratislava, Slovakia, klacsova@fpharm.uniba.sk*
[2]*ALBA Synchrotron, BL11 – NCD-SWEET, Cerdanyola del Vallés, Barcelona, Spain*

The interaction between antiviral compounds and host cell membranes is a critical factor in determining drug efficacy and understanding their molecular mechanisms of action. In the present study, we investigate the effect of four potent antiviral agents: GC376, 13b-K, Calpain inhibitor XII, and N3, on model lipid systems. These compounds are primarily recognized for their inhibition of the SARS-CoV-2 main protease (M^{pro})[1], and their amphiphilic nature suggests partitioning into lipid environments[2]. To account for the structural diversity of biological membranes, we utilized two distinct lipid systems: the lamellar-forming dipalmitoylphosphatidylcholine (DPPC), representing stable bilayer regions, and the H_{II}-prone palmitoyloleoylphosphatidylethanolamine (POPE), which mimics membranes with high intrinsic curvature and a tendency for non-lamellar structures. A small- and wide- angle synchrotron X-ray scattering (SAXS/WAXS) was employed to elucidate the effect of antivirals on lipid packing and polymorphic phase behavior as a function of temperature and drug-to-lipid ratio. Moreover, WAXS analysis revealed concentration-dependent crystallization, identifying the solubility limits of antivirals within the membrane core. Our results provide new insights into the potential of M^{pro} inhibitors to alter viral entry or budding processes, suggesting the possibility of a dual mechanism of action[3].

1. Agost-Beltrán, L., Hoz-Rodriguez, S., Bou-Iserte, L., *et al.*, 2022, Molecules 27:2523.
2. Klacsová, M., Čelková, A., Búcsi, A., *et al.*, 2022, Coll. Surf. B: Biointerfaces 220:112918.
3. Baglivo, M., Baronio, M., Natalini, G., *et al*., 2020, Acta Biomed. 91:161-164.

Acknowledgment: *This work was funded by the Ministry of Education, Research, Development and Youth of the Slovak Republic, projects VEGA 1/0305/24 and APVV-21-0108. The experiments were performed at BL11-NCD beamline (proposal 2022025753) at Alba Synchrotron with the collaboration of Alba staff.*

Stability Assessment of Engineered Flagellar Filaments for Biosensing Applications

Sara Sardou[1], Ivana Milosević [2], Éva Tóth[1], Vasa Radonić[2], Hajnalka Jankovics[1]

[1]*Research Institute of Biomolecular and Chemical Engineering, Bio-Nanosystems Laboratory/ Faculty of Engineering, University of Pannonia, Chemical Engineering and Material Sciences Doctoral School, Veszprém, Hungary, Sardou.sarah.blida@gmail.com*
[2]*BioSense Institute, University of Novi Sad, Novi Sad, Serbia*

Salmonella flagella are sophisticated macromolecular nanomachines that serve as essential locomotive and sensory organelles. Beyond their natural biological roles, their inherent structural stability and self-assembling nature make them potential candidates for advanced biosensing platforms, offering an alternative for traditional biorecognition molecules like antibodies. While proteins are attractive for high-specificity binding, incorporating them into stable layers for field sensing remains a significant challenge. This study addresses this matter by assessing the stability of genetically engineered flagellin variants within a mixed-polymer system.

The aim of this study is to evaluate the structural integrity of these engineered constructs through a comparative study against wild-type (WT) *Salmonella* flagellar filaments and antibodies. Our assessment utilizes Circular Dichroism (CD) spectroscopy to monitor conformational integrity and proteolytic assays to determine resistance to enzymatic degradation and other stress factors. By assessing the surface stability of the immobilized filaments as well, this research aims to demonstrate that engineered flagella can overcome the limitations of current protein-based sensors, providing a robust, high-specificity solution for the detection of contaminants in demanding in-field applications.

Acknowledgment: *This project was supported by 2024-1.2.6-EUREKA-2024-00001 from the National Research, Development and Innovation center.*

Aptamer-Based Imaging Approaches for Cancer Detection

Viktória Szebellaiová[1], Miroslav Gál[1], Alexandra Poturnayová[2]

[1]*Faculty of Chemical and Food Technology, Slovak University of Technology, Department of Inorganic Technology, Bratislava, Slovakia, viktoria.szebellaiova@stuba.sk*
[2]*Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia*

The high mortality rate associated with oncological diseases represents a global health challenge and highlights the need for the development of new and more sensitive diagnostic methods. One promising approach involves the use of fluorescence and confocal microscopy in combination with aptamers. Aptamers are short oligonucleotides capable of specifically binding to their target molecules and are widely used in biomarker detection and targeted delivery of therapeutic agents. In addition to optical imaging techniques, atomic force microscopy (AFM) provides detailed characterization of the morphological and mechanical properties of cells at the nanoscale level. This study focuses on the application of these imaging methods and on the optimization of sample preparation for confocal microscopy in order to visualize the interactions between selected DNA aptamers and a chosen cancer cell line. The optimization process investigated three key factors: buffer composition, incubation time, and aptamer concentration. The results indicate that a buffer enriched with $CaCl_2$ and $MgCl_2$ leads to a higher intensity of interactions between aptamers and cells. Furthermore, increased aptamer concentration and longer incubation times were found to enhance the observed interactions. The combination of confocal and atomic force microscopy provides complementary information that contributes to improved visualization and understanding of aptamer–cell interactions in cancer research.

Acknowledgment: *This research was supported by the projects grant VEGA 1/0157/24, APVV-24-0491 and EULiST 101124310.*

Generation Gap in Microbial Societies: Do Older Bacteria Communicate Differently Than Younger Ones?

Ágnes Ábrahám[1], László Dér[1], Anna Urbán[1], Péter Galajda[1], Krisztina Nagy[1,2]

[1]*Biophysics Institute, HUN-REN Biological Research Centre, Szeged, Hungary, abraham.agnes@brc.hu*

[2]*Institute of Physics, University of Szeged, Department of Experimental Physics, Szeged, Hungary*

In microbial communities cell-cell interactions are very common and can appear in several forms. Quorum sensing (QS) is a cell density-based complex communication system that relies on small diffusible molecules produced and sensed by bacteria. Once the concentration of these molecules reaches a certain threshold, cells synchronize their gene expression patterns. This process has an important role in coordinated cell behaviour, such as the production of virulence factors and biofilm formation[1].

Although QS is a mechanism that enforces homogeneous group behavior, heterogeneities on the single-cell level may appear. We used the mother machine microfluidic device to examine QS in *Pseudomonas aeruginosa*. The construction of the device made it possible to track cell lineage information through time-lapse microscopy[2].

We found that even genetically identical cells show variations in their QS states[3]. A possible source of heterogeneity could be bacterial aging. Therefore, we compared characteristic cellular parameters of the continuously aging (old-pole-inheriting) mother population to the younger cells present in the device. Our results show that cell size and division time depend on age, however changes in fluorescence intensity – the marker of the quorum response of the cells – follow similar dynamics for the older mother cells and their young relatives.

1. Miller, M. B., 2001, Quorum sensing in bacteria, Annu. Rev. Microbiol., 55:165-199.
2. Wang, P., Robert, L., Pelletier, J. *et al.*, 2010, Robust growth of *Escherichia coli*, Current Biology, 20: 1099-1103.
3. Ábrahám, Á., Dér, L., Csákvári, E. *et al.*, 2024, Single-cell level LasR-mediated quorum sensing response of *Pseudomonas aeruginosa* to pulses of signal molecules, Scientific Reports, 14:16181.

Acknowledgment: *This work was funded by the NKFI-1 ADVANCED 150958, by the National Research, Development and Innovation Office, Hungary.*

Protein Self-Assembly at the Molecular Scale: From Interactions to Phase Separation

Valeria Vetri[1]

[1]*Department of Physics and Chemistry Emilo Segrè, University of Palermo*

Protein self-assembly links molecular interactions to emergent collective behavior across length scales, from transient complexes to phase-separated states. A central challenge in biophysics is to understand how specific interaction patterns encode diverse assembly pathways.

In this context, nature provides a blueprint for multifunctional materials, where a limited set of components, such as proteins, generates complexity through hierarchical organization. Local physicochemical conditions, including hydration, polarity, and conformational fluctuations, modulate interaction networks and guide the transition from early associations to condensed protein phases.

The combination of classical spectroscopy and quantitative fluorescence microscopy offers both molecular sensitivity and spatial resolution, enabling the onset and evolution of protein-rich assemblies to be tracked in detail. Connecting microscopic interactions to emergent behavior helps reveal general physical principles of self-assembly, providing a framework to rationalize biological organization and guide the design of biomimetic materials.

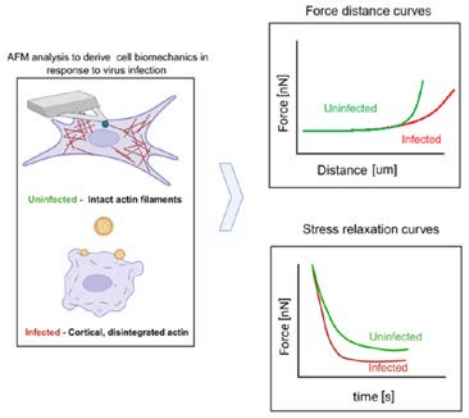
Acknowledgment: *This work received funding from MUR - PRIN PNRR - LLIPS (Liquid-Liquid Phase Separation Dynamics in Biomimetic Compartments) (P20228CCLL), MUR, PNRR-M4C2, ECS_00000022, spoke 3 – and NEST', code PE0000021, Spoke 1. Internal funding of University of Palermo is also acknowledged.*

Softening of Vero Cells Induced by Measles Vaccine Virus

Alexander Einschütz López[1], Johanna Bacher[2], Luis Ponce[1], José Toca-Herrera[1]

[1]*Institut für Biophysik, NWNR, Universität für Bodenkultur Wien, Muthgasse 11, 1190 Vienna, Austria.; alexander.el@boku.ac.at*
[2]*Institut für Bioverfahrenstechnik, DBL, Universität für Bodenkultur Wien, Muthgasse 18, 1190, Vienna, Austria*

The quantitative analysis of biophysical changes caused by viral infection is still in its early stages. In this study, we investigated the mechanical response of Vero cells following exposure to a Measles Vaccine Virus (MVV), employing atomic force microscopy (AFM) alongside confocal microscopy. We performed AFM force-distance measurements in the perinuclear region to assess the elastic and viscoelastic properties of infected cells. Within 24 hours post-infection (hpi), cells infected at a multiplicity of infection (MOI) of 0.5, exhibited a ~35% reduction in median Young's modulus compared to uninfected controls, indicating significant cellular softening. Shifts in viscoelastic parameters were also observed, including decreased relaxed modulus E0 and reduced viscosities η1 and η2, comparable to those measured in cells treated with the actin depolymerization agent cytochalasin-D. Complementarily, confocal microscopy showed a MOI-dependent cytoskeletal reorganization characterized by redistribution of F-actin and changes in filament morphology. These findings suggest that remodeling and actin organization influences the viscoelastic behavior of cells during infection. Overall, this study provides a straightforward method to characterize viral cell infection combining AFM and confocal microscopy.



Acknowledgment: *This research was funded by the COMET-Funding Program, managed by the Austrian Research Promotion Agency FFG and by the Austrian Science Fund (FWF project W1224 – Doctoral Program Biomolecular Technology of Proteins). This research was partially funded by the Austrian Science Fund (FWF): Grant-DOI 10.55776/P35777. The authors thank Lukas Schrangl for reading the manuscript, and Amsatou Andorfer Sarr and Valerie Wagner for technical assistance.*

Study of Collagen in Ligament Flavum by Polarized Second Harmonic Generation Microscopy

Damjan Stojić[1], Marko Radulović[2], Vojislav Bogosavljević[2], Mihailo Rabasović[3], Jelena Sopta[4]

[1]*Institute of Biophysics/Faculty of Medicine, University of Belgrade, Belgrade, Serbia, damjan.stojic@med.bg.ac.rs*
[2] *Clinic for Neurosurgery / University clinical center of Serbia, Belgrade, Serbia*
[2]*Clinic for Neurosurgery, University Clinical Center of Serbia, Belgrade, Serbia*
[3]*Institute of Physics Belgrade, University of Belgrade, Belgrade, Serbia*
[4]*Institute for Pathology/Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

Introduction: Ligamentum flavum (LF) is a well-defined anatomo-functional structure containing elastic and collagen fibers. Elastic fibers decrease with age and in pathological processes in the spine, and they are replaced by fibrous tissue.

Material and Methods: This study included 20 patients who underwent surgery at the Neurosurgery Clinic in 2025. year of spinal stenosis (SS) and disc herniation (LDH). LF materials obtained from patients during surgery were subjected to histopathological analyses. All tissue samples were fixed in 10% buffered formalin immediately after removal, automatically prepared and embedded in paraffin blocks for routine sectioning and staining with hematoxylin and eosin. Tissue samples were analyzed by optical microscopy. Fields of interest for analysis were identified based on histological characteristics. In marked fields, 198 images were separated for the collagen condition analysis using nonlinear laser-scanning microscopy and more precise polarized second-harmonic generation microscopy. Within each, they are divided into 6 healthy ligament flavum images and a group of 6 ligament flavum injured images. A group consists of three ROI, each containing 0° and 90° polarisation-angle images, which yield the corresponding β coefficient. With such a coefficient, the arrangement of the collagen molecules is defined. Also, for image analysis, CurveAlign 5.0 is used.

Results: We correlated collagen parameters obtained from our images with the patient's sex, age, weight and Body Mass Index (BMI), lesion localization, the presence of stenosis and disc herniation and the presence of comorbidities, cigarette smoking and exercise. A strong correlation between the β coefficients and BMI was confirmed ($p= 0.000006$). Also, there is a statistically significant correlation between the β coefficients and the patient's mass ($p= 0.0085$).

Conclusion: Since BMI is a potential risk factor for degenerative diseases of the locomotor system, our results suggest a link between the degree of collagen regulation, BMI, and degenerative changes in the spinal column.

Quantum Techniques for Biomedical Imaging

Filip Krajinić[1], Marija Ćurčić[1] and Brana Jelenković[1]

[1]*Institute of Physics University of Belgrade*

We present principles and techniques for imaging using non-classical properties of light-quantum correlation and entanglement. The use of quantum light promises to overcome some fundamental limitations of classical techniques for imaging, primarily signal-to-noise ratio at very low intensity of illuminating light, important for high contrast imaging of cells with lower threshold for photobleaching and thermal damage. The advancements and limitations of two quantum methods, quantum imaging with undetected photons and ghost imaging will be discussed, and results of these methods will be presented.

Optical Waveguides Deformed by Guided Light

Gergely T. Iványi[1,2], Cyril Slabý[3,4], Jana Kubacková[4], Alena Strejčková[5], Zuzana Jurašeková[3], Zoltán Tomori[4], Andrej Hovan[3], Lóránd Kelemen[1], Gaszton Vizsnyiczai[1], Gregor Bánó[3]

[1]*HUN-REN Biological Research Centre, Szeged, Institute of Biophysics, Szeged, Hungary, ivanyi@gergely@brc.hu*
[2]*Doctoral School of Multidisciplinary Medical Sciences, University of Szeged, Szeged, Hungary*
[3]*Department of Biophysics, Faculty of Science, P. J. Šafárik University in Košice, Košice, Slovak Republic*
[4]*Institute of Experimental Physics SAS, Department of Biophysics, Košice, Slovak Republic*
[5]*Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovak Republic*

Radiation pressure of light allows us to exert forces and trap microscopic objects with highly focused laser beams in the form of optical tweezers. In principle, light also exerts mechanical forces in curved optical waveguides as photons alter their direction and momentum. While these optical forces are typically negligible due to the rigidity of conventional materials, we demonstrate that soft photopolymer optical waveguides can undergo significant mechanical deformation driven by light momentum. Using two-photon polymerization direct laser writing, we fabricate micron-scale curved nanowire waveguides that exhibit large-scale movements when guiding light. An analytical optomechanical model that considers the mechanical properties of the photopolymer waveguide accurately predicts the observed shape deformations and their power-dependent behavior. Our findings pave the way for the development of light-driven micro-actuators, offering fresh opportunities for photonic and microelectromechanical systems.

Acknowledgment: *This work was supported by the Slovak Research and Development Agency, grants APVV-21-0333, 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. G.V. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00290/21/11). G.T.I. was supported by the National Research, Development and Innovation Office under the University Research Scholarship Program (EKÖP- 24-3-SZTE-332). This work was supported by the National Research, Development and Innovation Office, Hungary, under grant number FK 138520.*

Force and Solvent Effect on Protein Unfolding

Luis N. Ponce-Gonzalez[1], Ansgar Anders Schäfer[1], José L. Toca-Herrera[1]

[1]*Institut für Biophysik, Universität für Bodenkultur, NWNR, Vienna, Austria, luis.ponce-gonzalez@boku.ac.at*

Proteins are essential biopolymers in living organisms, responsible for structural, catalytic and regulatory functions. These activities are closely related to the protein three-dimensional structure.[1] Therefore, many bulk experiments have been focused on the disruption of the structural organization varying temperature, pH, salt, denaturant, and solvent conditions. For instance, ethanol can solubilize proteins, disturbing intramolecular H-bonding as well as hydrophobic interactions that keep the native conformation stable. In this matter, atomic force microscopy (AFM) allows to study single molecule probing under these conditions, helping elucidate the energetic landscape of different unfolding pathways.[2]

In this work, we studied the force and solvent effect on the mechanical unfolding of the titin I27 domain. For this aim, we measured force-distance and force-time curves at different pulling speeds and ethanol-PBS mixtures using AFM. The main results show that the unfolding force increases with increasing pulling rate and decreases with increasing ethanol concentration. Interestingly, the solvent effect on the unfolding time was only noticeable at low pulling speed.

1. Jones, C.M., 1997, J. Chem. Educ. 74(11):1306.
2. Gil-Redondo, J.C., Weber, A., Toca-Herrera, J.L., 2022, Microsc. Res. Tech. 85(8):3025-3036.

Acknowledgment: *This research was partly funded by the Austrian Science Fund (FWF), grant number P35777-B.*

The Role of Non-coding RNAs in Nucleolar Reorganization Under Stress

Anastasiia Gavrilova[1], Viktor Smirnov[1], Alexander Fonin[1]

[1]*Institute of Cytology, Russian Academy of Sciences, Department of biocondensates and membraneless organelles of the cell, Saint-Petersburg, Russia, asultanbekova@incras.ru*

Under heat shock or extracellular acidosis, nucleoli transform into A-bodies—amyloid-like assemblies containing non-coding RNA (rIGSRNA) and proteins in an amyloid conformation.

rIGSRNAs undergo liquid-liquid phase separation (LLPS) and sequester amyloidogenic proteins. During heat shock, A-bodies contain rIGSRNA transcribed from the region 16 kbp from the rRNA transcription start site (rIGSRNA16), while under acidosis—in the region 28 kbp (rIGSRNA28). Using bioinformatics methods and RT-PCR, increased rIGSRNA expression was observed in MCF-7 cells following heat shock and acidosis. Localization was confirmed by in situ fluorescent hybridization (FISH). Both ncRNAs were transcribed *in vitro*, isolated, and purified via exclusion chromatography. Absorption spectroscopy, circular dichroism, small-angle X-ray scattering, and atomic force microscopy revealed high conformational heterogeneity of both rIGSRNA types. Notably, rIGSRNA28 was more ordered, stable, and conformationally homogeneous compared to rIGSRNA16. The data correlated with bioinformatics analysis, indicating that rIGSRNA16 contains significantly more low-complexity regions than rIGSRNA28. These findings suggest distinct roles for rIGSRNA16 and rIGSRNA28 in the biogenesis and function of A-bodies: rIGSRNA16 mainly acts as a scaffold molecule, while rIGSRNA28 plays an active functional role.

Acknowledgment: *This work was funded by the Russian Science Foundation grant 25-75-20023 (headed by A. V. Fonin).*

Biophysical Approaches to Stress: Tracking Xenobiotic Toxicity in Fertilized Fish Eggs

Dragana Bartolić[1], Miloš Mojović[2], Mira Stanković[1], Miloš Prokopijević[1], Đura Nakarada[2], Dunja Banđur[1], Dušan Nikolić[1], Zoran Marković[3], Sandor Kasas[4], Sladjana Spasić[1], Branka Živanović[1], and Ksenija Radotić[1]

[1]*University of Belgrade - Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, Kneza Višeslava 1, Belgrade, 11030, Serbia, dragana.bartolic@imsi.bg.ac.rs*

[2]*University of Belgrade, Faculty of Physical Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia*

[3]*University of Belgrade Faculty of Agriculture: Belgrade, Serbia, RS*

[4]*Laboratory of Biological Electron Microscopy, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland*

The primary mechanism underlying environmental xenobiotic embryotoxicity centers on the uncontrolled generation of reactive oxygen species (ROS) and the subsequent induction of intracellular oxidative stress. Resolving these highly transient free radical networks in complex biological systems requires an advanced biophysical approach capable of cross-correlating spin-adduct kinetics with downstream physiological and phenotypic outcomes. Here, the multi-scale toxicological cascade induced by Bisphenol A (BPA) in fertilized egg cells is resolved by linking Electron Paramagnetic Resonance (EPR) spin-trapping, 3D EPR Imaging (EPRI), and real-time localized fluorescence spectroscopy to optical nanomotion detection (ONMD). Using cyclic Nitrone spin probes, membrane fluidity and intracellular redox status was quantified and spatially mapped under a low-power microwave regime to reconstruct radical accumulation "hot-spots" within the yolk sac and chorion membrane while maintaining embryo viability. Localized fiber-optic fluorescence spectroscopy captures real-time changes in endogenous NADH autofluorescence, tracking immediate BPA-induced intracellular energetic depletion. Quantitative modeling reveals that ONMD directly monitors these bioenergetic shifts through sub-resolution cellular oscillations, which systematically cease upon viability loss. Specifically, a second-order polynomial function effectively describes the mechanical dose-response curve of carp (*Cyprinus carpio*) eggs, identifying a critical xenobiotic concentration threshold of maximum mechanical activity that strictly precedes complete nanomechanical failure. These findings demonstrate that localized radical surges and concurrent bioenergetic collapse drive the complete failure of cellular mechanical oscillations, providing critical mechanistic insights into early embryotoxicity and a powerful biophysical framework for aquatic risk assessment.

Acknowledgment*: This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Contract grant numbers 451-03-33/2026-03/200053, 451-03-34/2026-03/200146, 451-03-33/2026-03/200146.*

SCIENTIFIC SESSION IX

NANOTECHNOLOGY AND TARGETED THERAPEUTICS

Qualitative and Quantitative Insights Into Dendritic Nanoparticle–Blood Interactions: A Comparison of Titration and Separate-aliquot Designs

Patrick Mydla[1], Simon Suty[1], Adriana Klukova[1], Daniel Maas[1], Tomas Jasenovec[2], Jana Radosinska[2], Iveta Waczulikova[1]

[1]*Faculty of Mathematics, Physics and Informatics, Comenius University Bratislava, Department of Nuclear Physics and Biophysics, Bratislava, Slovakia*
[2]*Faculty of Medicine, Comenius University Bratislava, Institute of Physiology, Bratislava, Slovakia*

Rheological assessment of nanoparticle–blood interactions is sensitive to experimental design, particularly when evaluating concentration-dependent effects. We compared two approaches for measuring how structurally distinct dendritic nanoparticles (NPs) alter whole-blood viscosity and flow behaviour across shear rates from 1 to 1 000 s^{-1}. The first approach used a titration-style design, in which incremental aliquots of NP colloidal solution were sequentially added to the same blood sample. Despite the confounding of dose, elapsed time, and repeated shear loading, this method revealed clear, NP-specific, dose-dependent changes in viscosity and shear stress, supporting its utility for qualitative or exploratory screening. The second approach used independent blood aliquots, each exposed to a single NP concentration and measured once, eliminating shear-history effects and yielding cleaner, more reproducible quantitative datasets, albeit at the cost of higher blood consumption. Rheological trends showed meaningful associations with selected haematological and coagulation parameters, indicating that viscosity-based measurements can complement routine blood analyses when evaluating NP-induced alterations. Together, these findings show that titration designs efficiently identify whether different NP types modulate whole-blood rheology, while separate-aliquot designs remain preferable for rigorous quantitative characterisation.

Acknowledgment: *This work was funded by Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic and Slovak Academy of Science under the project No. 1/0634/25; and by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00375. The authors would like to thank Dr. Zuzana Simonikova and Dr. Peter Bartek and the teams of Prof. Jean-Pierre Majoral, Prof. Maria Bryszewska and Prof. Dzmitry Shcharbin.*

Simulation and Experiments of Microelectrode Geometry Effects on Dielectrophoretic Particle Collection in Microfluidic Devices

Máté Farkas[1,2], Sándor Valkai[1], András Dér[1]

[1]*Institute of Biophysics, HUN-REN Biological Resource Centre, Szeged, Hungary*
[2]*Doctoral School of Multidisciplinary Medical Sciences, University of Szeged, Szeged, Hungary*

In a non-uniform electric field, particles show dielectrophoretic (DEP) behavior. This is a phenomenon where particles are attracted or repelled by the electric field depending on their electrical properties, size, the surrounding medium, and the frequency of the applied field. These effects are called positive or negative dielectrophoresis, corresponding to attraction toward or repulsion from the electrodes, respectively. With the right frequency, the particles can be manipulated, separated, or collected, based on their characteristics. We designed and simulated three types of electrodes: "fishbone," "snake," and "sine" structures, and integrated them into microfluidic channels. The electrodes were designed to direct the micron/submicron-sized particles by positive DEP force to the center of the channel, enabling their specific collection and concentration at the outlet. For comparison, we calculated the DEP force for each electrode design, and simulated the particle trajectories within the device. Additionally, we fabricated these devices and experimentally measured their particle collecting efficiency.

Lignin-based Nanoparticles Within Poloxamer Hydrogels for Cancer Treatment

Miljan Barić[1], Dragica Spasojević[1], Jasna Simonović Radosavljević[1], Nevena Preradović[1], Marijana Stojanović[2], Jovana Rajić[2], Martin Kreuzer[3], Tanja Dučić[3], Ksenija Radotić[1]

[1]*Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*
[2]*Institute for Biological Research "Siniša Stanković" - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*
[3]*ALBA-CELLS Synchrotron, MIRAS Beamline, Cerdanyola Del Vallès, Barcelona, Spain*

Nature-derived nanomaterials are biocompatible, biodegradable, non-toxic, and inexpensive materials with broad applicability in biomedicine[1]. Lignin is the most abundant aromatic biopolymer in the world. Due to its complex and recalcitrant structure, and low solubility in water and common organic solvents, it is often more practical to use a synthetic lignin such as DHP (dehydrogenated polymer), a lignin model compound produced through the enzymatic synthesis from coniferyl alcohol[2,3]. In this study, DHP nanoparticles were obtained via hydrothermal synthesis in the presence of ethylenediamine. The obtained nanoparticles were mixed with poloxamer 407 as a carrier and used on cell cultures of the human adenocarcinoma cell line A549. Intracellular changes in biomolecules were analyzed using Synchrotron Radiation Fourier Transform Infrared Spectroscopy (SR-FTIR). Compared to untreated cells, cells treated with DHP nanoparticles in poloxamer showed alterations in biologically relevant macromolecules, evident in characteristic spectral regions. These results provide a strong basis for further studies to confirm the anticancer effect of lignin-based nanoparticles and elucidate the underlying mechanism of action.

1. Ali D. A., Mehanna M.M. 2022, Int. J. Biol. Macromol. 221: 934-953.
2. Radotić K., Spasojević D., Zmejkoski D., 2023, Lignin-based material for biomedical applications: Basic requirements and properties, in: K. Joseph, R. Wilson, G. George, and S. Appukuttan (eds.) Lignin-based Materials: Health Care and Medical Applications, pp. 85-105, The Royal Society of Chemistry.
3. Spasojević D. Prodanović, O., Mutavdžić, D., Šekuljica, N., Jovanović, J., Maksimović, V., Radotić, K. 2023, Biotechnol. J. 18: 2300312.

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contracts 451-03-33/2026-03/200053 and 451-03-33/2026-03/200007.*

Antioxidant Activity of *Pinus nigra* and *Juniperus communis* Extracts: Toward Nanomaterial Applications and Redox Modulation

Anabela Dragićević[1,2], Đura Nakarada[1], Smilja Marković[2], Nevena Preradović[3], Nina Dominiković[4], Zoran Stanimirović[4], Miloš Mojović[1]

[1]*Center for Physical Chemistry of Biological Systems, BioScope Labs, Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia, anabela.dragicevic@itn.sanu.ac.rs*

[2]*University of Belgrade - Institute of Technical Sciences of SASA, Serbia,*

[3]*Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, University of Belgrade, Department of Life Sciences, Belgrade Serbia*

[4]*Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia*

Zinc oxide (ZnO) nanoparticles are widely applied in biomedicine and catalysis owing to their high surface-to-volume ratio and tunable physicochemical properties; however, improving their biocompatibility and antioxidant functionality remains a key challenge. Conifer-derived extracts, rich in phenolic compounds and essential oils, represent sustainable bioactive resources for the green synthesis and surface functionalization of ZnO, enabling development of biocompatible composite nanosystems. In this study, needles of *Pinus nigra* and *Juniperus communis* were lyophilized, ground, and extracted in distilled water and 50% ethanol by ultrasonic extraction. As the first phase, antioxidant activity of the extracts was evaluated by EPR spectroscopy, monitoring scavenging capacity against DPPH, $OH^{\bullet}$ and $O_2^{\bullet-}$ radicals. The results demonstrated a pronounced decrease in EPR signal intensity, with hydroethanolic *Juniperus communis* extracts showing the highest antioxidant potential. In the next phase, these extracts will be employed as stabilizing and antioxidant-enhancing agents for ZnO nanoparticles, with the aim of creating hybrid nanosystems that combine the intrinsic properties of ZnO with the bioactivity of plant-derived compounds. Such biocompatible composites are expected to find application in biomedicine, biosensor development, and in improving the redox status of pollinators.

1. Bayrami, A., Ghorbani, E., Rahim Pouran, S., Habibi-Yangjeh, A., Khataee, A., & Bayrami, M. (2019). Enriched zinc oxide nanoparticles by Nasturtium officinale leaf extract: Joint ultrasound-microwave-facilitated synthesis, characterization, and implementation for diabetes control and bacterial inhibition. *Ultrasonics sonochemistry*, *58*, 104613.

Acknowledgment: *This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers 451-03-33/2026-03/200146, 451-03-34/2026-03/200146, 451-03-34/2026-03/200143 and 451-03-33/2026-03/200175, and by the Science Fund of the Republic of Serbia, project Waste2ProtectBees No. 5455.*

Effects of Boron- and Nitrogen-Doped Carbon Dots on *Triticum aestivum* L. Growth and Oxidative status

Dunja Bandur[1], Miloš Mojović[2], Manuel Algarra[3], Đura Nakarada[2], Mira Stanković[1], Miloš Prokopijević[1], Ksenija Radotić[1], Olivera Prodanović[1], Nevena Surudžić[1], Dragana Bartolić[1]

[1]*University of Belgrade - Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, Kneza Višeslava 1, Belgrade, 11030, Serbia, dunja.bandjur@imsi.bg.ac.rs*
[2]*University of Belgrade, Faculty of Physical Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia.*
[3]*INAMAT[2] - Institute for Advanced Materials and Mathematics. Department of Science. Public University of Navarre. Campus de Arrosadia, 31006 Pamplona, Spain.*

Carbon dots (CDs) are nanomaterials with high stability and excellent biocompatibility, making them well suited for biological applications. Boron-doped and nitrogen/boron co-doped carbon dots (B-CDs and N,B-CDs) were synthesized via a hydrothermal method and characterized by fluorescence, electron paramagnetic resonance (EPR), X-Ray (XRD) and X-Ray Photoelectron (XPS) spectroscopy to assess their optical, surface, structural and radical-related properties. This study investigated the influence of B-CDs and N,B-CDs on germination and early development of wheat (*Triticum aestivum* L.). Wheat seeds were treated with CDs (1, 5, 10 mg mL^{-1}) and grown hydroponically for 7 days under a 16 h/8 h photoperiod. The biochemical (total phenolic content, TPC, and radical scavenging assay with DPPH) and morphological (length and dry-to-fresh weight ratio, DW/FW) parameters of roots and shoots were determined. Physicochemical characterization revealed that heteroatom doping strongly affects interactions between CDs and plant systems. The treatments significantly altered DW/FW in roots and shoots, reflecting changes in water status and dry matter accumulation. N,B-CDs showed higher DPPH scavenging activity than B-CDs, with maximum at 10 mg mL^{-1}, while TPC increased significantly in shoots across all treatments, compared to the control. These results highlight the potential of B-CDs and N,B-CDs as functional agents for enhancing antioxidant defense and physiological performance in crops.

Acknowledgment: *This work was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Contract grant numbers 451-03-33/2026-03/200053, 451-03-34/2026-03/200146, 451-03-33/2026-03/200146. M.A. is grateful for financial support from the Spanish Ministry of Science and Innovation (MCIU/AEI/10.13039/501100011033/FEDER, UE) through projects PID2021-122613OB-I00.*

Revealing Thermal Management in the Natural World by Holography

Katarina Nestorovic[1,2], Olga Vrbaški[3], Darko Vasiljević[3], Luka Krstic[4], Bojana Bokić[3], Branko Kolarić[3,5]

[1]*Military Technical Institute, Ratka Resanovica 1, 11030 Belgrade, Serbia, katarina.nestorovic993@gmail.com*
[2]*Military Academy, University of Defense, Veljka Lukica Kurjaka 33, 11042, Belgrade, Serbia*
[3]*Nanophotonics Laboratory, Photonics Center, Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia, olga.vrbaski@gmail.com, darko@ipb.ac.rs, bojana@ipb.ac.rs*
[4]*Faculty of Physics, University of Belgrade, Cara Dusana 13, 11000 Belgrade, Serbia, luka.krstic@ff.bg.ac.rs*
[5]*Micro and Nano Photonics Materials Group, University of Mons, Avenue Maistriau 19, B-7000 Mons, Belgium, Branko.KOLARIC@umons.ac.be*

The patterns of different scales influence and shape thermal management, making them essential for the survival of various biological species. Man-made photonic structures have prioritized long-range periodicity to control and shape their thermal and optical response. In contrast, biological systems operate under a fundamentally different design principle: short-range order nanostructures and corrugations that govern optical functionality rather than global crystallinity. The article presents a holographic study of various natural photonic systems (insects and butterflies), particularly the synergy of optical qualitative phase imaging with quantitative holotomography (HT) to reveal the thermal properties of these structures. The presented research opens up a new way to reveal the effect of complex geometry on thermal response in situ.

Single-Photon Detection of Quantum Emitters: Towards Biophysical Sensing

Katarina Žikić[1], Sergei Sobolev[1], André Stefanov[1]

[1]*Institute of Applied Physics, University of Bern, Department of Biomedical Photonics, Bern, Switzerland, katarina.zikic@unibe.ch*

Understanding biophysical processes at the single-particle level requires optical tools of exceptional sensitivity, ones capable of detecting and characterizing individual quantum emitters.

Quantum emitters, such as organic molecules, nitrogen-vacancy (NV) centers in diamond, and semiconductor quantum dots, emit light one photon at a time. This quantized emission, verified through the second-order correlation function $g^2(\tau)$, enables true single-particle sensitivity. Gold nanoparticles, while not single-photon emitters themselves, can enhance and localize fluorescent signals, further boosting detection sensitivity. Combined with time-resolved fluorescence lifetime spectroscopy, these tools reveal photophysical properties invisible to conventional microscopy.

Such techniques have direct biophysical relevance: fluorescence lifetime measurements enable single-molecule FRET, resolving conformational dynamics in proteins and nucleic acids1,2. NV centers in nanodiamonds, biocompatible and photostable, open the door to intracellular quantum sensing, including thermometry, magnetometry, and nanoscale field imaging inside living cells[3].

We present experimental characterization of quantum emitters and discuss how this quantum optical toolkit translates toward biological and medical applications.

1. Agam, N., et al., 2023, Nature Methods 20:523
2. Chung, S., et al., 2012, Proc. Natl. Acad. Sci. USA.
3. Chu, J., et al., 2021, ACS Sensors 6:2391

Acknowledgment: *This work was funded by the European Innovation Council under the Horizon Europe framework programme through a HORIZON-EIC2022-PATHFINDERCHALLENGES-01-06 project "ARTEMIS – Molecular Materials for on-chip integrated quantum light sources" – GA n. 101115149.*

A Standardized Magnetic Separation Workflow to Reduce Batch-to-Batch Variability in Functionalized Nanoparticle Production

Barnabás Tóth[1], Dr. László Forgách[1], Dr. Domokos Máthé[1]

[1]*Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary, toth.barnabas1@stud.semmelweis.hu*

Good Manufacturing Practice (GMP) provides the regulatory framework for modern pharmaceutical production. In nanoparticle synthesis, batch-to-batch variability in parameters such as hydrodynamic size, polydispersity index, and zeta potential can limit reproducibility.

For magnetic nanoparticles, these variations may alter in vivo behavior and introduce MRI signal artifacts. Standardization of manufacturing parameters and elimination of uncontrolled variables are therefore essential for robust standard operating procedures (SOPs).

Phase separation is a routine step when handling ferromagnetic nanoparticles, however, it commonly relies on centrifugation or commercial magnetic racks that may be costly, sample-limited, or poorly adaptable to nonstandard workflows. To address this, we developed a compact, low-cost magnetic separation device using open-source design tools, consumer-grade 3D printing, and neodymium magnets. The magnet arrangement generates a localized magnetic field gradient enabling rapid and stable immobilization of ferromagnetic nanoparticles from suspensions in standard 1.5 mL laboratory tubes.

The device enables reliable static phase separation without centrifugation and allows straightforward supernatant removal. Ongoing work focuses on quantitative performance analysis, including evaluation of separation time, recovery efficiency, hydrodynamic diameter, polydispersity index, and zeta potential to assess the device's impact on reproducibility.

Acknowledgment: *Part of the research leading to these results was funded by HCEMM, a Teaming grant associated with the European Molecular Biology Laboratories, from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No 739593. DM has received financial support from Semmelweis University with grants under STIA-KFI-2020 and STIA-KFI-PoC-2023. Part of the investigation was supported by the Hungarian government grant of Artificial Intelligence National Laboratory (MILAB) RRF-2.3.1-21-2022-00004 and by the grant of Future Health Industry Competence Centre of Semmelweis University 2022-1.1.1-KK-2022-00005. This research was supported by the National Research, Development, and Innovation Fund (Hungary), within the TKP2021-NVA-14 project. Part of the work was also supported in the cadre of the iMatrix consortium at H2020-JTC2022 ERA-NET-Neuron. This work was also partly funded by grants from the Hungarian National Research, Development, and Innovation Office (Thematic Excellence Program, TKP-BIOImaging, financed under the 2020-4.1.1-TKP2020 funding scheme, Investment to the Future 2020.1.16-Jövő-2021-00013 and TKP2021-EGA-23). DM and LF were supported by the Hungarian Government (National Research, Development and Innovation Office) with Research Hungary Grant No. HUN-REN RGH151414.*

Antioxidant Activity and Colloidal Stability of Chia Mucilage-Coated Cr_2O_3 Nanoparticles as Redox Modulators: An EPR and Zeta Potential Study

Sara Lukač[1], Đura Nakarada[2], Magdalena Stevanović[1], Miloš Mojović[2]

[1]*Group for Biomedical Engineering and Nanobiotechnology, Institute of Technical Sciences of SASA, Kneza Mihaila 35/IV, 11000 Belgrade, Serbia*
[2]*Center for Physical Chemistry of Biological Systems, BioScope Labs, Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11158 Belgrade, Serbia*

This study investigates the antioxidant activity and colloidal stability of chromium (III) oxide nanoparticles (Cr_2O_3 NPs) coated with chia mucilage, building upon previously established synthesis and biocompatibility findings. The nanoparticles were synthesized via chemical precipitation and thermal calcination, followed by surface functionalization to improve biological compatibility. Promising initial results required further evaluation of oxidative status and long-term stability as key parameters for potential therapeutic systems.[1] Using EPR spectroscopy, the radical-scavenging activity of uncoated and coated nanoparticles was quantified against DPPH radicals, while minimizing optical artifacts associated with strongly colored systems.[2] Both types of nanoparticles exhibited substantial antioxidant activity, with no significant difference between them. Zeta potential analysis confirmed excellent colloidal stability in aqueous media, with high negative surface charges (-29.5 mV and -31.0 mV), indicating resistance to aggregation via electrosteric stabilization. These findings suggest that chia mucilage-coated Cr_2O_3 nanoparticles form stable redox-active systems with potential to improve oxidative balance in sensitive biological and ecological environments, including pollinators. Future work will assess activity against additional reactive oxygen species and explore possible concentration-dependent pro-oxidant effects.[3]

1. Lukač, S.; Tomić, N.; Stojanović, Z.; Rajić, V.; Filipović, N.; Jović, M.; Stevanović, M. Synthesis, Characterisation, and Biological Assessment of Chromium Oxide Nanoparticles Coated with Chia Seed Mucilage Extract. Pharmaceutics 2025, 18 (1), 49. https://doi.org/10.3390/pharmaceutics18010049.
2. Yeo, J.; Shahidi, F. Critical Re-Evaluation of DPPH Assay: Presence of Pigments Affects the Results. J. Agric. Food Chem. 2019, 67 (26), 7526–7529. https://doi.org/10.1021/acs.jafc.9b02462.
3. Sotler, R. Prooxidant Activities of Antioxidants and Their Impact on Health. ACC 2019, 58. https://doi.org/10.20471/acc.2019.58.04.20.

Acknowledgment: *Funds for the realization of this work were provided by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers: 451-03-33/2026-03/200175, 451-03-33/2026-03/200146, 451-03-34/2026-03/200146, and by the Science Fund of the Republic of Serbia, project Waste2ProtectBees No. 5455.*

Hybrid Palladium-Tryptophan- Solid Lipid Nanoparticles: Synthesis, Characterization and Imaging Study

Anamarija Abu el Rub [1], Biljana Ristić [2], Sara Milošević [3], Radovan Dojčilović [1]

[1]*Center of Excellence for Photoconversion, Vinča Institute of Nuclear Sciences–National Institute of the Republic of Serbia, University of Belgrade, P.O. Box 522, Belgrade 11001, Serbia, anamarija.abuelrub@vin.bg.ac.rs*
[2]*Institute of Medical Research – National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia*
[3]*University of Belgrade – Faculty of Biology & Center for Electron Microscopy , Belgrade 11158, Serbia*

Due to a growing demand for advances in drug delivery, lipid-based nanoparticles are being extensively studied as the most promising candidates. Their attributes include high biocompatibility, the potential for various size and surface modifications, along with the capacity to carry both hydrophobic and hydrophilic drug components. We have developed hybrid lipid-metal nanoparticles (NPs) to be studied for use as antitumor agents. The hybrid NPs consist of Palladium nanoparticles functionalized with essential amino acid Tryptophan (PdTrp NPs) encapsuled into stearic acid based solid lipid nanoparticles (SLNPs). The PdTrp-SLNPs system has been synthesized via the electrospray method and characterized with various spectroscopy and microscopy techniques, including deep-UV (DUV) synchrotron microscopy. Both the photoluminescence spectroscopy and DUV microscopy studies have shown that the hybrid PdTrp-SLNPs have a higher retention of Tryptophan molecules, compared to Trp-SLNPs without the metal component. Pd-TRP SLNPs treatment of A375 cell lines have indicated a significant influence of the NPs on cell viability. Localization study of the hybrid systems in A375 cells by means of fluorescent microscopy confirmed a delivery of SLNPs inside the cells. The results indicate that the newly synthesized lipid-metal nanoparticles show a potential to be further studied for use as drug delivery systems.

1. L. Xu, X. Wang, Y. Liu, G. Yang, R.J. Falconer, C.-X. Zhao (2022) - Lipid Nanoparticles for Drug Delivery, Adv. NanoBiomed Res. 2, 2100109
2. J.-H. Lee, Y. Shin, W. Lee, K. Whang, D. Kim, L.P. Lee, J.-W. Choi, T. Kang (2016) - General and programmable synthesis of hybrid liposome/metal nanoparticles, Sci. Adv. 2 e1601838.

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project number 0302601.*

Exogenous Lung Surfactant as a Drug Delivery System: Structure and Thermodynamics

Daniela Uhríková[1], Rastislav Korfanta[1], Norbert Kučerka[1], Juan Carlos Martínez[2], Bruno Demé[3]

[1]*Faculty of Pharmacy, Comenius University Bratislava, Department of Physical Chemistry of Drugs, Bratislava, Slovakia, uhrikova@fpharm.uniba.sk*
[2]*ALBA Synchrotron, Cerdanyola del Vallés, Barcelona, Spain*
[3]*Institute Laue-Langevin, Grenoble, France*

Lung surfactant (LS) is a mixture of lipids (~90%) and ~10% of specific surfactant-associated proteins. LS lines the interior of the lung alveoli and acts to lower interfacial tension. The absence of LS due to prematurity or its damage is treated with exogenous surfactant (ELS) in neonatal medicine and also for ventilated patients with Covid-19 sickness in experimental treatment. A more recent trend involves the idea that ELS could also be used as a delivery vehicle for pulmonary therapeutics. We studied structural and thermodynamical features of the interaction of ELS and selected drugs: N-acetylcysteine with antioxidant and anti-inflammatory properties; polymyxin B peptide antibiotic[1], used to treat infections caused by resistant Gram-negative bacteria; and corticosteroid budesonide[2]. The clinically used substitute surfactant Curosurf®, a porcine lung tissue extract, and a protein-free lipid mixture served as ELS. Findings of small-angle X-ray (SAXS), neutron scattering (SANS), and membrane diffraction (SAND) techniques allowed us to unravel structural changes in ELS induced by the drugs. Thermodynamical properties were monitored by changes in the surface charge of the lipid bilayer by measuring the zeta potential. The effect of drugs on the temperature of the gel-to-fluid phase transition of ELS was followed by differential scanning calorimetry. The mutual interactions evaluated indicate the applicability of the ELS-drug connection for the concept of combined therapy.

1. Královič-Kanjaková, N., Asi Shirazi, A., Hubčík, L., *et al.*, 2024, Langmuir. 40:6847-6861.
2. Keshavarzi, A., Asi Shirazi, A., Korfanta, R., *et al.*, 2024, Int. J. Mol. Sci. 25:2990.

Acknowledgment: *This work was funded by the Ministry of Education, Research, Development and Youth of the Slovak Republic, the VEGA project 1/0305/24. SAXS experiments were performed on the BL11-NCD beamline at ALBA Synchrotron with the collaboration of ALBA staff. SAND experiments were performed on the D16 instrument, ILL Grenoble, DOI: 10.5291/ILL-DATA.8-02-1032.*

Magnetically Responsive Biomaterials: Structural Modulation in Pathological Environments

Caterina Ricci[1]

[1]*Bometra Dept., UNIMI, Milan, Italy, caterina.ricci@unimi.it*

Magnetically responsive biomaterials are attracting growing interest due to their ability to interact with external magnetic fields and modulate their structural or functional properties in a controlled manner. These systems encompass both synthetic constructs, such as engineered nanoparticles, and biologically derived architectures capable of binding or mineralizing iron. Understanding how magnetic behavior influences the interplay between composition, nanoscale organization, and environmental conditions is essential for the rational design of advanced functional materials. Structural characterization techniques play a central role in this process, enabling the investigation of diverse systems ranging from protein-based nanocages[1,2] -with intrinsic iron-handling capabilities-to magnetically active scaffolds developed for tissue reconstruction[3]. By correlating structural features with magnetic response, it becomes possible to clarify the mechanisms underlying their functionality and to guide the development of next-generation biomaterials for biomedical and technological applications.

1. Mastrangelo, Eloise, and Flavio Di Pisa. "Structural biology of ferritin nanocages." FEBS letters.Navarro-Ortega, A., Acuña, V., Bellin, A., *et al.*, 2015, Sci. Total Environ. 503-504:3-9.
2. Ricci, C., Abbandonato, G., Giannangeli, M., Matthews, L., Almásy, L., Sartori, B., ... & Moroni, A. (2024). Ferritin at different iron loading: From biological to nanotechnological applications. International Journal of Biological Macromolecules, 276, 133812.Bianchi, E., Banobre-Lopez, M., Ruggeri, M., Del Favero, E., Vigani, B., Ricci, C., ... & Sandri, G. (2025). Magnetic scaffolds for the mechanotransduction stimulation in tendon tissue regeneration. Materials Today Bio, 32, 101699.

Acknowledgment: *We thank the Horizon TEN4CARE project (101178590).*

Linear Cell System for Drug Transport Studies

Matúš Gális[1], Jana Kubacková[2], Anita Benická[3], Alena Strejčková[3], Zuzana Naďová[1], Silvia Tomková[4], Veronika Huntošová[4], Gregor Bánó[1]

[1]*Department of Biophysics, Faculty of Science, P.J. Šafárik University, Jesenná 5, 040 01 Košice, Slovakia, matus.galis@student.upjs.sk*
[2]*Dept. of Biophysics, Institute of Experimental Physics SAS, Watsonova 47, 040 01 Košice, Slovakia*
[3]*Dept. of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia*
[4]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, P.J. Šafárik University, Jesenná 5, 040 01 Košice, Slovakia*

The transport of drugs to their target destination within the human body plays a crucial role in terms of therapeutic efficacy. For this reason, the ability to efficiently and accurately quantify these processes is of utmost importance. Various 3D systems composed of multiple cells, such as cell spheroids, serve as model systems for studying drug transport in tissues. Between the system of isolated cells adhered to the bottom of a Petri dish and a 3D multicellular system, there are two intermediate stages that remain less studied. Specifically, these involve a linear 1D chain of adjacent cells and a 2D plane filled with closely packed cells.

In our study, we decided to establish 1D conditions in which intercellular drug transport would occur in only one direction, which should facilitate the quantification of this process. It is hypothesized that a reliable description of intercellular drug transport in a single linear direction can contribute to a better understanding of transport within the actual microenvironment of living tissues. To create the linear multicellular systems, we employed the two-photon polymerization technique, which enabled us to fabricate photopolymer microstructures [1] in the form of microscopic longitudinal containers. Their specific shape allows for the placement of cells using optical tweezers closely adjacent to one another exclusively in a single direction. Within these microstructures, we assembled a chain of SKBR3 breast cancer cells, of which only one contained the drug hypericin. Subsequently, we monitored the intercellular transport of hypericin using confocal microscopy.

1. Vasylyshyn, T., Huntošová, V., Patsula, V., et al., 2025 Nanoscale 17, 5838–5857

Acknowledgment: *This work was supported by the Slovak Research and Development Agency, grants APVV-21-0333, 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. Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00007.*

Antioxidant Activity of Liposome-Encapsulated *Eryngium Amethystinum* Extract: Implications for Honey Bee Studies

Mladen Duran[1], Uroš Glavinić[2], Đura Nakarada[3], Biljana Kukavica[4], Relja Suručić[5], Biljana Tubić[6], Jovana Šešić[1], Ranko Škrbić[1,7,8], Zoran Stanimirović[2], Miloš Mojović[3]

[1]*Center for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina, mladen.duran@med.unibl.org*
[2]*Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia*
[3]*University of Belgrade, Faculty of Physical Chemistry, Belgrade, Serbia*
[4]*University of Banja Luka, Faculty of Natural Sciences and Mathematics, Banja Luka, Bosnia and Herzegovina*
[5]*University of Banja Luka, Faculty of Medicine, Department of Pharmacognosy, Banja Luka, Bosnia and Herzegovina*
[6]*University of Banja Luka, Faculty of Medicine, Department of Pharmaceutical Chemistry, Banja Luka, Bosnia and Herzegovina*
[7]*Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina*
[8]*Academy of Science and Arts of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina*

Encapsulation of plant-derived bioactive compounds improves stability, protects from degradation, and enables controlled release, enhancing pharmaceutical and nutraceutical applications. Liposomes are promising carriers for extracts with high antioxidant potential. In this study, liposomal encapsulation was applied to the methanolic leaf extract of *Eryngium amethystinum* L., a Balkan–Italian species traditionally used as a diuretic, antimalarial, and wild edible. Antioxidant activity of leaf, stem, and flower extracts was evaluated after ultrasound-assisted extraction using DPPH, superoxide ($O_2^{\bullet-}$), and hydroxyl ($^{\bullet}OH$) assays. The leaf extract showed the highest activity (55.03% DPPH, 86.67% $^{\bullet}OH$, 75.26% $O_2^{\bullet-}$) and was selected for encapsulation. Liposomes were prepared by thin-film hydration with ultrasonication and confirmed by DLS. The encapsulated extract retained notable activity (53.81% DPPH, 56.54% $^{\bullet}OH$, 45.49% $O_2^{\bullet-}$), although reduced, likely due to limited immediate availability within the vesicles. Despite this, significant antioxidant capacity was preserved, indicating protective effects and controlled release. These findings support the use of liposomal systems to enhance the applicability of *E. amethystinum* bioactives. Considering the impact of oxidative stress on honeybee health and colony losses, such formulations could be explored in future apicultural studies, including the AmE-711 honey bee embryonic cell line, as a model for evaluating cytoprotective potential.

1. Duran, M., Suručić, R., Tubić, B., & Škrbić, R. (2025). Ethnopharmacology, chemical composition and pharmacological activity of amethyst sea holly (Eryngium amethystinum L). *Scripta Medica*, *56*(4), 745-756.
2. Tatipamula, V. B., & Kukavica, B. (2021). Phenolic compounds as antidiabetic, anti-inflammatory, and anticancer agents and improvement of their bioavailability by liposomes. *Cell biochemistry and function*, *39*(8), 926-944.

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development and Innovation of the Republic of Serbia, contract numbers 451-03-33/2026-03/200053, 451-03-33/2026-03/200146, 451-03-34/2026-03/200146 and 451-03-34/2026-03/200143, and by the Science Fund of the Republic of Serbia, project Waste2ProtectBees No. 5455.*

SCIENTIFIC SESSION X

SYSTEMS BIOPHYSICS

Multiscale Synchronization in Beta-Cell Networks: Network Analysis of Cellular Oscillations and Secretion Dynamics

Uroš Barać[1], Andraž Stožer[2], Marko Gosak[1,2,3,*]

[1]*Faculty of Natural Sciences and Mathematics, University of Maribor, Department of Physics, Maribor, Slovenia*
[2]*Faculty of Medicine, University of Maribor, Institute of Physiology, Maribor, Slovenia*
[3]*Alma Mater Europaea, Maribor, Slovenia*
[*] *marko.gosak@um.si*

Pancreatic β-cells within the islets of Langerhans coordinate pulsatile insulin secretion essential for metabolic homeostasis. This collective behavior emerges from complex intercellular coupling and unfolds across multiple temporal scales, including slow metabolic oscillations, fast electrical bursts, and ultrafast action-potential spikes[1,2,3]. However, the mechanisms linking these dynamics to coordinated hormone secretion remain poorly understood and the experimental data alone can provide only limited insight. To address this problem, we developed a phenomenological computational model of β-cell activity. The model integrates slow metabolic oscillations described by a Kuramoto oscillator with fast electrical bursting modeled by the Izhikevich neuron model, while intercellular communication is represented through spatial gap-junction coupling between cells. In addition, a minimal spike-driven secretion module links electrical activity to probabilistic insulin release events. Our results show that metabolic oscillations determine the period of insulin pulses, whereas the number and organization of spikes within bursts regulate the amplitude of secretion. Network analysis further reveals that spike activity is highly localized, while slower oscillatory components exhibit longer-range coordination across the islet. Reduced intercellular coupling disrupts synchrony and abolishes pulsatile secretion, reproducing features associated with diabetic dysfunction.

1. Marinelli, I., Fletcher, P.A., Sherman, A.S., Satin, L.S., Bertram, R., 2021, Front. Physiol. 12:781581.
2. Šterk, M., Barać, U., Stožer, A., Gosak, M., 2023, Phys. Rev. E 108:054409.
3. Dolenšek, J., Pohorec, V., Sklemen, M.S., Gosak, M., Stožer, A., 2025, Acta Physiol. 241:e14261.

Central Regulation of the Cough Reflex

Michal Simera, Denisa Berikova, Alexandra Lukacova, Marcel Veternik, Lukas Martvon, Jakub Misek, Ivan Poliacek

Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Institute of Medical Biophysics, Mala Hora 4, 03601 Martin, Slovakia, michal.simera@uniba.sk

Basic neural circuits controlling breathing and respiratory reflexes such as coughing are located in the brainstem extending from the pons to the lower medulla [1]. The respiratory network has rhythmogenic capability at multiple, probably hierarchically organized levels. Dynamic interactions between brainstem respiratory and non-respiratory neurones including those in the primary site of respiratory rhythm generation - the pre-Bötzinger complex (pre BötC, containing also pacemaker inspiratory neurons), the rostral ventral respiratory group, the BötC, the pontine areas, the retrotrapezoid/parafacial respiratory group, and the solitary tract nucleus (NTS), generate the central respiratory pattern, but also other related behaviors such as cough. Important features of the respiratory neuronal network are reciprocal inspiratory-expiratory inhibition and reconfiguration. Such structural diversity, together with functional complexity and adaptability enables for a large flexibility of this system to variable physiological conditions [1]. Brainstem regions are interconnected and receive the peripheral information via NTS, which is target area of inputs from peripheral receptors located within the cardiovascular and respiratory systems. These mechanisms affect an autonomic motor output, produce reflex responses, control breathing and airway resistance. The cough modulating e.g. antitussive activities are mediated in various brainstem regions, but their contribution is not uniform [2].

1. Smith J.C., Abdala A.P., Rybak I.A., Paton J.F., 2009, Structural and functional architecture of respiratory networks in the mammalian brainstem. Philos Trans R Soc Lond B Biol Sci. 364 (1529), 2577-87.
2. Ohi Y., Kato F., Haji A., 2007, Codeine presynaptically inhibits the glutamatergic synaptic transmission in the nucleus tractus solitaries of the guinea pig. Neuroscience 146, 1425–1433.

Acknowledgment: *This work was funded by Slovak Biophysical Society, this work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0052.*

MEPP as a Structural Consequence of Thermodynamic Symmetry in Enzymatic Cycles

Andrej Dobovišek[1,2], Aleš Fajmut[1,3]

[1]*Faculty of Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia, andrej.dobovisek@um.si*
[2]*Faculty of Medicine, University of Maribor, Maribor Slovenia,*
[3]*Faculty of Health Sciences, University of Maribor, Maribor, Slovenia*

We present a theoretical framework that reinterprets the Maximum Entropy Production Principle (MEPP) not as a variational assumption, but as a structural consequence of thermodynamic symmetry in an enzymatic cycle. Using a classical two-state enzyme model consistent with Briggs–Haldane kinetics, we demonstrate that MEPP naturally emerges from symmetry and topology of the system.

At the microscopic level, the model exhibits a continuous symmetry in configuration space that generates a Lie group of infinitesimal transformations leaving entropy production invariant. This symmetry structure allows a Noether-type interpretation of MEPP, linking conserved quantities with entropy production in nonequilibrium enzymatic dynamics.

The model links thermodynamics, analytical mechanics, and biochemistry, indicating a symmetry-based foundation for MEPP applicable to enzymatic, molecular, and biological transport systems operating far from equilibrium.

Acknowledgment: *This work was co-financed by the Slovenian Research and Innovation Agency (ARIS) (research core funding P1-0055 and Contract No. SN-ZRD/22-27/0552).*

Engineering a Multi-Organ Microphysiological Platform with Integrated Impedance Monitoring for Quantitative Barrier Characterization

Luíza Santa Brígida de Barros Góes[1,2,3*], Sándor Valkai[1], László Dér[1], Krisztina Nagy[1], Ágnes Ábrahám[1], Emese Bélai[4], Pirity Melinda Katalin[4], Péter Galajda[1], András Dér[1], Mária A. Deli[1], Fruzsina R. Walter[1*], Emanuel Carrilho[2,3,*]

[1]*Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary; luizagoes26@usp.br*
[2]*São Carlos Institute of Chemistry, University of São Paulo 400, Brazil;*
[3]*National Institute on Science and Technology in Bioanalytics Lauro Kubota – INCTBio-LK Campinas SP, Brazil,*
[4]*Institute of Genetics, HUN-REN Biological Research Centre, Szeged, Hungary*

Two-dimensional *in vitro* systems fail to reproduce the structural complexity, barrier functionality, and inter-organ communication observed *in vivo*. To address these limitations, we engineered a multi-organ microphysiological system (MPS) integrating embedded electrodes for real-time electrical impedance monitoring, enabling quantitative assessment of barrier physiology across three interconnected compartments under independently controlled perfusion.

The platform was fabricated from polymethyl methacrylate (PMMA) sheets assembled with biocompatible adhesive layers and a thin polydimethylsiloxane (PDMS) gasket, ensuring reversible and leak-proof operation. Each chamber contains apical and basolateral compartments separated by a porous membrane supporting endothelial or epithelial barrier formation. Independent perfusion allows both shared and compartment-specific media conditions, enabling the study of systemic interactions and inter-compartmental modulation.

Electrode placement and geometry were optimized to ensure stable trans-endothelial electrical resistance (TEER) measurements under dynamic flow. This configuration permits longitudinal, non-invasive monitoring of barrier development, maturation, and functional coupling between organ compartments. Barrier performance was further validated through permeability assays and immunofluorescence, allowing direct correlation of electrical readouts with structural integrity.

Acknowledgment: *The project was supported by the Hungarian National Research Development and Innovation Office of Hungary (K143766, 2024-1.2.2-ERA_NET-2024-00018 and ADV153360 for F.R.W.). F.R.W. is also funded by the Lendület "Momentum" Research Grant (LP2025-22/2025). Also supported by the National Council for Scientific and Technological Development (CNPq), process no. 200562/2025-8.*

SCIENTIFIC SESSION XI

CELLULAR MECHANICS AND BIOPHYSICAL REGULATION

Why Extensile and Contractile Tissues Could be Hard to Tell Apart

Jan Rozman[1,2], Sumesh P. Thampi[3], Julia M. Yeomans[4]

[1]*Jožef Stefan Institute, Ljubljana, Slovenia, jan.rozman@ijs.si*
[2]*Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Slovenia*
[3]*Department of Chemical Engineering, Indian Institute of Technology, Madras, Chennai, India 60003610*
[4]*Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford, United Kingdom*

Active nematic models can explain the topological defects and flow patterns observed in epithelial tissues, but the nature of active stress—whether it is extensile or contractile, a key parameter of the theory—is not well established experimentally. Individual cells are contractile, yet tissue-level behaviour often resembles extensile nematics. To address this discrepancy, we use a continuum theory with two tensor order parameters that distinguishes cell shape from active stress. We show that correlating cell shape and flow, whether in coherent flows in channels, near topological defects, or at rigid boundaries, cannot unambiguously determine the type of active stress [1]. Our results demonstrate that simultaneous measurements of stress and cell shape are essential to fully interpret experiments investigating the nature of the physical forces acting within epithelial cell layers.

1. Rozman, J., Thampi, S. P., and Yeomans, J. M, 2025, arXiv:2511.07012.

Acknowledgment: *JR and JMY acknowledge support from the UK Engineering and Physical Sciences Research Council (Award EP/W023849/1) and ERC Advanced Grant ActBio (funded as UKRI Frontier Research Grant EP/Y033981/1). JR acknowledges financial support from the Slovenian Research and Innovation Agency (research project J1-70062, development funding pillar RSF-0106, and research core funding P1-0055). SPT thanks the Royal Society and the Wolfson Foundation for the Royal Society Wolfson Fellowship award and acknowledges the support of the Department of Science and Technology, India via the research grant CRG/2023/000169.*

Surface Tension-Driven Elasticity of Circular Epithelia

Urška Andrenšek[1,2], Matej Krajnc[2]

[1]*Faculty of mathematics and physics, University of Ljubljana, Slovenia, urska.andrensek@ijs.si*
[2]*Jožef Stefan Institute, Department of theoretical physics, Ljubljana, Slovenia*

During the life of animals, epithelial tissues undergo extensive deformations--first to form organs during embryogenesis and later to preserve integrity and function in adulthood. To what extent these deformations resemble that of non-living elastic materials is not well understood. We derive an elasticity theory of a circular epithelia, in which the mechanics of individual cells are dominated by differential interfacial tensions stemming from cell cortical tension and adhesion. The analytical derivations in the limit of small deformation are supported by numerical results of 2-dimensional vertex model. We observe a critical value of differential surface tension at which the tissue spontaneously wrinkles and analytically derive the dominant wavelength of deformation. We derive the critical torque to induce the wrinkling instability and its dominant deformation mode, where the apico-basal symmetry is broken by both the differential surface tension and the radius of the circular tissue. Our findings demonstrate how the interplay between cellular properties, such as surface tension, and the tissue-scale constraints, such as tissue curvature, give rise to emergent, tissue-scale instabilities.

Acknowledgment: *This work was funded by the Slovenian Research and Innovation Agency (ARIS).*

Tissue Fluidization Driven by Basal Self-propulsion in Three-dimensional Epithelial Monolayers

Toshikaze Chiba[1], Matej Krajnc[1]

[1]*Jožef Stefan Institute, Ljubljana, Slovenia, Toshikaze.Chiba@ijs.si*

Tissue fluidization in epithelial systems is governed by cell rearrangements that enable collective motion. While two-dimensional models have established that rigidity, controlled by the target shape index, regulates neighbor exchange, how this mechanism extends to three-dimensional (3D) epithelia remains unclear. In 3D monolayers, cells exhibit apico–basal asymmetry, and an additional geometric degree of freedom emerges through scutoid formation, allowing distinct neighbor relations between apical and basal surfaces. Here, we investigate how scutoids influence cell motility and diffusion using a self-propelled 3D vertex model with volume and area elasticity.

We show that the system undergoes a rigidity transition governed by the preferred cell area (S_0) and aspect ratio (ζ), which control packing constraints. In the rigid regime, neighbor exchanges are suppressed, whereas in the floppy regime, rearrangements are enhanced. By comparing scutoid-enabled dynamics with conventional CompleteT1 transitions, we find that scutoids systematically increase cell motility. This is demonstrated by a cage-relative displacement order parameter, which robustly distinguishes caged from motile states. Phase diagram analysis reveals that scutoid formation shifts the motile–caged boundary, particularly along the S_0 axis, indicating a geometric control of fluidization. Furthermore, analysis of apical and basal shape indices shows that deformation accumulates on the basal side in CompleteT1, whereas scutoids enable its relaxation through cell exchange. These results suggest that scutoid formation provides an additional pathway for rearrangement, thereby promoting diffusion and fluidization in 3D epithelial monolayers.

Acknowledgment: *This work was funded by the Slovenian Research and Innovation Agency (research project J1-60013, development funding pillar RSF0106, and research core funding P1-0055).*

Tissue Mechanics: From Epithelia to Tumors

Urška Andrenšek[1,2], Urban Železnik[1,2], Tanmoy Sarkar[1,3], and Matej Krajnc[1]

[1]*Jozef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia*
[2]*Faculty of Mathematics and Physics, University of Ljubljana, Jadranska 19, SI-1000 Ljubljana, Slovenia* [3]*School of Physics, Indian Institute of Science Education and Research Thiruvananthapuram, Maruthamala PO, Thiruvananthapuram, Kerala 695551, India*
matej.krajnc@ijs.si

Animal tissues acquire their shape through morphogenesis, a process in which cells organize, change shape, migrate, and interact to build a 3D architecture. To investigate the complex, multiscale mechanics underlying this process, we employ vertex models that represent individual cells as polyhedra defined by the positions of their vertices. Tissue dynamics are simulated by integrating equations of motion for these vertices, while the network of cell–cell neighbors is continuously updated to capture local topological rearrangements such as cell intercalations and divisions. In this talk, I will present three aspects of tissue mechanics. First, I will coarse-grain a 1D lateral vertex model to derive an epithelial elasticity theory grounded in discrete cell-scale mechanics, and compare the resulting behavior with that of non-living elastic plates [1]. Second, I will use a 2.5D vertex model of planar epithelia to investigate a rigidity transition, in which tissues shift from rigid to floppy states characterized by vanishing shear and bulk moduli [2]. Third, I will introduce a minimal 3D vertex model that captures how tumors develop lobulated shapes during growth [3]. Overall, this talk will highlight three key aspects of tissue mechanics—elasticity, rheology, and three-dimensional shape—demonstrating the power and versatility of vertex models across different geometrical settings as a leading computational framework in theoretical tissue mechanics.

1. U. Andrenšek, P. Ziherl, and M. Krajnc, *Wrinkling instability in unsupported epithelial sheets*, *Phys. Rev. Lett.* 130, 198401 (2023).
2. T. Sarkar and M. Krajnc, Reentrant *Rigidity Transition in Planar Epithelia with Volume- and Area Elasticity*, *Phys. Rev. Lett.* 136, 068404 (2026).
3. U. Železnik, M. Krajnc, and T. Sarkar, *Tissue-Intrinsic Shape Mechanics in Growing Pre-Migratory Tumor Spheroids*. arXiv: https://arxiv.org/abs/2408.07551

Acknowledgment: *This work was funded by the Slovenian Research and Innovation Agency (research projects J1-3009 and J1-60013, development funding pillar RSF0106, and research core funding P1-0055).*

Mechanistic Insights Into β-Casein–Curcumin Interactions: A Concentration- Based Approach

Antonella Macías[1], Zuzana Garaiová[1], Tibor Hianik[1]

[1]*Faculty of Mathematics, Physics and Informatics, Comenius University, Department of Nuclear Physics and Biophysics, Bratislava 84248, Slovakia, caza1@uniba.sk*

Cancer remains a global leading cause of death. Many bioactive compounds—including curcumin, a polyphenol with anticancer activity—are limited by poor aqueous solubility and low bioavailability[1]. In this work, β-casein—an amphiphilic milk protein[2]—was investigated as a nanocarrier for curcumin (β-CN@curcumin). Dynamic light scattering (DLS) revealed β-casein micellization at ~50 μM, forming stable assemblies of 20–30 nm. Curcumin alone aggregated in Na-phosphate buffer (10mM, pH 7), forming clusters of 200–300 nm above ~37 μM. Interaction studies via DLS, UV–VIS, and fluorescence spectroscopy—using a bidirectional concentration approach—showed that β-casein encapsulates curcumin within micellar regime (≥50 μM), preventing its intrinsic aggregation in aqueous buffer. Cluster formation appeared as deviations from Beer–Lambert behavior at ~83 μM (absorbance) and ~50 μM (fluorescence) of curcumin and corresponded to a transition from micellar dimensions to larger structures. These findings define the β-casein micellar loading limit for curcumin as ~50–83 μM, which overlaps with reported therapeutically relevant plasma concentrations[1]. Stern–Volmer analysis yielded a quenching constant of ~1.0×10^5 M^{-1}, indicating strong molecular association between curcumin and β-casein within the micellar regime. These findings provide mechanistic insight into β-casein-mediated encapsulation and support its potential as a biocompatible nanocarrier for hydrophobic anticancer compounds.

1. Salem, M.; Rohani, S.; Gillies, E. R. Curcumin, a promising anti-cancer therapeutic: a review of its chemical properties, bioactivity and approaches to cancer cell delivery. RSC Advances 2014, 4, 10815–10829.
2. Chen, X.; Fan, R.; Wang, Y.; Munir, M.; Li, C.; Wang, C.; Hou, Z.; Zhang, G.; Liu, L.; He, J. Bovine milk β-casein: Structure, properties, isolation, and targeted application of isolated products. Comprehensive Reviews in Food Science and Food Safety 2024, 23, e13311.

Acknowledgment: *This work was funded by Science Agency VEGA, project No. 1/0554/23.*

Environmental Stress-Induced Changes in Microalgal Properties and Behavior

N. Ivošević DeNardis[1], N. Novosel Vlašić[1], T. Mišić Radić[1], M. Levak Zorinc[1], J. Zemła[2], M. Lekka[2], I. Vrana[1], B. Gašparović[1], D. Kasum[1], T. Legović[3,4], P. Žutinić[5], M. Gligora Udovič[5]

[1]*Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia, e-mail: ivosevic@irb.hr*
[2]*Institute of Nuclear Physics Polish Academy of Sciences, Kraków, Poland,*
[3]*Libertas International University, Zagreb, Croatia,*
[4]*OIKON-Institute for Applied Ecology, Zagreb, Croatia,*
[5]*Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia*

Global climate change and human activities, such as heavy metal and plastic pollution, seriously threaten aquatic ecosystems. Despite their small biomass, marine microalgae are essential primary producers and form the base of the food web in aquatic systems. Many studies have verified that environmental stressors affect community algal composition and abundance, but responses at the single-cell level remain insufficiently explored. This research links the cell surface properties and behavior with abiotic and anthropogenic stressors to better understand survival and acclimation strategies.

The study investigated three microalgal species with unique cellular barrier structures using a comprehensive biophysical approach under controlled laboratory conditions. The results demonstrate that cell responses are both species-specific and stressor-specific. Under three selected conditions, toxic cadmium concentrations, decreased temperature, and decreased salinity, flagellate microalgal species showed decreased motility and increased cell stiffness, accompanied by alterations in surface hydrophobicity, which often indicated a shift toward a dormant state despite increased physiological activity. In contrast, diatoms with rigid cell walls showed greater tolerance than green algae [1-3]. The observed changes in microalgal surface properties and behavior, compared to the control group, confirm the usefulness of these characteristics as sensitive indicators of cellular stress in aquatic systems.

1. Novosel, N., et al. 2022, J. Appl. Phycol. 34, 1293–1309.
2. Novosel, N., et al. 2022, J. Appl. Phycol. 34, 243–259.
3. Novosel, N., et al. 2020, J. Appl. Phycol. 32, 4057-4067.

Acknowledgment: *Funded by the Croatian Science Foundation ("From algal cell surface properties to stress markers for aquatic ecosystems") and the European Union – NextGenerationEU.*

FTIR Spectroscopy – Derived Insights into Cell Wall Remodeling in Twining Stems of *Dioscorea balcanica*

Jasna Simonović Radosavljević[1], Ksenija Radotić[1], Jelena Bogdanović Pristov[1], Gregory Mouille[2], Aleksandra Mitrović[1]

[1]*University of Belgrade-Institute for Multidisciplinary Research, National Institute of the Republic of Serbia, Belgrade, Serbia, jasna@imsi.bg.ac.rs*
[2]*INRA, Institut Jean-Pierre Bourgin, UMR1318 INRAAgroParisTech, ERL3559 CNRS, Saclay Plant Sciences, 78026 Versailles, France*

Structural adaptations of plant cell walls are essential for accommodating mechanical strain in twining stems of liana species. In this study, Fourier transform infrared (FTIR) microspectrometry was used to investigate compositional and structural changes in different cell wall types (parenchyma cells and xylem vessels) in straight and twisted internodes of *Dioscorea balcanica*.

FTIR analysis of parenchyma cell walls showed a decrease in cellulose and xyloglucan content, together with an increase in xylan and lignin with modified organization in twisted internodes, reflecting changes in cell wall mechanical properties, including increased flexibility and elasticity. In contrast, xylem vessel cell walls exhibited a different trend, characterized by reduced lignin, cellulose, and xylan content, and increased xyloglucan levels. These changes suggest decreased rigidity and increased extensibility, enabling vessels to withstand mechanical stress without collapse.

Spectral differences in the fingerprint region (800-1800 cm^{-1}) confirmed that distinct cell wall components contribute differently to the mechanical adaptation of tissues to stem twining. The results demonstrate that parenchyma cells and xylem vessels exhibit complementary structural responses: strengthening and elastic adjustment in parenchyma, and enhanced flexibility in vessels, indicating tissue-specific mechanisms of biomechanical adaptation.

Acknowledgment: *This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No. 451-03-33/2026-03/200053*

Cryoprotectant Concentration Reveals Hidden Functional Stress in Cryopreserved Human Keratinocytes

Katarína Čechová[1], Patrick Mydla[1], Iveta Štibrániová[2], Paulína Gálfiová[3], Iveta Waczulíková[1], Libuša Šikurová[1]

[1]Faculty of Mathematics, Physics and Informatics, Comenius University Bratislava, Department of Nuclear Physics and Biophysics, Bratislava, Slovakia
[2]Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia
[3]Department of Histology and Embryology, Faculty of Medicine, Comenius University Bratislava, Bratislava, Slovakia

Cryopreservation is widely used for long-term storage of biological material, yet freezing–thawing exposes cells to osmotic imbalance, membrane phase transitions, and cryoprotectant-induced toxicity. Despite the widespread use of dimethyl sulfoxide (DMSO), the relationship between cryoprotectant concentration and post-thaw cellular functionality remains insufficiently understood.

Here we investigated concentration-dependent biophysical responses of human keratinocytes cryopreserved with DMSO. Cells were stored at −80 °C using four concentrations (1.8%, 2.2%, 5%, and 10%) and analyzed after thawing using complementary functional and ultrastructural approaches assessing membrane fluidity, transmembrane potential, viability, proliferation, and cellular morphology.

Low DMSO concentrations (1.8–2.2%) provided insufficient cryoprotection and resulted in apoptotic-like morphology and reduced proliferative capacity. In contrast, 10% DMSO yielded the highest immediate viability (97.9%) but induced mitochondrial swelling, membrane depolarization, and nuclear lobulation, indicating early cellular stress. Cells preserved with 5% DMSO maintained relatively intact ultrastructure while exhibiting persistent membrane hyperpolarization. A strong negative correlation between DMSO concentration and membrane fluidity ($r = -0.84$) revealed nonlinear membrane responses to cryoprotectant exposure.

These findings show that morphological preservation after cryopreservation can mask substantial functional cellular stress and identify membrane dynamics as a critical determinant of post-thaw cellular stability.

Acknowledgment: *This work was funded by the Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic and the Slovak Academy of Sciences under project No. 1/0634/25*

Comparative Evaluation of Different Neural Network Architectures in Rectal Tumor Diagnosis on CT Images

Aleksandra Bibić[1], Zorica Nestorović[1], Edib Dobardžić[2]

[1]*Faculty of Medicine, University of Belgrade, Department of Biophysics, Belgrade, Serbia, aleksandra.bibic@med.bg.ac.rs*
[2]*Faculty of Physics, University of Belgrade, Belgrade, Serbia*

Background: The aim of this study was to investigate the application of neural networks for detecting rectal tumor regions in patients using computed tomography (CT) images following neoadjuvant chemoradiotherapy.
Materials and methods: The study included 138 patients, while two CT scans with a healthy rectum were included to balance the dataset, resulting in a total of 3566 CT images. We extracted CT slices showing the rectum and analyzed pixel values along the contours and within the segmented rectal region. We determined tissue mass density using calibration curves and applied statistical distribution functions to account for variations in rectal volume. We selected the most informative features using feature selection and dimensionality reduction methods, namely Mutual Information (MI) and Principal Component Analysis (PCA) and we used them for training and testing different neural network architectures. We trained neural network architectures such as n–2n–n–1 and n–2n–4n–2n–n–1, where n is the number of input features (ranging from 3 to 7), using batch sizes of 1, 11, 107, and 1069.
Results: We observed that the highest accuracy (~79%) was achieved by a network with a 4–8–4–1 architecture, batch size 1069, and 4 PCA-selected features. The highest sensitivity (~85%) was obtained with 6–12–24–12–6–1 and 4–16–8–4–1 networks, using 6 PCA-selected and 4 MI-selected features, respectively. We recorded the highest specificity (~80%) for the 4–8–4–1 network (batch size 1069, 4 PCA-selected features).
Conclusion: These results demonstrate that neural networks can accurately differentiate tumor from healthy rectal regions, achieving high sensitivity and specificity.

AUTHOR INDEX

Authors listed alphabetically by last name. Page numbers indicate where their abstract(s) appear in this volume.