10th Regional Biophysics Conference

&

15th International Summer School of Biophysics

August 26-30, 2024

Split, Croatia

BOOK OF ABSTRACTS

http://rbc2024.biofizika.hr

Editors:

Sanja Dolanski Babić
Maja Dutour Sikirić
Višnja Stepanić
Marija Raguž
Nadica Maltar Strmečki
Nadica Ivošević DeNardis

ISBN 978-953-7941-53-6 (online)

http://rbc2024.biofizika.hr

10th Regional Biophysics Conference

&

15th International Summer School of Biophysics

August 26-30, 2024

University of Split, School of Medicine
Split, Croatia

Scientific Committee

Sanja Dolanski Babić (Croatia)
Maja Dutour Sikirić (Croatia)
Višnja Stepanić (Croatia)
Miloš Mojović (Serbia)
Jure Derganc (Slovenia)
Beata Bugyi (Hungary)
Magdalena Majekova (Slovakia)
Rainer Schindl (Austria)

Organizing Committee

Marija Raguž (Croatia), Chair of 10th Regional Biophysics Conference
Nadica Maltar Strmečki (Croatia), Chair of 15th International Summer School of Biophysics
Nadica Ivošević DeNardis (Croatia)
Lara Jurković (Croatia), webmaster

10th Regional Biophysics Conference

&

15th International Summer School of Biophysics

Organized by

Sponsors

Dear participants!

We are pleased to welcome you to the 10th Regional Biophysics Conference and the 15th International Summer School of Biophysics at the University of Split School of Medicine, Split, Croatia.

We have put together a stimulating scientific program consisting of plenary lectures, invited talks, oral and poster presentations that we believe will inspire all participants. The program offers an excellent opportunity for learning and is designed to encourage interaction and discussion in the rapidly expanding field of biosciences and provide new knowledge gained about various biophysical phenomena.

The purpose of this joint event is to share the latest technological developments in various biophysical and related fields of research; stimulate innovative research; educate early career researchers, postdoctoral fellows, and graduate students, as well as facilitate future scientific collaborations among participants.

This event also intends to provide a forum for the dissemination of the state-of-the-art science and places great emphasis on building and strengthening research collaborations and working with instrument manufacturers to present new methodological and technological discoveries to a large community of scientists and potential customers.

The event is organized by Croatian Biophysical Society, University of Split School of Medicine, Split and Ruđer Bošković Institute, Zagreb.

Thank you for attending our 10th Regional Biophysics Conference and 15th International Summer School of Biophysics, and sharing your expertise!

We encourage you to learn, network, and enjoy all that Split has to offer - from the beautiful landscape, unique history and architecture to the famous Mediterranean cuisine and friendly people.

Sincerely,

Marija Raguž, Chair of 10th Regional Biophysics Conference

Nadica Maltar Strmečki, Chair of 15th International Summer School of Biophysics

Program

Monday, August 26, 20024

16.00 – 18.00 Registration

17.30 – 18.00 Opening

18.00 – 18.45 PL1 **Antonio Šiber** (Institute of Physics, Zagreb, HR)
Elastic design and large deformations of pollen grains

18.45 – 21.00 Welcome Reception

Tuesday, August 27, 2024

Session 1 ***Membranes and membrane proteins***
Chairs: Miklós Kellermayer, Kata Sára Haba

9.00 – 9.45 PL2 **Elena Pohl** (University of Veterinary Medicine Vienna, AT)
Uncoupling in mitochondria: the role of SLC25 superfamily proteins

9.45 – 10.10 IL1 **Danijela Bakarić** (Ruđer Bošković Institute, Zagreb, HR)
The revival of UV-Vis spectroscopy in lipid membrane research

10.10 -10.35 IL2 **Rainer Schindl** (Medical University of Graz, Graz, AT)
Structural insights into the mechanism of store-operated calcium channels

10.35 – 11.00 *Coffee Break*

11.00 – 11.25 IL3 **Uroš Tkalec** (University of Ljubljana, Ljubljana, SI)
Transport phenomena at liquid-crystal-water interfaces

11.25 – 11.50 IL4 **Stefania Abbruzzetti** (Università di Parma, Parma, IT)
Targeting microorganisms with PDI

11.50 – 12.05 SO1 **Cristiano Viapiani** (Università di Parma, Parma, IT)
Molecular basis for the nitric oxide sensitivity of a blue fluorescent protein

12.05 – 12.20 SO2 **Karol Ciepluch** (Jan Kochanowski University in Kielce, Kielce, PL)
Molecular insight into bacterial membrane disruption by dendritic nanoparticles - a pathway for antibacterial proteins

12.35 – 12.50 SO4 **Jordi Faraudo** (ICMAB-CSIC, Barcelona, ES)
Understanding the molecular origin of the effect of surfactants on enveloped viruses: the case of SARS-CoV-2

12.50 – 13.05 SO5 **Magdalena Majekova** (Centre of Experimental Medicine v.v.i. SAS, Bratislava, SK)
Modulation places for ATP binding – possible targets for allosteric activation

13.05 – 14.00 *Lunch*

Session 2 ***Biomechanics and mechanobiology***
Chairs: Nadica Ivošević DeNardis, Milan Žižić

14.10 – 14.55 PL3 **Miklós Kellermayer** (Semmelweis University, Budapest, HU)
Exploring viruses by single-molecule biophysics

14.55 – 15.20 IL5 **Antonella Battisti** (CNR and Scuola Normale Superiore, Pisa, IT)
Twisting and Turning: Fluorescent Molecular Rotors as Viscosity Sensors

15.20 – 15.45 IL6 **Nenad Pavin** (University of Zagreb, Faculty of Science, Zagreb, HR)
Mechanobiology of the Mitotic Spindle

15.45 – 16.15 *Coffee Break*

16.15 – 18.15 ***Poster Session A (P1 – P27)***

Wednesday, August 28, 2024

Session 3 ***Bio(inspired) materials & Computational approaches and machine learning***
Chairs: Rainer Schindl, Antonija Tomić

9.00 – 9.45 PL4 **Ranieri Bizzarri** (University of Pisa, Pisa, IT)
The different perspective of the Brownian scale and how biophysics targets it

9.45 – 10.10 IL7 **Tamás Fekete** (HUN-REN, Institute of Biophysics, Szeged, HU)
Microfabricated structures to solve biological problems

10.10 -10.35 IL8 **Andrej Korenić** (University of Belgrade, Belgrade, RS)
Integrating Machine Learning in Photonics: Advancements in Signal Analysis and Optical Data Interpretation

10.35 – 11.00 *Coffee Break*

11.00 – 11.15 SO6 **Nadica Ivošević DeNardis** (Ruđer Bošković Institute, Zagreb, HR)
Biocompatible reconstructed marine based vesicles as an alternative drug

delivery system

11.15 – 11.30 SO7 **Tea Mišić Radić** (Ruđer Bošković Institute, Zagreb,HR)
Nanoplastics and microalgae: nanostructural, nanomechanical, and antioxidant response of marine diatom Cylindrotheca closterium

11.30 – 11.45 SO8 **Andrej Dobovišek** (University of Maribor, Maribor, SI)
Enzymatic organization studied by the Maximum Entropy Production Principle

11.45 – 12.00 SO9 **Gabriel Žoldák** (CIB, TIP-UPJS, Košice, SK)
Stable substructures in proteins and the principle of minimum frustrations

12.00 – 12.15 SO10 **Jan Zmazek** (University of Maribor, Maribor, SI)
Machine Learning-Assisted Optimization of Guideline-Directed Therapy for Heart Failure

12.15 – 12.30 SO11 **Marko Gosak** (University of Maribor, Maribor, SI)
Multicellular Dynamics in Beta Cell Networks: Insights from Multicellular Imaging, Phenomenological Models, and Network Analyses

12.30 – 13.00 **Short talks - Bursaries**
Chair: Sanja Dolanski Babić
Kata Sara Haba, Milan Žižić, Nevena Preradović, Katarina Meštrović, Anais Biquet Bisquert, Viktoria Fedorova

13.00 – 14.00 *Group Photo & Lunch*

14.10 – 14.55 *RBC Advisory Board Meeting*

15.00 – 22.00 *Excursion and conference dinner*

Thursday, August 29, 2024

Session 4 ***Molecular biophysics 1***
Chairs: Srdjan D. Antić, Nevena Preradović

9.00 – 9.45 PL5 **Andrej Vilfan** (Jožef Stefan Institute, Ljubljana, SI)
Physics of Biological and Artificial Cilia

9.45 – 10.10 IL9 **Gregor Posnjak** (Ludwig-Maximilian-University Munich, Munich, DE)
DNA origami-based crystals for photonics

10.10 -10.35 IL10 **Erik Sedlák** (Pavol Jozef Šafárik University, Košice, SK)

Design of alternative genetically encoded photosensitizers

10.35 – 11.00 *Coffee Break*

11.00 – 11.25 IL11 **Katalin Goda** (University of Debrecen, Debrecen, HU)
Crosstalk between nucleotide and substrate binding in ABCB1 and ABCG2

11.25 – 11.50 IL12 **Zuzana Bednarikova** (Slovak Academy of Sciences, Košice, SK)
A lever-like anti-Aβ fibrils potential of azobenzene molecules

11.50 – 12.05 SO12 **Andrea Antošova** (Slovak Academy of Sciences, Košice, SK)
α-lactalbumin amyloid fibrillization dependence on Hofmeister anions in acidic pH

12.05 – 12.20 SO13 **Kristina Serec** (University of Zagreb, School of Medicine, Zagreb, HR)
Let's cut things short: A comparative study of short- and long-chain DNA in the presence of manganese cations

12.20 – 12.35 SO14 **Aleš Fajmut** (University of Maribor, Maribor, SI)
Modelling the role of cyclic guanosine monophosphate (cGMP) in modulating the contractility of vascular smooth muscles

12.35 – 12.50 SO15 **Veronika Bukina** (Jožef Stefan Institute, Ljubljana, SI)
Structural motif stability of bacteriophage MS2 RNA pack-aging signals upon changes in their flanking sequence

13.00 – 14.00 *Lunch*

Session 5 ***Neurobiophysics***
Chairs: Andrej Vilfan, Katarina Meštrović

14.10 – 14.55 PL6 **Srdjan D. Antić** (University of Connecticut School of Medicine, CT, USA)
Glutamate-Mediated Dendritic UP States in Cortical Pyramidal Neurons – Voltage Imaging and Biophysical Model

14.55 – 15.20 IL13 **Tony Schmidt** (Medical University of Graz, Graz, AT)
Photoactive Organic Semiconductors for Neurostimulation: In Vivo Application and Mechanistic Insights

15.20 – 15.45 IL14 **Marko Daković** (University of Belgrade, Belgrade, RS)
Diffusion tensor imaging in investigation of changes in brain infrastructure in neurological diseases

15.45 – 16.00 SO16 **Tomislav Stankovski** (Cyril and Methodious University in Skopje, Skopje, MK)

Neural Cross-Frequency Coupling Functions: An Application to Sleep

16.00 – 16.30 *Coffee Break*

16.30 – 18.30 ***Poster Session B (P28 – P52)***

Friday, August 30, 2024

Session 6 ***Molecular biophysics 2***
Chairs: ***Magdalena Majekova,*** **Anais Biquet Bisquert**

9.00 – 9.45 PL7 **Alexandra Zahradníková** (Slovak Academy of Sciences, Bratislava, SK)
Emerging ryanodine receptor function under the light of its molecular structure

9.45 – 10.10 IL15 **Chris Oostenbrink** (BOKU University, Vienna, AT)
Free energies from molecular dynamics simulations: acceleration of sampling in conformational and chemical space

10.10 – 10.40 *Coffee Break*

10.40 – 11.00 *Awards*

11.00 – 11.20 **Closing 10th RBC2024 & 15th International Summer School of Biophysics**

Abstracts

Plenary lectures (PL)

PL1 *Elastic design and large deformations of pollen grains*

Antonio Šiber

Institute of Physics, Bijenička cesta 46, Zagreb, Croatia
e-mail: asiber@ifs.hr

Biological shells often need to function on a border of stability, i.e. need to be sufficiently strong to protect their interior, yet sufficiently labile to activate and release the interior once they reach a suitable environment. For example, in case of viruses this happens once they enter the cell, and in the case of pollen grains, once they land on a stigma of a flowering plant. In order to function properly these shells must conform to specific mechanical requirements. These include the resistance of shells to the pressure from the inside, as is the case e.g. in the case of bacteriophage which densely pack their DNA pushing against the virus protein coating, but also to the pressure from the outside, as is the case in pollen grains which crumple and deform upon desiccation. Pollen grains contain male plant genetic material encapsulated in a hard protective shell. In addition to hard parts, shell also consists of flexible, soft regions—apertures. Thanks to the existence of this elastic inhomogeneity and the specific shape and distribution of the apertures, many pollen grains can fold in (infold) upon dehydration, protecting thus the sensitive interior (see Fig. 1). This mechanical design, however, may also lead to bursting of the apertures and the leakage of the pollen proteins in the atmosphere. Infolding pathways of pollen grains are investigated by studying elastic deformations of inhomogeneous thin shells. Different pathways are governed by the interplay between the elastic properties of the hard and soft regions of the pollen shell and by the aperture shape, number, and size. The regions of mechanical parameters of the pollen grain which lead to complete closure of all apertures, thus reducing water loss and presenting viable solutions to the infolding problem are delineated [1]. The bursting of the grain once it swells enough in humid atmosphere is shown to be a rapid transition at the critical point when the aperture can no longer sustain the internal pressure [2].

Theoretical calculation of the process of a regular closure of the pollen grain. The grain elongates as the apertures close.

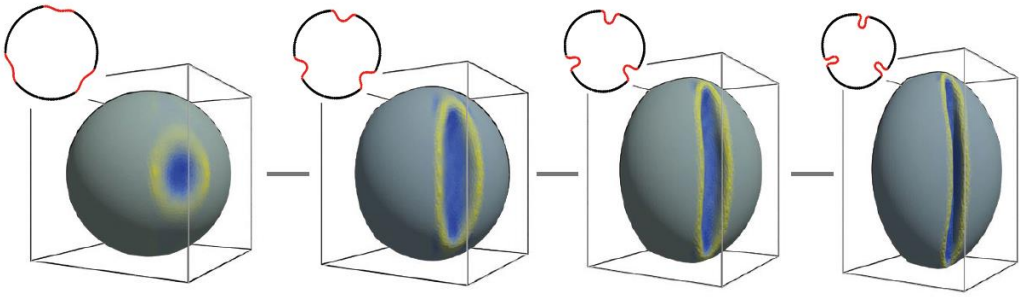


Figure 1: Theoretical calculation of a regular closure of the pollen grain upon desiccation, adapted from [1]. The equatorial cross sections of the shapes are shown in the upper-left corner of the boxes containing the three-dimensional images of the numerically simulated pollen grains. The apertures are denoted by red lines in the cross sections. The loss of the internal volume of the grain (desiccation) increases from left to right [1].

References

[1] A. Božič, A. Šiber, Proc. Natl. Acad. Sci. U.S.A. 117, 26600 (2020).
[2] A. Božič, A. Šiber, Biophys J 121, 782 (2022).

PL2 ***Uncoupling in mitochondria: the role of SLC25 superfamily proteins***

Elena E. Pohl

Physiology and Biophysics, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine, 1210 Vienna, Austria
e-mail: elena.pohl@vetmeduni.ac.at

The main source of ATP in aerobic organisms is oxidative phosphorylation. Mitchell's chemiosmotic theory predicts that the proton leak across the inner mitochondrial membrane, which is not coupled to ATP synthesis, would cause the uncoupling of respiration. UnCoupling Protein 1 (UCP1) provides such a proton pathway. In the presence of long-chain fatty acids UCP1, dissipates the proton gradient from ATP production as heat, a process known as non-shivering thermogenesis in mammalian brown adipose tissue. Mitochondrial uncoupling is becoming increasingly interesting as a basis for the therapeutic intervention in pathological conditions such as obesity, inflammatory, neurodegenerative and ischemic diseases. The mechanism of action of the classical synthetic uncouplers, such as DNP, FCCP and CCCP in mitochondria is also related to their protonophoric activity. However, the recently discovered discrepancy between the proton transport rates of some uncouplers and their ability to stimulate mitochondrial respiration has led to a new hypothesis suggesting the involvement of several mitochondrial substrate transporters of the SLC25 superfamily in proton transport. The underlying molecular mechanism is under debate and will be discussed in my lecture.

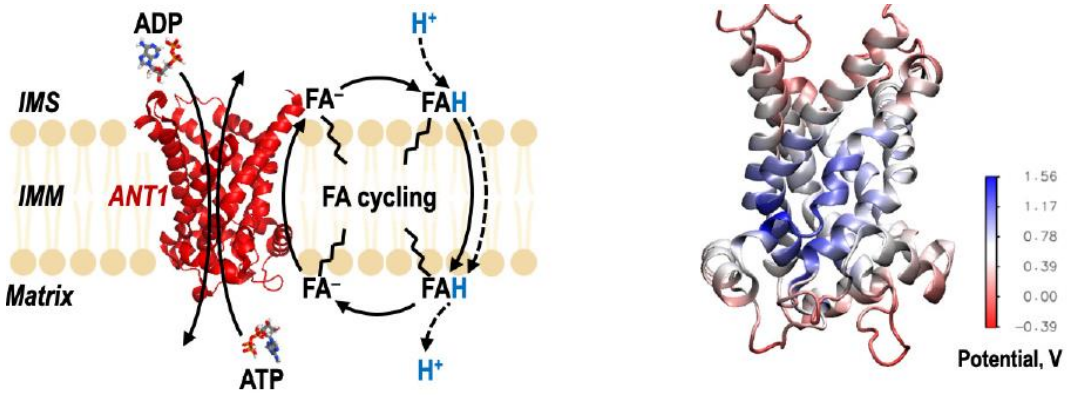


Figure 1: (Left) The FA cycling model, in which ANT1 is proposed to facilitate the transport of FA anion across the inner mitochondrial membrane (IMM) from the mitochondrial matrix to the intermembrane space (IMS). (Right) The calculated electric surface potential of ANT1.

Acknowledgements

This research was supported by the Austrian Science Fund and European Union's Horizon 2020 research and innovation program.

PL3 ***Exploring viruses by single-molecule biophysics***

Miklós Kellermayer[1,2], Dominik Sziklai[1], Dorottya Mudra[1], Luca Annamária Kiss[1], Zsombor Lohinai[1], Bernadett Pályi[3], Zoltán Kis[3,4], Gabriella Csík[1], Levente Herényi[1], Bálint Kiss[1,2]

[1]Department of Biophysics and Radiation Biology,
[2]HUN-REN-SE Biophysical Virology Group, Semmelweis University, Budapest, Hungary
[3]National Biosafety Laboratory, National Public Health Center, Budapest, Hungary
[4]Department of Medical Microbiology, Semmelweis University, Budapest Hungary
e-mail: kellermayer.miklos@semmelweis.hu

The advancement of single-molecule biophysics has led to unequaled possibilities in exploring viruses. By employing single-molecule methods here we investigated the life cycle of the dsDNA bacteriophage T7 and the nanoscale biophysical properties of the enveloped ssRNA virus SARS-CoV-2 and its variants. We used AFM, TIRF and phase contrast microscopies to image and mechanically manipulate individual virions and bacterial host cells.

T7 virions attached reversibly to the *E. coli* membrane through two-dimensional diffusion [1]. Stable anchoring was achieved by spatially isotropic binding. T7 infection led to the launch of an irreversible program in the host in three steps: a) bacterial surface roughening; b) membrane bleb formation (**Figure 1.a**); c) host cell lysis followed by the release of phage progeny. DNA ejection from T7 could be evoked *in vitro*, by photothermal excitation, only partially, suggesting that genome release is mechanically controlled, possibly to prevent premature delivery of host-lysis genes.

Unfixed SARS-CoV-2 showed a dynamic surface brush due to the rapid spike motion (**Figure 1.b**). Virions were compliant and able to recover from extreme mechanical stress [2]. Alpha and delta variants had significantly smaller radii than the wild type, hence they displayed increased specific surface, which likely contributes to their greater infectivity.

In summary, single-molecule biophysical approaches allow us to uncover unique mechanistic details of the remarkable srategies viruses employ throughout their life cycles.

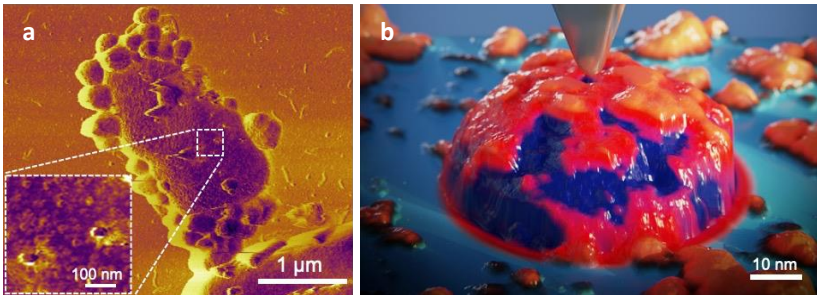


Figure 1: a. AFM image of an *E. coli* bacterium fixed with glutaraldehyde after 60 min of T7 infection [2]. **Inset**, T7 virions on the bacterium surface. **b.** Bird's-eye view of a SARS-CoV-2 virion, generated from a 3D-rendered AFM image of the virus and an artist's view of an AFM cantilever tip [2].

Acknowledgments

Supported by a grant (K143321) from the Hungarian National Research, Development and Innovation Office.

References

[1] Kiss B, Kiss LA, Lohinai ZD, Mudra D, Tordai H, Herenyi L, Csík G, Kellermayer M. *Int J Mol Sci.* **23**,11252, 2022.
[2] Kiss B, Kis Z, Pályi B, Kellermayer MSZ. *Nano Lett.* 21, 2675-2680, 2021.

PL4 L*everaging the E222Q replacement to generate novel reversibly switchable fluorescent aequorea victoria proteins for super-resolution imaging*

B. Storti[1], G. Lo Gerfo[1], S. Civita[2,3], R. Nifosì[1], S. Abbruzzetti[2], C. Viappiani[2], A. Diaspro[3,4], P. Bianchini[3], Ranieri Bizzarri[1,5]

[1]NEST Istituto Nanoscienze-CNR and Scuola Normale Superiore, Piazza San Silvestro 12, 56127 Pisa, Italy
[2]Dept. of Mathematical, Physical and Computer Sciences, University of Parma, Parco Area delle Scienze, 7/A, 43124 Parma, Italy
[3]Nanoscopy and NIC@IIT, CHT Erzelli, Istituto Italiano Tecnologia, Via Enrico Melen 83, Building B, 16152, Genoa, Italy
[4]DIFILAB Department of Physics, University of Genoa, Via Dodecaneso 33, 16146, Genoa, Italy
[5]Dept. of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Via Roma 65, 56126 Pisa, Italy

Reversibly photoswitchable fluorescent proteins (RSFPs) admirably combine the genetic encoding of fluorescence with the ability to repeatedly toggle between a bright and dark state, adding a new temporal dimension to the fluorescence signal. Accordingly, in the last years RSFPs have paved the way to novel applications in cell imaging that rely on their reversible photoswitching, including many super-resolution techniques such as F-PALM, RESOLFT, and SOFI that provide nanoscale pictures of the living matter. Yet many RSFPs have been engineered by a rational approach only to a limited extent, in absence of clear structure-property relationships that in most cases make anecdotic the emergence of the photoswitching. We have discovered E222Q replacement is a single photoswitching mutation since it restores the intrinsic cis-trans photoisomerization properties of the chromophore in otherwise non-switchable Aequorea proteins of different color and mutation pattern (Q-RSFPs) [1,2]. Our findings link indissolubly photoswitching and Q222 presence, by a simple yet elegant scenario: largely twisted chromophore structures around the double bond (including hula-twist configurations) are uniquely stabilized by Q222 via H-bonds. Likely, these Hbonds subtly modulate the electronic properties of the chromophore, enabling the conical intersection that connects the excited cis to ground trans chromophore [1]. Remarkably, analysis of photoswitching by fast spectroscopy revealed multiphase kinetics related to the peculiar protonation pattern of the protein. By applying E222Q to simple derivatives of the Enhanced Green Fluorescent Protein we generated a palette of green and yellow emitting Q-RSFPs that are tailored to several techniques addressing intracellular settings at nanoscale, such as photochromic FRET/anisotropy, SOFI, and F-PALM.

Acknowledgments

This research has been partially funded by the project PRIN 2022RRFJC4 "Novel protein-based Genetically-Encoded Fluorescent Indicators (GEFI) for Functional Super-Resolution Imaging of Biomolecular Activities in Living Cells [GEFInder]"

References

[1] Storti, B.; Margheritis, E.; Abbandonato, G.; Domenichini, G.; Dreier, J.; Testa, I.; Garau, G.; Nifosi, R.; Bizzarri, R. Role of Gln222 in Photoswitching of Aequorea Fluorescent Proteins: A Twisting and H-Bonding Affair? *ACS chemical biology* **2018**, *13*, 2082-2093, doi:10.1021/acschembio.8b00267.

[2] Bizzarri, R.; Serresi, M.; Cardarelli, F.; Abbruzzetti, S.; Campanini, B.; Viappiani, C.; Beltram, F. Single aminoacid replacement makes Aequorea victoria fluorescent proteins reversibly photoswitchable. *J Am Chem Soc* **2010**, *132*, 85-95, doi:10.1021/ja9014953.

PL5 ***Physics of Biological and Artificial Cilia***

Andrej Vilfan

Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
e-mail: andrej.vilfan@ijs.si

Cilia are cellular appendages that beat in an asymmetric fashion in order to transport the surrounding fluid. Their functions reach from the swimming of protozoa to mucous clearance in airways and establishment of body laterality in embryonic development. We will start with the question what the theoretical limits on the efficiency of ciliary propulsion are and how close cilia are to achieving it. We numerically determined the ciliary beating pattern that gives the maximum hydrodynamic efficiency and showed that both the shape of the beat and the metachronal coordination show striking similarity with the patterns found in nature [1]. Alternatively, chemical receptors located on beating cilia can achieve a significantly higher sensory efficiency than similar receptors on a flat surface [2]. However, the fact that metachronal waves lead to minimum dissipation does not yet explain how this dynamical state is achieved. To understand that, we studied a minimal model taking into account near-field effects and showed that the effective interaction between cilia at close distances becomes non-reciprocal: one cilium has a stronger influence on the phase of its neighbour than vice versa. This asymmetry can explain how metachronal waves in a finite group of cilia emerge and spread across the surface in a short time [3]. Furthermore, the efficient fluid trasport by cilia has inspired several concepts for biomimetic artificial cilia. We will frist look at magnetically driven cilia [4] and then discuss recent attempts to build synthetic cilia from protein constituents [5,6].

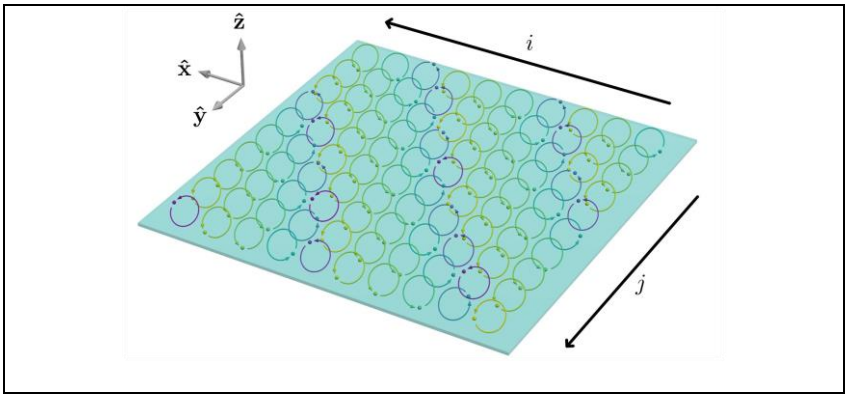


Figure 1: Metachronal wave emerging in a model of a ciliated surface

References

[1] N. Osterman and A. Vilfan, Proc. Natl. Acad. Sci. USA 108 (2011), 15727-15732.
[2] D. Hickey, A. Vilfan and R. Golestanian, eLife 10 (2021) e66322.
[3] D. Hickey, R. Golestanian and A. Vilfan, Proc. Natl. Acad. Sci. USA 120 (2023) e2307279120.
[4] M. Vilfan, A. Potočnik, B. Kavčič, N. Osterman, I. Poberaj, A. Vilfan and D. Babič, Proc. Natl. Acad. Sci. USA 107 (2010) 1844-1847.
[5] I. Guido, A. Vilfan, K. Ishibashi, H. Sakakibara, M. Shiraga, E. Bodenschatz, R. Golestanian and K. Oiwa, Small 18 (2022) 2107854.
[6] A. Vilfan, S. Subramani, E. Bodenschatz, R. Golestanian and I. Guido, Nano Lett. 19 (2019) 3359-3363.

PL6 *Glutamate-Mediated Dendritic UP States in Cortical Pyramidal Neurons – Voltage Imaging and Biophysical Model*

Srdjan D. Antić

University of Connecticut School of Medicine, Farmington, CT, 06030, USA
e-mail: antic@uchc.edu

The electrical signal serves as the fundamental physical medium for information processing within the mammalian brain. In neurons, both input and output are primarily electrical. In the first stage of neuronal electrical signaling, synaptic inputs undergo integration within individual dendritic branches. The resultant depolarizations from numerous branches converge onto the neuronal cell body, stimulating the axon (which is attached to the cell body) and eliciting a pattern of spikes (neuronal output). In the field of cortical cellular physiology, much effort has been directed towards investigating the thick apical dendrites of pyramidal neurons. However, our focus here is on the thin dendrites of pyramidal cells, including basal, oblique, and tuft dendrites. We aim to discuss a relatively novel form of electrical signal, known as the "NMDA spike," which is specific to these branches. Basal, oblique, and apical tuft dendrites receive a high density of glutamatergic synaptic contacts. Synchronous activation of 10-50 glutamatergic synapses triggers a local dendritic regenerative potential, termed the NMDA spike or plateau. This potential is characterized by a significant local amplitude (40-50 mV) and a long duration, lasting up to several hundred milliseconds. Utilizing “dendritic voltage imaging”, our investigation seeks to explore the actual voltage waveforms in distal dendrites receiving glutamatergic inputs. We aim to specify the dendritic voltage-gated and ligand-gated conductances that collectively contribute to the generation of glutamate-mediated spikes. The NMDA plateau potentials, when initiated in proximal segments of basal dendrites, frequently induce the neuronal cell body into a sustained depolarized state, reminiscent of a cortical “UP state”. At each dendritic initiation site (basal, oblique, and tuft), an NMDA spike creates favorable conditions for causal interactions among active synaptic inputs, including spatial or temporal binding of information, as well as processes of short-term and long-term synaptic modifications (e.g., long-term potentiation or long-term depression). Due to their robust amplitudes and durations, local dendritic NMDA spikes constitute the cellular substrate for multisite independent subunit computations, thereby enhancing the computational power and repertoire of cortical pyramidal cells. We propose that NMDA spikes are likely to play significant roles in cortical information processing in awake animals (e.g., spatiotemporal binding, working memory) and during slow-wave sleep (e.g., neuronal UP states, consolidation of memories).

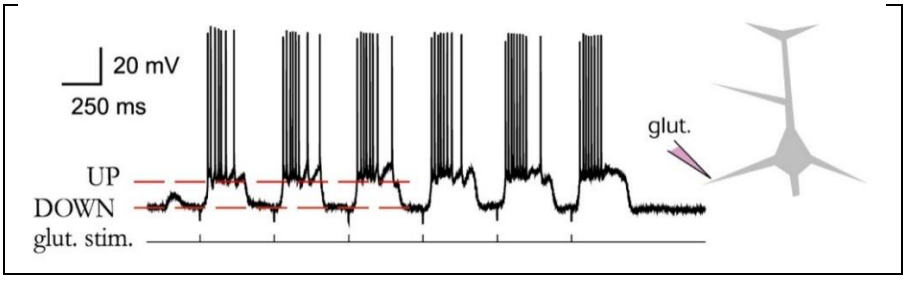


Figure 1: One dendritic branch drives the neuronal UP state.

Acknowledgments

Cure Alzheimer’s Fund

References

[1] PP. Gao, et al., J Neurophysiol. 2021 Jan 1; 125(1):23-42.
[2] SD. Antic, et al., J Neurosci Res. 2010 Nov 1; 88(14):2991-3001.

PL7 *Emerging ryanodine receptor function under the light of its molecular structure*

Alexandra Zahradníková

Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, 84505 Bratislava, Slovakia
e-mail: alexandra.zahradnikova@savba.sk

Ryanodine receptors (RyRs) are central to the excitation-contraction coupling in skeletal and cardiac muscle cells but their function is not fully understood yet, despite the copious structural and functional data. Here we have combined methods of bioinformatic analysis and statistical thermodynamics to create a model of RyR gating that would define the mechanism for the differences between the cardiac (RyR2) and skeletal isoform (RyR1) in regulation of their activity by Ca^{2+} and Mg^{2+} ions.

Analysis of allosteric pathways [1] was applied to RyR1 and RyR2 channels in closed, open, and inactivated states. Altogether, three allosteric pathways were identified. The activation pathway (Pathway 1) connected the Ca^{2+} binding activation site and the channel gate and led through the ATP binding site. The divalent ion binding inhibition site was connected with the same channel gate by two pathways. The intra-monomeric inactivation pathway (Pathway 2) led from the EF-hand region through the ATP binding site and partially overlapped with Pathway 1. The inter-monomeric inactivation pathway (Pathway 3), which was absent in closed channels, led from the EF-hand region to the gate through the S23 segment of a neighbor monomer, and its intensity was dependent on the strength of interaction between the monomers. Importantly, Pathway 3 was virtually absent in RyR2 structures due to a specific configuration of the EF-hand region.

These allosteric pathways were introduced into a RyR gating model of the Monod-Wyman-Changeux type [2]. The model contained two ion-binding regulatory sites on each monomer: the activation site, at which Ca^{2+} binding was a positive and Mg^{2+} binding was a negative allosteric effector of channel opening [3], and the inhibition site, at which both Ca^{2+} and Mg^{2+} ions were equally positive allosteric effectors of inactivation [4], with the same binding affinity in both RyR isoforms. Additionally, allosteric interaction between the two sites [5] was allowed. The resulting model had three macrostates – the closed, open, and inactivated, with probability dependent on ionic conditions. The probability of the occurrence of macrostates was derived using statistical thermodynamics [6]. The model was fitted against the available data for open probability of RyR1 and RyR2 at different $[Ca^{2+}]$ and $[Mg^{2+}]$, in the absence and presence of ATP. All data could be approximated well by the model with parameters that differed between RyR1 and RyR2 in the allosteric strength of Pathway 3, and in which the allosteric strength of Pathway 1 was increased by the presence of ATP and weakly depended on the species and RyR isoform. All other parameters of the model could be kept constant and identical for RyR1 and RyR2 under all examined conditions. These results revealed how utilization of structural information can greatly facilitate the understanding the ion channel operation.

Acknowledgments

Supported by the Slovak Research and Development Agency APVV-21-0473 and APVV-23-0545.

References

[1] J. Wang, A. Jain, L.R. McDonald, C. Gambogi, A.L. Lee, N.V. Dokholyan, Nat Commun 11 (2020) 3862.
[2] I. Zahradnik, S. Gyorke, A. Zahradnikova, J Gen Physiol 126 (2005) 515-27.
[3] A. Zahradnikova, I. Valent, I. Zahradnik, J Gen Physiol 136 (2010) 101-16.
[4] D.R. Laver, T.M. Baynes, A. F. Dulhunty, J. Membrane.Biol 156 (1997) 213-229.
[5] F.T. Horrigan, R.W. Aldrich, J Gen Physiol 120 (2002) 267-305.
[6] B. Iaparov, I. Baglaeva, I. Zahradnik, A. Zahradnikova, Front Physiol 12 (2022) e805956.

Invited lectures (IL)

IL1 T*he revival of UV-Vis spectroscopy in lipid membrane research*

Danijela Bakarić

Division for Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia
e-mail: danijela.bakaric@irb.hr

The self-organization glycerophospholipids in aqueous medium results in the formation of liposomes, spherical structures built from one lipid bilayer (unilamellar vesicles, LUVs) or several of them (multilamellar vesicles, MLVs). Depending on the concentration of lipids, nano- to micrometer dimensions of LUVs and especially of MLVs, make the obtained suspension cloudy. Numerous structural, thermoanalytical, and spectroscopic techniques are usually used in their characterization, among which, due to the absence of chromophores in glycerophospholipids with saturated hydrocarbon chains, UV-Vis spectroscopy was mostly neglected. In recent years we have shown that by multivariate analysis of temperature-dependent UV-Vis spectra (Fig. 1), solely based on the change in turbidity of suspensions, i.e. without a signal in the usual sense of the word, it is possible to determine not only the temperature of the main phase transition of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) ($T_m \approx 41$ °C) but also of its significantly weaker pretransition ($T_p \approx 34$ °C) [1]. Further, this lecture will demonstrate not only how UV-Vis spectroscopy can be used in the determination of temperature-dependent change in the DPPC lipid bilayers thickness [2], but also in the identification of structural changes on the surface of multilamellar aggregates constituted from 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) lipids that could not be observed with other techniques until now [3].

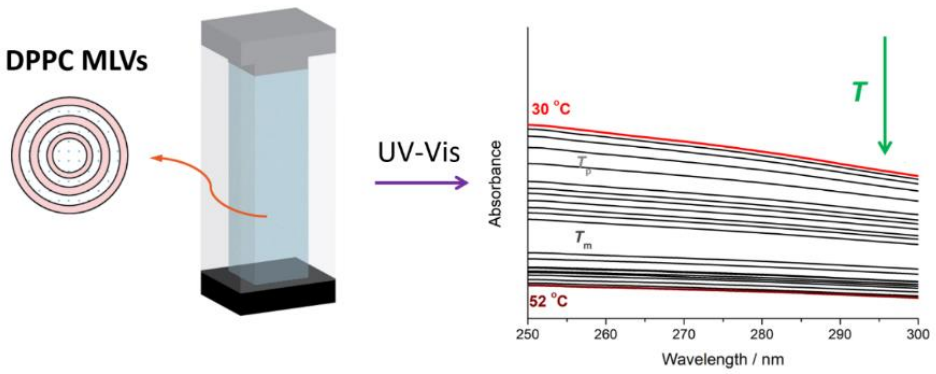


Figure 1: Temperature-dependent UV-Vis spectra of MLVs constituted from DPPC

Acknowledgments

This paper was supported by the Croatian Science Foundation through financing the project UIP-2020-02-7669 (“Model of demyelination on a molecular scale at physiological and pathological conditions”, DEMYMOLSCALE).

References

[1] P. Maleš, Z. Brkljača, D. Domazet Jurašin, D. Bakarić, Spectrochim. Acta A 272 (2022) 121013, 7.
[2] P. Maleš, B. Pem, D. Petrov, D. Domazet Jurašin, D. Bakarić, Soft Matter 18 (2022) 6703-6715.
[3] P. Maleš, M. Butumović, I. Erceg, Z. Brkljača, D. Bakarić, Biochim. Biophys. Acta – Biomembr. 1865 (2023) 184072, 9.

IL2 ***Structural insights into the mechanism of store-operated calcium channels***

Rainer Schindl

Medical University of Graz, Austria
e-mail: rainer.schindl@medunigraz.at

The single-pass transmembrane protein Stromal Interaction Molecule 1 (STIM1), located in the endoplasmic reticulum (ER) membrane, has two primary functions: sensing ER-Ca2+ concentration and directly binding to the store-operated Ca2+ channel Orai1 to activate it when Ca2+ levels decrease. When the ER-Ca2+ concentration is high, the ER-luminal STIM1 domain remains monomeric. However, once Ca2+ stores are depleted, STIM1 undergoes di-/multimerization. This multimerization is crucial for exposing the C-terminal binding site of STIM1, which interacts with Orai1 channels. Mutations that destabilize the resting configuration of STIM1 can lead to tubular aggregate myopathy. Despite its importance, the structural basis of the luminal association sites has been elusive.
Here, we present our findings using a combined approach of molecular dynamics (MD) simulations, purified proteins, and live cell techniques. We identified two critical di-/multimerization segments: the α7 helix and the adjacent region near the α9 helix in the sterile alpha motif (SAM) domain. I will discuss how interactions between the SAM domains of STIM1 monomers are vital for the protein's multimerization and activation.

Acknowledgment

Supported by the Austria Science funds to Rainer Schindl , P32778.

IL3 ***Transport phenomena at liquid-crystal-water interfaces***

Uroš Tkalec,[1,2,3] Rok Štanc,[1] Yang Xu[4], Meng Zhang[4], Xiaoguang Wang[4,5]

[1]Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[2]Faculty of Natural Sciences and Mathematics, University of Maribor, Maribor, Slovenia
[3]Department of Condensed Matter Physics, "Jožef Stefan" Institute, Ljubljana, Slovenia
[4]Department of Chemical and Biomolecular Engineering, Ohio State University, Columbus, OH, USA
[5]Sustainability Institute, Ohio State University, Columbus, OH, USA
e-mail: uros.tkalec@mf.uni-lj.si

The development of open surface microfluidic systems that enable orthogonal control of liquid mobility, mass transfer and chemical composition is crucial for devising the next generation of microfluidic platforms to be used in chemical, environmental and biomedical applications. Pulsatile or continuous release of chemicals is important for several potential applications, such as mechanical actuation, programmed chemical reactions, and treatment of various diseases [1]. To achieve these goals, we have designed a new class of open surfaces based on liquid crystals (LC), which are characterised by outstanding slipperiness, physical and chemical stability, and self-healing capabilities and can be applied to a variety of substrates [2]. Our results show that cargo release from an LC surface to a water droplet is strongly influenced by both the mesogenic orientational order and the droplet's wetting ridge. Mass transfer can be triggered by a programmable LC phase transition, charge imbalance and droplet impact [3-5]. Overall, the findings reveal novel liquid-to-liquid transport mechanisms that will broaden the range of printable inks for additive manufacturing, and could find applications in tissue regeneration, material synthesis and drug delivery.

Acknowledgments

This work is supported by the Startup Fund of The Ohio State University, National Science Foundation (NSF) collaborative research project CMMI-2227991, and core funding P1-0055 and research project J2-50092 from the Slovenian Research and Innovation Agency (ARIS).

References

[1] Y. K. Kim, X. Wang, O. Mondkar, E. Bukusoglu, N. L. Abbott. Nature 557 (2018), 539-544.
[2] Y. Xu et al. Science Advances 7 (2021) eabi7607.
[3] Y. Xu et al. Advanced Materials 34 (2022) 2108788.
[4] A. Borbora et al. Advanced Materials 35 (2023) 2302264.
[5] Y. Xu, M. Zhang, R. Štanc et al. To be published.

IL4 ***Targeting microorganisms with PDI***

Stefania Abbruzzetti[1], Andrea Mussini[1], Pietro Delcanale[1], Santi Nonell[2], Cristiano Viappiani[1]

[1]Dipartimento di Scienze Matematiche, Fisiche e Informatiche, Università di Parma - Parma, Italy
[2]Institut Quimic de Sarrià, Universitat Ramon Llull, Barcelona, Spain
e-mail: stefania.abbruzzetti@unipr.it

Photosensitizing molecules have been at the basis of photodynamic inactivation (PDI) since its early development in the 1950s. The photosensitizer (PS) is one of the three main PDI components, in addition to visible light and molecular oxygen. The therapy is an innovative methodology based on the administration of the PS to « undesidered »cells: when irradiated with visible light of appropriate wavelength, the photo-activated molecule starts a cascade of molecular transitions leading to cytotoxic effects promoted by the formation of singlet oxygen in proximity of targeted cells. The features of this approach make it interesting in fighting not only cancer cells but also bacteria and viruses, providing a possible alternative strategy against two relevant health emergencies, antibiotic resistance and deficiency of antiviral drugs.

Therefore, it is crucial developing supramolecular constructs as modular platforms, not only able to carry the pro-drug (PS), increasing its bio-availability, but also endowed with selectivity capabilities.

Hence, the aim of the herein research project is to create all-in-one multi-functional bio-molecules to be used in PDI treatments against microorganisms, with targeting, imaging and photosensitizing features.

IL5 ***Twisting and Turning: Fluorescent Molecular Rotors as Viscosity Sensors***

Antonella Battisti

Nanoscience Institute – CNR and Scuola Normale Superiore, p.zza S. Silvestro 12, 56127 Pisa, Italy

e-mail: antonella.battisti@nano.cnr.it

Intracellular viscosity, a critical parameter within cellular microenvironments, deeply influences the biological processes. From molecular diffusion to protein folding, the physical properties of the intracellular domain play a pivotal role in cellular function and health. Viscosity directly affects the microenvironment regulation (*e.g.* enzyme kinetics, protein-protein interactions, and molecular transport in the cytoplasm), features different organelles and can also bring some pathological implications, since altered intracellular viscosity is associated with diseases such as cancer, neurodegeneration, and metabolic disorders. Quantifying cellular viscosity changes can shed light on metabolic processes and provide valuable diagnostic insights.[1]

To unravel these intricate dynamics, researchers have turned to fluorescent probes, a powerful toolset that allows real-time monitoring of viscosity changes within living cells. Among these, fluorescent molecular rotors (FMRs) turned out to be suitable in probing several intracellular properties and processes.[2,3] FMRs generally consist of three main components: an electron donor unit, an electron acceptor unit and an electron-rich spacer that connects the previous units together. Upon photoexcitation, FMRs undergo a twisted intramolecular charge transfer (TICT) dynamic, producing an excited state whose energy is generally lower than that of the locally excited state. From the TICT state, energy can be released as red-shifted emission or non-radiatively. Since the dynamics of the TICT event are strongly solvent-dependent, the emission profile of the rotor can then be relevant in terms of energy, quantum yield, intensity, or lifetime, making this class of molecules particularly sensitive as viscosity probes. In combination with the phasor approach,[4] a graphical method that allows visualization of lifetime distributions, differences, and changes after a fluorescence lifetime imaging (FLIM) acquisition, FMRs express great potential as markers for the quantitative assessment of intracellular properties. Despite the well-known limitations due to photobleaching, cytotoxicity and probe specificity, FMRs are a top pick for real-time cellular imaging and microviscosity mapping.

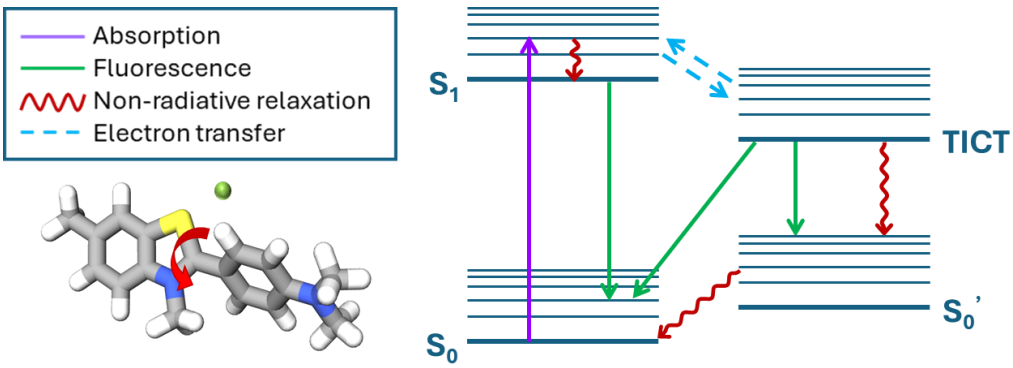


Figure 1: Jabłoński diagram showing some of the possible electronic transitions for a representative FMR.

References

[1] E. O. Puchkov, Biochemistry (Moscow) Supplement Series A: Membrane and Cell Biology 7 (2013) 270-279.
[2] M. A. Haidekker, *et al.* Organic & Biomolecular Chemistry 5 (2007) 1669-1678.
[3] S. C. Lee, *et al.* Chemistry – A European Journal 24 (2018) 13706-13718.
[4] S. Ranjit, *et al.* Nature Protocols 13 (2018) 1979-2004.

IL6 ***Scaling of mitotic spindle across eukaryotes is driven by pushing forces between chromosomes***

Nenad Pavin

University of Zagreb, Faculty of Science, Zagreb, Croatia
e-mail: npavin@phy.hr

Eukaryotes divide their genomes using the mitotic spindle, a molecular micro-machine built upon largely conserved biochemical and biophysical principles. While spindle size varies about 100-fold across eukaryotic organisms, genome size changes more than 10,000-fold. However, it is unknown how spindle biomechanics adapts to greatly varying genome sizes. Here, we reveal a spindle scaling law across eukaryotes, where the metaphase plate diameter shows a power-law scaling with genome size. Experiments on human cells, where ploidy and chromosome stiffness were manipulated, suggested that spindle size and shape result from chromosome crowding within the metaphase plate. We introduce a theoretical model in which chromosomes compete for space by pushing against each other, thereby determining the spindle width. The model predicts spindle widening under external compression and an asymmetric shape of spindle poles when the metaphase plate is off-centered, which we confirmed by squeezing the cells and removing a centrosome. Taking a broader perspective, we explain the spindle scaling law across eukaryotes by chromosome pushing forces. Thus, our work identifies a conserved mechanism of spindle adaptation to genome size from yeasts to plants and animals.

IL7 ***Microfabricated structures to solve biological problems***

T. Fekete, G. Vizsniczai, S. Valkai, G. T. Iványi, D. Petrovszki, M.A. Deli, L. Kelemen, A. Dér

HUN-REN Biological Research Centre of Szeged, 6726 Szeged Temesvári krt. 62.
e-mail: fekete.tamas@brc.hu

Microfabricated structures can be prepared for specific biological tasks with processes that are based primarily on single or multiphoton absorption. With single photon absorption (SPA) mostly 2D patterns can be polymerized, using mask lithography or direct laser writing (DLW) [1,2]. On the other hand, exploiting multiphoton absorption (most often two photon absorption, TPA), polymer solidification happens only in small-volume voxels, resulting in complex 3D structures with submicron-size features [3, 4, 5, 6].

In the recent years our Institute demonstrated the applicability of 2D and 3D structures in solving biological problems. Complex biosensors in the form of an integrated Mach-Zehnder interferometer [1] was used to determine the SARS-CoV-2 Spike protein penetration through barrier forming cells with sub-μg/mL resolution. A similar device was used to detect low concentrations of bacteria (100 CFU/mL) with a microfluidic chamber-integrated optical fiber exploiting light scattering by the bacterial cells that were concentrated over the optical fiber using a fishbone-like electrophoretic microelectrode array [2].

Polymer microtools made by two-photon polymerization and manipulated with optical tweezers were also designed for specific biological tasks. The Young's modulus of the membrane of Barrier-forming living endothelial cells were measured with low loading rates and indentations as never before [4]. The adhesion force between the endothelial cell and the tripeptide glutathione covalently linked to a microtool we also measured [5]. We were able to adhere a single living cell to an intricate mobile polymer microtool and visualize its mitochondria in 3D with isotropic resolution via multiview microscopy [3]. Recently, we presented a new approach to bind cells to microtools using deformable structures. The cells are attached to the microstructure making use of their elasticity and can be detached from the structures after being investigated.

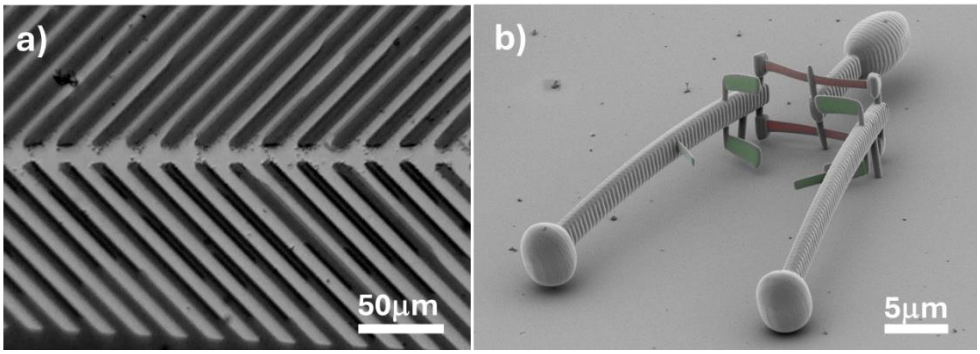


Figure 1: a) Fishbone-like electrophoretic microelectrodes made by DLW and lift-off technique. b) Flexible polymer cell

References

[1] D. Petrovszki, FR Walter, JP Vigh et al. Biomedicines, 10 (2022) 188.
[2] D. Petrovszki, S Valkai, E Gora et al. Microelectronic Engineering, 239 (2021) 111523.
[3] G. Vizsnyiczai, A Buzas, BL Aekbote et al. Optics Express, 11 (2020) 945-962.
[4] I. Grexa, T Fekete, J Molnar et al. Micromachines 11, (2020) 882
[5] T. Fekete, M Meszaros, Z Szegletes et al. ACS Applied Materials & Interfaces 13 (2021) 39018-39029.
[6] G. T. Iványi, B Nemes, I Grof et al. Advanced Materials 36 (2024) 2401115.

IL8 ***Integrating Machine Learning in Photonics: Advancements in Signal Analysis and Optical Data Interpretation***

Andrej Korenić

The Centre for Laser Microscopy, Institute for Physiology and Biochemistry "Ivan Djaja", Faculty of Biology, University of Belgrade, Serbia
e-mail: andrej.korenic@bio.bg.ac.rs

The fusion of machine learning (ML) with photonics has revolutionized optical systems by improving functionality and performance, especially in cases where traditional models are inadequate to explain complex and intractable relationships between inputs and outputs. Recent advances go beyond early ML applications such as genetic algorithms for pattern recognition and image reconstruction. They now include solving large datasets and inverse problems, demonstrating ML's capabilities in data classification and structural analysis. This development has led to breakthroughs in the fields of nanomaterials, cell classification, and super-resolution microscopy.

In the field of super-resolution microscopy, ML has made a decisive contribution to improving image resolution beyond traditional limits. One example of this is the development of Rationalized Deep Learning (rDL) for structured illumination microscopy and Lattice Light Sheet Microscopy (LLSM). This approach, which requires only minimal high-resolution target images, optimizes the imaging of living cells, and achieves unprecedented improvements in resolution.

At the same time, advances in label-free imaging have achieved high accuracy in cell classification through a flow-cytometry method that utilizes the photonic time-stretch concept and deep learning. This technique achieves high accuracy in label-free cell classification by capturing and analysing biophysical cell features, setting new standards in high-throughput quantitative imaging.

These developments emphasize the central role of ML in pushing the boundaries of photonics, leading to innovative solutions that improve the capabilities of optical systems and signal analysis.

IL9 *DNA origami-based crystals for photonics*

Gregor Posnjak

Faculty of Physics and CeNS, Ludwig-Maximilian-University Munich, Munich, Germany
e-mail: gregor.posnjak@lmu.de

Self-assembly is a promising approach to structuring matter on the scale of hundreds of nanometers, however many non-close packed architectures that are interesting for applications, have proven to be difficult if not impossible to realise experimentally [1]. I will present how we utilised the unique assembly properties of DNA origami to overcome the challenges of manufacturing a diamond lattice, which is one of the structures with the potential for widest photonic band gaps[2].

DNA origami uses DNA strands as programmable matter that self-assembles into pre-designed shapes on the scale of tens of nanometers[3, 4]. This is achieved through the predictable binding of Watson-Crick pairs on a set of short (~20 – 50 nucleotides) DNA "staples", which bind complementary sections of a long (~8000 nucleotides) single-stranded DNA and fold it into the desired shape. These structures with almost arbitrary shapes can be functionalised with addressable DNA overhangs that can control their polymerization into 3D lattices. We used DNA origami to design a tetrapod monomer with the tetrahedral symmetry needed for assembling a diamond lattice (Fig. 1A,B). After folding and purification, the tetrapods form 5 - 50 µm crystals with a periodicity of 170 nm that can be conformally coated with high refractive index materials to open a photonic band gap in the UV. Optical reflection measurements confirmed strong reflection in the range 300 – 350 nm, that was redshifted with increasing thickness of the TiO_2 coating.

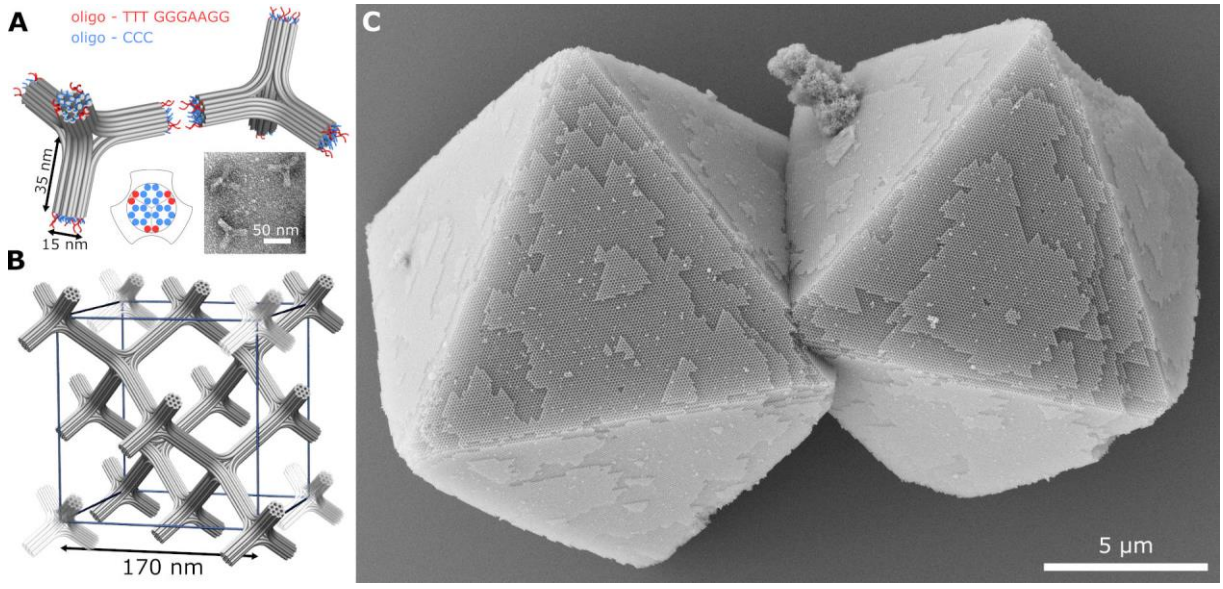


Figure 1: A) DNA origami tetrapods (TEM image in the right inset) with selective binding strands (red), positioned in a pattern (left inset) to ensure 60° rotation between neighbouring tetrapods, which is needed for diamond cubic lattices. B) DNA origami tetrapods, crystallized in a diamond lattice with 170 nm periodicity. C) SEM image of two octahedral diamond crystals, assembled from DNA origami tetrapods.

References

[1] C. A. Mirkin, S. H. Petrosko, *ACS Nano*. 17 (2023) 16291–16307.
[2] G. Posnjak *et al.*, *Science*. 384 (2024) 781–785.
[3] P. W. K. Rothemund, *Nature*. 440 (2006) 297–302.
[4] S. M. Douglas *et al.*, *Nature*. 459 (2009) 414–418.

IL10 ***Design of alternative genetically encoded photosensitizers***

Erik Sedlák[1,2]

[1]Center for Interdisciplinary Biosciences, Technology and Innovation Park, P.J. Šafárik University, Jesenná 5, 041 54 Košice, Slovakia
[2]Department of Biochemistry, Faculty of Science, P.J. Šafárik University, Moyzesova 11, 041 54 Košice, Slovakia
e-mail: erik.sedlak@upjs.sk

Flavin mononucleotide (FMN) is a highly efficient photosensitizer (PS) yielding singlet oxygen ($^1O_2$). However, its $^1O_2$ production efficiency significantly decreases upon isoalloxazine ring encapsulation into the protein matrix in genetically encoded photosensitizers. Typical examples are flavoproteins such as miniSOG in which $^1O_2$ production efficiency of FMN decreases upon binding to protein by more than 10-fold [1]. This is a result of the quenching of flavin cofactor triplet state due to isoalloxazine ring interactions with the surrounding amino acids [1]. Consequently, the conventional approach to increase $^1O_2$ production efficiency is to reduce interactions of amino acids with the isoalloxazine ring [2]. We believe that this approach has a critical limitation related to a weakening of a PS affinity to a protein. By contrast, our approach towards increasing of $^1O_2$ production efficiency is based by introducing oxidation prone amino acid close to the isoalloxazine ring selected in such way that will have minimal effects on protein-cofactor interactions in the native state of the flavoproteins. However, dissociation of FMN will be actively triggered upon irradiation with blue light *via* (mutated) amino acid(s) oxidation. As a proof of concept, we prepared three variants of the LOV2 domain of *Avena sativa* (AsLOV2), namely V416C, T418C, and V416C/T418C, on which we showed feasibility of this approach. In fact, effective $^1O_2$ production strongly correlated with the efficiency of irradiation-induced FMN dissociation (wt<V416C<T418C<V416C/T418C) [3].

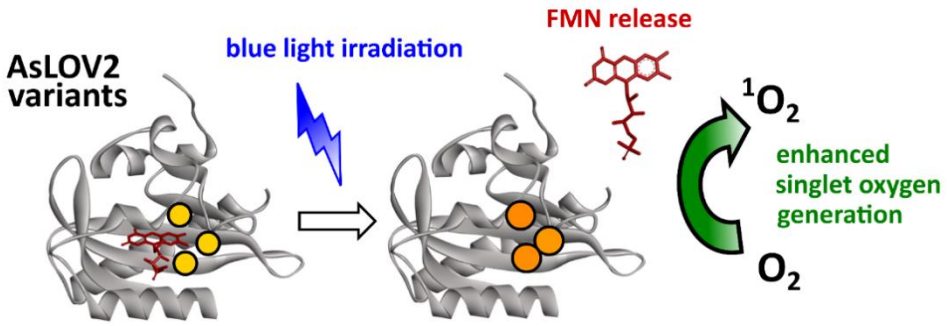


Figure 1: Schematic summary of the design strategy.

Acknowledgments

This research was funded by the Slovak Research and Development Agency (project APVV-20-0340), the Program EXCELES of National Plan of recovery and resilience Ministry of Education, Youth and Sports of the Czech Republic (NPO-NEURO-EXCELLES grant LX22NPO5107), the Grant Agency of Charles University (grant 359521), and the Czech Academy of Sciences (RVO61388971)

References

[1] M. Petrenčáková, F. Filandr, A. Hovan, G. Yassaghi, P. Man, ..., G. Bánó, E. Sedlák, Sci. Rep. 10 (2020) Art.# 4119.
[2] M. Westberg, M. Etzerodt, P.R. Ogilby, Cur. Opin. Struct. Biol. 57 (2019) 56-62.
[3] K. Felčíková, A. Hovan, M. Polák, D.S. Loginov, V. Holotová, ..., G. Bánó, E. Sedlák, Protein Sci. (2024) (in press).

IL11 *Crosstalk between nucleotide and substrate binding in ABCB1 and ABCG2*

Katalin Goda,[1] Zsuzsanna Gyöngy,[1] Nimrah Ghaffar,[1,2] Zsuzsanna Ritter,[1,2] Gábor Mocsár,[1] Szabolcs Tarapcsák,[1] Thomas Stockner,[3] Gergely Szakács[4]

[1]Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[2]Doctoral School of Molecular Cell and Immune Biology, University of Debrecen, Debrecen, Hungary
[3]Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria
[4]Institute of Cancer Research, Medical University of Vienna, Vienna, Austria
e-mail: goda@med.unideb.hu

ABCB1 and ABCG2 are exporter type ABC proteins that can expel numerous chemically unrelated xeno- and endobiotics from cells. When expressed in tumor cells and tumor stem cells, they may cause multidrug resistance contributing to the failure of chemotherapy. A better understanding the molecular mechanism of these active transporters may provide new therapeutic targets to improve the treatment of drug resistant tumors. According to the "alternating access" model membrane transporters fluctuate between inward-facing (IF) and outward-facing (OF) states, in which the centrally located substrate-binding site is intermittently accessible from the cytosolic or from the extracellular side of the membrane. The energy requirement of the conformation changes and the uphill transport of substrates is covered from ATP hydrolysis by two structurally symmetric nucleotide binding sites (NBS). However, molecular details orchestrating substrate translocation and ATP hydrolysis remain elusive in these ABC transporters.

Here we developed fluorescence-based assays to investigate the affinity of ABCB1 and ABCG2 to transported drugs and nucleotides at distinct steps of their catalytic cycle in live or semi-permeabilized cells. Using conformation-sensitive antibodies selectively recognizing the IF conformation of ABCB1 and ABCG2 we have found that the switch to the OF conformation is induced by nucleotide binding. The IF to OF transition is accelerated by substrates and hindered by known inhibitors. We also demonstrated that the high-to-low switch of substrate binding affinity coincides with the IF to OF transition in case of both transporters. Low substrate binding persists in the vanadate-trapped post-hydrolysis state, indicating that dissociation of the ATP hydrolysis products is required to reset the high substrate affinity IF conformation.

To study the coupling between ATP hydrolysis and substrate transport ABCB1 variants carrying mutations in conserved residues of one or both NBSs were analysed. Bilateral NBS mutants were found completely inactive. Unilateral NBS mutations leading to about 10-fold increase of ATP affinity resulted in complete inactivation of ABCB1, while a comparable decrease of ATP-affinity was tolerated and these mutants showed significant ATP hydrolytic activity and transport function. Detailed analysis of unilateral NBS mutants suggested that the wild-type catalytic site can hydrolyse ATP in repeated cycles without hydrolysis at the other NBS.

Acknowledgments

The work was supported by Hungarian National Science and Research Foundation (OTKA) grant K124815 and GINOP-2.2.1-15-2017-00079.

References

[1] Z. Gyöngy, G. Mocsár, É. Hegedűs, T. Stockner, Z. Ritter, L. Homolya, A. Schamberger, T. I. Orbán, J. Remenyik, G. Szakács and K. Goda. eLife (2023) 12: e83976.
[2] O. Barsony, G. Szaloki, D. Turk, S. Tarapcsak, Z. Gutay-Toth, Z. Bacso, I. Holb, L. Szekvolgyi, G. Szabo, L. Csanady, G. Szakacs, K. Goda. Sci Rep (2016) *6*: 24810.
[3] K. Goda, Y. Dönmez-Cakil, S. Tarapcsák, G. Szalóki, D. Szöllősi, Z. Parveen , D. Türk , G. Szakács, P. Chiba, T. Stockner. PLoS Genet. (2020) 16(10): e1009016

IL12 ***A lever-like anti-Aβ fibrils potential of azobenzene molecules***

Z. Bednarikova[1], D. Niedzialek[2], G. Wieczorek[2], P. Kozminski[3], Z. Gazova[a]

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

[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

[3]Centre of Radiochemistry and Nuclear Chemistry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland

e-mail: bednarikova@saske.sk

Amyloid fibrils formed by amyloid β (Aβ) peptides represent a key neuropathological feature of Alzheimer's disease (AD), a rapidly growing incurable neurological disease. As a result, there is a pressing need for innovative treatment strategies. We have employed the lever-like potential of azobenzene molecules, capable of reversible photo-induced isomerization between the cis (nonplanar, metastable) and trans (planar, thermodynamically stable) conformations, to dissociate fibrillar aggregates of $A\beta_{42}$ peptide. We have integrated *in vitro*, *in silico*, and cell assays to study the efficacy of azobenzene-based compounds, consisting of one or two molecules of azobenzene linked by DTPA. The DTPA-linked azobenzene dimer was able to dissociate Aβ fibrils in low micromolar concentrations, with dissociation activity increasing 10-fold upon photo-induced isomerization. Importantly, the DTPA-linked azobenzene dimer and monomer were able to dissociate the Aβ fibrils into non-cytotoxic species. Based on our *in silico* results, we assume that the cis-trans switch of the initially excited azo-molecule put mechanical stress on the β-strands which increase the potential of compounds to dissociate of the Aβ fibrils.

Acknowledgments

This work was supported by the Slovak Research and Development Agency under the Contract nos. APVV-18-0284 and APVV-22-0598; Slovak Grant Agency VEGA 02/0176/21; Mobility grant PAS-SAS-2022-13; National Science Centre OPUS16 number 2018/31/B/ST4/03809 and the Operational Programme Integrated Infrastructure, the project „DIAGNAD“, ITMS: 313011T553, co-funded by ERDF.

IL13 ***Photoactive Organic Semiconductors for Neurostimulation: In Vivo Application and Mechanistic Insights***

Tony Schmidt,[1] Marta Nowakowska,[2] Anna Tvrdoňovác,[3] Marie Jakešová,[3] Eric Daniel Głowacki,[3] Rainer Schindl

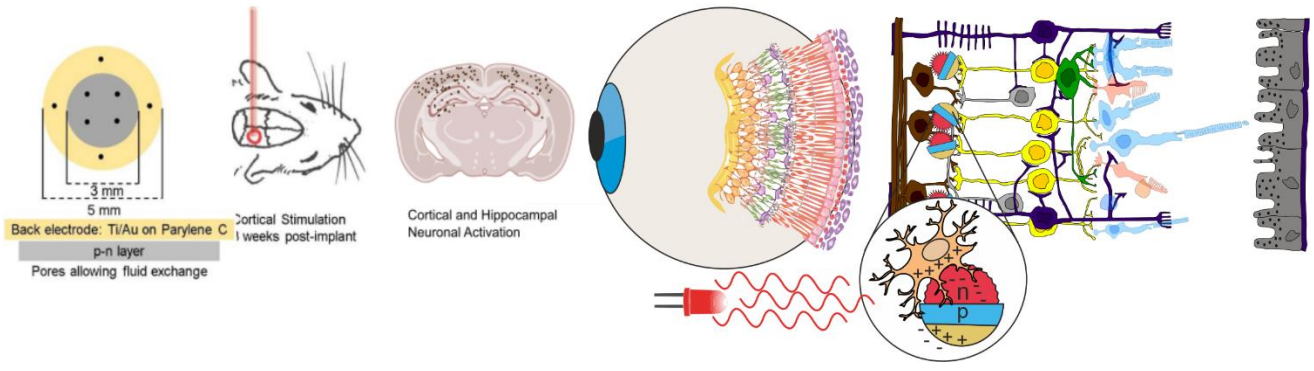
[1]Gottfried Schatz Research Center, Chair of Biophysics, Medical University of Graz, Neue Stiftingtalstraße 6, 8010 Graz, Austria
[2]Research Unit of Experimental Neurotraumatology, Department of Neurosurgery, Medical University Graz, Auenbruggerplatz 2.2, 8036, Graz, Austria
[3]Bioelectronics Materials and Devices Laboratory, Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 61200 Brno, Czech Republic
e-mail: tony.schmidt@medunigraz.at

The frontier of neurostimulation is being redefined through the innovative use of photoactive organic semiconductors, offering a novel approach to traditional treatments. Our previous research has successfully demonstrated the modulation of ion channel gating and neuronal stimulation *in vitro* through photocapacitive stimulation with a light-sensitive photovoltaic device. Our current research encompasses the development and application of organic electrolytic photocapacitors (OEPCs) and miniaturized versions of light-active microparticles, exploring their potential in both the neurostimulation *in vivo* and the treatment of degenerative eye diseases. By integrating these technologies, we aim to unlock new methodologies to activate nervous tissues with light-controlled electrical stimulation.

Our studies demonstrate the effectiveness of OEPCs in modulating neural activity within the brain of adult rats, through targeted cortex stimulation. In particular, the application of OEPCs has shown a significant up-regulation of c-Fos, indicating neuronal activity without eliciting reactions from surrounding tissues or the immune system. These findings underscore the biocompatibility and functional sustainability of OEPCs as neural implants, highlighting their potential for chronic applications in neurostimulation.

In parallel, we work with injectable organic microparticles for vision restoration in degenerative eye diseases, such as age-related macular degeneration (AMD), which presents a new approach to bioelectronic interfaces. By focusing on the precise modulation of retinal neurons, these microparticles facilitate targeted stimulation, bypassing degenerated photoreceptors and offering a promising avenue for vision restoration.

Our ongoing research will elucidate the mechanisms underlying the interaction between these novel organic semiconductors and biological systems, with the aim of redefining and optimizing their application for clinical use.



IL14 *Diffusion tensor imaging in investigation of changes in brain infrastructure in neurological diseases*

Marko Daković

University of Belgrade-Faculty of Physical Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia
e-mail: marko@ffh.bg.ac.rs

Diffusion tensor imaging (DTI) is an advanced technique of magnetic resonance imaging (MRI) that employs anisotropy of diffusion of water molecules to map spatial distribution of nerve tracts in brain and spine. In addition it enables estimation of diffusion parameters, such as fractional anisotropy (FA) and radial diffusivity (RD), which are correlated with level of integrity of nerve tracts [1] and enable tracing of their changes in various neurological diseases.

The technique uses several algorithms for the reconstruction of 3D distribution of tracts, from the simplest deterministic tractography, which relies on user definition of starting and ending points, to more sophisticated and time consuming probabilistic tractography that uses probability maps of distribution of white matter.

The changes in DTI parameters have been reported for numerous neurological diseases, from the “common” one like migraine [2] to the rare amyotrophic lateral sclerosis (ALS). However, most frequently DTI has been employed in assessment and follow-up of changes in multiple sclerosis (MS), where decrease of FA parameter in tracts pinpoints to degradation of myelin sheet [3]. While the changes in MS affect multiple tracts, in epilepsy the changes in DTI parameters were found to be restricted to hippocampal region of brain [4]. Similarly, the localized decrease of FA and RD parameters was found in corticospinal tract and spine of ALS patients [5]. DTI characteristics of Alzheimer disease are widespread depression of FA and mean diffusivity parameters that worsens with disease progression.

Nevertheless, the DTI can not be used as reliable standalone technique for characterization and follow-up of neurological disorders. However, in combination with techniques of functional and structural MRI, represents powerful tool for investigation of neurological disease.

Acknowledgments

This work was financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers 451-03-65/2024-03/200146.

References

[1] Heckel A, Weiler M, Xia A, Ruetters M, Pham M, et al. Peripheral Nerve Diffusion Tensor Imaging: Assessment of Axon and Myelin Sheath Integrity. PLOS ONE 10(6) (2015) e0130833.
[2] Petrušić, I., Daković, M., Kačar, K. *et al.* Migraine with aura and white matter tract changes. *Acta Neurol Belg* 118, 485–491 (2018).
[3] Sbardella E, Tona F, Petsas N, Pantano P. DTI Measurements in Multiple Sclerosis: Evaluation of Brain Damage and Clinical Implications. Mult Scler Int. (2013) 671730.
[4] Ahmadi ME, Hagler DJ Jr, McDonald CR, Tecoma ES, Iragui VJ, Dale AM, Halgren E. Side matters: diffusion tensor imaging tractography in left and right temporal lobe epilepsy. AJNR Am J Neuroradiol. (2009) 30 1740-1747.
[5] Li J, Pan P, Song W, Huang R, Chen K, Shang H. A meta-analysis of diffusion tensor imaging studies in amyotrophic lateral sclerosis. Neurobiol Aging (2012) 33 1833-1838.

IL15 *Free energies from molecular dynamics simulations: acceleration of sampling in conformational and chemical space*

Chris Oostenbrink

BOKU University, Institute for Molecular Modeling and Simulation, Muthgasse 18, 1190 Vienna, Austria
Christian Doppler Laboratory for Molecular Informatics in the Biosciences, BOKU University, Austria
e-mail: chris.oostenbrink@boku.ac.at

Molecular dynamics simulations offer insight into molecular properties at a resolution that is often not accessible experimentally. Moreover, using robust statistical mechanics, we gain access to thermodynamic properties like the free energy of processes. Physically relevant free-energy differences can be calculated from simulations of unphysical reference states. Over the years, we have successfully applied the one-step perturbation method by the judicious design of reference states. To ensure overlap between the reference state and the end states enveloping distribution sampling (EDS) was developed over a decade ago [1]. Here, the reference state is constructed from the end states directly, potentially maximizing the overlap in sampling. By accelerating the EDS potential [2] improved sampling of the end-states becomes feasible. Various applications in drug-design settings [3] show that the accelerated EDS approach (A-EDS) can also enhance the sampling of orthogonal degrees of freedom [4] and may furthermore be used as a chemostat. This opens the way to applications in virtual screening or the sampling of water molecules in the active site of protein-ligand complexes [5].

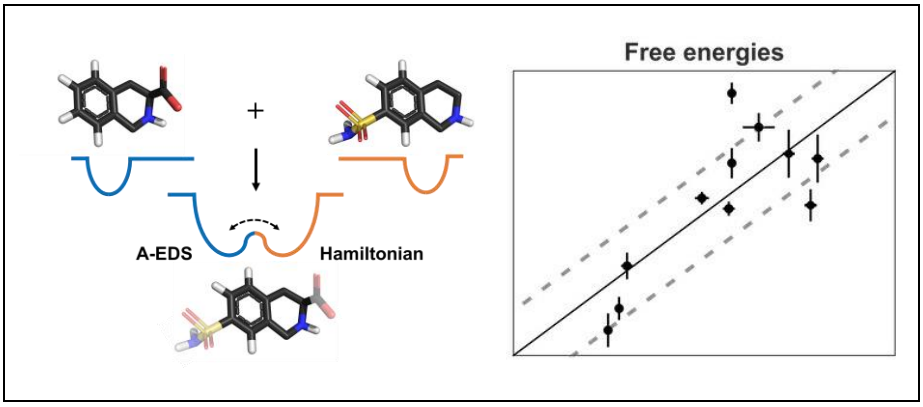


Figure 1: Accelerated Enveloping Distribution Sampling combines multiple molecules into one reference state, allowing us to compute the relative binding free energy of several molecules in one simulation

Acknowledgments

Financial support by the Austrian Federal Ministry for Digital and Economic Affairs, the National Foundation for Research, Technology and Development and the Christian Doppler Research Association is gratefully acknowledged.

References

[1] C.D. Christ, and W.F. van Gunsteren. J. Chem. Phys., 2007, 126, 184110. doi: 10.1063/1.2730508
[2] J.W. Perthold and C. Oostenbrink. J. Phys. Chem. B, 2018, 122, 5030 – 5037. doi: 10.1021/acs.jpcb.8b02725
[3] J.W. Perthold, D. Petrov, C. Oostenbrink. J. Chem. Inf. Model., 2020, 60, 5395 – 5406. doi: 10.1021/acs.jcim.0c00456/
[4] O. Gracia Carmona and C. Oostenbrink. J. Chem. Inf. Model., 2023, 63, 197 – 207. doi: 10.1021/acs.jcim.2c01272
[5] O. Gracia Carmona, M. Gillhofer, L. Tomasiak, A. de Ruiter, C. Oostenbrink. J. Chem. Theory Comput., 2023, 19, 3379–3390. doi: 10.1021/acs.jctc.3c00109

Selected orals (SO)

SO1 *Molecular basis for the nitric oxide sensitivity of a blue fluorescent protein*

Cristiano Viappiani,[1] L. Bellanova,[1] Keren Morreale,[1] Arne Franzen,[2] Beatrix Santiago-Schübel,[3] V. Svensson, [4] T. Drepper, [4] Stefano Bruno,[5] Antonio Scarano,[5] Stefania Abbruzzetti,[1] Pietro Delcanale,[1] Riccardo Nifosì,[6] Thomas Gensch[2]

[1]Dipartimento di Scienze Matematiche, Fisiche e Informatiche, Università di Parma, Parma, Italy
[2]Institute of Biological Information Processing (IBI-1: Molecular and Cellular Physiology), Forschungszentrum Jülich, Jülich, Germany
[3]Zentralinstitut für Engineering, Elektronik und Analytik, ZEA-3, Forschungszentrum Jülich, Jülich, Germany
[4]Institute for Molecular Enzyme Technologie (IMET), Heinrich-Heine-University Duesseldorf, Germany
[5]Dipartimento di Scienze degli Alimenti e del Farmaco, Università di Parma, Parma, Italy
[6]NEST, CNR-Istituto Nanoscienze and Scuola Normale Superiore, Pisa, Italy
e-mail: cristiano.viappiani@unipr.it

Genetically encoded fluorescent sensors for the visualization of nitric oxide (NO) at the singe cell level are still poorly developed. [1] We have recently reported the NO sensitivity of the blue-emitting fluorescent protein mTagBFP2. Protein mutants and mass spectrometry suggested that S-nitrosylation of Cys residues is at the basis of the observed reduction in emission intensity and lifetime in response to NO exposure. The potential of this GES for monitoring intracellular NO was shown on HeLa cells transiently expressing mTagBFP2. [2] In this work, we further investigate the molecular basis for the observed NO sensitivity.
From the experimental point of view, we have performed a more accurate study on the dynamics of S-nitrosylation of Cys residues in mTagBFP2, using the NO donor MAHMA nonoate. The resulting changes in fluorescence emission intensity and lifetime were monitored as a function of time to retrieve a better estimate of the dissociation constant and of the reaction kinetics. We have also tested the response of mTagBFP2 to the presence of the NO donors S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP). Fluorescence quenching and mass spectrometry experiments on mTagBFP2 mutants containing only one of the Cys residue show that two out of the three Cys residues in mTagBFP2 are responsible for the changes in fluorescence emission. Further experiments on bacterial cells expressing mTagBFP2 and its mutants, are underway. We have also investigated possible mechanisms of fluorescence loss upon S-nitrosylation including perturbations in the protein structure, leading to increased chromophore flexibility and subsequent enhancement of non-radiative decay. Additionally, Förster resonance energy transfer (FRET) to the newly acquired NO group(s) is considered, as their absorption spectrum, characterized by n → π* and π → π* bands peaking at 545 and 335 nm respectively, partially overlaps with the emission spectrum of mTagBFP2. Through molecular dynamics simulations, we address the structural alterations upon NO binding and calculate the geometric factors necessary to accurately estimate FRET efficiency. Finally, we discuss how our theoretical and computational findings align with experimental measurements on mTagBFP2 and other FP variants.

Acknowledgments
Funded by the European Union – Next Generation EU-PRIN 2022PNRR P2022F4WR8_001 “A molecular platform for intracellular nitric oxide sensing”.

References

[1] Eroglu, E.; Charoensin, S.; Bischof, H.; Ramadani, J.; Gottschalk, B.; Depaoli, M. R.; Waldeck-Weiermair, M.; Graier, W. F.; Malli, R., Genetic biosensors for imaging nitric oxide in single cells. Free Radic. Biol. Med. 2018, 128, 50-58.
[2] Montali, C.; Abbruzzetti, S.; Franzen, A.; Casini, G.; Bruno, S.; Delcanale, P.; Burgstaller, S.; Ramadani-Muja, J.; Malli, R.; Gensch, T.; Viappiani, C., Nitric Oxide Sensing by a Blue Fluorescent Protein. Antioxidants 2022, 11, (11), 2229

SO2 *Molecular insight into bacterial membrane disruption by dendritic nanoparticles - a pathway for antibacterial proteins*

Karol Ciepluch[1], Małgorzata Łysek-Gładysinska[1], Kinga Skrzyniarz[1], Samuel Takvor-Mena[2,3,4], Karolina Lach[1], Óscar Barrios-Gumiel[2,3,4], Jesús Cano[2,3,4], Javier Sanchez-Nieves[2,3,4]

[1]Division of Medical Biology, Jan Kochanowski University in Kielce, Uniwersytecka Street 7, 25-640 Kielce, Poland
[2]Department of Organic and Inorganic Chemistry, Research Institute in Chemistry "Andrés M. del Río" (IQAR), University of Alcalá, 28805 Alcalá de Henares, Spain
[3]Networking Research Center for Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Av. Monforte de Lemos, 3-5, 28029 Madrid, Spain
[4]Ramón y Cajal Institute of Health Research, IRYCIS, Ctra. de Colmenar Viejo, Km. 9, 28034 Madrid, Spain
e-mail: karol.ciepluch@ujk.edu.pl

Bacterial resistance to antibiotics poses a serious challenge with increased morbidity and mortality. Gram-negative bacteria from the ESKAPE group are particularly problematic due to their virulence factors and the presence of an outer membrane (OM). The outer bacterial membrane of drug-resistant bacteria constitutes a significant barrier to many antimicrobials. Therefore, the development of new antibacterial agents focuses primarily on damaging the outer bacterial membrane of Gram-negative bacteria. Among many membrane-disrupting substances, cationic dendritic systems are the most promising. They have the ability to damage the bacterial outer membrane and create a path for antimicrobial proteins (lysozyme or phage endolysin), which usually have no chance to cross this obstacle and reach the peptidoglycan and degrade it. Therefore, several biophysical methods have been used to study the interactions of dendritic polymers from the bacterial outer membrane. The use of carbosilane dendrimers shows that the mechanism of action depends on the type of dendrimer and the lipid composition of the membrane. We also demonstrate that changes in membrane fluidity and endolysin permeability by methylimidazolium and pyridylimidazolium dendrimers may play a greater role in the antimicrobial activity compared to cell membrane damage caused by positively charged dendrimers. The complex formed from endolysin and carbosilane dendrimers may be an excellent alternative to other antimicrobial agents focused on only one resistance mechanism.

Acknowledgments

This work has been supported by grants PID2020-112924RB-I00 (MINECO, Spain) and PIUAH21/CC-040 (UAH-AE-2017-2). CIBER-BBN is an initiative funded by the VI National R-D-I Plan 2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions, and financed by the Instituto de Salud Carlos III (Spain) with assistance from the European Regional Development Fund. This study was supported by Polish National Science Centre research grant No. 2021/43/D/NZ6/00560.

References

[1] K. Skrzyniarz, D. Kuc-Ciepluch, M. Lasak, M. Arabski, J. Sanchez-Nieves, K. Ciepluch, Biomaterial Science, 11 (2023), 6421-6435.
[2] K. Skrzyniarz, S. Takvor-Mena, K. Lach, M. Łysek-Gładysińska, O. Barrios-Gumiel, J. Cano, K. Ciepluch, Journal of Colloid and Interface Science, (2024) accepted.

SO4 ***Understanding the molecular origin of the effect of surfactants on enveloped viruses: the case of SARS-CoV-2***

Jordi Faraudo, Marc Domingo

Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus UAB, E-08193 Bellaterra, Spain
e-mail: jfaraudo@icmab.es

Surfactants are commonly used as disinfecting agents against all sorts of pathogens, including enveloped viruses (such as those responsible for cold, flu and COVID-19). However, there is a lack of understanding of the molecular mechanisms of the inactivation of these viruses by surfactants. In a recent work [1], we have combined all atomic MD simulations of the viral envelope and coarse grain (CG) modelling of a full virion to identify the mechanisms underlying the possible inactivation of the SARS-CoV-2 virus by different types of surfactants. Our theoretical predictions motivated detailed experimental tests [2] that confirmed the proposed mechanisms. Overall, we found [1] that ionic surfactants have only a small impact over the virus envelope, being inserted into the envelope without dissolving it or generating pores. However, ionic surfactants strongly interact with the spike protein of the virus (responsible for its infectivity), easily covering it and inducing its collapse over the envelope surface of the virus. Anionic surfactants such as SDS have the strongest effect, by blocking the receptor binding domain of the Spike protein. These results have important consequences for the design of surfactants as virucidal agents, suggesting that the optimal strategy should be to focus on molecules strongly interacting with the spike protein

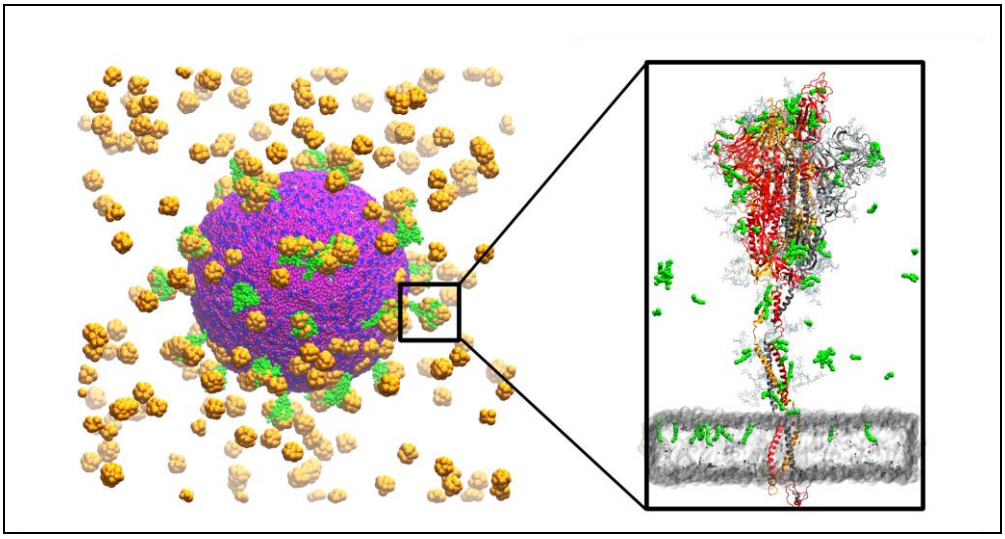


Figure 1: Simulation snapshots showing the CG model of a full SARS-CoV-2 virion surrounded by surfactant micelles and the atomistic model of its viral envelope patch including a Spike protein.

Acknowledgments

This work was supported by MCIN/AEI/10.13039/501100011033 agency through Grant PID2021-124297NB-C33 (JF) and FPI Grant No. PRE2020-093689 (MD).

References

[1] M. Domingo, J. Faraudo, *J. Chem. Phys.* **158**, 114107 (2023).
[2] H. Mateos, A. Mallardi, M. Camero and G. Lanave, J. Colloid Interface Sci, **662**, pp- 535-544 (2024).

SO5 *Modulation places for ATP binding – possible targets for allosteric activation*

Magdalena Majekova,[1] V. Heger,[1] B. Benesova,[1] P. Rezbarikova[1], L. Horakova[1]

[1]Centre of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia
e-mail: magdalena.majekova@savba.sk

SERCA (Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-ATPase) is a transmembrane protein that is critical in maintaining calcium homeostasis within cells. In numerous cases, SERCA activity has been observed to be disrupted during the development of chronic diseases. Therefore, compounds that can enhance the expression or activity of SERCA may prove to be useful in treating such diseases. Compounds of natural origin have been proved both as inhibitors as well as activators of SERCA activity. We examined possible binding sites of [6]-gingerol, resveratrol, and ellagic acid able to increase SERCA activity [1].

The docking and induced fit studies were performed by the Molecular Operating Environment (MOE 2020.0901) modeling program, using a triangle matcher and the London dG score for basic docking and the GBVI/WSA DG score for the induced-fit refinement of the geometry. Ligands were observed for protonation under a physiological pH. Ellagic acid was treated as a dianion.

We found that the SERCA activation with [6]-gingerol, resveratrol, and ellagic acid may be associated with binding to amino acid Glu439, related to one of the modulatory places of ATP in SERCA.

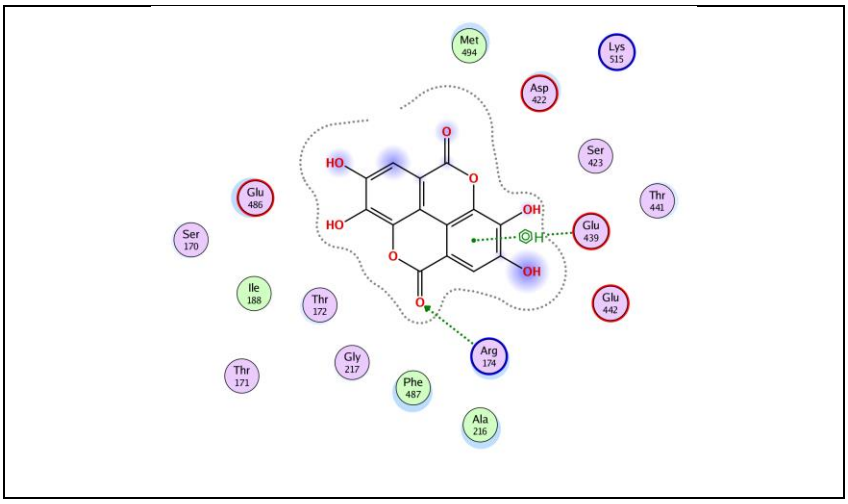


Figure 1: Position of ellagic acid bound in SERCA (E2P model, pdb code 2zbe).

Acknowledgments

This research was funded by Slovak National grant APVV-20-0543, VEGA 2/0113/21, VEGA 2/0063/22 and VEGA 2/0103/22.

References

[1] V. Heger, B. Benesova, M. Majekova, et al. *Preprints* **2023**, https://doi.org/10.20944/preprints202308.0834.v1.

SO6 ***Biocompatible reconstructed marine based vesicles as an alternative drug delivery system***

Nadica Ivošević DeNardis[1]*, Tea Mišić Radić[1], Maria Klacsová[2], Daniela Uhríková[2], Ruža Frkanec[3], Joanna Zemła[4], Malgorzata Lekka[4], Martin Lukeš[5], Ondrej Prášil[5], Krysztina Sebők Nagy[6], Tibor Páli[6], Katarina Lisac[1], Marcela Chovancová[2], Karlo Ojdanić[7], Lucija Horvat[1], Petra Peharec Štefanić[7], Kristina Smokrović[1], Nadica Maltar Strmečki[1], Dijana Pavlović Saftić[1], Ivo Piantanida[1], Tanja Matijević Glavan[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia, [2]Faculty of Pharmacy, Comenius University Bratislava, Bratislava, Slovakia, [3]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia, [4]Institute of Nuclear Physics Polish Academy of Sciences, Kraków, Poland, [5]Institute of Microbiology of the Czech Academy of Science, Centre of Algatech, Czech Republic, [6]HUN-REN Biological Research Centre, Szeged, Hungary, [7]Faculty of Science, University of Zagreb, Croatia

*e-mail: ivosevic@irb.hr

The development of drug delivery systems using renewable and environmentally friendly materials is an important part of biotechnology. Therefore, we are investigating vesicles composed of microalgae cells that are bioinspired and subjected to hypoosmotic stress. A comprehensive biophysical characterization was performed to unreveal structural features, surface properties, biocompatibility, and drug delivery ability. The vesicles have different shapes and sizes, and their membranes contain carotenoids, which may have antioxidant properties, as well as free products of chlorophyll degradation. Compared to lipids, especially polar lipids, they contain a higher concentration of proteins and a balance between saturated and unsaturated fatty acids, indicating hydrophilic properties. Crucially, the vesicles do not trigger cytotoxic or immunogenic reactions and are biocompatible. The results show that the non-covalent binding of the vesicles to the membrane enables the transport of oligonucleotides, glycopeptides as drug models. In addition, hydrated, lyophilized material can spontaneously and reversibly form into vesicles, which guarantees the long-term stability of the material. This study suggests that biocompatible reconstructed vesicles could be a viable option for pharmaceutical applications in the future.

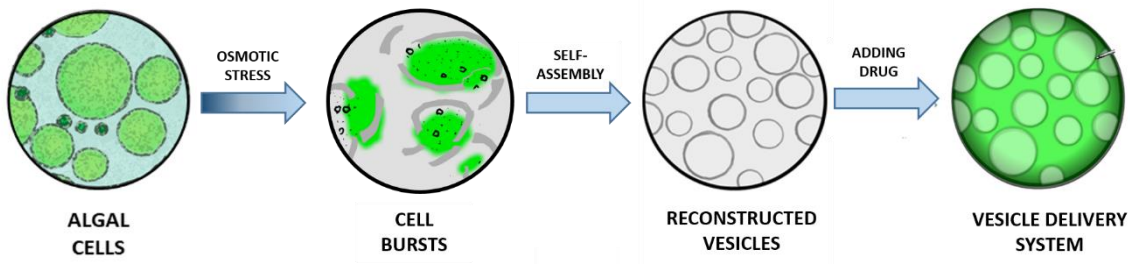


Figure 1. Proposed mechanism: from algal cells to drug modified vesicles.
(adapted from video: https://www.youtube.com/watch?v=l0pQnmjXI74, Ivošević DeNardis 2022).

Acknowledgments

This study was conducted as part of the "Research network V4-Croatia for the development of novel drug carriers from algae" (ID 22220015) which was supported by the International Visegrad Fund.

References

[1] Levak Zorinc M., Demir-Yilmaz I., Formosa-Dague, C., Vrana, I., Gašparović, B., Horvat, L., Butorac, A., Frkanec, R., Ivošević DeNardis, N., Reconstructed membrane vesicles from the microalga *Dunaliella* as a potential drug delivery system, Bioelectrochemistry 150 (2023) 108360.

[2] Ivošević DeNardis, N., Pletikapić, G., Frkanec, R., Horvat, L. Vernier, P. T. From algal cells to autofluorescent ghost plasma membrane vesicles. Bioelectrochemistry 134 (2020) 105724, 8.

SO7 *Nanoplastics and microalgae: nanostructural, nanomechanical, and antioxidant response of marine diatom Cylindrotheca closterium*

T. Mišić Radić[1], P. Vukosav[1], B. Komazec[2], C. Formosa-Dague[3], D. Domazet Jurašin[1], P. Peharec Štefanić[2], A. Čačković[1], K. Juraić[1], N. Ivošević DeNardis[1]

[1]Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia
[2] Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia
[3]Toulouse Biotechnology Institute, Université de Toulouse, Institut National des Sciences Appliquées, Institut National de la Recherche Agronomique et Environnementale, Centre National de la Recherche Scientifique, Toulouse, France
e-mail: tmisic@irb.hr

This study aimed to investigate the impact of polystyrene nanoplastics (PS NPs) on the nanostructural, nanomechanical and antioxidant responses of the marine diatom *Cylindrotheca closterium*. The results showed that both types of PS NPs, amine-modified (positively charged) and carboxyl-modified (negatively charged), regardless of their surface charge, inhibited the growth of *C. closterium* during short-term exposure. However, prolonged exposure to both types of PS NPs did not significantly inhibit growth, possibly due to the detoxification effect of microalgal extracellular polymers (EPS). Exposure to both types of PS NPs above their respective concentrations, which resulted in a 50% growth reduction (EC50) showed phytotoxic effects, primarily attributed to excessive generation of reactive oxygen species, leading to increased oxidative damage to lipids and changes in antioxidant enzyme activities. In addition, diatoms exposed to nanoplastics showed a significant decrease in cell wall rigidity, possibly making the cells more vulnerable. Atomic force microscopy images showed that positively charged PS NPs predominantly adhered to the cell surface, while both types of PS NPs were incorporated into the EPS, which serves as a protective barrier for the cells. Considering that the microalgal EPS serves as a vital food source for phytoplankton grazers and higher trophic levels, the incorporation of NPs into the EPS and their interactions with algal cell walls may have implications for the stability and functioning of marine ecosystems.

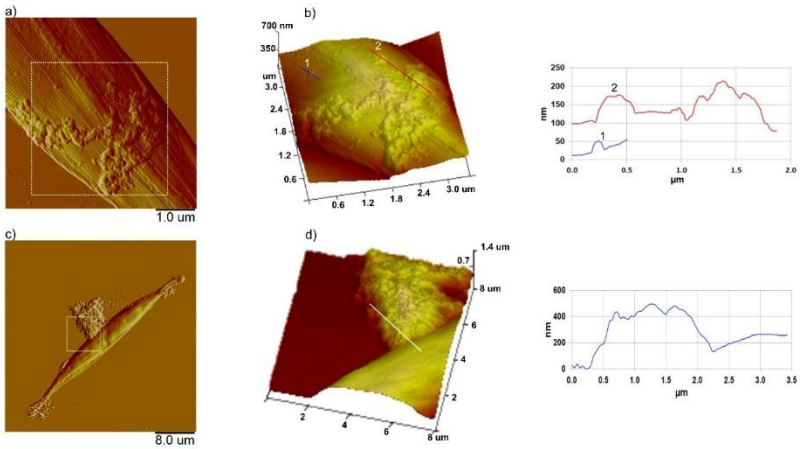


Figure 1: AFM images of a *Cylindrotheca closterium* cell exposed to amine-modified (a, b) and carboxyl-modified polystyrene nanoplastics (c, d) with vertical profiles along the indicated lines (Mišić Radić et al. 2022).

Acknowledgments
This work was supported by the project "From algal cell surface properties to stress markers for aquatic ecosystems" (CELLSTRESS, IP-2018-01-5840), funded through the Croatian Science Foundation.

References

[1] T. Mišić Radić, P. Vukosav, B. Komazec, C. Formosa-Dague, D. Domazet Jurašin, P. Peharec Štefanić, A. Čačković, K. Juraić, N. Ivošević DeNardis, Water, 14, 2163 (2022). https://doi.org/10.3390/w14142163

SO8 ***Enzymatic organization studied by the Maximum Entropy Production Principle***

Andrej Dobovišek [1,2], T. Blaževič [1], S. Kralj [1,3], A. Fajmut [1,4]

[1]University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška 160, Maribor, Slovenia
[2]University of Maribor, Faculty of Medicine, Taborska ulica 8, Maribor, Slovenia
[3]Jozef Stefan Institute, Jamova 39, Ljubljana, Slovenia
[4]Faculty of Health Sciences, University of Maribor, Žitna ulica 15, Maribor, Slovenia
e-mail: andrej.dobovisek@um.si

This study conducts a theoretical investigation to clarify the mechanisms and conditions driving the transition between two distinct enzymatic organizations: soluble enzyme cascades and enzyme complexes. We explore a scenario where this transition occurs spontaneously as concentrations of metabolic intermediates within the cascade are significantly altered by concurrent processes, reaching levels where effective catalysis and metabolic regulation become impossible [1, 2]. We propose a mathematical model that considers a metabolic intermediate in the enzyme cascade as a central control parameter, with the Maximum Entropy Production Principle (MEPP) serving as the foundational theoretical framework for thermodynamic analysis.

A detailed thermodynamic analysis is performed on the optimal steady-state performance of both the enzyme cascade and the enzyme complex. For both enzymatic organizations, we compare optimal values of crucial reaction parameters and thermodynamic quantities, including the rate of entropy production, rate constants, Shannon information entropy, kinetic stability, and elasticity coefficients. Two critical values of the control parameter are predicted, at which both types of enzymatic organization are equally probable. We demonstrate that the rate of entropy production, kinetic stability, and elasticity coefficients exhibit discontinuities at critical points, suggesting that the transition between the two enzymatic organizations can be characterized as a discontinuous first-order phase transition.

References

[1] S. Schoffelen, B. Baric, J. C. M van Hest, Soft Matter 8 (2012) 1736-1746.
[2] M. Pröschel, R. Detsch, A. R. Boccaccini, U. Sonnewald, Front. Bioeng. Biotechnol. 3 (2015) 1-13.

SO9 *Stable substructures in proteins and the principle of minimum frustrations*

Gabriel Žoldák

Centrum interdisciplinárnych biovied, Technologický a inovačný park Univerzita P.J. Šafárika, Trieda SNP 1, 040 11 Košice, Slovensko
e-mail: gabriel.zoldak@upjs.sk

Specific proteins, like translation factors, must operate efficiently in diverse microenvironments, such as the ribosome and cytoplasm. The diverse environmental conditions present distinct stability obstacles, which are inherent to the process of evolution. In my recent analysis, I investigated the hypothesis that studying frustration within and between protein domains can provide insights into the fundamental mechanisms that control protein stability and functionality in different operational microenvironments. The impact of contact frustration is frequently disregarded due to various other molecular factors, and it remains uncertain what role, if any, it plays.

The frustration analysis was conducted by utilizing the stand-alone frustration analysis R-package (https://github.com/proteinphysiologylab/frustratometeR) [1-3]. A thorough examination of frustration was carried out. This method categorizes individual contacts based on their frustration index values, differentiating between contacts that are significantly dissatisfied ("highly frustrated") and those that are relatively satisfied ("minimally frustrated") compared to other options in the same area. Contacts not falling into these extreme categories are considered "neutral." Contacts that are minimally frustrated, meaning they have low energy compared to other decoy energies, indicate that different combinations of amino acids in the same position may not be as favorable for correct folding. On the other hand, highly frustrated contacts with a higher level of native energy suggest that most alternative pairings would be more advantageous for folding. The frustration index quantifies the level of favorability of a specific interaction compared to all potential interactions in that location while being adjusted for the variability of that distribution.

The existence of strongly conflicted interactions between protein domains can indicate potential problems for the coordination and effectiveness of the protein, potentially resulting in specific changes in its shape. By identifying these crucial interactions, the analysis enhances our comprehension of the structural and functional interplay among various proteins, including IF2 [4]. The investigation emphasizes the importance of frustration analysis, particularly for proteins that operate in extreme conditions or are designed to function in diverse environments. I hypothesize that this concept can be utilized in the misfolding of pathological proteins due to the interaction between the inherent tendency of the specific local sequence to aggregate.

Acknowledgments

This work was supported by the research grant from the Slovak Grant Agency VEGA No 1/0024/22.

References

[1] M. Jenik, R. G. Parra, L. G. Radusky, A. Turjanski, P. G. Wolynes, D. U. Ferreiro, Nucleic Acids Research 40 (2012), W348–W351
[2] R.G. Parra, N.P. Schafer, L.G. Radusky, M.Y. Tsai, A.B. Guzovsky, P.G. Wolynes, D.U. Ferreiro, Nucleic Acids Res. 44 (2016), W356-60.
[3] A.O. Rausch, M.I. Freiberger, C.O. Leonetti, D.M. Luna, L.G. Radusky, P.G. Wolynes, D.U. Ferreiro, R.G. Parra, FrustratometeR: Bioinformatics. 37 (2021), 3038-3040.
[4] V. Džupponová, N. Tomášková, A. Antošová, E. Sedlák, G. Žoldák, Int J Mol Sci. 24 (2023) 6787.

SO10 ***Machine Learning-Assisted Optimization of Guideline-Directed Therapy for Heart Failure***

Jan Zmazek

University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška cesta 160, 2000 Maribor
e-mail: jan.zmazek@um.si

Heart failure is a significant public health problem, affecting millions of people worldwide and accounting for a substantial proportion of hospital admissions and healthcare costs [1]. Despite advances in the management of heart failure, the morbidity and mortality associated with this condition remain high. One approach to improve outcomes for patients with heart failure is guideline-directed therapy, which involves the use of evidence-based treatments to reduce symptoms, prevent hospitalization, and improve survival [2]. Regardless, heart failure treatment implementation is insufficient despite ESC guidelines, with a significant number of patients not receiving medications at target doses or not at all [3]. The ongoing efforts to bridge the gap between guidelines and clinical practice include the development of tools to assist with guideline implementation, such as decision support systems. However, in Slovenia and many other countries, the implementation of such systems is stagnant due to the use of various electronic health record systems among hospitals and clinics, making it difficult to integrate and analyze data across different facilities. To overcome this problem, we aimed to develop a machine learning algorithm that can be applied to electronic health records from separate databases, providing a unified analysis. We present a software package implementing natural language processing algorithms and other machine learning methods trained on discharge letters of heart failure patients from a Slovenian hospital. Sections of unstructured textual data of electronic health records are categorized into standardized medical concepts using document classification methods. Using named entity recognition and in conjunction with information extracted from unstructured data, the algorithm extracts key clinical parameters, such as prescribed therapy, comorbidities, and clinical outcomes. The algorithm's accuracy is tested against manually extracted and labeled data. Ultimately, the algorithm is used to estimate the prognosis of heart failure patients, identify patients not receiving guideline-directed therapy, and analyze factors associated with non-adherence. The findings aim to inform the development of targeted interventions to improve adherence to ESC guidelines, ultimately improving patient outcomes and reducing healthcare costs.

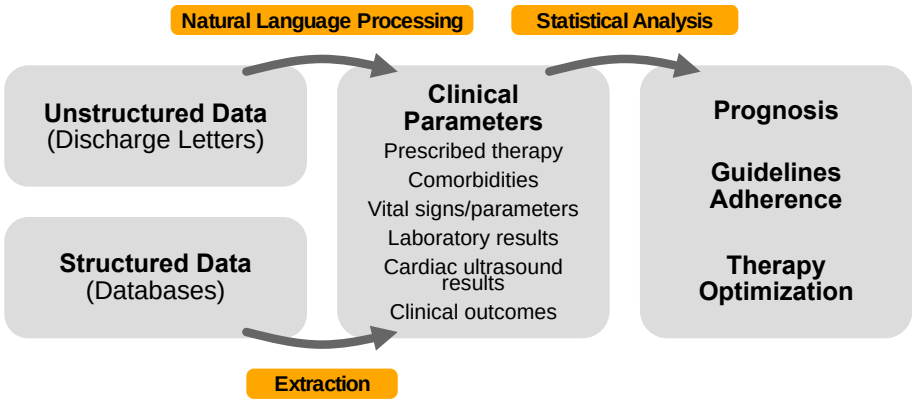


Figure 1: Structure of software implementation of the algorithm.

References

[1] V. L. Roger, Circ Res 128 (2021) 1421-1434.
[2] G. M. C. Rosano *et al.*, Eur J Heart Fail 23 (2021) 192-156.
[3] T. A. McDonagh *et al.*, *Eur Heart J* 42 (2021) 3599–3726.

SO11 ***Multicellular Dynamics in Beta Cell Networks: Insights from Multicellular Imaging, Phenomenological Models, and Network Analyses***

M. Gosak,[1,2,3*] M. Šterk,[1,2,3] U. Barać[1], A. Stožer[2]

[1]Faculty of Natural Sciences and Mathematics, University of Maribor, Slovenia
[2]Faculty of Medicine, University of Maribor, Slovenia
[3]Alma Mater Europaea – ECM, Slovenia
[*]e-mail: marko.gosak@um.si

Islets of Langerhans are multicellular networks in which several hundred of beta cells work in synchrony to produce secretory pulses of insulin, a hormone crucial for controlling metabolic homeostasis. Their collective rhythmic activity is facilitated by intercellular coupling and affected by their multimodal activity due to networked feedback interactions of various oscillatory subsystems as well as by their functional heterogeneity. Consequently, the multicellular dynamics of beta cell populations is far from elemental and is characterized by complex patterns of activity. In this contribution I will present how we explore these intricacies by combining high-resolution confocal laser-scanning imaging in acute pancreatic tissue slices, with computational modelling and network science approaches [1]. Construction of functional beta cell networks reveals that their structure is stimulation-dependent [2], modulated by pharmacological agents such as antidiabetics [3,4], and heavily influenced by the dynamical compound being analysed, i.e., high oscillatory electrical activity vs. slow metabolic activity [5]. Furthermore, utilizing phenomenological models we were able not only to identify which physiological determinants need to be considered in the model to achieve good agreement between the model and experimental results, but also gain a more profound mechanistic understanding of the complexities in intra- and inter-cellular activities within islets. Specifically, as the main factors driving good consistency between model predictions and experimental data, heterogeneity in cellular metabolism, K_{ATP} channel conductivity, and intercellular coupling have been identified, which, in addition to electrical cell-to-cell interactions, must also encompass metabolic ones [6]. Finally, we argue that the proposed computational approaches hold relevance for comprehending the intricacies of collective cellular activity across diverse contexts, where the assessment of multicellular dynamics can be achieved through suitable imaging techniques.

References

[1] A. Stožer, M. Šterk, E.P. Leitgeb, R. Markovič, M.S. Klemen, C.E. Ellis, L.K. Bombek, J. Dolenšek, P.E. MacDonald, M. Gosak, Frontiers in Endocrinology 13 (2022) 922640.
[2] M. Šterk, J. Dolenšek, M.S. Klemen, L.K. Bombek, E.P. Leitgeb, J. Kerčmar, M.S. Rupnik, M. Perc, A. Stožer, M. Gosak, Biophysical Journal 122 (2023) 784.
[3] M. Šterk, L.K Bombek, M.S. Klemen, M.S. Rupnik, M. Marhl, A. Stožer, M. Gosak, PLoS Computational Biology 17 (2021) e1009002.
[4] E.P. Leitgeb, J. Kerčmar, L.K. Bombek, V. Pohorec, M.S. Klemen, M. Rupnik, M. Gosak, J. Dolenšek, A. Stožer, Frontiers in Endocrinology 14 (2024) 1315520.
[5] J. Zmazek, M.S. Klemen, R. Markovič, J. Dolenšek, M. Marhl, A. Stožer, M. Marhl, Frontiers in Physiology 12 (2021) 612233.
[6] M. Šterk, U. Barać, A. Stožer, M. Gosak, Physical Review E 108 (2023) 054409.

SO12 ***α-lactalbumin amyloid fibrillization dependence on Hofmeister anions in acidic pH***

A. Antosova, M. Gancar, J. Marek, Z. Gazova

Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia

e-mail: antosova@saske.sk

Protein aggregation is a hallmark of more than fifty protein conformational amyloid disorders. α-lactalbumin (α-LA), 123 amino acids-consisting globular protein, is an interesting model protein for amyloid aggregation study due to its ability to form a molten globular state. The presence of salts can readily control the amyloid aggregation of proteins. Using a multi-technique approach, we have compared the effect of 300 mM salts (Na_2SO_4, CH_3COONa, NaH_2PO_4, NaBr, $NaNO_3$, $NaClO_4$) on the amyloid fibril formation of α-LA in the acidic pH. The aggregation kinetics, the secondary structure content, and amyloid fibrils' morphology have been studied using ThT fluorescence, FT-IR and CD spectroscopy, and atomic force microscopy. We discovered that the effect of anions on the kinetic parameters of α-LA amyloid formation and the morphology of its fibrils depends on the salt's position in the Hofmeister series. These findings are essential to uncover the general mechanism of amyloid fibrillation and the possible future application of amyloid aggregates in biotechnology.

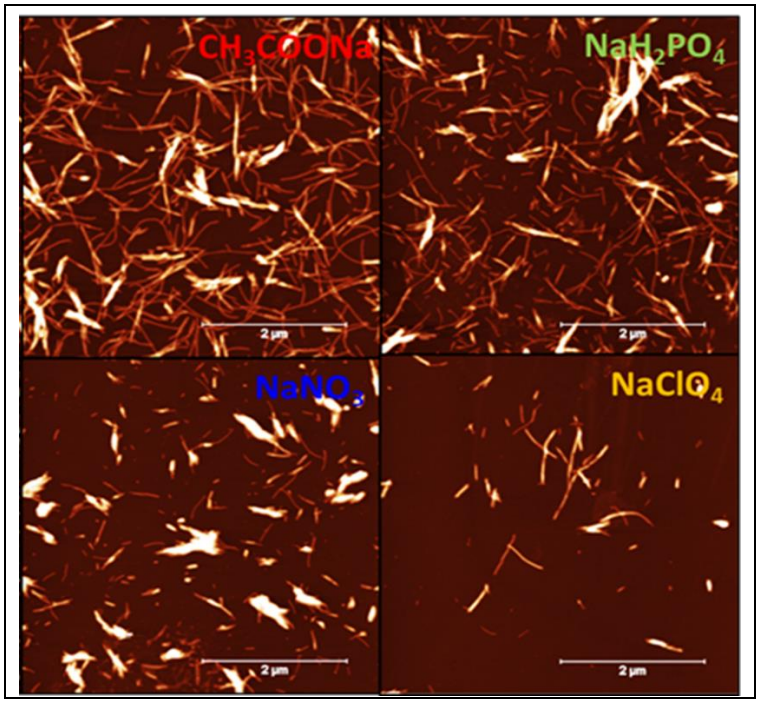


Figure 1: Atomic force microscopy (AFM) images of α-LA amyloid structures prepared in 300 mM salts. All images are 5x5 µm with a scale bar of 2 µm.

Acknowledgments

This work was supported by the Slovak Research and Development Agency under Contract no. APVV-22-0598 and Slovak Grant Agency VEGA 2/0176/21, 02/0164/22 and BIOVID-19 (ITMS2014+ 313011AVG3) supported by OPII and funded by the ERDF.

SO13 ***Let's cut things short: A comparative study of short- and long-chain DNA in the presence of manganese cations***

Kristina Serec, Antonia Lovrenčić, Josip Basić, Martin Bobek, Lucija Gojmerac, Sanja Dolanski Babić

Department of Physics and Biophysics, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia
e-mail: kristina.serec@mef.hr

The behaviour of double-stranded DNA is intricate, influenced by various factors including the type, valence, and concentration of positive counterions surrounding it, alongside polyion length and concentration. Understanding the behaviour of DNA under diverse environments improves our fundamental comprehension of DNA physics, crucial for biological functioning and carries implications for a wide array of applications spanning from biotechnology to nanotechnology [1,2].

Over the last years our research has been dedicated to exploring vibrational dynamics of long-chain DNA, characterized by an average fragment length of 4 μm, within both single and mixed ion atmospheres [1-5]. The research focused on elucidating the impact of cations on DNA structure under semi-dilute DNA conditions, maintaining a constant DNA fragment length and concentration throughout our studies. In our current research endeavour, we adopt a contrasting perspective, focusing instead on the influence of DNA fragment length and concentrations, thereby exploring the influence of cations on the DNA structure in two distinct regimes: semi-dilute and dilute.

In this study, we employ FTIR spectroscopy to investigate the dynamic and structural aspects of short-chain DNA thin films with the estimated DNA fragment length of 5-17 nm (15-50 bp) in the presence of divalent manganese counterions. The significantly shorter DNA fragment length, several nanometres in size compared to the polydisperse long-chain fragments of 4 μm, enables precise control over various solution regimes through manipulation of DNA concentration. Ion atmosphere has been controlled by the counterion to phosphate molar concentration ratio *r,* which varied between 0.1 and 2. To potentially distinguish between the semi-dilute and dilute solution regimes, we prepared three distinct concentrations of short-fragment DNA: 5 g/L, 1 g/L, and 0.1 g/L.

Our research showed that highly hydrated DNA thin films have the ability to capture various solution regimes as evidenced by the distinct spectral features displayed by manganese counterions. Specifically, Mn^{2+} counterions exhibit notable changes at DNA concentrations ≤ 1 g/L, suggesting a modified screening effect of Mn^{2+} counterions in dilute regimes. Furthermore, a comparative analysis between short-chain DNA and our previously acquired data on long-chain DNA in the presence of manganese cations [4] provides a fresh perspective on the interaction between DNA dynamics and its environmental factors.

References

[1] K. Serec, N. Šegedin, M. Krajačić and S. D. Babić, Appl. Sci., 11, (2021) 2360
[2] K. Serec, S. D. Babić, Appl. Sci., 11, (2021) 5895
[3] K. Serec, S. D. Babić, R. Podgornik, and S. Tomic, Nucleic Acids Res 44, (2016) 8456
[4] S. D. Babić and K. Serec, Spectrochim Acta A Mol Biomol Spectrosc 241, (2020) 118646
[5] K. Serec, S. D. Babić, and S. Tomić, Spectrochim Acta A Mol Biomol Spectrosc 268, (2022) 120663

SO14 *Modelling the role of cyclic guanosine monophosphate (cGMP) in modulating the contractility of vascular smooth muscles*

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

[1]Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor
[2]Faculty of Health Sciences, University of Maribor
e-mail: ales.fajmut@um.si

The synthesis of cyclic guanosine monophosphate (cGMP), facilitated by nitric oxide (NO) through its action on soluble guanylate cyclase (sGC), triggers the activation of cGMP-dependent protein kinase (PKG). This intricate process regulates cytosolic calcium ($[Ca^{2+}]_i$) signaling and modulates Ca^{2+}-(de)sensitization within the contractile apparatus, affecting both the encoding and decoding aspects of the $[Ca^{2+}]_i$ signal. More than ten substrates phosphorylated *in vivo* by PKG have been identified, with many playing roles in $[Ca^{2+}]_i$ dynamics. Building on previous models [1, 2] that highlighted the influence of cGMP on $[Ca^{2+}]_i$ regulation via mechanisms such as large-conductance Ca2+-activated K+ channels (BKCa), Ca^{2+}-dependent Cl^- channels (ClCa), Na^+/Ca^{2+} exchanger (NCX), $Na^+/K^+/Cl^-$ cotransport (NKCC), and Na^+/K^+-ATPase (NKA), we introduce four novel mechanisms through which cGMP acts: sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), plasma membrane Ca^{2+}-ATPase (PMCA), inositol 1,4,5-trisphosphate receptor channels type 1 (IP3R1), and myosin light chain phosphatase (MLCP), the latter being critical for Ca^{2+}-desensitization [3]. All these mechanisms, except for the activation of ClCa channels by cGMP/PKG, lead to a decrease in $[Ca^{2+}]_i$ and vasorelaxation. The excessive activation of ClCa channels by high cGMP levels, though, could potentially cause cell depolarization, VOCC activation, increased $[Ca^{2+}]_i$, and possible vasoconstriction [4]. Given that high cGMP levels do not induce contraction, this suggests that the impact of Cl- channels might be negligible or that desensitization of the contractile apparatus at elevated $[Ca^{2+}]_i$ is a crucial mechanism for vasorelaxation. We will investigate these hypotheses through a complex theoretical model that simulates the dynamic interactions of these mechanisms, aiming to elucidate the comprehensive role of cGMP in VSM contractility. Additionally, the model will be used to assess the contribution of each cGMP/PKG-dependent mechanism in modulating the $[Ca^{2+}]_i$ signal and its subsequent role in vasorelaxation. This approach will provide comprehensive insights into the dynamic functioning of the system, enabling the differentiation between essential and non-essential mechanisms and, finally, identifying potential targets for pharmacological intervention.

References

[1] J.C.B. Jacobsen, C. Aalkjær, H. Nilsson, V.V. Matchkov, J. Freiberg, N.-H. Holstein-Rathlo, American Journal of Physiology-Heart and Circulatory Physiology 293(1) (2007) H215-H228.
[2] A. Kapela, A. Bezerianos, N.M. Tsoukias, Journal of theoretical biology 253(2) (2008) 238-260.
[3] A. Fajmut, "Molecular mechanisms and targets of cyclic guanosine monophosphate (cGMP) in vascular smooth muscles" in *Muscle Cell and Tissue - Novel Molecular Targets and Current Advances* (K. Sakuma, Ed.), IntechOpen, London, 2021, p. 1.

SO15 ***Structural motif stability of bacteriophage MS2 RNA pack-aging signals upon changes in their flanking sequence***

V. Bukina[1,2], A. Božič[1]

[1]Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia
[2]Faculty of Mathematics and Physics, University of Ljubljana, Jadranska ulica 19, 1000 Ljubljana, Slovenia
e-mail: veronika.bukina@ijs.si

The RNA motifs can be responsible for specific important functions. For instance, in bacteriophage MS2, they serve as packaging signals (PSs), which play a crucial role in binding viral coat proteins during capsid assembly. This work aims to analyse motif structural stability on the example of the well-studied MS2 virus. As a consequence of current experimental studies, which reveal the multiple putative PS sites across the MS2 RNA genome, we focus on 14 RNA motifs of the virus, including the most widely known TR hairpin. The study of motif stability involves manipulating the flanking sequences surrounding them and comparing various measures calculated from their secondary structure, such as structure probability, ensemble defect, and Shannon entropy. To verify the tertiary structures of these RNA segments, the oxDNA software is employed. Our results show the stability of certain motifs regardless of the presence or absence of sequences around them, even when the nucleotide content of flanking sequences is randomized. Conversely, other unstable motifs are stabilized by genome or flanking sequences that tell us about the importance of the particular structure as well as of the genome sequence. The outcome of this work is beyond viral PS structural stability, extending to applications in RNA binding protein (RBP) studies and more.

SO16 ***Neural Cross-Frequency Coupling Functions: An Application to Sleep***

Tomislav Stankovski

Faculty of Medicine, Ss. Cyril and Methodius University, Skopje 1000, North Macedonia
Department of Physics, Lancaster University, Lancaster LA1 4YB, United Kingdom
e-mail: t.stankovski@medf.ukim.edu.mk

Coupling functions describe the detailed information about the functional mechanisms underlying the interactions and prescribe the physical rule specifying how an interaction occurs [1]. The functional mechanisms can also describe in detailed the causality and the information flow between nonlinear dynamical systems. By focusing on dynamical oscillators and how they interact, we investigated the interactions in the brain. In particular, we used a method based on dynamical Bayesian inference [2] in order to model and reconstruct the coupling functions from data of interacting oscillations. The method accounts also for potential time-varying dynamics and noise interferences, similar to those encountered in biological systems [3,4]. We focus on phase dynamics interactions between brainwave oscillations, extracted from EEG recordings, thus studying the neural cross-frequency coupling functions [5-7]. The effectiveness of the method is demonstrated on analysis of delta-alpha coupling function during whole night sleep, thus observing how this cross-frequency coupling changes during the different sleep stages [8]. The results (Fig. 1) demonstrated that the delta-alpha coupling function was increasing gradually from Awake to NREM3 (non-rapid eye movement), but only during NREM2 and NREM3 deep sleep it was statistically significant. The form of the coupling functions during NREM2 and NREM3 stages had characteristic shape, like a wave along the delta phase axis, pointing that this coupling is predominantly resulting from a direct influence from delta to alpha oscillations. The presented sleep application demonstrates the advantages of using coupling functions for quantifying the mechanisms of directional information flow in neural interactions.

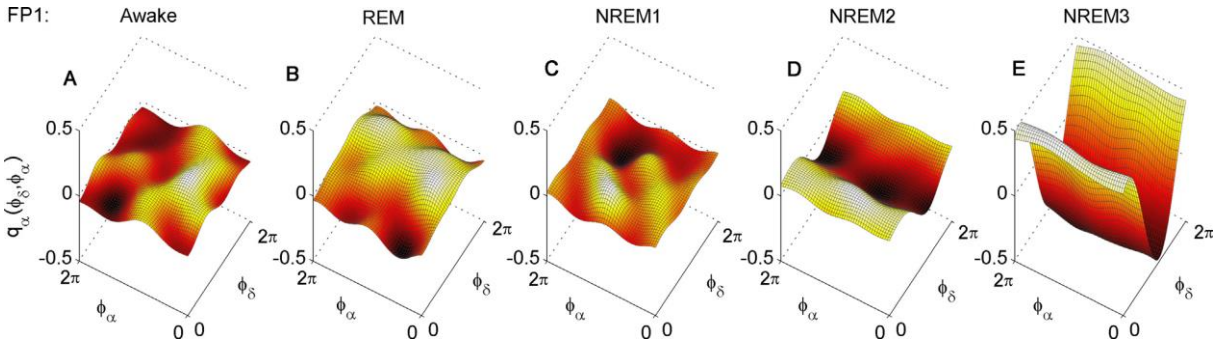


Figure 1: The δ-to-α neural coupling functions for the five different sleep stages in the sleep cycle.

References

[1] Stankovski, T., Pereira, T., McClintock, P. V., & Stefanovska, A. (2017). *Rev. Mod. Phys.*, 89(4), 045001.
[2] Stankovski, T., Duggento, A., McClintock, P.V., & Stefanovska, A. (2012). *Phys. Rev. Lett.*, 109(2), 024101.
[3] Stankovski, T. (2017). *Phys. Rev. E*, 95(2), 022206.
[4] Lukarski, D., Stavrov, D., & Stankovski, T. (2022). *Biomed. Signal Process. Control.*, 71, 103152.
[5] Stankovski, T., Ticcinelli, V., McClintock, P. V., & Stefanovska, A. (2017). *Front. Syst. Neurosci.*, 11, 33.
[6] Stankovski, T., et al. (2016). *Philos. Trans. Royal Soc. A.,* 374(2067), 20150186.
[7] Lukarski, D., Petkoski, S., Ji, P., & Stankovski, T. (2023). *Chaos*, 33(10)
[8] Manasova, D., & Stankovski, T. (2023). *Neuroscience*, 523, 20-30.

Posters (P)

P1 *Elongation index derivative as a new mechanobiological parameter in Diabetes mellitus patients*

Mihajlo D. Radmilović,[1] Vesna Lj. Ilić[2], Drenka Trivanović[2], Ana Petakov[3], Katarina Lalić[3,4], Mihailo D. Rabasović[1], Aleksandar J. Krmpot[1], Ivana T. Drvenica[2]

[1]Institute of Physics Belgrade, University of Belgrade, Pregrevica 18, Belgrade, Serbia
[2]Institute for Medical Research, National Institute of Republic of Serbia, University of Belgrade, Dr. Subotića 4, Belgrade, Serbia
[3]Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Center of Serbia, Dr. Subotića 13, 11000 Belgrade, Serbia
[4]Faculty of Medicine, University of Belgrade, Dr. Subotića 8, 11000 Belgrade, Serbia
e-mail: mihajlor@ipb.ac.rs

Erythrocyte deformability plays pivotal role in blood circulation in Diabetes Mellitus (DM) patients [1,2]. Comparative examination of erythrocyte mechanical properties in DM patients and healthy donors was conducted. We mainly focused on calculating the Elongation Index (*EI*) which was obtained from diffraction images, measured by using ektacytometry. No statistically significant difference in *EI* values on specific physiologically relevant pressures (3Pa or at half maximal value of deformability curve) between DM and control was found, and we introduced the first derivative of deformability curve (d*EI*/d*SS*) as a measure for assessing erythrocyte response to deformation (*SS* denotes shear stress in Pa). Significantly higher values of d*EI*/d*SS* at the half maximum value of the deformability curve were noted in healthy people comparing to DM patients, indicating a slower erythrocyte response to shear stress in DM. Scatter plot analysis exhibited a linear decline in the d*EI*/d*SS* ratio with increasing shear stress, suggesting reduced erythrocyte responsiveness to higher shear stress, particularly evident in DM. Although preliminary, this investigation indicates that d*EI*/d*SS* may offer valuable insights into the hemorheological alterations of DM and enhance comprehension of erythrocyte mechanobiological behavior under different shear stress conditions. Correlations between the proposed measure of erythrocyte mechanical properties and established clinical indicators of DM and its complications such as serum cholesterol and creatinine, will be further explored to gain understanding of the potential application of d*EI*/d*SS* for improved diagnosis and/or patient care.

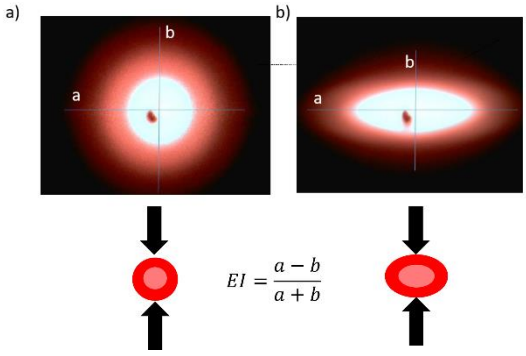


Figure 1: Diffraction images of erythrocytes passing through narrow channel measured by ektacytometry: a) without deformation and b) erythrocytes with maximal deformation shear stress ~ 18 Pa. "a" and "b" represents deformation axis from which *EI* was calculated.

References

[1] J. Kobayashi, K. Ohtake, I. Murata, K. Sonoda, Nitric Oxide 129 (2022) 25-29.
[2] S. Lee, M.Y. Lee, J.S. Nam, S. Kang, J.S. Park, S. Shin, S, C. W. Ahn, K.R. Kim, Diabetes Technology & Therapeutics 17 (2015) 1-8

P2 *When Biological Clocks Lose their Rhythm: Understanding Dysfunction in Populations of Multimodal Biological Oscillators*

U. Barać[1], M. Perc[1,2,3,4,5], M. Gosak[1,2,6]

[1]Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor, Koroška cesta 160, Maribor, Slovenia
[2]Alma Mater Europaea, Slovenska ulica 17, 2000 Maribor, Slovenia
[3]Department of Medical Research, China Medical University Hospital, China Medical University, Taichung, Taiwan
[4]Complexity Science Hub Vienna, Josefstädterstrasse 39, 1080 Vienna, Austria
[5]Department of Physics, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul, Republic of Korea
[6]Institute of Physiology, Faculty of Medicine, Taborska ulica 8, University of Maribor, Maribor, Slovenia
e-mail: uros.barac1@um.si

In real-life systems, macroscopic functionality arises from intricate interactions among microscopic components. These systems, ranging from biological networks to technological infrastructures, are vulnerable to both internal failures and external disturbances. Within this realm, a specialized subfield investigates the critical shift towards macroscopic inactivity in coupled oscillator networks—the aging transition [1]. The phenomenon explores how the global activity of coupled oscillators deteriorates with increasing fraction of inactivated elements and is particularly relevant in biological systems research, where the collective function often arises from the interactions of individual oscillatory units that can progressively malfunction under pathological conditions. Till today, research endeavours were focused on ensembles of coupled limit-cycle [2] or excitable [3] oscillators, but in biological systems the oscillatory activity is often more complex. Namely, many systems demonstrate multimodal activity, characterized by oscillations occurring on diverse timescales and interdependent behaviors. Such rhythmic activity is usually generated by a complex interplay of different oscillator subsystems that are driven by different mechanisms but are intertwined with each other. Amidst the process of pathogenesis, wherein individual cells experience dysfunction, it is possible that only specific subsystems, e.g., the slow ones, are affected by the disease, which, however, affects the overall rhythmic activity and function. To this end, we constructed a two-layered phenomenological network model, with each layer representing its own oscillatory subsystem, one slow and the other fast. Within individual layers, oscillators interact with each other, while the interlayer interactions are encompassed by the modulation of the activity of the fast oscillatory layer by the slow oscillators. Employing this multiplex and multimodal model, we proceeded to methodically examine the impact of oscillator inactivation within the slow oscillatory layer on the macroscopic activity of the fast oscillatory layer. Specifically, we investigate the effects of coupling strengths and the ratio of inactive oscillators on the average global amplitude and frequency of the fast system signal. Our numerical analyses unveil intriguing behaviours intricately reliant on the interplay between the fraction of inactivated units and the coupling strengths among both types of oscillators. We show that under specific coupling schemes the inactivation of the slow subsystem can lead to a loss in synchronization of the slow components and thus enhanced activity of the fast population which on the global level showcases itself as an increase in the signal frequency. We hope that these insights will improve our understanding of the robustness and fragility of oscillatory biological networks, such as populations of muscle and endocrine cells or neuronal ensembles.

References

[1] W. Zou, D. V. Senthilkumar, M. Zhan, J. Kurths, Physics Reports **931** (2021) 1-72.
[2] K. Daido, N. Nakanishi, Phys. Rev. Lett. **93** (2004) 104101.
[3] U. Barać, M. Perc, M. Gosak, Chaos **33** (2023) 043120.

P3 ***Influence of high cholesterol content and charged lipids on electroformation of giant unilamellar vesicles from damp films***

I. Mardešić, Z. Boban, M. Raguž

Department of medical physics and biophysics, University of Split School of Medicine, Split, Croatia
e-mail: imardesi@mefst.hr

Giant unilamellar vesicles (GUVs) are frequently used as membrane models in studies on membrane properties [1]. They are most commonly produced using the electroformation method. However, there are a variety of parameters that can influence the successfulness of the preparation. The traditional electroformation protocol was found to be incompatible with lipid mixtures containing a high concentration of cholesterol (Chol). This is due to the precipitation of Chol into anhydrous Chol crystals during the formation of dry lipid film. When the film is rehydrated, these anhydrous Chol crystals do not participate in the formation of the bilayer, resulting in a lower Chol concentration in the vesicle bilayers compared to the Chol concentration in the initial lipid solution [2]. The presence of charged lipids also has a negative impact on vesicle formation [3]. Modelling membranes with such a high Chol concentration and charged lipids is important for groups like ours that study the properties of the eye lens plasma membranes or for groups performing atherosclerosis research. Primarily because of the problem of artefactual Chol demixing, we modified the electroformation protocol by incorporating rapid solvent exchange, ultrasonication, plasma cleaning and spin coating techniques to reproducibly produce GUVs from damp lipid films. In addition to solving the Chol demixing artifact, we expected to successfully produce GUVs from lipid mixtures containing charged lipids using this modified protocol. In our samples the mixing ratio of Chol/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS)/(POPC + Chol) was ranging from 0 to 2.5 and 0 to 1.5, respectively. A high yield of GUVs was obtained for all samples. The average diameter and yield of GUVs decreased with increasing Chol content. A higher POPS content also reduced the GUVs yield.

References

[1] Ertel, A.; Marangoni, A.G.; Marsh, J.; Hallett, F.R.; Wood, J.M. Mechanical Properties of Vesicles. I. Coordinated Analysis of Osmotic Swelling and Lysis. Biophys. J. 1993, 64, 426–434, doi:10.1016/S0006-3495(93)81383-3.
[2] Baykal-Caglar, E.; Hassan-Zadeh, E.; Saremi, B.; Huang, J. Preparation of Giant Unilamellar Vesicles from Damp Lipid Film for Better Lipid Compositional Uniformity. Biochim. Biophys. Acta - Biomembr. 2012, 1818, 2598–2604, doi:10.1016/j.bbamem.2012.05.023.
[3] Boban, Z.; Mardešić, I.; Subczynski, W.K.; Raguz, M. Giant Unilamellar Vesicle Electroformation: What to Use, What to Avoid, and How to Quantify the Results. Membranes (Basel). 2021, 11, 860, doi:10.3390/membranes11110860.

P4 ***Prediction of the biomechanical drivers of cell delamination in multi-layered epithelial tissues using a dynamic 3D vertex model***

Somiealo Azote,[1] Clementine Villeneuse[2], Matthias Rübsam [3], Elizabeth Lawson-Keister [1], Sara A. Wickström[2], Carien M. Niessen[3], M. Lisa Manning [1]

[1]Syracuse University, Syracuse, New York, 13244, USA
[2]Max Planck Institute for Molecular Biomedicine, 48149 Münster, Germany
[3]Department of cell Biology of the skin, University Hospital, University hospital, Cologne University, Germany
e-mail: sazoteep@syr.edu

Stratified epithelial tissues, such as skin and gut, comprise major organs in humans and perform multiple functions, including serving as a barrier to mechanical insults and pathogens. They are constantly self-renewing: balancing stem cell proliferation in the basal layer with a tightly controlled differentiation program in which cells move upward while undergoing stepwise transcriptional and cell shape changes to form the distinct suprabasal layers. Understanding how mechanosensitive mechanisms at the scale of molecules couple to the collective mechanical behavior of the self-renewing tissue, allowing cells to move upward across the sharp basal-suprabasal boundary to regulate stratified tissue homeostasis, is thus essential. Although some delamination events are coupled to cell division, we first focus on the simpler case where delamination occurs in the absence of cell division, and develop a biomechanical model to investigate several experimentally driven hypotheses for what drives delamination those cases: i) changes in the adhesion of basal cells to extra cellular matrix in the basement membrane, ii) local fluidization of surrounding tissue due to fluctuations or nearby cell divisions, or iii) cell autonomous changes to cell-cell adhesion and cortical tension. We investigate these hypotheses using computational simulations of a novel dynamic 3D Vertex model of stratified epithelia, recently developed in our group. Experimental data from the developing mammalian epithelium in the Niessen and Wickström labs have identified specific changes to the transcriptome of cells committed to delamination. Many of these changes are associated with cell-cell and cell-substrate adhesion pathways. We incorporate them in the computational models as changes to the model parameters describing heterotypic and homotypic cell-cell interfacial tensions and adhesion to a fiber network substrate. We make quantitative predictions for cell shapes, delamination probabilities, and delamination speeds that we compare directly to experiments, in both control and perturbed systems, in order to determine how different mechanisms are driving delamination.

Acknowledgments

This work is supported by grants from NSF (Grant NSF-CMMI-1334611 to Lisa Manning) and from Future Professor Postdoctoral Fellowship from Syracuse University award to Somiealo Azote.

References

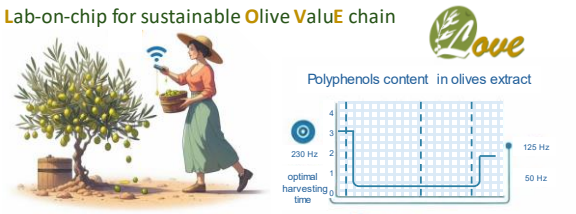
[1] Zhang, Tao, and J. M. Schwarz, *Physical Review Research* 4.4 (2022), 043148.
[2] Villeneuve, Clémentine, et al., *Nature Cell Biology* (2024), 1-12

P5 ***Polyphenols as Olive Quality Markers: a LOVE-ly Lab-on-Chip for Sustainable Olive Value Chain***

G. Tori,[1] M. Gagliardi,[2] A. Bianchi[3], M. Cecchini,[2] C. Sanmartin[3], A. Battisti[2]

[1]NEST, Scuola Normale Superiore, p.zza S. Silvestro 12, 56127 Pisa (Italy)
[2]NEST, Nanoscience Institute – CNR and Scuola Normale Superiore, p.zza S. Silvestro 12, 56127 Pisa (Italy)
[3]DISAAA, Università di Pisa, v. del Borghetto 80, 56124 Pisa (Italy)
e-mail: antonella.battisti@nano.cnr.it

Polyphenols are bioactive compounds that offer a multitude of potential health benefits, principally due to their antioxidant effect. These compounds combat cell damage caused by oxidative stress and have been linked to a reduced risk of developing chronic conditions such as type 2 diabetes, cardiovascular diseases, cancer, cognitive disorders.[1] They also play a crucial role in plant defense mechanisms, where they serve as secondary metabolites that shield plants from ultraviolet radiation and pathogen aggression. Olive fruits are rich in some specific polyphenols such as hydroxytyrosol, tyrosol, oleuropein and caffeic acid, and their overall concentration can be utilized to assess ripeness.[2] An optimal ripening degree corresponds to high-quality fruits, which, in turn, contribute to the production of premium olive derivatives. However, detection of polyphenol content is still somewhat tricky, and requires expensive and/or time-consuming methods such as mass spectrometry, chemiluminescence, electroanalysis, chromatography. Contrasting the current approach of collecting samples, transporting them to analytical labs, and waiting for results, the LOVE project (Lab-on-chip for sustainable Olive ValuE chain) proposes a simplified, cost-effective, and time-efficient strategy for evaluating the phenolic content in olives. This innovative approach relies on an acoustic sensor with an optimized surface, enabling rapid detection of polyphenols in olive extracts. Some of the more represented phenols in olive products were selected as standard references to test the sensor, then samples of olive derivatives extracted from different olive cultivars were analysed to correlate their phenol content with the results of conventional analyses. Drawing an analogy to similar achievements in the wine industry,[3] this platform could revolutionize olive quality assessment by providing real-time, in situ measurements during the ripening process. Ultimately, it aims to enhance farming efficiency and elevate the overall quality of olive production.



Acknowledgments

Project PRIN 2022 - Cod. 2022M4WB3M (LS9) LOVE – "Lab-on-chip for sustainable Olive ValuE chain: development of a lab-on-chip for detecting polyphenolic compounds in olive and its derivatives" - CUP B53D23017860006, funded by the European Union – Next Generation EU in the context of the Italian National Recovery and Resilience Plan, Mission 4/ Component 2/ Investment 1.1, "Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)"

References

[1] A. Rana, *et al.* J Food Biochem 46 (2022) e14264.
[2] M. D. P. Fernández-Poyatos, *et al.* Foods 10 (2021) 380.
[3] M. Gagliardi, *et al.* Nanomaterials 12 (2022) 166.

P6 ***New Era in Precision Treatment of Pancreatic Cancers: Electrically-Controlled Release of Hydrogel-conjugated Chemotherapeutics***

Christian Bayer[1], Helena Saarela[2], Marle Vleugels[2], Donghak Byun[2], Iwona Bernacka Wojcik[2], Tobias Abrahamsson[2], Nikolaus Poremba[3], Sebastian Hecko[3], Martin Wilkovitsch[3], Hannes Mikula[3], Tonny Bosman[4], Nasssim Ghaffari Tabrizi-Wizsy[5], Rainer Schindl[1], Daniel Simon[2], Theresia Arbring Sjöström[2], Johannes Bintinger[2], Linda Waldherr

[1]Gottfried Schatz Research Center – Division of Medical Physics and Biophysics Medical University of Graz, Graz, 8010 Austria

Laboratory of Organic Electronics Department of Science and Technology Linköping University, Norrköping, 60174 Sweden

[3]Institute of Chemical Technologies and Analytics Technische Universität Wien, Vienna, 1060 Austria

[4]SupraPolix, Eindhoven, 5612 AX Netherlands

[5]Otto Loewi Research Center – Division of Immunology Medical University of Graz, Graz, 8010 Austria

e-mail : christian.bayer@medunigraz.at

Numerous cancer treatments, involving chemotherapy, face challenges with their effectiveness, primarily stemming from problems related to drug delivery efficiency and systemic toxicity. Our focus lies on addressing the complex challenge of combating hard-to-treat cancers, with a specific emphasis on Pancreatic Adenocarcinoma (PAAD). In this research, we present a novel approach that utilizes iontronic devices to achieve precise and controlled delivery of a specific triggering molecule into a hydrogel containing a chemotherapeutic drug covalently bound within the gel. Inside this hydrogel, the triggering molecule initiates a "click-to-release" (C2R) reaction, enabling the electrically controlled release of the drug directly at the tumor site. Our primary goal is to enhance the effectiveness of the released drugs by accurately and efficiently targeting the tumor site, thereby optimizing their anti-cancer impact and reducing side effects. The components of the C2R system were tested on multiple cancer cell lines to assess their IC_{50} concentrations and toxicity levels. Simultaneously, the rates of iontronically-mediated trigger molecule delivery were evaluated using two different ion exchange membranes. Subsequently, we conducted experiments to test the iontronic-controlled C2R of potent chemotherapy drugs and hydrogel prototypes in two distinct cancer models: a 2D cell culture system and a 3D *in vivo* tumor model, the chick chorioallantoic membrane (CAM) model. We successfully performed an iontronically controlled C2R of a potent drug from its prodrug from. Precise control over the activation and deactivation of the system was successfully achieved, allowing the effective regulation of cancer cell death. These results underscore the significant potential of this approach and lay the groundwork for further pre-clinical investigations into a novel therapeutic strategy for PAAD.

This project has received funding from the European Union's Horizon Europe Research and Innovation Programme under Grant Agreement No 101099963.

P7 *Maximum entropy production and Shannon information entropy in an enzyme cascade and enzyme complex*

T. Blaževič,[1] A. Dobovišek[1,2]

[1]Faculty of Natural Sciences and Mathematics, University of Maribor,
Koroška cesta 160, 2000 Maribor, Slovenia
[2]Faculty of Medicine, University of Maribor, Taborska ulica 8, 2000 Maribor, Slovenia
e-mail: tina.blazevic@student.um.si

In cellular environments, enzymatic reactions are characterized as open and non-equilibrium systems that exchange matter and energy with their surroundings. These systems are known to spontaneously organize into non-equilibrium steady states, characterized by maximal entropy production rate. Our study provides a detailed thermodynamic analysis of two regulatory mechanisms: an enzyme cascade, controlled by two distinct enzymes, and a reaction facilitated by an enzyme complex, where multiple enzymes function as a single biological catalyst [1,2].
Initially, we identify the most probable thermodynamic state for each system through the Maximum Entropy Production Principle (MEPP), allowing us to pinpoint the most probable reaction parameters [3]. Our analysis reveals that, in the case of the enzyme cascade, variables such as reaction rate, Shannon information entropy, and system stability are influenced by the concentration of the intermediate product within the cascade. Conversely, for reactions mediated by enzyme complexes, these parameters appear independent of intermediate product concentration. Furthermore, we employ theoretical models to investigate spontaneous transitions between these two regulatory mechanisms based on variations in intermediate product concentration within enzyme cascade. Our findings indicate conditions under which one type of regulation may be thermodynamically favored over the other.
This work not only advances our understanding of the thermodynamics of enzymatic reactions within cellular systems but also sheds light on the conditions under which certain regulatory mechanisms are preferred. These insights might have significant implications for both our theoretical understanding of biological systems as well as development of biotechnological applications.

References

[1] M. Pröschel, R. Detsch, A. R. Boccaccini, U. Sonnewald, Front. Bioeng. Biotechnol. 3 (2015) 1-13.
[2] S. Schoffelen, B. Baric, J. C. M van Hest, Soft Matter 8 (2012) 1736-1746.
[3] A. Dobovišek, M. Vitas, T. Blaževič, R. Markovič, M. Marhl, A. Fajmut, , Int. J. Mol. Sci. 24, 8734 (2023).

P8 *Insights into the Alzheimer's disease specific pre-aggregation conformation of monomeric tau proteins using conformational antibodies*

Ondrej Cehlar,[1] Stefana Njemoga,[1] Rostislav Skrabana[1], Adam Polak[1], Radovan Dvorsky[1], Lubica Fialova[1], Petra Majerova[1], Viliam Volko[2], Jozef Hritz[2,3], Pavel Kaderavek[2], Branislav Kovacech[1]

[1]Institute of Neuroimmunology, Laboratory of Structural Biology of Neurodegeneration, Slovak Academy of Sciences, Bratislava, Slovakia
[2]CEITEC MU, Brno, Czech Republic
[3]Faculty of Science, Department of Chemistry, Masaryk University, Brno, Czech Republic
e-mail: ondrej.cehlar@savba.sk

A key yet unresolved question of the pathogenesis of Alzheimer's disease (AD) and other tauopathies is the cause and the mechanism of the transition from the unstructured monomeric tau protein to the insoluble filaments deposited in the brain tissue. In the physiological state, tau protein exists as a conformational ensemble of interconverting structures and on the scale of transition from monomeric through oligomeric and filamentous species we can observe conformations reacting with specific antibodies, mainly with DC11, which is able to specifically discriminate between tau proteins isolated from healthy brain and tau proteins isolated from the brain of AD patient. The antibody recognizes also the recombinant truncated tau proteins up to the shortest fragment tau321-391 [1]. It was found that conformational antibodies DC11 and MN423 have catalytic pro-aggregatory effects in tau aggregation assay, whereas the antibody DC8E8 has inhibitory effects on tau filament formation [2]. This may imply possible mechanism of induction of pathological tau conformation, in which the antibody prepared against pathological tau imprints the pathological conformation into the physiological tau proteins in solution and therefore speeds up the tau aggregation. The information about conformational epitopes of these antibodies is therefore of high significance. To further uncover the binding mode of the conformational antibody DC11, we have performed NMR epitope mapping using ^{13}C, ^{15}N labelled tau321-391 and tau297-391 (dGAE) and recombinantly prepared Fab fragment of DC11 antibody. The overlay of HSQC spectra showed the region of tau between residues 370-390 to be affected by the binding of DC11, i.e., its C-terminal region. However, previous studies suggest the importance of region 321-325 for the interaction of tau with DC11 antibody. We have further characterized the influence of DC11 Fab binding on the non-epitope tau residues using NMR relaxation measurements of ^{15}N labelled tau dGAE.
The results highlight the importance of the R' region of tau, that was recently shown to be important also for tau interaction with microtubules [3]. This sequence forms the interface of rigid filament core and flanking fuzzy C terminal segment in solved tauopathy filaments.

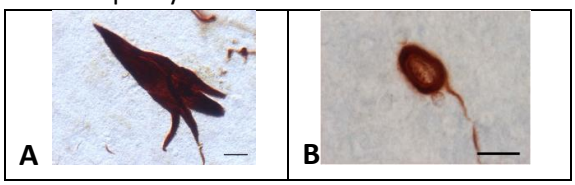


Figure 1: DC11 stains tau present in the AD brain: (**A**) neurofibrillary tangles (scale bar: 10 µm) and (**B**) ghost tangles (scale bar 20 µm).

Acknowledgments

This work was supported by the European Union's Horizon Europe program under the grant agreement No. 101087124 (ADDIT-CE). This work was supported by research grants APVV 21-0479, VEGA 2/0125/23 and 2/0141/23, and by iNEXT-Discovery, grant number 871037, and MSCA-RISE 873127, both funded by the Horizon 2020 program of the European Commission. This work was also supported by the CA21160 - ML4NGP.

References

[1] Vechterova, L.; Kontsekova, E.; Zilka, N.; Ferencik, M.; Ravid, R.; Novak, M. Neuroreport, 14 (2003),87-91.
[2] Kontsekova, E.; Zilka, N.; Kovacech, B.; Skrabana, R.; Novak, M. Alzheimers Res Ther, 6 (2014), 45.
[3] El Mammeri, N., Dregni, A. J., Duan, P., Wang, H. K., Hong, M. Sci. Adv., 8 (2022), 4459.

P9 ***Electrochemical and EPR Characterization of Stem Cells for Regenerative Medicine Applications***

Marko Daković,[1] Đura Nakarada,[1] Aleksandar Savić,[2] Dijana Mitić,[3] Aleksandra Pavićević,[1] Zorica Mojović[4], Miloš Mojović[1]

[1]Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
[2] Maecenium and Perun Biosciences, Houston, TX 77406, United States
[3]Implant-Research Center, Faculty of Dental Medicine, University of Belgrade, Rankeova 4, 11000 Belgrade, Serbia
[4]Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia
e-mail: marko@ffh.bg.ac.rs

Stem cell research has emerged as a promising avenue for regenerative medicine, offering potential solutions for various medical conditions, particularly wound healing, such as burns, where conventional treatments often fall short. Central to this field is the exploration of stem cells and stem cell-conditioned media, which have shown remarkable therapeutic potential [1,2]. Stem cell-conditioned media contain a complex array of bioactive molecules secreted by stem cells during their growth, including growth factors, cytokines, and extracellular vesicles. These factors play critical roles in modulating cellular behavior, promoting tissue repair, and stimulating regeneration. As research in this area progresses, stem cell-conditioned media are becoming increasingly recognized for their versatility and efficacy in various applications, driving further exploration and development in regenerative medicine.

This study focuses on the utilization of a combined Electrochemistry and Electron Paramagnetic Resonance (EPR) spectroscopy approach for the rapid and comprehensive characterization of stem cells. Real-time electrochemical assessment has been shown to be a valuable tool for monitoring dental pulp stem cell (DPSC) health, offering a faster alternative to traditional viability assays. Since redox processes play a vital role in cell regeneration and are indicative of cellular health and function, EPR spectroscopy has been utilized by means of investigating the capacity of DPSC to scavenge free radical species, especially ROS and RNS.

Obtained results have shown that the integration of electrochemical and EPR techniques is a powerful toolkit for the comprehensive characterization of stem cells, shedding light on their physiological conditions and potential applications in wound healing and tissue regeneration. Moreover, our findings suggest that this approach can be extended to study other types of stem cells and various pathological conditions, offering novel pathways for the development of innovative regenerative therapies.

Acknowledgments

This work was financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers 451-03-65/2024-03/200146, 451-03-66/2024-03/200146 and the Science Fund of the Republic of Serbia - Program IDEJE, contract number 7750038.

References

[1] P. Jayaraman, P. Nathan, P. Vasanthan, S. Musa, V. Govindasamy, Cell Biology International 37 (2013) 1122–1128.
[2] Y. An, S. Lin, X. Tan, S. Zhu, F. Nie, Y. Zhen, L. Gu, C. Zhang, B. Wang, W. Wei, D. Li, J. Wu, Cell Proliferation, 54 (2021) 1-12.

P10 *The Role of Magnesium Cations in Mitigating Ionizing Radiation Damage to DNA: Insights from FTIR Spectroscopy*

Sanja Dolanski Babić, Nikola Šegedin, Antonia Lovrenčić, Josip Basić, Martin Bobek, Lucija Gojmerac, Kristina Serec

Department of Physics and Biophysics, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia
e-mail: kristina.serec@mef.hr

The presence of cations such as Mg can play a significant role in reducing the harmful effects of ionizing radiation on biological systems [1]. Cations like magnesium can mitigate irradiation damage through various mechanisms, stabilize DNA structures by binding to the phosphate backbone, thereby shielding it from direct radiation-induced damage and influence the repair mechanisms within cells, enhancing the efficiency of DNA damage repair processes post-irradiation [2,3].

This study explores the impact of ionizing radiation on DNA in the presence and absence of magnesium ions utilizing the FTIR spectroscopy. Solutions containing DNA at various concentrations, along with different concentrations of magnesium cations were prepared and irradiated with different absorbed doses of Co-60 beams used by the Gamma Knife Icon build on the Perfexion platform, a device typically utilized in brain radiosurgery. For the irradiation purposes, the DNA solution was placed at the custom 3D printed insert, made of tissue-equivalent plastics, employed with the PMMA phantom and positioned at the device's isocentre. In addition, to account for the effects of polyion length, we studied long chain DNA, from 2 to 20 kilobase pairs (kbp) with an average length of 4 micrometers (μm), and short-chain DNA samples spanning from 15 to 50 base pairs (bp), estimated to be between 5 and 17 nanometers (nm) in length. DNA ion atmosphere has been controlled by the counterion to phosphate molar concentration ratio r, which varied between 0.01 and 2. Following irradiation, the samples were immediately transferred to an ice bath to halt any further denaturation process.

References

[1] T. Theophanides and J. Anastassopoulou, Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 52 (2017) 1030-1040.
[2] K. Sofińska, N. Wilkosz, M. Szymoński, and E. Lipiec, Molecules 25 (2020) 561-581.
[3] K. Serec, S. D. Babić, and S. Tomić, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 268 (2022) 120663-120673.

P11 ***The role of ionic liquids' cations in the stability and aggregation of proteins***

D. Fedunova,[1] Z. Bednarikova[1], A. Antosova[1], V. Vanik[1], J. Marek[1], M. Gancar[1], M. Guncheva[2], Z. Gazova[1]

[1]Institute of Experimental Physics, Slovak Academy of Sciences, Kosice, Slovakia
[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
e-mail: fedunova@saske.sk

Amyloid fibrils have unique structural and mechanical properties that make them promising biomaterials for various applications in nanotechnology, tissue engineering, and drug delivery. Understanding the mechanism of amyloid fibril formation and developing strategies to control this process is of great interest. Solvent characteristics are among the critical factors affecting the protein self-assembly into amyloid fibrils. Ionic liquids (ILs), known as "designer solvents," are innovative solvents comprising organic cations and organic/inorganic anions, offering adjustable physicochemical properties via compositional variations. Previously, the anionic component of inorganic salt-based solvents was considered to have a greater impact on protein aggregation, stability, or activity. However, ILs or other organic salts with large organic cations have been demonstrated to exert a comparable influence [1].

In this study, we investigated the impact of various ILs containing choline (CHOL) and n-alkyl imidazolium (IM) cations with different aliphatic and aromatic substituents on the amyloid aggregation of insulin (IN) and lysozyme (LZ). Our findings demonstrate that ILs can enhance or reduce protein stability, affect fibril formation kinetics, and induce the formation of distinct fibril morphologies based on cation concentration and hydrophobicity. We have shown that at lower concentrations of ILs, up to 50 mM, ILs enhance the activity of LZ while preserving its native structure and enhancing its thermal stability, with the order of efficacy being benzyl > propyl > allyl substituents. However, a decline in thermal stability and activity is observed at higher concentrations. ILs composed of n-alkyl imidazolium cations [n = 4, 6, 8, 10] were observed to accelerate or inhibit IN amyloid aggregation's kinetics depending on concentration and n-alkyl chain length. The resulting fibrils exhibited a needle-like morphology with varying heights, lengths, and propensity for lateral association or clustering. ILs cations interact with the amyloid aggregation-prone hydrophobic region of the protein, with the affinity increasing with the n-alkyl chain increase. Overall, IL effects result from a delicate interplay between specific and non-specific protein-ion, ion-water interactions, and long-range electrostatic shielding. Harnessing ILs as modulators of protein amyloid aggregation holds the potential for enhancing biotechnological applications of fibrils and advancing the understanding of amyloid aggregation mechanisms in complex solvents like ILs.

Acknowledgments

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0598; Slovak Grant Agency VEGA 02/164/22, 02/0176/21 and the Operational Programme Integrated Infrastructure, the project "NANOVIR ", ITMS: 313011AUW7, co-funded by ERDF.

References

[1] T. Takekiyo, Y. Yoshimura, Biophys. Rev. 10 (2018) 853 -860.

P12 *GLIONICs Precision Chemotherapy for Enhanced Tumor Treatment*

F. Telebar-Žbulj[1,2], V. Handl[1], I. Bogdan[1], I. Bernacka Wojcik[3], H. Saarela[3], M. Nowakowska[5], S. Erschen[1], A. Gorischek[1], T. Tomin[4], T. Arbring Sjöström[3], J. Bintinger[3], S. Ropele[6], M. Üçal[5], D. T. Simon[3], R. Schindl[1], L. Waldherr[1,2]

[1]Gottfried Schatz Research Center – Biophysics, Medical University of Graz, 8010 Graz, Austria
[2]BioTechMed-Graz, Austria, Auenbruggerplatz 30, 8036 Graz, Austria
[3]Laboratory of Organic Electronics, Linköping University, 60174 Norrköping, Sweden
[4]Institute of Chemical Technologies and Analytics, Technische Universität Wien, 1060 Vienna, Austria
[5]Research Unit of Experimental Neurotraumatology, Medical University of Graz, 8010 Graz, Austria
[6]Division of General Neurology, Medical University of Graz
e-mail: franciska.telebar@medunigraz.at

Administering chemotherapeutics locally and over extended periods has shown great promise in enhancing the potency of established cancer treatments. We utilize iontronic tools for the continuous administration of anti-tumor drugs, achieving sustained high local drug concentrations at the tumor site over several days. We showed that this drug concentration profile effectively diminishes tumor size compared to conventional drop-casting methods in *in-vitro*[1] and *in-vivo*[2] models.

We have developed a C6 Glioblastoma (GMB) rat brain model. Using stereotactic surgery, we implant a catheter intracranially into the right parietal cortex[3]. After allowing the complete wound healing process, we inject C6 cells through the catheter using a syringe pump. Tumor growth is monitored via MRI imaging every 2-3 days. Once the tumor reaches approximately 3 mm in diameter, we initiate treatment with the Glioblastoma Iontronic (GLIONC) pump.

Our rat model closely mimics the human condition, exhibiting inter- and intra-tumoral heterogeneity. We have established markers to distinguish between tumor tissue (GFAP, Ki67) and healthy brain tissue (PLP, NeuN) and observed significant macrophage infiltration within the tumor. Additionally, we have developed a pharmacokinetic method to track the drug in various tissues, including the DNA and cytosolic fractions. Our experiments have shown that the GLIONC pump can effectively operate within our setup in the rat brain, maintaining stable current traces in a sleeping rat. We further demonstrated stable drug delivery using the GLIONIC device with KCl and Gemcitabine in an awake rat for 24 hours.

Our future goal is to establish a wireless set-up in the Rat Glioblastoma model, performing short-term delivery for pharmacokinetic studies and long-term constant delivery for brain tumor treatment.

References

[1] Waldherr, L. *et al.* Targeted Chemotherapy of Glioblastoma Spheroids with an Iontronic Pump. *Adv Mater Technol* **6**, 2001302 (2021).
[2] Handl, V. *et al.* Continuous iontronic chemotherapy reduces brain tumor growth in embryonic avian in vivo models. *J Control Release* **369**, 668–683 (2024).
[3] Üçal, M. *et al.* Rat Model of Widespread Cerebral Cortical Demyelination Induced by an Intracerebral Injection of Pro-Inflammatory Cytokines. *JoVE (Journal of Visualized Experiments)* e57879 (2021) doi:10.3791/57879.

P13 *Strucural analysis of the EB1 protein*

Péter Gaszler[1,2], János Erostyák[3,4], Andrea Vig[1], Tony Wesely[1], Fruzsina Szász[1], Mónika Ágnes Tóth[1,2], Rauan Sakenov[1], Beáta Bugyi[1,2]

[1]Department of Medical Biology, Medical School, University of Pécs, Szigeti Street 12, H-7624 Pécs, Hungary
[2]The Expert Committee of Physics and Astronomy, Spectroscopy Committee, Regional Committee of The Hungarian Academy of Sciences at Pécs, Pécs, Hungary
[3]Department of Experimental Physics, Faculty of Sciences, Univ. of Pécs, Ifjúság Street 6, H-7624 Pécs, Hungary
[4]János Szentágothai Research Centre, University of Pécs, Pécs, Ifjúság Street 20, H-7624 Pécs, Hungary
e-mail: peter.gaszler@aok.pte.hu

EB1 (End-binding protein 1, also known as MAPRE1) is a member of the +TIPS (microtubule plus-end tracking proteins) family. Its N-terminal calponin homology (CH) domain binds to microtubules and actin filaments, potentiating EB1 as a cytolinker of the two polymer systems. The C-terminus (EBH-tail) acts as a structural scaffold for dimerization and +TIP interactions. Our study aims to investigate EB1's structural properties and its capacity to form droplets via LLPS (liquid-liquid phase separation) or aggregates.

We recombinantly produced the EB1 protein along with its N- and C-terminally truncated fragments. Initially, we assessed the protein's structural characteristics using in silico methods. We then measured its aggregation propensity using the aggregation index from absorption spectra [1]. Protein stability was investigated using fluorescence spectroscopy (tryptophan) and differential scanning calorimetry.

In contrast to a previous study [2], we have not yet observed LLPS of EB1, but we have observed the formation of aggregates. The fluorescence intensity of buried tryptophan in the CH domain increases during denaturation. This phenomenon can be explained by a relatively rare occurrence where the indole nitrogen participates in an NH . . . π- hydrogen bond with the ring of another aromatic residue [3]. The CH domain of EB1 exhibits lower stability (41°C) compared to the C-terminal CH domains of utrophin (57°C or 71.5°C) [4].

We discovered the concentration-dependent aggregation capability of EB1 and the unusual increase in the intensity of Trp emission during denaturation within its CH domain. This provides a valuable tool for probing its denaturation. Furthermore, we identified the CH domain's low stability. In the subsequent studies, we aim to explore how these structural features correlate with EB1's activity.

Acknowledgments

Supported by the ÚNKP-22-3-II-PTE-1607 New National Excellence Program of The Ministry for Culture and Innovation. We thank József Mihály and István Földi (Biological Research Centre, Szeged) for the EB1 plasmids. J. E. was supported by TKP2021-EGA-17 provided by the National Research, Development and Innovation Fund of Hungary, financed under the EGA 17 funding scheme.

References

[1] Katayama DS, Nayar R, Chou DK*, et al.* Solution behavior of a novel type 1 interferon, interferon-tau. J Pharm Sci 2005;94(12):2703-15.
[2] Song X, Yang F, Yang T*, et al.* Phase separation of EB1 guides microtubule plus-end dynamics. Nat Cell Biol 2023;25(1):79-91.
[3] Nanda V, Brand L. Aromatic interactions in homeodomains contribute to the low quantum yield of a conserved, buried tryptophan. Proteins 2000;40(1):112-25.
[4] Bandi S, Singh SM, Mallela KM. The C-terminal domain of the utrophin tandem calponin-homology domain appears to be thermodynamically and kinetically more stable than the full-length protein. Biochemistry 2014;53(14):2209-11.

P14 ***Single-cell classification based on label-free high-resolution optical data of cell adhesion kinetics***

Kinga Dora Kovacs[1,2], Balint Beres[1,3], Nicolett Kanyo[1], Balint Szabó[2], Beatrix Peter[1], Szilvia Bősze[4], Inna Szekacs[1], Robert Horvath[1]

[1]Nanobiosensorics Group, Institute of Technical Physics and Materials Science, HUN-REN Centre for Energy Research, Konkoly-Thege út 29-33, H-1120 Budapest, Hungary
[2]Department of Biological Physics, Eötvös University, Budapest, Hungary
[3]Department of Automation and Applied Informatics, Faculty of Electrical Engineering and Informatics, Budapest University of Technology and Economics, Műegyetem rkp. 3., H-1111 Budapest, Hungary
[4]HUN-REN-ELTE Research Group of Peptide Chemistry, Hungarian Research Network, Eötvös Loránd University, 1117 Budapest, Hungary
e-mail: kovacskingadora@gmail.com

Selecting and isolating various cell types is a critical procedure in many applications, including immune therapy, regenerative medicine, and cancer research. Usually, these selection processes involve some labeling or another invasive step potentially affecting cellular functionality or damaging the cell. In the current proof of principle study, we first introduce an optical biosensor-based method capable of classification between healthy and numerous cancerous cell types in a label-free setup. We present high classification accuracy based on the monitored single-cell adhesion kinetic signals. We developed a high-throughput data processing pipeline to build a benchmark database of ~4500 single-cell adhesion measurements of a normal preosteoblast (MC3T3-E1) and various cancer (HeLa, LCLC-103H, MDA-MB-231, MCF-7) cell lines. Several datasets were used with different cell-line selections to test the performance of deep learning-based classification models, reaching 70- 83% depending on the classification task. Beyond testing these models, we aimed to draw interpretable biological insights from their results; thus, we applied a deep neural network visualization method (grad-CAM) to reveal the basis on which these complex models made their decisions. Our proof-of-concept work demonstrated the success of a deep neural network using merely label-free biosensor kinetic data to classify single mammalian cells into different cell types. We propose our method for label-free single-cell profiling and in vitro cancer research involving adhesion. The employed label-free measurement is noninvasive and does not affect cellular functionality. Therefore, it could also be adapted for applications where the selected cells need further processing, such as immune therapy and regenerative medicine.

P15 ***Simulation of the Effect of Various Phosphodiesterase 5 Inhibitors on the Production of Cyclic Guanosine Monophosphate***

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

[1]Department of Physics, Faculty of Natural Sciences and Mathematics, University of Maribor
[2]Faculty of Health Sciences, University of Maribor
e-mail: katarina.mestrovic1@student.um.si

Phosphodiesterases (PDE) are a large family of enzymes whose function is the degradation of two key signaling molecules, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The discovery that sildenafil, a potent PDE-5 inhibitor, effectively relaxes not only pulmonary arteries but also the blood vessels of the corpus cavernosum led to its successful application in treating erectile dysfunction [1]. Since PDE-5 is also expressed in smooth muscle cells of the kidneys, bladder, and pancreas, the clinical applications of PDE-5 inhibitors could extend beyond just pulmonary hypertension and erectile dysfunction. Consequently, other PDE-5 inhibitors, such as vardenafil, tadalafil, and avanafil, have been developed. Their efficacy is significantly influenced by their potency of inhibition of PDE-5 and their pharmacokinetic properties, including absorption and elimination rates, bioavailability, and peak plasma concentration. Furthermore, their selectivity for PDE-5 is critical in minimizing the side effects of treatment. Our research examines the impact of PDE-5 inhibitors (sildenafil, vardenafil, tadalafil, and avanafil) on cGMP production and degradation in vascular smooth muscle cells. We have upgraded an existing mathematical model for cGMP production [2] by incorporating a pharmacokinetic model for the oral administration of PDE5 inhibitors and their inhibitory effect on PDE-5 activity. This enhancement enables us to investigate the temporal effects of these inhibitors on cGMP concentration in vascular smooth muscle cells, considering both the constant influx and the degradation of nitric oxide (NO). Our findings reveal that avanafil most significantly increases cGMP production in vascular smooth muscle cells due to its pharmacokinetic properties and potent inhibition of PDE-5. The simulations suggest that the synergistic effect of PDE-5 inhibitors with a constant NO influx enhances cGMP production, indicating that combining NO-donor drugs with PDE-5 inhibitors might significantly amplify vascular smooth muscle relaxation.

References

[1] B. Zhu, S. J. Strada, Current topics in medicinal chemistry 7 (2007), 437-454.
[2] J. Yang, J.W. Clark, R.M. Bryan, American journal of physiology. Heart and circulatory physiology 289 (2005), 886-897.

P16 ***Identifying a new aggregation hotspot in Alzheimer's disease: Opportunities for drug discovery***

Michal Nemergut,[1,2] Jiri Damborsky[1], Martin Marek[1]

[1]Loschmidt Laboratories, Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
[2]Center for Interdisciplinary Biosciences, Technology and Innovation Park, P. J. Safarik University in Kosice, Trieda SNP 1, 04011 Kosice, Slovakia
e-mail: michal.nemergut@upjs.sk

Apolipoprotein E (ApoE) ε4 genotype is the most prevalent risk factor for late-onset Alzheimer's Disease (AD) [1]. Although ApoE4 differs from its non-pathological ApoE3 isoform only by the C112R mutation, the molecular mechanism of its proteinopathy is unknown. Here, we reveal the molecular mechanism of ApoE4 aggregation using a combination of experimental and computational techniques, including X-ray crystallography, site-directed mutagenesis, hydrogen-deuterium mass spectrometry (HDX-MS), static light scattering and molecular dynamics simulations. Treatment of ApoE ε3/ ε3 and ε4/ ε4 cerebral organoids with tramiprosate was used to compare the effect of tramiprosate on ApoE4 aggregation at the cellular level [2].

We found that C112R substitution in ApoE4 induces long-distance (>15 Å) conformational changes leading to the formation of a V-shaped dimeric unit that is geometrically different and more aggregation-prone than the ApoE3 structure. AD drug candidate tramiprosate and its metabolite 3-sulfopropanoic acid induce ApoE3-like conformational behavior in ApoE4 and reduce its aggregation propensity. Analysis of ApoE ε4/ε4 cerebral organoids treated with tramiprosate revealed its effect on cholesteryl esters, the storage products of excess cholesterol. Our results connect the ApoE4 structure with its aggregation propensity, providing a new druggable target for neurodegeneration and ageing.

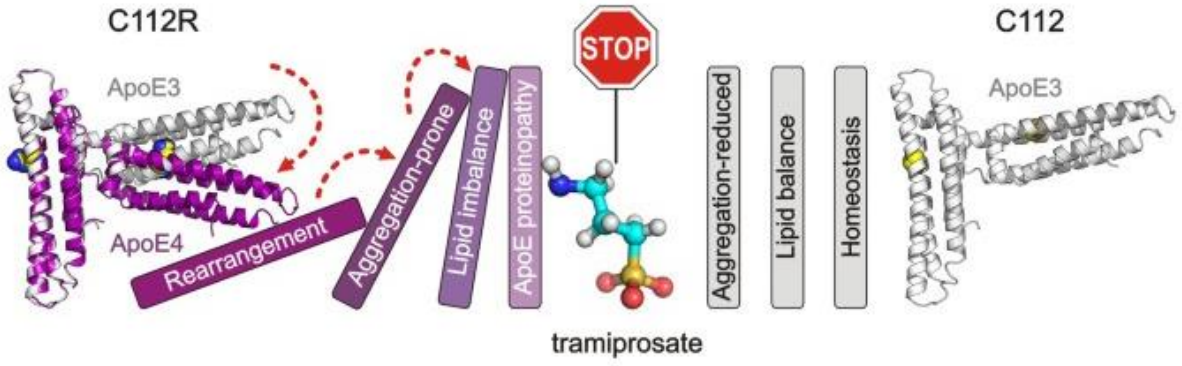


Figure 1: Domino-like effect of ApoE C112R mutation and its reduction by tramiprosate.

Acknowledgments
This work was supported by research grants from the Slovak Research Grant Agency KEGA No. 004UPJŠ-4/2024.

References

[1] S. Sadigh-Eteghad, M. Talebi and M. Farhoudi, Neurosci., 17 (2012) 321–6.
[2] M. Nemergut, SM. Marques, L. Uhrik, T. Vanova, M. Nezvedova et al., Mol. Neurodegener. 18 (2023) 38.

P17 ***Light-evoked channel activity using photolipids***

Rohit·Yadav, Jürgen Pfeffermann, Nikolaus Gössweiner-Mohr, Peter Pohl

Johannes Kepler University, Linz, Austria
e-mail: peter.pohl@jku.at

Light-induced modulation of membrane mechanical properties has many applications. For example, lipids with azobenzene moieties enable the assembly or disassembly of lipid domains (1, 2). By reducing membrane thickness and altering spontaneous membrane curvature, these lipids force a light-driven increase in the open probability of model mechano-sensitive channels (3). Rapid switching of the photolipids leads to depolarizing optocapacitive currents, which in turn trigger action potentials in excitable cells (4). Part of the effect that photo-susceptible lipids have on cell excitability is due to their ability to alter membrane tension. An increase in membrane tension can trigger the opening of mechanosensitive channels. The facilitated ion flux may also depolarize excitable cells, thereby triggering action potentials (4). The activity of voltage-gated potassium channels is also modulated by photolipid excitation. To this end, we recorded the light-evoked increase in open probability of purified and reconstituted potassium ion channels. The transient nature of the increase in membrane tension allowed us to attribute part of the effect to altered mechanical properties of the bilayer. Our study paves the way for light-guided investigations of the effects of mechanical stimuli in complex environments.

Acknowledgments

Supported by: Austrian Science Fund (FWF, award TAI 181 to PP)

References

[1] A. Saitov, S. A. Akimov, T. R. Galimzyanov, T. N. Glasnov, P. Pohl, Ordered Lipid Domains Assemble via Concerted Recruitment of Constituents from Both Membrane Leaflets. Phys. Rev. Lett. 124, 108102 (2020).

[2] A. Saitov, M. A. Kalutsky, …, P. Pohl, Determinants of Lipid Domain Size. Int. J. Mol. Sci. 23, 3502 (2022).

[3] J. Pfeffermann, B. Eicher, …, P. Pohl, Photoswitching of model ion channels in lipid bilayers. J. Photochem. Photobiol. B: Biol. 224, 112320 (2021).

[4] C. A. Z. Bassetto, J. Pfeffermann, …, P. Pohl, Photolipid excitation triggers depolarizing optocapacitive currents and action potentials. Nature Communications 15, 1139 (2024).

P18 ***Inhibition of human insulin amyloid aggregation: Origanum vulgare constituents' interplay***

M. Gancar,[1] S. B. Fialova,[2] E. Kurin,[2] S. Dokupilova,[2] M. Nagy,[2], Z. Gazova[1]

[1]Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice
[2]Faculty of Pharmacy, Comenius University Bratislava, Odbojarov 10, 832 32 Bratislava, Slovakia
e-mail: gancar@saske.sk

Exogenous insulin, utilized as a therapeutic agent for diabetes, can form insoluble deposits containing amyloid fibrillar structures near the administration site [1]. One promising therapeutic approach involves inhibiting amyloid formation and/or enhancing the clearance of amyloid aggregates. Small natural molecules, abundant in medicinal plants, show potential as inhibitors of amyloid formation [2].

Extracts from *Origanum vulgare* are rich in flavonoid and phenolic compounds and have demonstrated antimicrobial, antiparasitic, antioxidant, anti-inflammatory, and antispasmodic activities both *in vitro* and *in vivo* [3]. This study evaluated the anti-amyloid activity of the *Origanum vulgare* water extract (LYO), its predominant constituents identified by mass spectrometry (rosmarinic acid, lithospermic acid (LA), luteolin-7-diglucuronide (L7dG), origanol A (OA)) and their mixtures against human insulin. Thioflavin T fluorescence was utilized to quantify the anti-amyloid efficacy (IC50 values) and track the process of amyloid aggregation (kinetic parameters). Additionally, atomic force microscopy (AFM) was employed to visualize the studied samples (see Fig. 1), and *in silico* calculations were conducted to understand observed synergistic/antagonistic behavior. LA and L7dG emerged as the most potent individual inhibitors. Their mixture, LA:L7dG, retained the activity of its individual constituents. Surprisingly, the mixture LA:OA displayed significant synergistic interplay, while OA was found to be ineffective when tested individually. Interestingly, none of the studied compounds or mixtures approached the anti-amyloid activity of LYO in terms of kinetics inhibition.

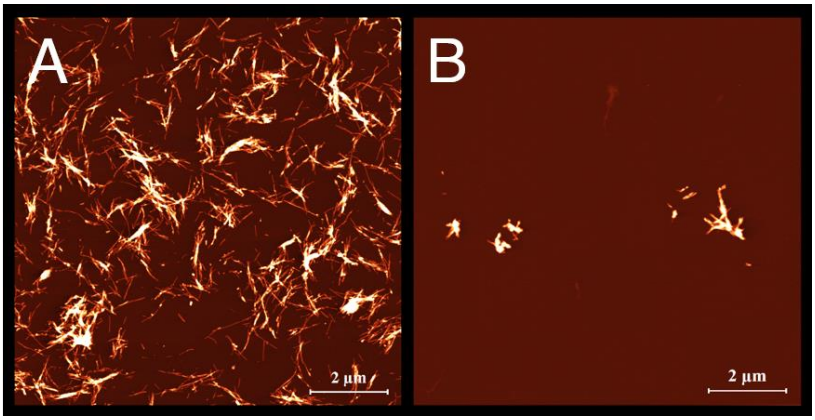


Figure 1: AFM images of (A) human insulin amyloid aggregates without treatment and (B) aggregates formed in the presence of 1mM LYO (*Origanum vulgare* water extract). The images are unfiltered, 10x10 µm, and contain a scale representing 2 µm.

Acknowledgments

This work was supported by the Slovak Research and Development Agency under Contracts no. APVV-22-0598 and APVV-18-0284, Slovak Grant Agency VEGA 2/0176/21 and 02/0164/22.

References

[1] Alam, P. *et al*., *Arch. Biochem. Biophys*. **621**, 54–62 (2017).
[2] Vus, K. *et al*., *J. Mol. Liq.* **276**, 541–552 (2019).
[3] Pezzani, R. *et al*., *Phytochem. Rev.* **16**, 1253-1268 (2017).

P19 ***Ferrocene-containing pyrazole/pyrimidine analogues of curcumin as modulators of amyloid β peptide aggregation with a potential in anticancer treatment***

K. Siposova,[1] V. Huntosova,[2], V. Kovac[3]

[1]Department of Biophysics, Institute of Experimental Physics SAS, Watsonova 47, Kosice, Slovakia
[2]Center for Interdisciplinary Biosciences, Technology and innovation park, P.J. Safarik University in Kosice, Jesenna 5, Kosice, Slovakia
[3]Laboratory of Organic Chemistry, University of Zagreb, Faculty of Food Technology and Biotechnology, Pierotti Str. 6, Zagreb, Croatia
e-mail: siposova@saske.sk

Current technological and medical progress has significantly prolonged the average lifespan of the population and demographic aging as a worldwide process is observed. However, the older population suffers from dementia. Among the plethora of various neurodegenerative disorders and dementia, the most common is Alzheimer's disease, which affects 50-60% of people with dementia (AD)[1,2]. There are various hypotheses represented by numerous targets, which are believed to affect the progression of the disease. Besides the degradation of the brain, the typical pathogenic hallmark of AD is the presence of extracellular senile plaques (composed of amyloid β fibrils) and intracellular neurofibrillary tangles (formed by τ protein aggregates). Numerous efforts have been devoted to finding compounds that can interfere with the amyloid aggregation of proteins, including curcumin. However, due to the low bioavailability of curcumin, structural modifications seem to be a very important approach to improve stability and pharmacological activity. Motivated by this, new ferrocene-containing pyrazole (compounds C1-C4) and pyrimidine analogues (compounds C5, C6) of curcumin have been synthesized. Atomic force microscopy and fluorescence analysis have been utilized to investigate the amyloid aggregation of the Aβ peptide in the presence of newly synthesized compounds C1-C6. The experimental data have shown that they possess the ability to affect Aβ fibrillogenesis in a dose-dependent manner. The formation of Aβ peptide amyloid fibrils and the disassembly of preformed fibrils is significantly affected by each of the tested compounds, however, the strongest effect is observed for pyrimidine derivatives, indicating their structure-dependent anti-amyloidogenic activity. The apparent half-maximal inhibiting (IC_{50}) and half-maximal disassembly (DC_{50}) values of compounds were found to be in the micromolar concentration range. Analysis of bioactivity and cell metabolic activity by MTT assay, Western blot, fluorescence microscopy, and flow cytometry revealed that these compounds can effectively inhibit cancer cell proliferation depending on the concentrations used. However, the pyrimidine derivatives C5 and C6 as well as the pyrazole derivative C3 show the ability to induce apoptosis in glioblastoma cells at much lower concentrations than the compounds C1, C2, and C4, making them promising for the treatment of cancer.

Acknowledgments

This work has been fully supported by Croatian Science Foundation under the project IP-2020-02-9162 and by grants from MVTS SAS-MOST JRP 2021/2 (AZCAI) and Slovak Grant Agency VEGA (No. 2/0034/22).

References

[1] D.J. Selkoe, P.J. Lansbury, Jr. Alzheimer's Disease Is the Most Common Neurodegenerative Disorder. In: Siegel GJ, Agranoff BW, Albers RW, et al., editors. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition. Philadelphia: Lippincott-Raven, 1999
[2] S. Long, C. Benoist, W. Weidner. World Alzheimer Report 2023: Reducing dementia risk: never too early, never too late. London, England: Alzheimer's Disease International, 2023

P20 ***Invitation to enhance capacities for technology transfer, company building and innovations in the field of ICT in the Danube region***

Višnja Stepanić, Anja Barešić, Ilona Kulikovskikh, Tomislav Šmuc

Laboratory for machine learning and knowledge representation, Rudjer Boskovic Institute, Zagreb, Croatia
e-mail: visnja.stepanic@irb.hr

The CapTTict project aims to bridge the innovation gap and enhance technology and knowledge transfer in ICT across nine Danube region countries. The initiative targets synergies, cooperation, and business support deficiencies affecting SMEs, which are essential for valorising research from high educational institutions (HEIs) and public research organizations (PROs) and enabling SMEs to develop high-value products. CapTTict has three specific objectives: i) enhancing technology transfer (TT) capacities at HEIs/PROs, ii) leveraging European Digital Innovation Hubs (EDIHs) and the European Enterprise Network (EEN), and iii) creating ICT access in non-EU countries for TT/RDI cooperation and Smart Specialisation Strategies (S3) development. Activities for enhancing TT capacities include establishing guidelines for effective TT, organizing workshops, setting up the Danube Digital Accelerator, and promoting offerings of EDIHs and the EEN through a digital tool.

The poster will introduce the project in short with the aim to promote it and call teams for joining the international Danube Incubation and Acceleration Programme (www.irb.hr/capttict). The teams should provide science-based ICT solutions at the minimal TRL3 level for current key S3 issues. The overall purpose is to create a robust ecosystem that supports the introduction of new technologies and the establishment of spinoffs/startups, and to facilitate the integration of ICT solutions while fostering collaboration and innovation across the region.

LinkedIn: https://www.linkedin.com/company/capttict/posts/?feedView=all

Figure 1: Countries participating in CapTTict. The consortium gathers 16 partner organizations with Czech Technical University in Prague as a lead partner. Croatian participants are RBI, Mreža znanja and HAMAG-BICRO.

Acknowledgments

The project DRP0200048 CapTTict is funded by the Interreg Danube program (2021-2027).

P21 ***Advancing Biotechnology: Exploring DNA-Surface Interactions via Surface-Enhanced Raman Spectroscopy***

Daria Stoia[1,2], Monica Potara[2], Marc Lamy de la Chapelle[3], Monica Focsan[1,2]

[1]Biomolecular Physics Department, Faculty of Physics, Babes-Bolyai University,1 M. Kogalniceanu Street, 400084, Cluj-Napoca, Romania
[2]Nanobiophotonics and Laser Microspectroscopy Centre, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, 42 Treboniu Laurian Street, 400271, Cluj-Napoca, Romania
[3]Institut des Molécules et Matériaux du Mans (IMMM - UMR6283), Université du Mans, avenue Olivier Messiaen, 72085 Cedex 9 Le Mans, France
e-mail: daria.stoia@ubbcluj.ro

As biotechnology advances, numerous researchers are now focusing on studying how DNA behaves at interfaces. The interaction between DNA and the substrate is pivotal in facilitating DNA hybridization at these interfaces. Surface-Enhanced Raman Spectroscopy (SERS) emerged as a potent tool for detecting and identifying chemical or biological substances at low concentrations, owing to its remarkable sensitivity, distinctive fingerprint spectra, and instantaneous detection capabilities. Nonetheless, ensuring the reproducibility and consistency of SERS measurements within the realm of SERS sensors presents a significant challenge.

In this study, we implemented a specialized measurement protocol on a commercial SERS gold substrate obtained from Hamamatsu Photonics, employing single-stranded and hybridized DNA at an excitation of 633 nm. The protocol involves the binding of thiol-modified polyA to the gold substrate and the acquisition of 4 SERS maps across the substrate's entire surface. Within each map, 5 spectra are recorded to compute the average SERS signal. 4 different lengths of polyA (5, 10, 15, and 20 bases respectively) have been analyzed. Moreover, the hybridization with unmodified polyT (5, 10, 15, and 20 bases) was analyzed for 4 different concentrations of polyT: 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M. Throughout the collection and analysis of a substantial dataset of spectra, utilizing the intensity of adenine ring breathing SERS band, we assess the reproducibility and repeatability of the substrate. This protocol holds promise for applications to other substrates, facilitating precise evaluation of their sensing capabilities.

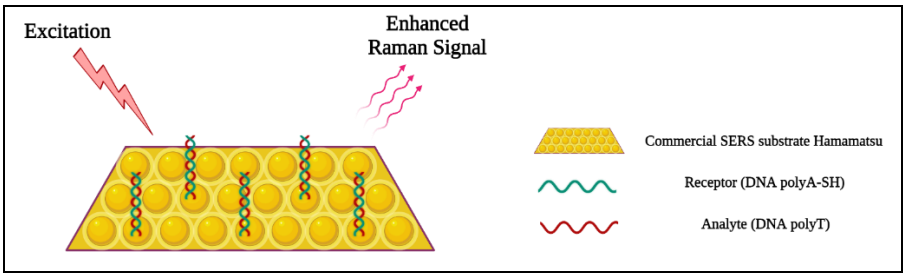


Figure 1: Illustration depicting the functionalization of a commercial gold SERS substrate with thiol-modified polyA oligonucleotides, followed by the hybridization of polyT strands. The interaction highlights the substrate's capability for DNA hybridization studies using Surface-Enhanced Raman Spectroscopy.

Acknowledgments

This work was funded through the project "Plasmon mediated biology: Exploitation of plasmonics to investigate and enhance biological processes and application to biomedical issues (acronym: BioPlasmonics" funded by European Union – NextGenerationEU and Romanian Government, under the National Recovery and Resilience Plan for Romania, contract no760037/23.05.2023, cod PNRR-C9-I8-CF-199/28.11.2022, through the Romanian Ministry of Research, Innovation and Digitalization, within Component 9, Investment I8.

P22 *Position of the C-terminal α-helix could be important for understanding the molecular mechanism of cellular oxidative stress sensing by KEAP1*

Antonija Tomić

Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia
e-mail: atomic@irb.hr

The KEAP1–NRF2 pathway regulates cellular responses to oxidative and electrophilic stresses [1]. Under normal conditions, level of NRF2 is low due to its binding with KEAP1, which targets it for ubiquitination and suppresses cytoprotective gene expression [2]. When exposed to either oxidative or electrophilic stress, KEAP1's cysteine residues undergo post-translational modifications, and the mode of NRF2 binding to KEAP1 changes disabling ubiquitination of NRF2. As a consequence, NRF2 accumulates in the cytoplasm and migrates to the nucleus to activate cytoprotective gene expression. Experimental studies showed that KEAP1 utilizes four specific cysteine residues (C226, C613, and C622/624) for sensing H_2O_2, establishing an elaborate fail-safe mechanism [3]. Three of these four cysteines are located in the C-terminal α-helix of the KEAP1 monomer, while C226 is situated in the IVR domain. The assumption is that the C-terminal α-helix and IVR domain need to come into close proximity to allow cysteine bridge formation under the oxidation and disable formation of the KEAP1–NRF2 complex structure adequate for the NRF2 ubiquitination.

Although the X-ray structure of the entire KEAP1 dimer is still not available, X-ray structures of its domains have been solved. Using the recently predicted AlphaFold 3D structure of the KEAP1 monomer and the X-ray structure of the BTB domain dimer (pdb: 7EXI), the 3D structure of the KEAP1 dimer was built. To understand how the oxidative stress influences the KEAP1 structure I performed a series of different molecular dynamics (MD) simulations with particular focus on the orientation of the C-terminal α-helices. During simulations, no spontaneous approach of any of two of the mentioned four Cys residues was observed that would enable formation of the disulfide bridge under oxidative stress conditions. Additionally, adaptive steered MD simulations on a truncated KEAP1 system showed that the work required to bring the C-terminal α-helix closer to the IVR domain is around 80 kcal/mol. Therefore, this KEAP1 structure does not offer comprehensive understanding of its response to the oxidative stress. Considering the lower confidence score predicted by AlphaFold for the C-terminal α-helix compared to the more structured parts of the protein, we built a KEAP1 dimer structure with an alternate position for the C-terminal α-helix that could better explain KEAP1's answer to the oxidative stress as determined by Suzuki et al.

Acknowledgments

Author acknowledge the supercomputer Supek (https://www.srce.unizg.hr/en/advanced-computing) for computer time.

References

[1] a) N. Wakabayashi et al., Nat. Genet. 35 (2003) 238–245, b) K. Taguchi et al., Genes Cells. 16 (2011) 123–40, and c) Q. Ma, Annu. Rev. Pharmacol. Toxicol. 53 (2013) 401–26.
[2] L. Baird and M. Yamamoto, Mol. Cell. Biol. 40 (2020).
[3] T. Suzuki et al., Cell Reports 28 (2019) 746-758.

P23 *Stability of the radical in the P-type ferryl intermediate of cytochrome c oxidase*

A. Tomkova,[1] M. Fabian,[2] D. Jancura[1]

[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.
e-mail: adriana.tomkova1@student.upjs.sk

Respiratory cytochrome c oxidase (CcO) catalyzes the reduction of molecular dioxygen to water by electrons supplied by ferrocytochrome c. Four electrons, required for the full reduction of O_2 to H_2O, are transferred sequentially from cytochrome c via four metal centers, copper A (Cu_A), heme *a*, heme *a*$_3$ and copper B (Cu_B). Cu_A is the first electron acceptor from cytochrome c. The electron flow then continues in CcO to heme *a* and finally to the catalytic heme a_3-Cu_B center. At this heme a_3-Cu_B center the reduction of dioxygen takes place. Reduction of O_2 proceeds through a sequence of several oxy intermediates of the heme a_3-Cu_B center. These intermediates are determined by the number of electrons and protons delivered into the catalytic center [1]. In one of them, produced by the reaction of two-electron reduced CcO with O_2, the free radical at the catalytic center is also formed. In this state, the iron of heme *a*$_3$ is in the ferryl state and very likely Tyr244 (bovine CcO numbering), located in the proximity of the center, is oxidized to a neutral radical [Fe_{a3}^{4+}=O Cu_B^{2+} YO$^{\bullet}$]. For historical reasons this ferryl intermediate is called P_M state.

However, several published studies indicated that this radical migrates the large distances from the catalytic center of CcO. The Result of this migration is oxidative damage of the enzyme. In spite of importance of this radical in catalysis of respiratory oxidases the lifetime of this radical has not been established [2, 3, 4]. To answer this question, we have developed a protocol to estimate its lifetime in the P_M form. This method is based on one-electron reduction of the P_M form. In case that radical is present, the product of this reduction is spectrally different second ferryl form (F type). However, if the radical already migrated from the catalytic center, then reduction of this intermediate results in the formation of fully oxidized CcO. Consequently, if this one-electron reduction is performed at different times after the formation of the P_M the generated fraction of the F form represents population in which the radical is still present.

This kind of measurements have been performed on purified bovine CcO at pH 8.0 and at 10 and 20°C. At both temperatures, the loss of the radical from the catalytic center occurs on time scale of minutes. The time constant of migration increases with the rise of the temperature from 15 minutes at 10 °C to 7 minutes at 20 °C. Data also indicated that at 37 °C, the time constant should be about 100 seconds. This relatively long lifetime indicates that under physiological turnover conditions, the escape of the radical from this catalytic center is very unlikely. It can only occur if there is a very limited access of external electron donors.

Acknowledgments

This work was supported by VEGA, 0028/22.

References

[1] Wikstrom, M., Krab, K., and Sharma, V., Chem. Rev., 118 (2018), 2469-2490.
[2] Musatov, A., Hebert, E., Carroll, C. A., Weintraub, S. T., and Robinson, N. C., Biochemistry, 43 (2004), 1003-1009.
[3] Musatov, A., and Robinson, N. C., Free Radic Res, 46 (2012), 1313-1326.
[4] Lemma-Gray, P., Weintraub, S. T., Carroll, C. A., Musatov, A., and Robinson, N. C., FEBS Lett, 581 (2007), 437-442.

P24 *Enhancing staphylokinase properties through directed evolution*

M. Tomková[1], M. Štulajterová[2], Ľ. Ambro[1], V. Hovanová[1], E. Sedlák[1]

[1]Center for Interdisciplinary Biosciences, Technology and Innovation Park, P. J. Šafárik University, Jesenná 5, 041 51 Košice, Slovakia
[2]Department of Biophysics, Faculty of Science, P. J. Šafárik University, Jesenná 5, 041 51 Košice, Slovakia
e-mail: maria.tomkova@upjs.sk

Directed evolution represents a powerful strategy for the development of therapeutic proteins with improved properties [1]. We employed ribosome display to improve a thrombolytic agent, staphylokinase (SAK). SAK is a single-chain extracellular protein secreted by *Staphylococcus aureus*. It initiates the fibrinolytic cascade to help invading bacteria move deeper into the tissues. As a result of its thrombolytic properties and high fibrin specificity, it is considered a promising new thrombolytic agent. However, not all SAK features are optimized for their practical applications, leaving room for improvement. Engineering the affinity and stability of SAK could increase its residence time on plasmin, thus reducing the severity of side effects after its application.

We have successfully implemented the selection of SAK variants towards plasmin by ribosome display and after the first round of display we proved that it is feasible to evolve SAK by this technology. Evolution techniques require several consecutive rounds. We completed six rounds of display, which yielded SAK variants with hot spot amino acid substitutions, suggesting their involvement in SAK-plasmin interaction, with notable prevalence of the K121E substitution by the 6th round.

We expressed and purified the SAK mutant harboring the K121E substitution and evaluated its activity and thermostability. While its activity was slightly compromised, differential scanning calorimetry (DSC) revealed a slight improvement in its thermostability compared to wild-type SAK. Our preliminary findings suggest that ribosome display-based evolution shows potential for enhancing SAK properties, although comprehensive biophysical characterization of selected mutants is pivotal for the validation of evolved variants of SAK.

Acknowledgments

This work was supported by Slovak Research and Development Agency through the project APVV-20-0340 and by the grant agency of the Ministry of Education, Science, Research, and Sport of the Slovak Republic, grant no. VEGA 1/0074/22.

References

[1] Ch. Zahnd, P. Amstutz and A. Plückthun. Nature Methods, 4 (2007), 269-279.

P25 *Cellular effects of the R196H cytoskeletal β-actin mutation causing baraitser-winter syndrome*

A. Varga,[1] É. Gráczer,[1] T. Bozó[1], K. Pászty[1], Á. Altorjai[1], J. N. Greve[2], N. Di Donato[3], M. Kellermayer[1]

[1]Semmelweis University, Department of Biophysics and Radiation Biology, Budapest, Hungary
[2]Hannover Medical School, Institute for Biophysical Chemistry, Hannover, Germany
[3]Hannover Medical School, Department of Human Genetics, Hannover, Germany
e-mail: matkovicsne.andrea@med.semmelweis-univ.hu

Actin, one of the most abundant proteins in eukaryotic cells, is the building block of the actin cytoskeleton. Dynamic reorganization of the actin cytoskeleton, regulated by actin-binding proteins (ABPs), is important for several cellular processes such as division, adhesion, migration, and contraction. There are six actin isoforms, two of which are expressed in non-muscle cells, called cytoskeletal β- and γ-actin [1]. Germline mutations in β-actin are almost exclusively associated with Baraitser-Winter Cerebrofrontofacial syndrome (BWCFF). The most frequently affected actin residue is R196, with the mutation R196H being the most common. Patients carrying this mutation show BWCFF-specific craniofacial malformations, muscular hypotonia, moderate to severe intellectual disability and delayed speech [2]. We analyzed the cellular effects of the R196H mutation by using patent-derived fibroblasts. First, we compared the F-actin content of wild-type and mutant cells and found that the mutation reduced the amount of filamentous actin two-fold. The actin filament polymerizing and stabilizing drug, jasplakinolide increased the amount of F-actin in mutant cells to the level observed in wild type cells in the absence of jasplakinolide. We observed a different localization pattern of actin fibers upon mutation by using confocal microscopy. Super-resolution (STED) microscopy imaging indicated that the structure of the filaments is also changed. We also compared the elastic modulus of patient-derived fibroblasts by using atomic force microscopy (AFM), which decreased ten-fold upon mutation. By using recombinant cytoskeletal actin, we compared the structures of the wild type and the R196H variant in the presence of phalloidin and found no differences in either monomer-monomer distance or half-helical pitch of the actin calculated from AFM images. We also investigated how the mutation affects the reorganization of actin by using a cell stretcher system (Cytostretcher LV, Curi Bio Inc.). We applied uniaxial stretch on the fibroblasts with a magnitude of 30% strain for 15 minutes. We observed thinner actin fibers upon mutation in the direction of the applied stretch. Localization of cofilin, which plays a role in the depolymerization and reorganization of the filaments, was changed upon stretch and showed less cofilin in the periphery of mutant fibroblasts. Taken together, the R196H mutation decreases the stability of actin filaments in patient-derived fibroblasts and increases the dynamic reorganization of actin. The amount and stability of the filaments can be increased by actin polymerizing and stabilizing drugs, such as jasplakinolide and phalloidin.

Acknowledgments

The research was supported by the European Joint Programme on Rare Diseases.

References

[1] Perrin BJ, Ervasti JM. Cytoskelet Hoboken NJ. (2010) 67(10):630–634.
[2] Rivière JB, Van Bon BWM, Hoischen A, Kholmanskikh SS, O'Roak BJ, Gilissen C, et al. Nat Genet. (2012) 44(4):440–444.

P26 *Local Drug Delivery for Optimized Combinatory Cancer Treatment*

Vito Telebar-Zbulj[1,2,3], Franciska Telebar-Zbulj[1,2], Rainer Schindl[1], Martina Schweiger[3], Julia Kargl[4] Linda Waldherr[1,2]

[1]Gottfried Schatz Research Center – Biophysics, Medical University of Graz, 8010 Graz, Austria
[2]BioTechMed-Graz, Austria, Auenbruggerplatz 30, 8036 Graz, Austria
[3]Institute of Molecular Biosciences, University of Graz, 8010 Graz, Austria
[4]Otto Loewi Research Center – Division of Pharmacology, Medical University of Graz, 8010 Graz, Austria
e-mail: vito.zbulj@medunigraz.at

Combining chemotherapy and immunotherapy offers a powerful strategy against various cancers. However, systemic chemotherapy can disrupt the immune system, reducing the efficacy of immunotherapy. Locally delivered chemotherapy, administered through specialized devices, addresses this issue by targeting the tumor site directly and minimizing systemic side effects [1,2]. This continuous localized approach allows for precise, on-demand administration of chemotherapeutics over a desired period. Combined with immune checkpoint inhibitors (ICIs), we hypothesize that the controlled nature of the local release allows us to expand on the local limitations of the device and ignite a systemic adaptive immune response against metastasis.

This study aims to investigate the mechanisms behind this combined local therapy approach. We propose the integration of local chemotherapy with precise temporal control and ICIs. With this we anticipate superior treatment outcomes compared to current methods. In preliminary experiments, we assessed the effects of gemcitabine, oxaliplatin, and doxorubicin on a fibrosarcoma MCA207 cell line. To simulate metastasis, we developed a mouse model with MCA207 tumors in both flanks. We plan to implant commercially available pumps subcutaneously on the dorsal side of the mice, directing the outlet to one tumor site. The mice will subsequently be treated with multiple regimens of combined chemoimmunotherapy. We will monitor survival, tumor growth and immune cell populations of both the local and distal tumors.

We expect our experiments to reveal the optimal timing for combining chemo- and immunotherapy. The immune response elicited by this combined treatment should affect both the local and the untreated tumors. Consequently, we anticipate observing significant immune cell infiltration in both tumors.

References

[1] Chao Wang, Jinqiang Wang, Xudong Zhang, Shuangjiang Yu, Di Wen, Quanyin Hu, Yanqi Ye, Hunter Bomba, Xiuli Hu, Zhuang Liu, Gianpietro Dotti, And Zhen Gu, Science Translational Medicine, 2018, Issue 429
[2] Verena Handl, Linda Waldherr, Theresia Arbring Sjöström, Tobias Abrahamsson, Maria Seitanidou, Sabine Erschen, Astrid Gorischek, Iwona Bernacka-Wojcik, Helena Saarela, Tamara Tomin, Sophie Elisabeth Honeder, Joachim Distl, Waltraud Huber, Martin Asslaber, Ruth Birner-Grünberger, Ute Schäfer, Magnus Berggren, Rainer Schindl, Silke Patz, Daniel T. Simon, Nassim Ghaffari-Tabrizi-Wizsy, Journal of Controlled Release, 2024, Volume 369, Pages 668-683

P27 ***Exploring Novel Approaches for Breast Cancer Detection via Machine Learning Algorithms: Assessing Metabolomics and Spectroscopy for Non-Invasive Diagnosis***

Markus Zetes[1], Stefania D. Iancu[1], Carmen Socaciu[2], Nicolae Leopold[1]

[1]Faculty of Physics, Babes-Bolyai University, 1 Kogalniceanu, 400084, Cluj-Napoca, Romania
[2]Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, 400372 Cluj-Napoca, Romania
e-mail: markus.zetes@ubbcluj.ro

Breast cancer (BC) stands as one of the most prevalent public health concerns for women. Timely detection and precise prognosis are critical for mitigating cancer recurrence and improving patient outcomes. This study focuses on evaluating two distinct methods for BC detection utilizing metabolites extracted from urine biofluids: High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) serves as a robust metabolic tool, while surface-enhanced Raman spectroscopy (SERS) is an emerging technique for detecting various cancer types by analysing metabolites adsorbed to metal nanoparticles. The aim of this research is to assess the potential of these methods as non-invasive and accessible platforms for early BC diagnosis and prognosis. This assessment involves analysing MS and SERS data obtained from urine samples using diverse supervised and unsupervised machine learning (ML) algorithms.

Urine samples were collected from BC patients (n=53) and healthy controls (n=22) matched for age and body-mass index. HPLC-MS analysis was conducted to identify and quantify the metabolites present in the BC and healthy urine samples. We pre-processed the HPLC-MS metabolomics and SERS datasets by normalizing them to vector unity and employing unsupervised-learning feature reduction through principal component analysis (PCA). The reduced datasets were then utilized in random forest (RF), support vector classifier (SVC), Logistic Regression (LR) and Extreme Gradient Boosting (XGBoost) algorithms for classifying BC and healthy control samples. A 5-fold cross-validation was used for training and testing the data.

For the MS metabolomics dataset, dimensionality reduction using PCA retained the first 21 PCs, explaining 98% of the variance. The XGBoost model achieved the highest classification accuracy and F1-score, with mean cross-validation accuracy and F1-scores of 96% and 97%, respectively, and test accuracy and F1-score of 91% and 93%. Dimensionality reduction of the SERS data retained the first 15 PCs, explaining 90% of the variance. The SVC model showed promising results, with mean cross-validation accuracy and F1-score of 84% and 88%, respectively, and test accuracy and F1-score of 87% and 91%.

The presented results show promise for early BC diagnosis research. Despite dataset imbalances, the results demonstrate no overfitting, as dimensionality reduction and close accuracy and F1-scores between training and testing sets indicate good generalization of algorithms to new data. Future prospects include combining the two datasets and employing machine and deep learning algorithms to establish correlations between SERS spectra peaks and variations in the concentration of typical BC metabolites.

Acknowledgments

S.D.I. highly acknowledges a fellowship supported by Babeș-Bolyai University, under contract numbers SRG-UBB-32922/22.06.2023

P28 *Molecular dynamics study of antimicrobial peptide TSO8 from Taenia solium*

A. Begić, [1] M. Maleš[2], L. Zoranić[1]

[1]University of Split, Faculty of Science, Ruđera Boškovića 33, 2100 Split, Croatia
[2]University of Split, Faculty of Maritime Studies, Ruđera Boškovića 37, 2100 Split, Croatia
e-mail: abegic@pmfst.hr

Antibacterial resistance remains a major problem in need of answering in recent decades. Antimicrobial peptides (AMPs) play an important role in non-specific defence systems of almost all organism, and as such represent a promising alternative to classical antibiotic treatment [1]. An often-overlooked source of AMPs are helminth parasites. Due to the adaptability of helminths to different environments throughout their life cycles, which enables them to modulate the hosts' immune responses, AMPs derived from parasites could offer promising leads for drug development [2].

This study focuses on TSO8 peptide identified in the helminth parasite *Taenia solium*. TSO8 contains 39 amino acids, with a net charge of +15.9 at pH 7. It has a specific structure with its N-terminal being α-helical with initial GW sequence, and its C-terminal having a coil like structure containing a disulfide bridge. To assess the atomic details of its mode of action, TSO8 and four specific fragments, along with a linearized peptide obtained by replacing cysteine residues with alanine, were investigated through molecular dynamics simulations in various environments. Results of simulations in water elucidated the importance of the initial GW sequence in preserving the helical content and are in line with previous findings [3]. Simulations of single peptides placed near a model of Gram-negative bacterial membrane, which consisted of POPE and POPG lipids in the ratio 3 :1, highlighted two distinct contributions. The N-terminal α-helix stands out as crucial for the initial electrostatic interaction with the membrane. On the other hand, the unstructured C-terminal segment shows low affinity for membrane binding, which implies that its function might be emphasized in another phase of the peptide's antimicrobial activity. Additionally, the role of associations was tested in simulations of the multiple peptides in water and in the vicinity of the bacterial membrane. The results indicated that the peptides quickly associate in water, forming one large cluster while preserving the N-terminal α-helical structure. When interacting with the membrane, peptides bind swiftly, however they do not exhibit a tendency to cluster on the membrane surface. These findings underscore several important aspects of the mechanism of action of TSO8 peptide, providing a solid foundation for ongoing research involving the biological and biophysical characterization of TSO8 and other helminth antimicrobial peptides.

Acknowledgments

This research is partially funded by HRZZ IP-2022-10-8432. The molecular modelling was supported by the Centre for Advanced Computing and Modelling, University of Rijeka.

References

[1] T. Rončević, J. Puizina, and A. Tossi, "Antimicrobial Peptides as Anti-Infective Agents in Pre-Post-Antibiotic Era?" *Int J Mol Sci,* vol. 20, no. 22, Nov 14 2019, doi: 10.3390/ijms20225713.
[2] T. Rončević *et al.*, "Anisaxins, helical antimicrobial peptides from marine parasites, kill resistant bacteria by lipid extraction and membrane disruption," *Acta Biomaterialia,* vol. 146, pp. 131-144, 2022/07/01/ 2022, doi:10.1016/j.actbio.2022.04.025.
[3] H. Sato and J. B. Feix, "Peptide-membrane interactions and mechanisms of membrane destruction by amphipathic alpha-helical antimicrobial peptides," *Biochim Biophys Acta,* vol. 1758, no. 9, pp. 1245-56, Sep 2006, doi: 10.1016/j.bbamem.2006.02.021.

P29 ***Spatio-temporal dynamics of the proton motive force on single bacterial cells***

Anaïs Biquet-Bisquert[1], Baptiste Carrio[1], Nathan Meyer[1], Thales Fernandes[1], Manouk Abkarian[1], Axel Magalon[2], Ashley L. Nord[1], Francesco Pedaci[1]

[1]Centre de Biologie Structurale, Université de Montpellier, CNRS, INSERM. Montpellier, France
[2]Aix Marseille Université, CNRS, Laboratoire de Chimie Bactérienne (UMR7283), IMM, IM2B, 13402, Marseille, France
e-mail: anais.biquet@cbs.cnrs.fr

The proton motive force (PMF) is an essential electro-chemical potential established across the membranes of bacteria, mitochondria, and chloroplasts. It serves as a bioenergetic currency of living systems that powers an impressive range of physiological processes, including the synthesis of ATP and bacterial motility. While traditionally considered homeostatic, recent experiments have shown that the PMF exhibits rich temporal dynamics at the single-cell level. Rapid membrane depolarizations observed on single Escherichia coli cells [1], related to mechanosensing [2] are indications of a temporally dynamical PMF. Furthermore, polar clustering of respiratory complexes observed in E. coli suggests the possibility of spatial heterogeneity of the PMF at the single-cell level [3]. Such spatial heterogeneity has been recently shown along the inner membrane of individual mitochondria [4]. However, the spatial and temporal dynamical behavior of the PMF in bacteria remains poorly characterized and understood, especially at the single-cell level.

In this work, we characterized the dynamics of PMF on E. coli both temporally and spatially. We employed their flagellar motors as local sensors on the membrane, taking advantage from the linearity between their rotational speed and the PMF. We also use spatio-temporally structured laser excitation on single bacteria expressing the light-driven proton pump proteorhodopsin [5] to controllably trigger the production of an excess of PMF in space and time. We resolved temporal dynamics on the millisecond time scale and observed an asymmetrical capacitive response of the cell. Using localized perturbations on long filamentous cells, we found that the PMF is rapidly homogenized along the entire cell, faster than proton diffusion can allow. Instead, the electrical response can be explained in terms of electrotonic potential spread, similar to that observed in passive neurons and described by cable theory. This implies a global coupling between PMF sources and consumers in the bacterial membrane, excluding a sustained spatial heterogeneity while enabling fast temporal dynamics.

References

[1] Kralj, J. M., Hochbaum, D. R., Douglass, A. D., & Cohen, A. E. (2011). Electrical spiking in Escherichia coli probed with a fluorescent voltage-indicating protein. Science, 333(6040), 345-348.
[2] Bruni, G. N., Weekley, R. A., Dodd, B. J., & Kralj, J. M. (2017). Voltage-gated calcium flux mediates Escherichia coli mechanosensation. Proceedings of the National Academy of Sciences, 114(35), 9445-9450.
[3] Magalon, A., & Alberge, F. (2016). Distribution and dynamics of OXPHOS complexes in thebacterial cytoplasmic membrane. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1857(3), 198-213.
[4] Wolf, D. M., Segawa, M., Kondadi, A. K., Anand, R., Bailey, S. T., Reichert, A. S., ... & Shirihai, O. S. (2019). Individual cristae within the same mitochondrion display different membrane potentials and are functionally independent. The EMBO journal, 38(22), e101056.
[5] Walter, J. M., Greenfield, D., Bustamante, C., & Liphardt, J. (2007). Light-powering Escherichia coli with proteorhodopsin. Proceedings of the National Academy of Sciences, 104(7), 2408-2412.

P30 *Nanomechanical and Optical Properties of Human Dental Collagen in Type 2 Diabetes Mellitus*

Kata Sára Haba,[1,2] Sarolta Antal,[2] Róbert Szipőcs,[3] Miklós Kellermayer,[2] Dániel Végh,[1] Dóra Haluszka[2]

[1]Department of Prosthodontics, Semmelweis University, Budapest, Hungary
[2]Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary
[3]HUN-REN Wigner RCP, Budapest, Hungary
e-mail: haba.kata@semmelweis.hu

Dentin is a mineralized tissue that provides the bulk of the tooth, and it is most affected by oral complications associated with type 2 diabetes mellitus (T2DM). Since dental collagen receives its nourishment through the dental tubules in the pulp, it can become glycated by blood glucose, progressively leading to the formation and accumulation of advanced glycation end products (AGEs) [1]. Most AGE(s) possess intrinsic fluorescent properties. Our previous research showed, in an *in vitro* glycation model by using second harmonic generation (SHG) and atomic force-microscopy techniques, that the accumulation of AGEs on dental collagen influences the structural and mechanical properties of its fibres [2]. Dentin matrix displayed greater autofluorescence (AF) and SHG intensity, and elevated stiffness of intertubular dentin could be detected.

This study aims to examine the effects of T2DM on the nonlinear optical behaviour and nanomechanical properties of dental collagen.

Human teeth (n = 10) were extracted from patients with T2DM and from healthy controls (n = 8). Samples were demineralized, then sections of 60 μm thickness were sliced parallel to the vertical axis of the tooth. To obtain a global view of glycation, a Fluorescence in Vivo Imaging (FOBI) system was used. Then, to reveal the structural alteration of dental collagen, both AF and SHG signals were determined by nonlinear microscopy. Finally, surface topography measurements and nanoindentation tests were performed of the coronal region by using AFM.

FOBI measurements showed moderately increased autofluorescence signal dominating in periphery of coronal region. The intensity of AF and SHG signals were slightly increased in the T2DM group compared with the controls. In some T2DM samples significant structural rearrangements could be observed in the SHG images. Diabetic sections displayed lower values of Young's moduli compared to controls.

In this study we uncovered the optical and nanomechanical properties of dental collagen in demineralized diabetic tooth sections. While the average optical properties (AF, SHG) changed moderately**,** remarkable structural alterations were detected in the fibre arrangement, pointing at the possible decay of the dental collagen. In contrast to our expectations, the AFM-based nanoindentation tests showed decreased Young's moduli of diabetic collagen matrix. These results indicate that in diabetic individuals the dental collagen displays a modulated structure and elasticity, which may be caused by various degradation processes such as increased level of reactive oxygen species or elevated protease activity.

Acknowledgments: supported by TKP2021-EGA-23

References

[1] Matsuda, Y., et al., *Influence of Nonenzymatic Glycation in Dentinal Collagen on Dental Caries.* J Dent Res, 2016. **95**(13): p. 1528 - 1534.
[2] Haluszka, D., et al., *Nanomechanical and Nonlinear Optical Properties of Glycated Dental Collagen.* J Dent Res, 2022. **101**(12): p. 1510-1516.

P31 ***Molecular dynamics analysis of the enzyme PQQ-GDH in interaction with a carbon nanotube***

Lucia Buljac,[1] Željka Sanader Maršić,[1] Emmanuelle Bignon[2]

[1]Faculty of Science, University of Split, Croatia
[2]Universit´e de Lorraine and CNRS, UMR 7019 LPCT, F-54000 Nancy, France
e-mail: lbuljac@pmfst.hr

The enzyme pyrroloquinoline quinone-dependent glucose dehydrogenase, PQQ-GDH, is a classical quinprotein that oxidizes glucose using the cofactor pyrroloquinoline quinone (PQQ). The enzyme belongs to the group of oxidoreductases that have gained popularity in the development of enzymatic biosensors and biofuel articles. In this work, molecular dynamics simulations of monomeric and dimeric structures of the enzyme PQQ-GDH as well as simulations of the same enzyme in a complex with a short and infinitely long carbon nanotube (CNT) were analyzed. To check the conservation of the enzyme structure, analysis of the standard deviation of the displacement of the atoms from their reference positions were performed both for the proteins as a whole and for various selected subregions (cofactor, substrate). The results of the RMSD analysis showed the stability of the monomer structure, higher amplitudes of the movement of the glucose molecule and lower amplitudes of the cofactor PQQ in systems with CNT. Analysis of the distance of the cofactor PQQ and the glucose molecule from the active site amino acid, His144, showed that the cofactor fluctuates near the active site, while the glucose molecule is mostly in the active site only at the beginning of the simulations. To test the conservation of the active site of the enzyme, a root mean square fluctuation (RMSF) analysis of the amino acids of the active site and the binding site of the cofactor and the glucose molecule was performed. Stronger fluctuations of the amino acids Gln168 and Gln76, the binding site of glucose, were observed, while the amino acid of the active site, His144, showed negligible fluctuations.

References

[1] A. Oubrie et al, Structure and mechanism of soluble quinoprotein glucose dehydrogenase,The EMBO Journal, vol. 18, 1999.
[2] https://www.ks.uiuc.edu/Training/Tutorials/vmd/tutorial-html/vmd-tutorial-main.html
[3] M. Saeed et al, Assessment of antidiabetic activity of the Shikonin by allosteric inhibition of protein-tyrosine phosphatase 1B (PTP1B) using state of art: An in silico and in vitro tactics, Molecules, 2021.
[4] P. Rao et al, Revealing the molecular interplay of curcumin as Culex pipiens Acetylcholine esterase 1 (AChE1) inhibitor, Nature/Scientifc Reports, 2021.
[5] K. Czaja et al, Theoretical investigations on interactions of arylsulphonyl indazole derivatives as potential ligands of VEGFR2 kinase, International Journal of Molecular Sciences, 2020.

P32 ***The influence of pH on the G-quadruplex ↔ i-Motif ↔ DNA duplex transition***

Milena Kh. Badalyan, Ishkhan V. Vardanyan, Yeva B. Dalyan

Yerevan State University, Chair of Molecular Physics, Yerevan, Armenia
e-mail: milena.badalyan@ysu.am

It is known that in specific regions of double-stranded genomic DNA (such as telomeres, promoter regions of oncogenes, centromeres, etc.) there exist G-rich and C-rich short regular sequences capable of forming four-stranded G-quadruplex and i-Motif structures. Cells utilize the formation of such structures as molecular switches to regulate cellular processes under physiological conditions. The discovery of this fact has become the basis for the formation of a new direction in anti-tumor therapy, based on the targeted generation or disruption of G-quadruplex and i-Motif in selected DNA regions.

In this work, using circular dichroism and differential scanning calorimetry methods, we investigated structural transitions in a 22-mer segment of human telomeric DNA: the G-rich d[5'-A(GGGTTA)3GGG-3'] and the complementary C-rich d[3'-T(CCCAAT)3CCC-5'], as well as their 1:1 mixture depending on the pH of the environment. Studies were conducted at three physiological pH values (5.5, 6.5, and 7.0). The formation of G-quadruplex and i-Motif was monitored by circular dichroism spectra. Calorimetric melting curves of G-quadruplex, i-Motif and their 1:1 mixtures at various pH values were then recorded. The research results showed that upon mixing equimolar amounts of G-quadruplex and i-Motif, the formation of a duplex is enthalpically preferable at all pH values. However, in an acidic environment (pH 5.5) the i-Motif is enthalpically favorable and can coexist with the duplex. Increasing the pH to neutral reduces the enthalpy of melting of the i-Motif structure to zero, thereby shifting the equilibrium towards the duplex.

Acknowledgments

This work supported by the Science Committee of the Ministry of Education, Science, Culture, and Sport of the Republic of Armenia under grant no. 21T-1F115.

References

[1] Milena Kh. Badalyan, Ishkhan V. Vardanyan, Samvel G. Haroutiunian, and Yeva B. Dalyan. Structural Transitions in Complementary G-Rich and C-Rich Strands and Their Mixture at Various pH Conditions, ACS Omega 8 (49), 47051-47056, 2023

P33 ***Innovative Fabrication of Biocompatible Superparamagnetic Iron Oxide Nanoparticles for Enhanced Cancer Therapy***

R. Borlan,[1] R. Lapusan,[1,2] A. Balmus,[2] M. Muntean,[3] M. Focsan[1,2]

[1]Nanobiophotonics and Laser Microspectroscopy Centre, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, 42 Treboniu Laurian Street, Cluj-Napoca, Romania
[2]Biomolecular Physics Department, Faculty of Physics, Babes-Bolyai University, 1 Mihail Kogalniceanu Street, Cluj-Napoca, Romania
[3]Department of Cell and Molecular Biology, Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 8 Victor Babes Street, Cluj-Napoca, Romania
e-mail: raluca.borlan@ubbcluj.ro

Every year, cancer tragically claims the lives of 9.6 million individuals, highlighting the pressing need for groundbreaking therapies. Enter nanotechnology, a revolutionary field transforming cancer treatment by offering precise targeting and improved compatibility, sidestepping the limitations of conventional methods. At the heart of this innovation lie superparamagnetic iron oxide nanoparticles (SPIONs), hailed for their easy synthesis, adaptability, and magnetic properties.

Our study delves into the development of biocompatible SPIONs with heightened multimodal therapy capabilities. Through a refined precipitation technique, we crafted SPIONs with an average diameter of around 6 ± 1 nm and a positive zeta potential of 32 ± 3 mV. To enable seamless surface functionalization for therapeutic agent attachment, we employed a two-step adaptation to coat the SPIONs with bovine serum albumin (BSA). Validation through TEM imaging revealed a final diameter of 67 ± 9 nm, with zeta potential measurements pointing to a negative potential of -64 ± 2 mV.

But the innovation doesn't stop there. We further enhanced the therapeutic potential of these SPIONs by grafting them with curcumin, harnessing its dual role as a photosensitizer and antioxidant. This integration amplifies the nanoparticles' efficacy in targeted cancer treatment. Our validation methods, including UV-Vis and fluorescence spectroscopy analyses, confirm the successful grafting of curcumin onto BSA-coated SPION agents.

In summary, our study presents a novel approach in the realm of cancer therapeutics, utilizing cutting-edge nanotechnology to develop SPIONs with enhanced multimodal therapy capabilities.

Acknowledgments

Raluca Borlan is thankful for the 'Grant for young scientists', awarded by Babes-Bolyai University, contract number SRG-UBB 32995/23.06.2023.

P34 Assessment of biophysical properties of natural variants of staphylokinase

M. Štulajterová[1], Ľ. Ambro[2], M. Tomková[2], E. 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.
e-mail: monika.stulajterova@student.upjs.sk

Thrombosis represents one of the leading cause of mortality in developed countries [1]. Thrombolytic therapy employs medications to dissolve intravascular clots [2]. Prompt treatment with thrombolytic drugs can restore blood flow before major brain damage and improve recovery after stroke [3]. Ideally, thrombolytic agents should have high fibrin specificity, require low dosages, prevent bleeding complications and re-occlusions, and be non-antigenic [4]. Yet, current drugs have limited efficacy and significant hemorrhagic risks, suggesting room for improvement.
Staphylokinase (SAK) is a 136 amino acid -protein originating from lysogenic strains of Staphylococcus aureus [5]. SAK has a high tendency to turn inactive fibrin-bound plasminogen into its active form plasmin through formation of equimolar (1:1) complex with plasmin. SAK is a promising thrombolytic agent with properties of cost-effective production and negligible side effects [6].
Currently, three natural variants SAK 42D, SAK ϕC and SAK STAR have been characterized. These variants have a similar potential to activate plasminogen, but differ in thermostability [7]. We purified two variants SAK 42D and SAK STAR and performed their conformational and functional characterization by applying of circular dichroism, differential scanning calorimetry and chromogenic activity assay. We further analyzed a role of His-tag on the studied biophysical properties.

Acknowledgments

This work was supported by the Slovak Research and Development Agency (project APVV-20-0340) and Agency of Ministry of education, research, development and youth of the Slovak republic (VEGA 1/0074/22).

References

[1] D. Ashorobi, M. A. Ameer, R. Fernandez, StatPearls Publishing, Treasure Island (FL) (2019).
[2] M. U. Baig, J. Bodle, StatPearls Publishing, Treasure Island (FL) (2020).
[3] J. M. Wardlaw, V. Murray, E. Berge, G. J. Del Zoppo, Cochrane database of systematic reviews 7 (2014).
[4] D. Nikitin, S. Choi, J. Mican, M. Toul, W. S. Ryu, J. Damborsky, D. E. Kim, Journal of Stroke 23 (2021).
[5] L. T. Nguyen, H. J. Vogel, H. J., Scientific reports 6 (2016).
[6] R. Nedaeinia, H. Faraji, S. H. Javanmard, G. A. Ferns, M. Ghayour-Mobarhan, M. Goli, M. Ranjbar, Molecular Biology Reports 47 (2020) 819-841.
[7] S. Vanderschueren, F. Van de Werf, D. Collen, Fibrinolysis and Proteolysis 11 (1997) 39-44.

P35 ***Investigating cancer related K-Ras mutations using free energy calculations***

Viktorija Dujmović,[1,2] Dražen Petrov[1]

[1]Institute of Molecular Modeling and Simulation, the BOKU University, Vienna
[2]Faculty of Science, University of Split
e-mail: vdujmovic@pmfst.hr

K-Ras protein is a GTPase signaling protein with the main purpose of regulating cell proliferation and growth. The activation mechanism relies on the nucleotide guanosine triphosphate (GTP) molecule being bound and subsequently catalyzed to guanosine diphosphate (GDP), which then inactivates signaling. K-Ras is the most oncogenic Ras protein, with missense mutation causing pancreatic, colorectal, and lung cancer in the affected population, with a guanine-cytosine mutation at residue position 12 (G12C) being predominant. Understanding the mechanistic details of KRAS G12C activation is crucial for the development of targeted therapies that inhibit mutant protein and effectively treat cancers caused by it. While the mechanisms causing oncogenic transformation are not yet fully known, molecular dynamics (MD) simulations are a powerful computational tool that is able to give us insight into K-Ras binding and activation properties. Here, we employ free energy calculations to thermodynamically characterize K-Ras in the context of mutations, binding of GTP/GDP and binding of small compounds as potential inhibitors.

References

[1] Tatu Pantsar. The current understanding of KRAS protein structure and dynamics. Computational and Structural Biotechnology, Journal 18 (2020) 189-198.
[2] Anita de Ruiter, Chris Oostenbrink. Advances in the calculation of binding free energies. Current Opinion in Structural Biology, Volume 61 (2020) 207-212.

P36 *Conformational study of AD-associated protein tau*

Stefana Njemoga[1,2], Vojtech Spiwok[3], Ondrej Cehlar[1]

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
[2]Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
[3]Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague
e-mail: stefana.njemoga@savba.sk

Intrinsically disordered protein (IDP) tau is neuronal protein that regulates the stability and dynamics of microtubules. On the other hand, because of its structural flexibility, tau is likely to misfold and cause tau filaments to accumulate in Alzheimer's patients' brains [1]. Turning IDPs into druggable targets provides a great opportunity to extend the druggable target-space for novel drug discovery. Since, the most accurate way to describe IDPs is by providing their conformational ensemble, ensemble-based approach involving generation of realistic ensemble of conformers subjected to binding sites analysis is generally chosen as a drug design strategy. To this end, ensemble generators or molecular dynamics simulation could be used to produce the initial set of protein conformers. A common undesirable result of MD simulation is conformation trapping in specific energetic minimum, leading to insufficient conformation sampling. Biasing MD simulations by altering the potential energy function can help protein to overcome energetic barrier and efficiently explore conformational space. With this aim, we employed metadynamics simulations with two sets of collective variables (CVs), with the first set being CVs derived from alphafold [2], and second set of CVs describing the protein's secondary structural properties by stimulation of alpha-helix and anti-beta structure formation [3]. In addition, third set of CVs has been designed by t-distributed parametric time-lagged stochastic neighbor embedding (ptltSNE) machine learning method for future simulations [4]. By comparing results from different types of enhanced sampling simulations, we can select the most suitable method for conformational study of non-globular protein tau and obtain new metastable conformations of tau for our future experiments. The goal of the study is the generation of realistic conformational ensemble of protein tau in order to identify potential binding sites targetable by small molecules. Future plans will involve virtual screening of small molecules library with the ability to inhibit tau aggregation.

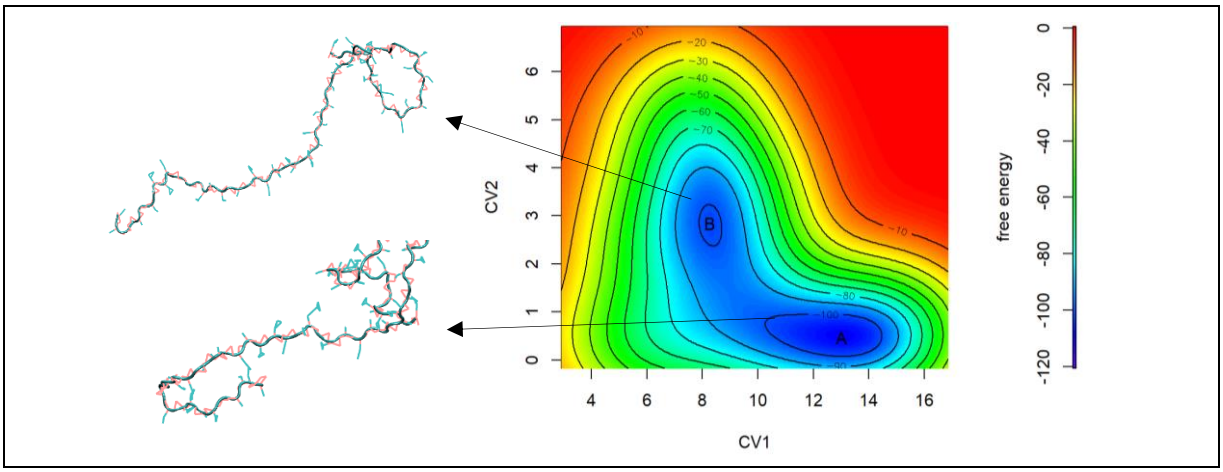


Figure 1: The analysis of the free energy landscape of protein tau from metadynamics simulation guided by a alphafold-derived collective variables and the two corresponding global minimum energy conformations of protein tau [5].

Acknowledgments

This work was supported by COST (European Cooperation in Science and Technology, COST Action CA21160), APVV 21-0479, VEGA 2/0125/23 and InterTAU.

References

[1] Braak H. et al., Journal of Neuropath. Exp. Neur. (2011), 70, 960–969.
[2] Spiwok V. et al., Frontiers in Mol. Biosc., (2022), p. 9.
[3] The PLUMED consortium. Nat. Methods, (2019) 16, 670.
[4] Spiwok V. & Kříž, P., Frontiers in Mol. Biocs. (2020), p. 7.
[5] Trapl D. & Spiwok V., The R Journal, (2022), 14(3):46-58.

P37 *Cholesterol Sensing in TRPC3: Insights from Photopharmacological and MD Studies*

H. Erkan-Candag,[1] D.J. Platero-Rochart[2], O. Tiapko[1], P.A. Sánchez-Murcia[2], T. Glasnov[3], T. Stockner[4], K. Groschner[1]

[1]Gottfried-Schatz Research Center, Medical Physics and Biophysics, Medical University of Graz, Austria, [2]Otto-Loewi Research Center, Medicinal Chemistry, Medical University of Graz, Austria, [3]Institute of Chemistry, University of Graz, Austria, [4]Institute of Pharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria.
e-mail: hazel.erkan@medunigraz.at

Cholesterol is crucial for membrane protein assembly and regulates the surface expression and activity of ion channels like Transient Receptor Potential Canonical 3 (TRPC3). Despite its importance, the precise mechanism of cholesterol's impact on TRPC3 function still remains elusive. Previous studies have highlighted cholesterol's involvement in modulating the activity of K^+ channels such as Kir2.1 and BK channels, underscoring its importance in cellular physiology (1, 2).

Recent single-particle cryo-EM studies have identified two essential lipid coordination sites, L1 and L2, on TRPC3 (3). To investigate the effects of these sites on TRPC3-cholesterol interactions, we employed a combination of all-atom molecular dynamics (MD) simulations, electrophysiological recordings, and photopharmacological tools. MD simulations revealed that cholesterol binding to TRPC3 is highly dynamic, with the sterol adopting multiple conformations rather than a single fixed position. These simulations identified the L1 site and the domain-swapped interface of the voltage-sensor-like domain (VSLD) as key regions with higher cholesterol residency time.

To investigate the principles of sterol regulation in TRPC3 with the precision of light, we introduced OptoChol-1, a photoswitchable lipid that mimics cholesterol. Photopharmacological experiments demonstrated that OptoChol-1 photocycling effectively regulates the basal activity of TRPC3 in HEK cells. Notably, the M586A mutation, predicted by molecular docking as a loss-of-function mutation for channel regulation via L1-bound sterols, showed a significantly reduced response to OptoChol-1. Conversely, the W334A mutation in the CRAC motif within the L1 site had minimal effect on OptoChol-1's impact. Moreover, the modulation of TRPC3 currents by OptoChol-1 photocycling appeared strictly dependent on the chemical structure of diacylglycerols bound to L2.

Our findings offer valuable insights into the structural basis of sterol sensing and the cellular regulation of TRPC3, and provide first evidence for channel regulation by cooperative lipidation events in the L1 and L2 regulatory site. These findings on cholesterol's role in TRPC channel modulation will pave the way towards new therapeutic strategies by targeting lipid-ion channel interactions.

References

[1] A. Rosenhouse-Dantsker, D. Mehta, I. Levitan, Compr Physiol. 2012;2(1):31-68
[2] I. Levitan, Y. Fang, A Rosenhouse-Dantsker, V. Romanenko, Subcell Biochem. 2010; 51:509-49
[3] C. Fan, W. Choi, W. Sun, J. Du, W. Lu, Elife. 2018;7.

P38 ***From marine lipids to marine vesicles: A preliminary study***

Maja Levak Zorinc[1], Ruža Frkanec[2], Blaženka Gašparović[1], Maja Dutour Sikirić, Nadica Ivošević DeNardis[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia
e-mail: maja.levak@irb.hr

The marine ecosystem is an important resource for bioactive substances and various materials that could be potentially used in medicine, nutrition and biotechnology. In recent decades, biomedicine has turned to the sea, in particular to find the source of natural organic materials that can serve as a matrix for the fundamental study of membrane-related processes. Studies on vesicle formation from the lipids extracted from the seawater are very scarce. Therefore, our aim is to prepare vesicles from extracted marine lipids and characterize their morphology, surface charge and lipid composition. We used marine lipid extracts from four sampling stations in the northern Adriatic Sea and prepared a unique lipid composite for which we will show a lipid profile. We apply an analogous protocol for the formation of liposomes by the thin film hydration method.

The vesicles were formed efficiently with diameters of up to 5 µm. DLS measurements revealed that the zeta potential is – 43 mV, indicating a stable vesicle suspension that persists over an extended period of time. The detailed structure and morphology of the marine lipid vesicles was imaged for the first time using atomic force microscopy (AFM). The vesicles imaged in air had a very defined, spherical shape. The average thickness of the dried membrane was 60 ± 30 nm, while the height was 2.5 ± 0.5 nm. When the vesicles were imaged in a liquid and adhered to a substrate, they acquired a dome-like to spherical shape with heights between 600 and 1000 nm.

This study contributes to the understanding of the spontaneous transformation of marine lipid matter through self-organization processes[1,2] at the various interfaces in aquatic systems.

References

[1] Moussai, N., Cansell, M., Denizot, A. Marinosomes, marine lipid-based liposomes: physical characterization and potential application in comestics*, Int. J. Pharm*. **242**, 2002, 361-365.
[2] Shabana, S.; Hamouda, H.I.; Hamadou, A.H.; Ahmed, B.; Chi, Z.; Liu. C. Marine phospholipid nanoliposomes: A promising therapeutic approach for inflammatory bowel disease: Preparation, safety, and efficacy evaluation. Colloid. Surf. B. Biointerfaces

P39 *Understanding of free radicals induced by ultrasonication and their effects on antioxidative capacity and microbial quality in tomato juice*

F. Markić,[1] K. Smokrović,[1] V. Stulić,[2] S. Pleslić,[3] M. Zadravec,[4] T. Vukušić-Pavičić,[2] N. Maltar-Strmečki[1]

[1]Ruđer Bošković Institute, Division of Physical Chemistry, Zagreb, Croatia
[2]University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia
[3]University of Zagreb, Faculty of Electrical Engineering and Computing, Zagreb, Croatia
[4]Croatian Veterinary Institute, Zagreb, Croatia
e-mail: Franka.Markic@irb.hr

Tomato (*Solanum lycopersicum L.*) is the one of the most worldwide used agricultural product [1]. Tomato and tomato-based products are rich in antioxidant compounds especially carotenoids (lycopene and β-carotenoids), ascorbic acid and phenolic compounds. These compounds play an important role in inhibiting radical activity in human organism by scavenging free radicals [2]. The main radicals are reactive oxygen species (ROS) such as superoxide anion, hydroxyl, peroxyl, alkoxyl radical, hydrogen peroxide, singlet oxygen and reactive nitrogen species (RNS). The development of non-thermal food processing shows an advantage of preserving food products without unfavourable effects on the main nutritional, chemical and physical properties which impact on quality of the final product, compared with conventional heat treatments [3]. Therefore, the aim of this study was to investigate influence of ultrasonication treatment duration on reactive oxygen species, antioxidative capacity and microbiological spoilage in tomato juice.

Tomato juice was prepared from fresh tomato plants (*Solanum lycopersicum L.*). The UWave-2000 Multifunctional Microwave Chemistry Reaction Workstation (Sineo) was used for ultrasonic treatment. X-band ESR spectra were recorded using Bruker Magnettech ESR5000 spectrometer. Radical formation was monitored on ESR by using 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine hydrochloride (CMH) spin trap.

In this study, the results show the reduction of the total number of the analysed microorganisms in ultrasonicated processed tomato juice. This effect was associated with the duration of ultrasonication (1, 3 and 5 minutes). The decrease in concentration of oxygen radicals detected by ESR spectroscopy have been observed in comparison with control sample. The effect of increase of antioxidative capacity determined by DPPH ESR assay confirms that the application of ultrasonic treatment as non-thermal method for food processing shows more advantages than traditional heat treatments [3].

Acknowledgments

This work has been fully supported by H2020 PRIMA Initiatives (The Partnership for Research and Innovation in the Mediterranean Area) as part of the project No. 2032 Functionalized Tomato Products (FunTomP).

References

[1] RG Borguini, EA Ferraz Da Silva Torres, Food Reviews International (2009) 313.
[2] MY Ali, AA Sina, SS Khandker, L Neesa, EM Tanvir, A Kabir, MI Khalil, SH Gan, Foods (2020) 10(1):45.
[3] A Starek, Z Kobus, A Sagan, B Chudzik, J Pawłat, M Kwiatkowski, P Terebun, D Andrejko, Scientific Reports (2021) 3488

P40 ***Expression, purification and identification of HPIV2 nucleoprotein oligomer in Escherichia coli***

Z. Matić[1], M. S. Khan[2], D. Vujaklija[2], S. Kazazić[2]

[1]Laboratory for electron spin spectroscopy, Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
[2]Laboratory for mass spectrometry and functional proteomics, Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
e-mail: zmatic@irb.hr

Human parainfluenza virus type 2 (HPIV2) belongs to the *Paramyxoviridae* viral family. This single-stranded negative-sense RNA virus has a genome comprising six genes: NP, P/V, M, F, HN, and L. The viral RNA is tightly bound by the nucleoprotein (NP), forming a highly organized helical ribonucleoprotein (RNP) filamentous structure [1]. When interacting with short RNAs, NP forms oligomeric structures. Specifically, HPIV2 NP oligomers consist of ring-like structures assembled from 13 protomers, with the protomer number being virus-specific [2].

Nanoscale self-assembled proteins serve as versatile platforms in various biomedical applications, including the development of new vaccine candidates [2]. Due to their nano-size, NP ring-like structures exhibit delivery system and adjuvant properties, making them promising candidates as noninfectious carriers of antigenic determinants [3].

In our study, we expressed, solubilized, and purified HPIV2 nucleoprotein from *Escherichia coli* to characterize it using transmission electron microscopy (TEM). TEM images revealed NP oligomeric structures consistent in size with those in the homology model based on the crystal structure of the closely related PIV5 nucleoprotein [4]. These HPIV2 NP oligomeric structures will serve as a vaccine carrier in the further development of new vaccine candidates against influenza infection.

Acknowledgments

This study was financially supported by the RAPTOVAX project (KK.01.1.1.04), a project co-financed by the Croatian Government and the European Union and by Structure characterization of HPIV2 nucleoprotein-based nanoparticles project, iNEXT-Discovery project financed by European Union.

References

[1] L. Bloyet, Viruses 13 (2021) 2465.
[2] M. Šantak, Z. Matić, Viruses 14 (2022) 521.
[3] V. Aida, V. C. Pliasas, P. J. Neasham, F. North, K. L. McWhorter, S. R. Glover, C. S. Kyriakis, Frontiers in Veterinary Science 8 (2021) 654289.
[4] M. Alayyoubi, G. P. Leser, C. A. Kors, R. A. Lamb, PNAS 112 (2015) E1799-E1792.

P41 *Quantum Chemical Docking of Quinuclidine-Based Carbamates Within the Active Site of Butyrylcholinesterase*

A. Mikelić,[1,2] I. Primožič,[1] T. Hrenar[1]

[1]Department of Chemistry, University of Zagreb Faculty of Science, Horvatovac 102a, Zagreb, Croatia

[2]Department for Veterinary Public Health, Croatian Veterinary Institute, Savska cesta 143, Zagreb, Croatia

e-mail: mikelic@veinst.hr

The aim of this research was to investigate the interaction of quinuclidine-based carbamates with the active site of butyrylcholinesterase by performing quantum chemical docking. Two newly developed methods were used: stochastic Monte Carlo sampling (QMMC) and tensor decomposition of *ab initio* molecular dynamics (AIMD) trajectories of a free (unbound) small molecule within the active site of the enzyme using our own program *qcc* [1].

In QMMC docking, the configurational space of each ligand was spanned in 3 translational, 3 rotational and all relevant torsional degrees of freedom whereas the protein was rigid during the docking process. For each generated configuration, single-point calculations were performed using the semi-empirical PM7 Hamiltonian.

The method of molecular docking by tensor decomposition of AIMD trajectories utilizes a previously published protocol for AIMD simulations [2]. Machine learning was applied to determine the point at which full configurational spaces were sampled for minimal simulation steps and dimensionality of the reduced trajectory (Figure 1).

After sampling the active site with AIMD or QMMC, Michaelis complexes with the lowest energy were selected for QM/QM geometry optimizations and harmonic frequency calculations using the two-layer method ONIOM(B3LYP-D3/6-31G(d):PM7).

The results obtained with the two methods will be compared based on the positioning of small molecules in the active site concerning the characteristic regions of butyrylcholinesterase: the catalytic triad, the acyl pocket, the choline-binding site, and the oxyanion hole.

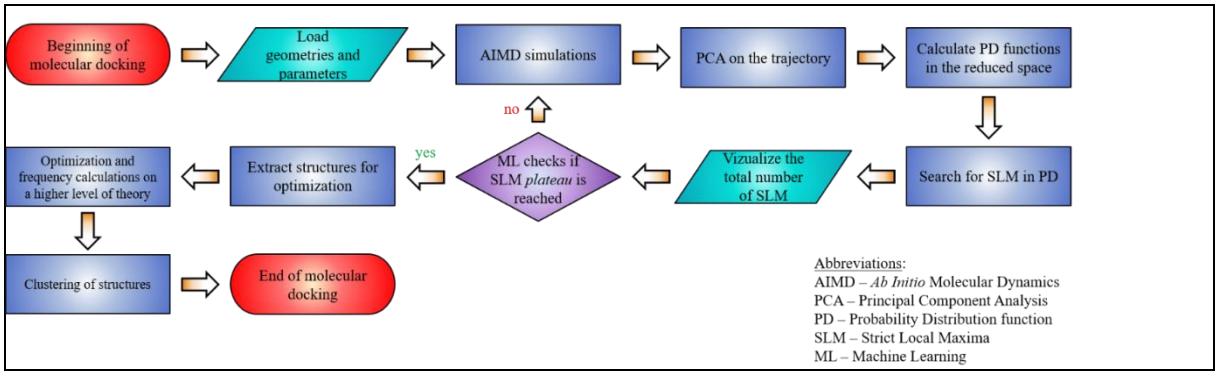


Figure 1: Flowchart of molecular docking by tensor decomposition of AIMD trajectories using ML.

Acknowledgments

This research was funded by the Croatian Science Foundation, grants: "*Young researchers' career development - Training New Doctoral Students*" (ESF-DOK-2018-09-3416), "*Activity and* in silico *guided design of bioactive small molecules*" (IP-2016-06-3775) and *Target-guided synthesis of cholinesterase inhibitors supported by machine learning* (IP-2022-10-9525).

References

[1] T. Hrenar, *qcc*, *Quantum Chemistry Code*, rev. 0.6826, 2024.

[2] T. Hrenar, I. Primožič, D. Fijan, M. Majerić Elenkov, *Phys. Chem. Chem. Phys.* **19** (2017) 31706.

P42 *Pharmacological profiles of the main compounds of Artemisia dracunculus*

A. Pujicic, A. Isvoran

Department of Biology-Chemistry, West University of Timisoara, 16 Pestalozzi, 300115 Timisoara, Romania
e-mail: andrijana.pujicic@e-uvt.ro

Artemisia dracunculus is a plant that is used in food industry and traditional medicine because its known antibacterial, antifungal, antihemintic, antiseptic, anti-inflammatory, antipyretic, carminative, digestive and stimulant activities. The aim of this study is to obtain the pharmacological profiles of the main compounds of *Artemisia dracunculus* by using a computational approach. Using the FooDB database we have identified the main organic compounds (with a content of at least 100 mg/100 g) in *Artemisia dracunculus*: caffeic acid, 17alpha-ethynyl estradiol, anethole, 1-methoxy-4-(2-propenyl) benzene, nerol, maltose, thujene and methyleugenol. The results obtained reveal that these compounds can inhibit organic anion and cation transporters, P-glycoprotein, and cytochromes involved in endo- and xenobiotic metabolism, and can produce skin sensitization and hepatotoxicity. In addition, 17alpha-ethynyl estradiol may lead to cardio- toxicity, respiratory toxicity, endocrine disruption, and reproductive toxicity. Anethole, caffeic acid and methyleugenol are the compounds that are also likely to produce reproductive toxicity. This information is important especially for those that are professionally exposed to higher quantities of these compounds.

Acknowledgments

The financial support to participate to this event was assured by the project „FSS: International student – next step in education"

References

[1] https://foodb.ca/ accessed in 02.05.2024
[2] Y. Hongbin, L. Chaofeng, S. Lixia, L. Jie, C. Yinchun, W. Zhuang, L. Weihua, L. Guixia, T. Yun, Bioinformatics 35 (2019) 1067-1069.
[3] X. Guoli, W. Zhenxing, Y. Jiacai, F. Li, Y. Zhijiang, H. Changyu, Y. Mingzhu, Z. Xiangxiang, W. Chengkun, L. Aiping, C. Xiang, H. Tingjun, C. Dongsheng, Nucleic Acids Res 49 (2021) 5-14.

P43 ***Exploring Biomedical Applications of BSA-Coated SPIONs Grafted with Curcumin: A Multimodal Approach for Targeted Cancer Therapy***

R. Lapusan,[1,2] R. Borlan,[2] O. Soritau,[3] M. Focsan[1,2]

[1] Nanobiophotonics and Laser Microspectroscopy Centre, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babes-Bolyai University, 42 Treboniu Laurian Street, Cluj-Napoca, Romania
[2] Biomolecular Physics Department, Faculty of Physics, Babes-Bolyai University, 1 Mihail Kogalniceanu Street, Cluj-Napoca, Romania
[3] Department of Radiobiology and Tumor Biology, Oncology Institute Prof. Dr. Ion Chiricuta, 34-36 Republicii Street, Cluj-Napoca, Romania
e-mail: radu.lapusan@ubbcluj.ro

The present study explored the biomedical applications of the bovine serum albumin (BSA) -coated superparamagnetic iron oxide nanoparticles (SPIONs) grafted with curcumin due to its well-known therapeutic properties. After a thorough in solution characterization, including the assessment of curcumin's antioxidant activity, these nanoparticles were incorporated into phantoms and imaged using dark field and fluorescence microscopy to assess their potential in medical imaging. The results demonstrated their suitability for medical applications, showing promising imaging capabilities within the phantoms.

A melanoma cell line was used for further *in vitro* testing. Viability tests were conducted to evaluate the compatibility of the free SPION versus BSA-coated SPION agents with biological systems. The findings indicated good compatibility of the BSA-coated agents, paving the way for further biomedical applications.

Furthermore, fluorescence *in vitro* imaging was performed to examine the cellular internalization of the nanoparticles. Interestingly, when cells were treated with BSA-coated curcumin-grafted SPIONs, significant cellular internalization was observed throughout the cell body, but not in the nucleus. Comparatively, cells treated with free curcumin showed penetration into the nucleus, confirming the successful functionalization of our BSA-coated SPIONs with curcumin. Additionally, it is noteworthy that the free form of SPIONs exhibited intrinsic fluorescence, which was observed *in vitro*. This intrinsic fluorescence adds another dimension to their potential applications, opening avenues for further research and development in biomedical imaging and therapy.

P44 *Copper-nitrocatechol interactions govern their toxic effects to microalgae*

S. Bakija Alempijević, I. Mihaljević, S. Frka, S. Strmečki Kos

Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
e-mail: saranda.bakija.alempijevic@irb.hr

Nitrocatechols (NCs) are monoaromatic atmospheric pollutants which account for a significant fraction of submicron atmospheric aerosols and water-soluble organic carbon [1] making them hazardous to humans and the environment. The interactions with various organic and inorganic compounds in natural waters determine the detrimental effects of organic pollutants on aquatic microalgae [2]. One of the most significant trace metals in the aqueous atmosphere phase is copper (Cu(II)) which affects radical production, redox processes, and the cycling of other metal ions in the atmosphere [3]. Cu(II) is one of the essential minerals for microalgae in both freshwater and seawater, although at high concentrations, its toxicity depends on complexation with organic molecules [4]. The aim of our research was to study if and how Cu-NCs interactions impact their binary toxic effects to microalgae.

We performed a chronic *in vivo* toxicity test with the unicellular green alga *Scenedesmus subspicatus* using the standardized method ISO 8692:2004 (AlgaeTox bioassay). We selected 4-nitrocatechol (4NC) and 4-methyl-5-nitrocatechol (4M5NC) as a representative model atmospheric NCs. We found that Cu(II), 4M5NC and 4NC individually were harmful to microalgae, with µM concentrations of IC_{50} values (the concentration at which 50% of the algal population died). Then, the microalgae's vitality was followed at various ratios of Cu(II) to 4NC or 4M5NC concentrations. Depending on the ratios, both Cu-4NC and Cu-4M5NC mixtures were toxic to algae. To explain such an effect, we have studied the formation of Cu(II)-NC complexes in aqueous solutions by the electrochemical method of square-wave voltammetry using a mercury drop working electrode, bearing in mind that mono- and di-catecholates, CuL and CuL_2^{2-}, are produced when Cu(II) combines with catechol (1,2-dihydroxybenzene) type ligands in aqueous solution [5]. When stable Cu(II)-4NC and Cu(II)-4M5NC complexes were formed, the microalgae increased their survival because complexation lessened the concentration of free (uncomplexed) Cu(II) and NCs in the binary mixture when compared to the effects of Cu(II) or NCs alone. We demonstrated that mutual interactions of organic and inorganic pollutants governed their bioavailability and therefore could be crucial for their (non)toxic effects on microalgae cells in aquatic systems.

Acknowledgments

We acknowledge HrZZ project BiREADI (IP-2018-01-3105) for financial support.

References

[1] S. Frka, M. Šala, H. Brodnik, B. Stefane, A. Kroflič, I. Grgić, Chemosphere, 299 (2022) 134381.
[2] Y.G. Tao, W. Li, B. Xue, J.C. Zhong, S.C. Yao, Q.L. Wu, Journal of Hazardous Materials, 261 (2013) 21-28.
[3] L. Deguillaume, M. Leriche, K. Desboeufs, G. Mailhot, C. George, N. Chaumerliac, Chemical Review, 105 (2005) 3388-3431
[4] W.G. Sunda, Frontiers in Microbiology, 3 (2012).
[5] M.J. Sever, J.J. Wilker, Dalton Transactions, (2004) 1061-1072.

P45 ***Effect of type 2 diabetes on the fracture resistance of human dentin***

Sarolta Antal,[1] Kata Sára Haba,[1,2] Gábor Szebényi,[3] Horváth Ildikó,[1] Miklós Kellermayer[1], Dóra Haluszka[1]

[1]Semmelweis University Institute of Biophysics and Radiation Biology, 1094 Budapest, Tuzolto u. 37-47.
[2]Semmelweis University Department of Prosthodontics, 1088 Budapest Szentkirályi utca 47.
[3]Budapest University of Technology and Economics Faculty of Mechanical Engineering, Department of Polymer Engineering, 1111 Budapest, Muegyetem rkp. 3
e-mail: antal.sarolta20@gmail.com

Dentin is a mineralized tissue that forms the bulk of tooth and consists of a mineralized collagen matrix containing a large number of small, parallel tubules. Non-enzymatic glycation, occurs when the aldehyde group of a reducing sugar (e.g., glucose, fructose) interacts with the free amino groups of a long-lived protein eventually forming advanced glycation end products (AGEs). The accumulation of AGEs is responsible for several complications in type 2 diabetes mellitus (T2DM) and is implicated in many age-related chronic diseases [1]. Considering that dental collagen displays no protein turnover, it is highly susceptible to the accumulation of AGEs due to elevated blood glucose, thus affecting its long-term structural stability. It is already known that T2DM negatively affects the root fracture resistance, which can be explained by low levels of calcium, phosphorous or strontium [2]. However, fraction tests under static force on diabetic coronal region of tooth samples have not been carried out yet. By exposing samples to static compression we can reveal the load bearing capacity of the tooth material. In this study, our focus lies in exploring the mechanical and structural changes induced in teeth by AGEs in T2DM.

For the static compression test normal (n = 18) and T2DM-affected (n = 13), extracted teeth with uncovered coronal region were embedded in epoxy resin in a circular plastic container. Coronal regions were compressed perpendicular to the occlusal surface with 2 mm/min load by using an 8 mm diameter spherical steel indenter until the first visible crack appeared, based on the recorded force vs. indentation curve. To detect the location, direction and size of failures, specimens were scanned before and after the mechanical tests with CT.

Regarding the mechanical test, the average fracture resistance in control teeth was twice as large as in diabetes-affected tooth samples. Based on the CT images following the fracture test, remarkable tooth material loss could be seen in the diabetes group, which indicates a higher fracture susceptibility. Further analysis showed that the majority of fractures was located in the approximal and occlusal surface of the teeth, but in the diabetes group these cracking lines were deeper and wider resulting a greater amount tooth material loss.

In our work we detected that the fracture susceptibility of human teeth affected by type 2 diabetes is drastically increased. These results confirmed that the accelerated glycation process in type 2 diabetes significantly altered the mechanical properties of tooth material. These findings also have clinical relevance and shed lights on different complication in oral health such as enhanced erosion, abfraction which eventually may lead to tooth loss.

Acknowledgments: supported by TKP2021-EGA-23

References

[1] Avery, N.C. and A.J. Bailey, Enzymic and non-enzymic cross-linking mechanisms in relation to turnover of collagen: relevance to aging and exercise. Scand J Med Sci Sports, 2005. **15**(4): p. 231-40.
[2] Saghiri, M.A., et al., The effect of diabetes on Fracture Resistance of Teeth: An in vitro study. Aust Endod J, 2021. **47**(3): p. 499-505.

P46 *CRISPR AMR DX: an optimised CRISPR/Cas12a-based multiplex biosensor for rapid diagnosis of enteric fever and antimicrobial resistance*

Mujar Minette Shalo[1], Jenny Molloy[1], Stephen Baker[2]

[1]University of Cambridge, Department of Chemical Engineering and Biotechnology
[2]University of Cambridge, Department of Medicine
e-mail: mms87@cam.ac.uk

Enteric fever is a common cause of morbidity and mortality in underdeveloped countries where it is endemic. Clinical management and control of the disease rely on prompt diagnosis and effective treatment. Nevertheless, current diagnostic methods are not sufficiently specific and sensitive while antimicrobial resistance is becoming widespread, limiting treatment efficacy. Significant delays to treatment are due to the widely-used blood culture diagnostics which take several days to return a result and have limited sensitivity of only 40–60% [1]. The emergence of novel molecular techniques such as Clustered regularly interspaced short palindromic repeats (CRISPR-Cas12a)-based diagnostics has improved the potential of point-of-care molecular diagnostics. Cas12a is an RNA-guided endonuclease that detects very specific DNA sequences. Hybridization to the target strand activates indiscriminate cleavage of a single-stranded reporter DNA in the reaction mix, releasing a fluorescent read-out[2] (Figure 1).

CRISPR AMR Dx seeks to engineer an affordable, sensitive multiplex Cas12a-based biosensor to diagnose enteric fever caused by typhoidal Salmonella and identify related antibiotic-resistant genes. Computational tools and molecular dynamic simulations will be used to inform the engineering of a thermostable Cas12a to improve its processivity and sensitivity. RNA guides specific to multiple bacteria and AMR targets will be predicted and optimised using machine learning algorithms. With the help of a collaborator, the improved assay will be integrated into a multichannel microfluidic device. This will enhance specificity and sensitivity while reducing complexity and the need for instrumentation, in line with the REASSURED criteria for point-of-care diagnostics.

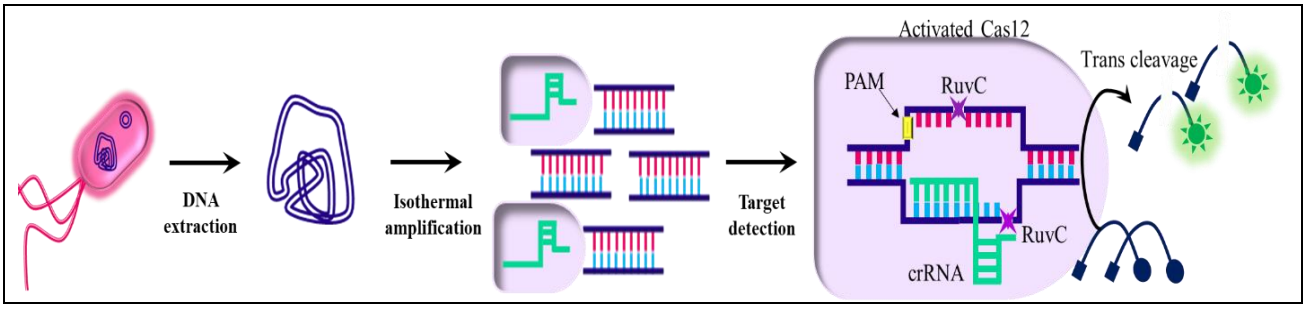


Figure 1: CRISPR-based diagnostics rely on collateral cleavage activity of Cas12a which cleaves a specific sequence and a ssDNA reporter producing a readable signal.

Acknowledgements

To Open Bioeconomy Lab (OBL) and the endeavour for an open, globally inclusive and equitable bioecconomy.

References

[1] Parry, C.M., Wijedoru, L., Arjyal, A. and Baker, S., 2011. The utility of diagnostic tests for enteric fever in endemic locations. Expert review of anti-infective therapy, 9(6), pp.711-725.
[2] Chen, J.S., Ma, E., Harrington, L.B., Da Costa, M., Tian, X., Palefsky, J.M., Doudna, J.A., 2018. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science 360, 436–439. https://doi.org/10.1126/science.aar6245.

P47 ***Determining hydration entropy of proteins and co-solutes utilizing microscale thermophoresis***

Simon van Impel, Oktavian Krawczyk, Christian Herrmann

Physical Chemistry I, Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, Germany
e-mail: simon.vanimpel@rub.de

The hydration of proteins has a major impact on their structure, stability, and functionality. The stability and structural integrity of proteins is based on relatively weak interactions between peptide surfaces, competing with interactions involving the solvent and co-solutes. The enthalpy/entropy compensation mechanism plays a crucial role in this process, yet its quantitative understanding remains elusive. Various physical and molecular factors contribute to the balance between the two thermodynamic driving forces, including polarity, viscosity, H-bonding propensity, and even molecular crowding and confinement.

This study focuses on examining the changes in the entropy of hydration water, distinct from bulk water. In particular, the type and concentration of the hydrated co-solute used should be analysed, as it has an impact on the perturbation of water available for hydration of proteins – and hence on protein stability and interactions.

Microscale Thermophoresis is traditionally utilized for titrating biomolecular interactions, such as protein and ligand or protein-protein interactions. Here we return to the physical principles of thermophoresis, specifically the Soret effect, which arises from the temperature dependence of free energy causing the diffusion of molecules in a temperature gradient [1]. To a large part this effect is controlled by the hydration entropy of the protein [2]. In order to make use of this in our experimental approach we had to define precisely the temperature gradient for the experimental setup of the thermophoresis equipment (Nanotemper Monolith®). We achieved this by exploiting the temperature sensitivity of fluorescent reference compounds.

With this we are equipped to measure the effects of various co-solutes on the thermophoresis of fluorescence molecules and to obtain values of hydration entropy of the first and second water layer of co-solute molecules. We find differences in the perturbation of water entropy (available for protein hydration) for co-solutes like urea, isopropanol, glycerol or NaCl. In light of the high concentration of similar co-solutes, biomolecules and proteins inside a living cell this is of particular interest for judging protein stability and interactions when life conditions and diluted solutions as in laboratory conditions are compared.

References

[1] Duhr, S., & Braun, D. (2006) *Physical Review Letters* **96** (2006) 16: 165901(4)
[2] Duhr, S., & Braun, D. (2006) *Proceedings of the National Academy of* Sciences **103** (2006) 52: 19678–19682

P48 ***SR-FTIR characterization of the hippocampus: the role of tenascin C in adult neurogenesis in the subgranular zone***

Milena Tucić[1], Andrej Korenić[1], Tanja Dučić[2], Pavle R. Andjus[1]

[1]Center for laser microscopy, Faculty of Biology, University of Belgrade, Belgrade, Serbia
[2]CELLS-Alba Synchrotron, Barcelona, Spain
e-mail: milena.tucic@bio.bg.ac.rs

Adult stem cell niches are specialized forms of extracellular matrix suitable for maintaining the development of newborn neurons. The process is regulated by internal and external factors, such as enriched environment (EE). The subgranular zone (SGZ) in the hippocampus is enriched with molecules such as tenascin C (TnC), a regulatory glycoprotein involved in the proliferation and migration of cells. The aim of this study was to examine how TnC affects the distribution of different biomolecules and the number of developing cells in the adult SGZ.

To directly characterize the molecular environment of the subgranular zone, synchrotron based FTIR spectroscopy was used. We analyzed the differences in the distribution of lipids, proteins and carbohydrates among four animal groups - TnC+/+ mice housed in standard conditions, TnC+/+ mice housed in EE, TnC-/- mice housed in standard conditions, TnC-/- mice housed in EE. Additionally, immunohistochemistry was used to estimate the number of proliferative cells and immature neurons in the same experimental groups.

The statistical analysis of FTIR spectra showed that TnC deficiency leads to decrease in the lipid composition and decreased phosphate content in the adult SGZ. On the other hand, the absence of TnC did not affect the number of proliferative cells. The number of immature neurons remained unaffected in the absence of TnC, while the length of the neuronal dendritic trees increased in TnC-/- mice.

This study reports the role of TnC in affecting the maturation of immature neurons during the adult hippocampal neurogenesis. Well-known stimulating effect of EE on adult neurogenesis lacks in the absence of TnC since the initial developmental stages of newborn cells are affected by region-specific biomolecular composition.

P49 *Spider silk protein-DNA bioconjugates binding functional ligands*

V. Fedorova[1], K. Siposova[1], M. Humenik[2]

[1]Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovak Republic

[2]Faculty of Engineering Science, University Bayreuth, Prof.-Rüdiger- Bormann-Str.1, D-95447 Bayreuth, Germany

e-mail: fedorova@saske.sk

Recombinant spider silk protein eADF4(C16) is known as one of the functional proteins, which undergoes autonomous self-assembly process resulting in formation of fibrillar nanostructures with extraordinary mechanical and physicochemical characteristics[1]. Moreover, the possibility of chemical modification of this protein using functional ligands such as DNA aptamers lays the foundation for processing new bio-based nanomaterials with bio-targeting functions[2].

Our aim was to synthesize and characterize bioconjugates composed of eADF4(C16) and aptamers TBA15 or TBA29, which specifically bind thrombin enzyme on active and regulatory site, respectivelly[3]. The first step of the synthesis was chemical modification of protein's N-terminus with an azide group and aptamers' 5' end with dibenzocyclooctyne moiety enabling "click" reaction of the modified reactants. Verification of these steps was performed by MALDI-TOF, HPLC and native gel electrophoresis.

Further experiments using biochemical/biophysical techniques, e. g. spectroscopy and atomic force microscopy showed that in the bioconjugates the ability of protein moiety to form nanofibrils by self-assembly was preserved, which could be controlled by experimental conditions like ion concentration (phosphate, Na^+, K^+, Cl^-) or temperature.

Subsequent addition of thrombin enzyme into solution with eADF4(C16)-TBAx bioconjugates and detailed description of the interactions between both components might result in forming of hierarchically organized bio responsive nanostructures with potential for biomedical applications for example as drug delivery systems with the possibility of controlled release of the ligand as well as the absence of dangerous side effects.

Acknowledgments

This work was supported by the grants VEGA 2/0034/22 and SK-DAAD 2024-2025.

References

[1] Humenik, M., & Scheibel, T.: *ACS nano*, *8*(2), 1342-1349 (2014).

[2] Humenik, M., Preiß, T., Gödrich, S., Papastavrou, G., & Scheibel, T.: *Materials Today Bio*, *6*, 100045 (2020).

[3] Lai, P. X., Mao, J. Y.,Unnikrishnan, B., Chu, H. W., Wu, C. W., Chang, H. T. & Huang, C. C. (2018). *Biomaterials science*, *6*(7), 1882–1891.

P50 ***Importance of synchrotron-based x-ray fluorescence microscopy in studies of distribution of nanoparticles in biological systems***

M. Žižić,[1,2] V. Bonanni,[1] and A. Gianoncelli[1]

[1]Elettra Sincrotrone Trieste, SS 14 km 163, 5 in Area Science Park, 34149 Basovizza, Trieste, Italy
[2]Institute for multidisciplinary research, University of Belgrade, Serbia
e-mail: milan.zizic@elettra.eu

Application of synchrotron-based x-ray fluorescence microscopy (XFM) in biological systems takes one of the leadership positions regarding studies of spatial distribution of essential elements at cellular level [1]. TwinMic beamline of Elettra synchrotron in Trieste hosts a twin x-ray microscope (Fig 1A) intended to investigate heterogeneous phenomena at microscopic length scale by XFM, irradiating samples with photons that belongs to low energy x-ray part of electromagnetic spectrum [2]. The implemented technique is based on interaction of soft x-rays with samples from different fields of biology, chemistry and medicine, whereby distribution of light elements at the cellular or tissue level with spatial resolution up to 100 nm can be determined. The completeness of problem solution is reached by simultaneously collected absorption and differential contrast phase imaging (scanning transmission x-ray microscopy) [3]. The combination of these two techniques provides chemical and morphological images of sample in parallel, with minimal exposure to radiation and without special requirements for sample preparation that include application of chemical agents. Applications in biology are multiple and comprise studies of samples from all biological kingdoms. Here, we are presenting selected results obtained by synchrotron radiation soft x ray microscopy in exploring cells exposed to different nanoparticles (NPs). Studies performed on fibroblast cells treated with Co-Fe NPs shed light to the fine line between benefits and toxicity of nanoparticles, that in dependence of concentration may induce positive but also irreversible negative consequence (Fig 1B). On the other side, it is presented how the absorption of soft energy x-rays can give an answer about the possibilities of substitution of commercially used fertilizers by NPs in a bid to promote plant growth in more efficient and ecologically more acceptable manner (Fig 1C). Finally, the capacity of biologically produced of Se nanoparticle by *Phycomyces blakesleeanus* mycelium has been elucidated and spatially resolved by using soft XFM(Fig 1D).

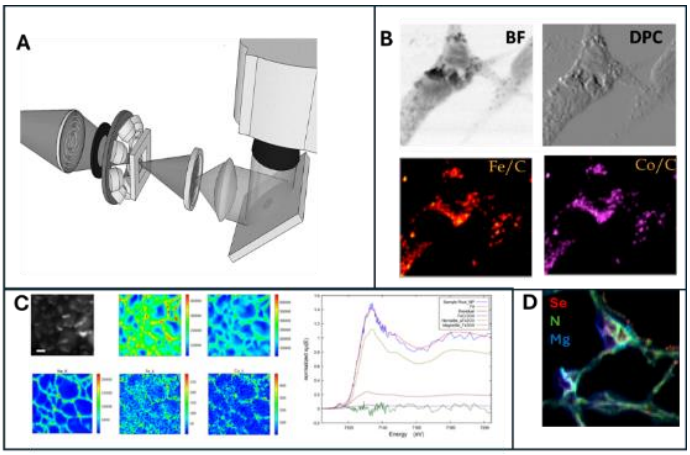


Figure 1: The blueprint of the important elements at end station of TwinMic. Absorption and chemical images of NPs treated fibroplast cells. XRF maps of light elements in root of zucchini with XANES spectra of: Cu standards and Cu inside the tissue. Comparative display of Se, N and Mg in the mycelium of the fungus *of P. blakesleeanus*.

References

[1] B. Kaulich, P. Thibault, A. Gianoncelli and M. Kiskinova, J. Phys.: Condens.Matter, 2011, 23, 083002.
[2] A. Gianoncelli, B. Kaulich, R. Alberti, T. Klatka, A. Longoni, A. De Marco, A. Marcello and M. Kiskinova, Nucl. Instrum. Methods Phys. Res., Sect. A, 2009, 608, 195–198.
[3] A. Gianoncelli, G. R. Morrison, B. Kaulich, D. Bacescu and J. Kovac, Appl. Phys. Lett., 2006, 89, 251117.

P51 *Determination of the antioxidant activity of the wild Pomegranate (Punica granatum L.) peel extract obtained by Supercritical CO_2 extraction*

N. Preradovic[1], Đ. Nakarada[2], M. Barić[1], K. Radotic[1], J. Simonovic Radosavljevic[1], M. Mojovic[2]

[1]Institute for multidisciplinary research, University of Belgrade, Kneza Višeslava 1, 11000 Belgrade, Serbia
[2]Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
e-mail: nevena@imsi.bg.ac.rs

Pomegranate (*Punica granatum* L.) fruit is a rich source of various bioactive compounds and has been used in traditional medicine for treating many medical conditions [1,2]. Pomegranate peel is also rich in bioactive compounds with phenolic being the primary source of its diverse health benefits, owing to their antioxidant, anti-inflammatory, and antimicrobial properties [1,2]. Despite this, pomegranate peel is often discarded by the food industry as fruit waste [3]. Also, a detailed study of its antiradical activity has never been conducted before. Samples were collected from the area of Mostar, Bosnia and Herzegovina.
Herein, the supercritical CO_2 extraction of wild pomegranate peel has been employed to obtain an extract rich in polyphenolic compounds. Supercritical CO_2 extraction, a green technology method, replaces classical solvent-based extraction, enabling the isolation of plant compounds in their purest form without degradation of the sample [4]. Owing to its high sensitivity and specificity, Electron paramagnetic resonance (EPR) spectroscopy has been utilized to access the extract's scavenging activity against free radical species (DPPH and hydroxyl radicals). The spin-trapping technique was employed to detect activity against short-lived hydroxyl radicals.
The results demonstrate that the pomegranate peel extract is a significant free-radical scavenger. It exhibited remarkable selectivity in eliminating hydroxyl radicals (71.3% compared to 23.6% for DPPH), which is particularly noteworthy as hydroxyl radicals are considered the most reactive oxygen radical species, biologically relevant, and responsible for a number of pathologies. These findings underscore the therapeutic potential of pomegranate peel in combating oxidative stress-related medical conditions but also suggest possibilities for further investigation into its mechanism of action and formulation into pharmaceutical or nutraceutical products.

Acknowledgments:

This work was financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract No. 451-03-66/2024-03/200053, 451-03-65/2024-03/200146 and 451-03-66/2024-03/200146

References:

[1] M. Pirzadeh et al., *Crit Rev Food Sci Nutr* 61 (2021), 982 -999
[2] M. Dikmen M et al., *J Med Food* 14 (2011), 1638-1646
[3] K. Kupnik et al. *Plants* 11 *(*2022), 928

Sponsor oral

Applying BioAFM to Study Structure and Mechanics of Biomaterials, Cells and Tissues in Life Science and Biomedical Research

Florian Kumpfe, Dimitar Stamov, Joan-Carles Escolano, Alexander Dulebo, André Körnig, Torsten Müller and Thomas Henze
JPK BioAFM, Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany
e-mail: Florian.Kumpfe@bruker.com

Atomic force microscopy (AFM) is a surface technique that can be successfully applied for comprehensive nanomechanical characterization of single molecules, cells and tissues, under near physiological conditions. Some of the current biomedical research trends feature development of novel nano- and biomaterials for regenerative medicine, tissue engineering, and sample diagnostics. Further advances in large biosample analysis and medical diagnostics are driven by the demand for mapping of biological and clinical samples that are often inhomogeneous, rough, and difficult to modify/adapt in their native state. Recent AFM developments have also led to unprecedented imaging rates in fluid, enabling temporal resolution on the sub-20-milisecond scale.

We will show several bioAFM applications demonstrating how high-speed AFM, with a temporal resolution on the second to millisecond scale, can be applied to resolve dynamic processes in biological systems. We will introduce the concept of automated large area multiparametric characterization of densely packed cell layers and highly corrugated tissue samples, where full automation, smart mechanical sample analysis, multiple scanner technology, and optical integration is critical for data throughput and reliable correlative microscopy. We will discuss how these developments, in combination with advanced optical microscopy techniques, can overcome the inherent drawbacks of traditional AFM systems for characterizing challenging biological samples.

PRESENTING AUTHOR INDEX