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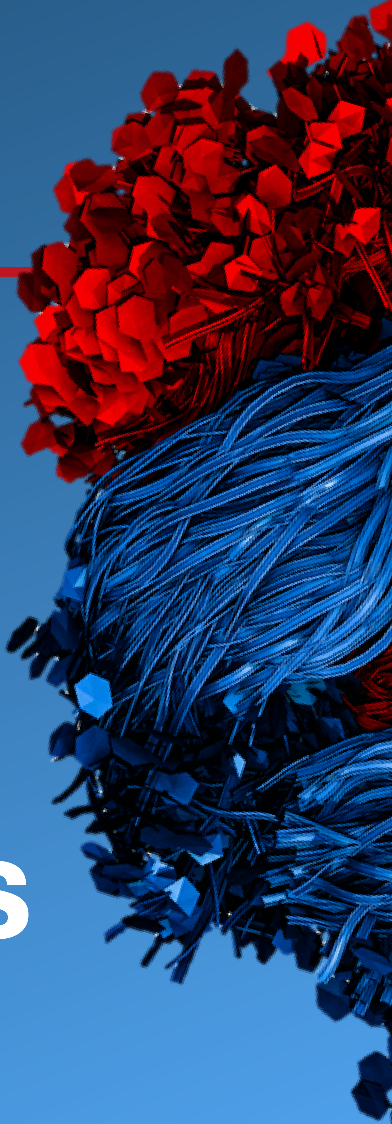
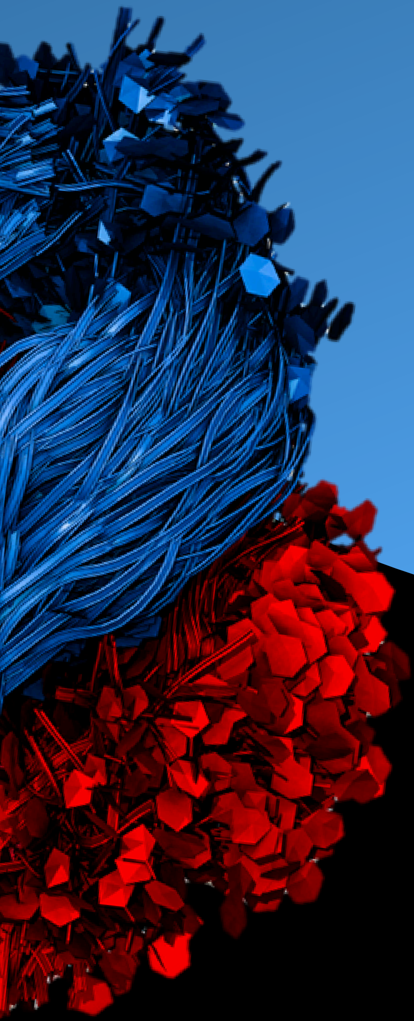


1st Meeting of Young Biophysicists

Ruđer Bošković Institute
Zagreb, Croatia
November 14, 2025



PROGRAMME AND BOOK OF ABSTRACTS

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November 14th, 2025
Ruđer Bošković Institute
Zagreb, Croatia

PROGRAMME
&
BOOK OF ABSTRACTS

Croatian Biophysical Society

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Preface

It is our great pleasure and honour to welcome all participants to the **1st Meeting of Young Biophysicists**, held on November 14, 2025, at the Ruđer Bošković Institute in Zagreb, Croatia. This newly established meeting is designed as a focused, one-day on-site event that brings together young researchers to present and discuss their work in a stimulating and friendly scientific atmosphere.

The meeting brings together PhD students and postdoctoral researchers working in various areas of biophysics: molecular biophysics, cell biophysics, membrane biophysics and computational biophysics. A total of 18 participants will present their research through oral presentations. By offering a dedicated platform for young biophysicists, the meeting aims to support their scientific development, enhance their presentation skills and encourage critical discussion of their results.

The **1st Meeting of Young Biophysicists** is held under the auspices of the **Croatian Biophysical Society**, **and the Ruđer Bošković Institute** whose support has been essential in launching this initiative. We hope that this first edition will lay the foundation for a continuing series of meetings for young biophysicists, strengthening the biophysics community in Croatia and beyond, fostering new collaborations and inspiring future research.

We would like to thank all participants, mentors, supporting institutions and sponsors for their contributions to this meeting and for helping to create an engaging environment for scientific exchange among young biophysicists.

Editors

PROGRAMME

08:30	**Registration**
09:00	**Opening** **Nives Novosel Vlašić & Lana Vujica, Ruđer Bošković Institute, Zagreb**
09:05	**Welcome Remarks** **Nadica Ivošević DeNardis, Ruđer Bošković Institute, Zagreb**
09:15	**Greta Grassmann, University of Rome, Sapienza University** Young section of the European Biophysical Societies' Association (yEBSA)
09:30	**Invited lecture** **Aleksandra Maršavelski, University of Zagreb, Faculty of Science** Integrative approaches in computational enzyme engineering: from sequence design to functional optimization
Membrane biophysics **Chair: Ana Sunčana Smith, Ruđer Bošković Institute, Zagreb**	
10:00	**Ana-Marija Milisav, Ruđer Bošković Institute, Zagreb** Antibiofilm Ag- and Cu-doped ZnO thin films with calcium phosphates
10:15	**Maja Levak Zorinc, Ruđer Bošković Institute, Zagreb** Marine-derived lipid vesicles: what secrets do they hold from sea to structure?
Cell biophysics **Chair: Maja Novak, University of Zagreb, Faculty of Science**	
10:30	**Adrian Perhat, Ruđer Bošković Institute, Zagreb** Temporal characterization of *in vitro* resistance to paclitaxel at the single-cell level
10:45	**Mario Đura, Ruđer Bošković Institute, Zagreb** Centrosome age asymmetry of chromosome unalignment in symmetrical cell divisions
11:00	**Iva Dundović, Ruđer Bošković Institute, Zagreb** Mapping the evolution of polyploidy in a model of intestinal regeneration

11:15	**Coffee break**
Computational Biophysics I **Chair: Višnja Stepanić, Ruđer Bošković Institute, Zagreb**	
11:45	**Antonia Matić, Ruđer Bošković Institute, Zagreb** The influence of a peptidase activity inhibitor on the stability of the DPP3-kelch complex
12:00	**Lucija Tomašić, University of Zagreb, Faculty of Science** Macro-karyotype approach reveals a universal selection principle across cancer types
12:15	**Shane Fiorenza, University of Zagreb, Faculty of Science** Synchronization of microtubule ends via length-dependent mechanisms regulates spindle dynamics
Computational Biophysics II **Chair: Piotr Nowakowski, Ruđer Bošković Institute, Zagreb**	
12:30	**Boris Gomaz, University of Zagreb, Faculty of Science** From motion to communication: tracking allosteric networks in purine nucleoside phosphorylase
12:45	**Jelena Matekalo, University of Split, Faculty of Science** Computational modeling of protein–metal nanoclusters for early diagnostic bioimaging
13:00	**Matej Kožić, University of Zagreb, Faculty of Science** Optimizing oligonucleotide selection for DNA data storage (ongoing research)
13:15	**Lunch Break + Group Photo**
Molecular Biophysics **Chair: Aleksandra Maršavelski, University of Zagreb, Faculty of Science**	
14:15	**Katarina Kajan, Ruđer Bošković Institute, Zagreb** Biophysical properties of transcription initiation during embryonic development
14:30	**Marta Bošnjaković, Ruđer Bošković Institute, Zagreb** High-resolution mapping of transcription start sites using CAGE-seq
14:45	**Anamarija Budimir, University of Split, Faculty of Science** Unstructured C-terminal in α-helical antimicrobial peptides modulates their activity

15:00	**Suzana Inkret, Ruđer Bošković Institute, Zagreb** Biomolecules and their role in improving properties of calcium phosphates/silver nanoparticles composite coatings on the titanium surface
15:30	**Best Talk Awards & Meeting Closure** **Nives Novosel Vlašić & Lana Vujica, Ruđer Bošković Institute, Zagreb**

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ORAL PRESENTATIONS

Young Section of the European Biophysical Societies' Association (yEBSA)

Greta Grassmann[1,2*]

[1]Department of Physics, Sapienza University of Rome, Italy
[2]Center for Life Nano & Neuro Science, Italian Institute of Technology, Rome, Italy
*greta.grassmann@uniroma1.it

yEBSA is a section of the European Biophysical Societies' Association [1], dedicated to supporting early-career researchers, including PhD students and postdoctoral fellows, with many initiatives also open to final-year Master's students.

Our main mission is to foster biophysics by building a strong network among young researchers and providing valuable resources for their careers.

To this end, we have launched the yEBSA online platform [2], where you can find a list of upcoming events (conferences, schools, etc.) and internships contributed by community members.

By joining the platform, you can:

- See which other members are attending an event and connect with them.
- Explore internship experiences shared by others and ask for insights directly.
- Contribute by sharing events, internships, and other opportunities to keep the platform dynamic and useful for all young biophysicists.

By registering on the website, you can also subscribe to the yEBSA mailing list, through which you will receive updates about yEBSA initiatives, including our upcoming online seminar series (starting in November) focused on career paths, funding opportunities, paper and CV writing, and presentation skills.

If you have any questions or suggestions, feel free to contact us at yEBSA@ebsa.org
or through our social media channels [3, 4, 5].

Acknowledgments
European Biophysical Societies' Association (EBSA)

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Integrative Approaches in Computational Enzyme Engineering: From Sequence Design to Functional Optimization

Aleksandra Maršavelski[1*]

[1]University of Zagreb, Faculty of Science, Department of Chemistry, Horvatovac 102a, 10000 Zagreb, Croatia
*amarsavelski.chem@pmf.hr

Modern enzyme engineering leverages integrative computational strategies to bridge the gap between sequence design and functional optimization. This talk explores how combining data-driven models and physics-based simulations accelerates the development of enzymes with improved stability, activity, and new functionalities, focusing on the enhancement of esterases that cleave ester bonds. Esterase optimization for polymer (polyester) degradation serves as a prime example, [1,2] alongside other impactful case studies. Machine learning mines sequence-function relationships to predict beneficial mutations, vastly reducing experimental screening. A structure-guided ML algorithm engineered a PET-degrading esterase ("FAST-PETase") with five synergistic mutations, yielding a robust variant [3]. Molecular dynamics (MD) simulations provide insight into enzyme flexibility and substrate binding, guiding rational improvements. MD-driven loop redesign of a polyester hydrolase enhanced catalytic efficiency, enabling faster PET degradation [4]. Ancestral sequence reconstruction (ASR) yields thermostable scaffolds with high activity [5]. ASR-derived enzymes exhibit superior stability, offering excellent starting points for further engineering.

Beyond optimizing natural enzymes, integrative approaches now enable *de novo* enzyme design. Advances in deep learning and generative protein design have produced new biocatalysts, exemplified by an artificial luciferase that rivals natural enzymes [6].

Keywords: Enzyme Engineering, Esterases, Computational Design, Machine Learning

Acknowledgments

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Antibiofilm Ag- and Cu-doped ZnO thin films with calcium phosphates

Ana-Marija Milisav[1*], Maja Mičetić[1], Pavo Dubček[1], Lamborghini Sotelo[2,3], Cristina Cantallops-Vilà[4], Tommaso Fontanot[5], Ina Erceg[1,5], Krunoslav Bojanić [1], Željka Fiket[1], Maja Ivanić[1], Silke Christiansen[3,5], Edwige Meurice[4], Tihomir Car[1], Maja Dutour Sikirić[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany
[3]Innovations-Institut für Nanotechnologie und Korrelative Mikroskopie, Forchheim, Germany
[4]CERAMATHS, Université Polytechnique Hauts-de-France, Cambrai, France
[5]Fraunhofer Institute for Ceramic Technologies and Systems IKTS, Forchheim, Germany
*amilisav@irb.hr

Bacterial biofilm formation on medical surfaces is a major challenge [1-2]. Antibacterial surface modification, particularly using nanostructured metal oxide thin films via magnetron sputtering, offers a promising solution due to controllable physicochemical properties [3]. This study, for the first time, compared Ag- and Cu-doped ZnO thin films and explored enhancing their biocompatibility through biomimetic calcium phosphate deposition.

Thin Ag- and Cu-doped ZnO films of varying compositions were prepared by simultaneous magnetron sputtering. Calcium phosphates were then biomimetically deposited to improve bioactivity. Physico-chemical characterization was done with grazing incidence small-angle X-ray scattering (GISAXS), X-ray diffraction (XRD), atomic force microscopy (AFM), helium-ion microscopy (HIM), scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS), and ion release was measured by inductively coupled plasma mass spectrometry (ICP-MS). Biological evaluations included human osteoblastic cell line (MG-63) viability (MTT assay) and *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm formation.

Results confirmed Ag and Cu nanoparticle formation, with size and shape dependent on composition. Increased Ag or Cu had opposite effects on nanoparticle structure, grain size, and water contact angle. EDS indicated mixed Ag/ZnO or Cu/ZnO grains, and XRD suggested Ag or Cu were incorporated into the ZnO structure. Calcium phosphate deposition reduced Ag, Cu, and Zn release, improved biocompatibility, especially for Ag-doped ZnO films, and enhanced biofilm prevention.

These findings highlight magnetron sputtering as a versatile and promising method for creating tailored coatings, not just for biomedical applications but for diverse fields requiring effective surface modification.

Keywords: Magnetron sputtering, calcium phosphates, copper oxide, zinc oxide, nanoparticles

Acknowledgments

This project was supported by the European Union under the Horizon 2020 Research and Innovation Programme under Marie Skłodowska-Curie Grant Agreement No. 861138.

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Marine-derived lipid vesicles: What Secrets Do They Hold from Sea to Structure?

Maja Levak Zorinc[1*], Blaženka Gašparović[1], Maja Dutour Sikirić[2],
Ruža Frkanec[3], Nadica Ivošević DeNardis[1]

[1]Division for Marine and Environmental Research, Ruđer Bošković Institute, Zagreb, Croatia
[2]Division of Physical Chemistry, Ruđer Bošković Institute, Zagreb, Croatia
[3]Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia
*mlevak@irb.hr

Marine lipids, although a minor component of dissolved organic matter in aquatic environments, exhibit long persistence and notable biophysical properties. However, their ability to form vesicular structures has received little attention. We prepared marine-based lipid vesicles from lipids isolated at two seawater depths (0 m and 20 m) using the thin-film hydration method, which enables spontaneous vesicle formation. A comprehensive multimethod approach, combining thin-layer chromatography, atomic force microscopy (AFM), and electrophoretic mobility measurements, was used to characterize lipid composition, vesicle morphology, mechanical properties such as elasticity, and interfacial characteristics such as adhesion. Our findings reveal oval vesicles a few micrometers in diameter that are negatively charged, indicating stability. Vesicles from both depths had similar elasticity (Young's modulus ~1.4 MPa) but differed in hydrophilicity: 0 m vesicles were distinctly hydrophilic, while 20 m vesicles showed mild hydrophobicity, suggesting a higher nonpolar lipid content. These results provide insight into vesicle structural features relevant to understanding interfacial transformations and the stability of marine lipids in their natural environments, potentially supporting their development as sustainable resources for future biotechnological applications.

Keywords: marine-based lipid vesicles, marine lipids, structural characterization

Temporal Characterization of *In Vitro* Resistance to Paclitaxel at the Single-Cell Level

Adrian Perhat[1*], Kruno Vukušić[1], Petar Škrobo[1], Iva M Tolić[1]

[1]Ruđer Bošković Institute, Bijenička cesta 54, 10 000, Zagreb
*aperhat@irb.hr

Resistance to chemotherapeutics is a major obstacle to successful cancer treatment, particularly in highly heterogeneous tumor types such as ovarian cancer. One of the most clinically used chemotherapeutic agents is the microtubule poison paclitaxel, whose antineoplastic activity is well characterized and partly related to the induction of mitotic spindle multipolarity and subsequent death of the progeny [1]. However, early cellular adaptations to paclitaxel at the spatiotemporal level remain poorly defined, even though these adaptations are crucial, as cells that survive initial treatment may give rise to resistant clones. To address this, we established long-term imaging methods that enable detection and characterization of surviving ovarian cancer cells following paclitaxel treatment. Live-cell imaging and subsequent analysis allowed tracking of multiple generations of treated cells and revealed heterogeneous mitotic phenotypes and outcomes, even among genetically near-identical daughter cells derived from the same mother cell. Preliminary data indicate that varying degrees of mitotic errors, correlating with the duration of mitosis, determine progeny survival and proliferative capacity. Furthermore, mitotic duration depends on paclitaxel concentration, with cells treated with 5 nM undergoing longer mitotic arrest than those treated with 2 nM, resulting in less proliferative progeny. To uncover the mechanisms underlying these observations, we developed an "endpoint immunofluorescence analysis" that enables fixation and staining of previously live-imaged cells of interest, allowing correlation of mitotic outcomes with protein expression and localization at the single-cell level. Collectively, these approaches might enhance our understanding of early cellular adaptations to paclitaxel and provide insights into the mechanisms driving resistance development.

Keywords: Paclitaxel, resistance, mitosis, ovarian cancer

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Centrosome age asymmetry of chromosome unalignment in symmetrical cell divisions

Mario Đura[1*], Alexandre Thomas[2], Patrick Meraldi[2], Iva Marija Tolić[1]

[1]Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
[2]Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva, Geneva, Switzerland
*mdjura@irb.hr

The unalignment of chromosomes during mitosis is a phenotype present in cancer cell divisions which leads to segregation errors and cancer progression [1]. It has been previously described that unaligned chromosomes more commonly accumulate on the mitotic spindle pole of the older of the two centrosomes [2]. The older centrosome is the centriole of the previous cell cycle, from which the new centrosome grew in the form of a procentriole [3]. This distinction is very apparent during the interphase of the cell cycle, but is just recently being investigated in greater detail during mitosis [4]. Currently, we are working on describing the possible mechanisms of how these asymmetries are caused and resolved – from the initial asymmetric positioning of centrosomes at nuclear envelope breakdown to the asymmetric congression of unaligned chromosomes. These mechanisms could help explain the ways in which mitotic errors arise in erroneous cancer cell divisions.

Keywords: Centrosome age, Chromosome unalignment, Asymmetry, Cell division, Centrosome positioning

Acknowledgments

This research is a part of the SNSF-HRZZ CentroPol Project, a Swiss-Croatian bilateral research collaboration.

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Mapping the evolution of polyploidy in a model of intestinal regeneration

Iva Dundović[1*], Iva M. Tolić[1]

[1]Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
*idundovic@irb.hr

Polyploidy is a state of multiple chromosome sets being present within a cell after a whole-genome duplication event, such as cytokinesis failure [1]. While such events are usually associated with somatic cells in cancer, recently they have been found in a model of healthy intestine undergoing regeneration [2]. Polyploid cells that appear in regenerating mouse small intestine (mSI) cysts survive for several generations, but it is unknown how they propagate [2]. Here we show that one mechanism promoting survival is centrosome loss, which happens quickly as the mSI cysts mature. This is supported by observations that induced tetraploids in cell lines lose centrosomes to prevent multipolar divisions [3]. Surprisingly, even the cells with extra centrosomes show a high fraction of bipolar divisions, indicating that high clustering efficiency further promotes survival of polyploid cells. Interestingly, in the population of dividing polyploids we notice hexaploids and octaploids, which suggests an attenuated tumor suppression activity of p53 or a lack of it in early cysts [4,5]. As polyploid cells that appear in regenerating mSI cysts either die or go senescent by the time the organoid is fully developed [2], it seems that centrosome loss and clustering are not sufficient to sustain proliferation and a new penalty is imposed, potentially led by the activation of p53 in developed organoids. Finally, we demonstrate that polyploids significantly increase the chromosome segregation error rate in early cysts, suggesting a contribution of polyploidy to oncogenic potential in chronic regeneration.

Keywords: mitosis, polyploidy, regeneration

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The Influence of a Peptidase Activity Inhibitor on the Stability of the DPP3-Kelch Complex

Antonia Matić[1*], Luka Petohleb[2], Antonija Tomić[1]

[1]Ruđer Bošković Institute, Zagreb
[2]University of Zagreb, Faculty of science, Department of Chemistry, Zagreb
*amatic@irb.hr

Dipeptidyl peptidase III (DPP3) is a zinc-dependent exopeptidase found in nearly all human tissues, involved in the degradation of small peptides. It plays an important role in physiological and pathological processes such as oxidative stress, blood pressure regulation, and pain modulation. DPP3's role in oxidative stress is linked to its direct interaction with Keap1 (Kelch-like ECH-associated protein 1), a key regulator of the cellular oxidative stress response, which functions via interaction with Nrf2 (Nuclear factor erythroid 2-related factor 2). DPP3 binds to the Kelch domain of Keap1 through its ETGE motif, located in a flexible loop in the protein's upper domain. Since Nrf2 binds to the same site, DPP3 competes with Nrf2 for Keap1 binding, promoting Nrf2 activation. This mechanism has been demonstrated in breast, lung, and colorectal cancer cells.

The aim of this study is to investigate the impact of a peptidase activity inhibitor bound in the active site of DPP3 on the stability of the DPP3-Kelch complex, using isothermal titration calorimetry and molecular dynamics simulations. Measurements were performed on wild-type DPP3 and its inactive form (E451A mutant), as well as their complexes with the peptide inhibitor IVYPW. The study examined how DPP3 inactivation either by mutation or inhibitor binding affects the detachment of the flexible loop containing the ETGE motif from the rest of the protein, which is a crucial step preceding the formation of the DPP3-Keap1 complex. Furthermore, the effect of inhibitor binding on the flexibility of the loop and on the interactions formed between ETGE motif residues and the rest of the protein was investigated.

Keywords: DPP3-Keap1 interaction, peptidase inhibition, oxidative stress

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Macro-karyotype approach reveals a universal selection principle across cancer types

Lucija Tomašić[1*], Shane A. Fiorenza[1], Thomas W. van Ravesteyn[2], Geert J.P.L. Kops[2], Nenad Pavin[1]

[1]Department of Physics, Faculty of Science, University of Zagreb, Bijenička cesta 32, 10000 Zagreb, Croatia
[2]Oncode Institute, Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW), and University Medical Center Utrecht, Utrecht, the Netherlands
*ltomasic@phy.hr

Aneuploidy, an abnormal number of chromosomes, is a defining feature of most cancers. These large-scale genomic alterations create immense diversity between cancers and patient prognosis, making it difficult to identify universal evolutionary principles. While specific chromosome changes are often linked to specific cancer types, general principles for how abnormal karyotypes evolve has remained unclear. To address this, we analyzed over 90,000 patient-derived cancer karyotypes using a new visualization approach and mathematical modeling. We find that, across cancer types, and even in yeast, aneuploid karyotypes overwhelmingly organize into what we call “binary karyotypes”, configurations built from only two chromosome copy numbers. Despite the vast combinatorial landscape of theoretically possible chromosome configurations, these states dominate empirical data, comprising more than three-quarters of observed karyotypes. Our model shows that this bias can be explained by a modest but consistent fitness advantage of binary karyotypes over other chromosome configurations. This principle also sheds light on how cancer cells tolerate stress responses such as those mediated by the tumor suppressor p53. In contrast to other karyotypes, binary karyotypes have a lower rate of TP53 inactivation. These results reveal binary karyotypes as a distinct evolutionary class of aneuploidy, defined not by individual chromosomes but by global organizational rules. This conserved principle offers a new perspective on genome evolution and potential strategies to target shared vulnerabilities in cancer.

Keywords: Aneuploidy, Cancer, Karyotype evolution

Acknowledgments
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Synchronization of microtubule ends via length-dependent mechanisms regulates spindle dynamics

Shane A. Fiorenza[1*], Sheba Cheeran[2,3], Elena Doria[2,3], Iva Tolić[4], Patrick Meraldi[2,3], Nenad Pavin[1]

[1]Department of Physics, Faculty of Science, University of Zagreb, Zagreb, Croatia
[2]Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[3]Translational Research Centre in Onco-hematology, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[4]Laboratory of Cell Biophysics, Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia
*sfiorenza@phy.hr

The mitotic spindle is a biomechanical structure that relies on a well-controlled geometry to accurately segregate genetic material. To this end, a myriad of feedback loops and regulatory mechanisms are employed by the spindle to self-assemble, self-regulate, and self-transform throughout the process of mitosis. One such mechanism is proteins that regulate the dynamics of microtubule plus-ends in a length-dependent manner, e.g., the kinesin-8 family. Another important mechanism is the continual treadmilling of microtubule segements towards the minus-end, a phenomenon referred to as poleward flux. Depletion of kinesin-8 at plus-ends leads to larger spindles and, counterintuitively, changes at minus-ends in the form of increased poleward flux velocity. How microtubule minus-ends respond to changes at the plus-end and the role of minus-end regulation within the spindle remains unclear. In this work, we use a combination of theory and experiment to study spindle dynamics with a focus on microtubule minus-end regulation. We find that having length-dependent mechanisms at both microtubule ends facilitates the synchronization of plus- and minus-end dynamics, explaining how depletion of plus-end-associated proteins can increase poleward flux velocity at the minus-end. Furthermore, our model predicts that plus- and minus-end depletions influence flux velocity in opposite ways, allowing spindle length and flux velocity to be varied independently of each other when both depletions are combined. We confirm these predictions with *in vivo* experiments. Taken together, our results suggest that the synchronization of plus- and minus-ends through length-dependent mechanisms facilitates new regulatory pathways within the mitotic spindle.

Keywords: mitotic spindle, microtubules, length regulation, analytic modeling

From Motion to Communication: Tracking Allosteric Networks in Purine Nucleoside Phosphorylase

Boris Gomaz[1,2*], Alessandro Pandini[3], Aleksandra Maršavelski[1], Zoran Štefanić[2]

[1]Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
[2]Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia
[3]Brunel University London, Deparment of Computer Science, Uxbridge UB8 3PH, United Kingdom
*bgomaz@chem.pmf.hr / bgomaz@irb.hr

Allostery plays a crucial role in enzyme regulation, yet the underlying communication pathways often remain hidden within the complexity of protein dynamics. In this work, we investigated purine nucleoside phosphorylase (PNP) from *Helicobacter pylori* [1] using microsecond-scale molecular dynamics simulations combined with advanced data analysis. We developed a method, **MDavocado**, [2] which follows time-dependent φ/ψ backbone angle variations for each amino acid, providing compact visualizations of conformational transitions throughout the simulation. This approach enables identification of dynamically active residues and regions involved in coordinated conformational changes. By applying circular correlation analysis and graph theory, we mapped residue–residue communication networks that propagate structural changes across the enzyme. The detected pathways are primarily located at dimer interfaces, within the central cavity, and near the active sites, suggesting potential routes of allosteric signal transmission. Our results provide new insights into the dynamic behavior and internal communication of PNP, demonstrating how combining molecular dynamics with network analysis can uncover the structural basis of allosteric regulation.

Keywords: allostery, *Helicobacter pylori*, Purine nucleoside phosphorylase, Ramachandran plot

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Computational Modeling of Protein–Metal Nanoclusters for Early Diagnostic Bioimaging

Jelena Matekalo[1*], Željka Sanader Maršić[1], Dusica Maysinger[2], Antoine Rodolphe[3]

[1]Department of Physics, University of Split
[2]Department of Pharmacology & Therapeutics, McGill University
[3]Institut Lumière Matière, University Claude Bernard Lyon 1
*jmatekalo@pmfst.hr

Ligand-protected metal nanoclusters (NCs) have gained growing interest due to their unique physicochemical properties, particularly their luminescence and potential for biomedical applications. Gold nanoclusters (AuNCs), composed of a few to several hundred atoms and stabilized by organic ligands, are ideal candidates for such studies owing to their high chemical stability, bright photoluminescence, and excellent biocompatibility [1]. The choice of ligands strongly influences their biological behavior and minimizes toxicity *in vivo*. However, the molecular mechanisms governing AuNC–protein interactions remain poorly understood, making atomic-level modeling essential for their safe and effective biomedical use [2]. In this work, molecular predocking was performed to investigate the interaction between interleukin-33 (IL-33) and AuNCs, a system with potential applications in bioimaging. As a next step, molecular simulations will be employed to explore the structural and optical properties of the conjugate [3]. To illustrate the computational workflow and validate the simulation approach, a simplified model system involving cadmium and tyrosine binding is presented. This research is conducted in collaboration with colleagues from the University of Lyon and the University of Montreal, whose experimental work complements the computational results. The cadmium–tyrosine model system represents an approximate depiction of the simulations that will follow for the IL-33 complex and AuNCs.

Keywords: computational simulations, metal nanoclusters, interleukin-33 (IL-33), bioimaging, QM/MM method

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Optimizing oligonucleotide selection for DNA data storage (ongoing research)

Matej Kožić[1*], Kaya Wernhart[2], Fabian Schroeder[2], Branimir Bertoša[1], Ivan Barišić[2]

[1]University of Zagreb, Faculty of Science, Department of Chemistry
[2]Eko Refugium d.o.o.
*mkozic@chem.pmf.hr

Information for all known life is encoded in DNA molecules. Optimized through two billion years of evolution, DNA is an attractive target for modern data storage solutions. For companies to be able to cold-store massive amounts of data for e.g. disaster recovery, archiving, etc., DNA data storage system would have to be optimized (among other things) in terms of cost, efficiency, and stability. For that reason, to effectively store data, a predetermined set of oligonucleotides with desired properties, an "oligolibrary", would be defined. Each oligo would map onto a packet of information (e.g. one byte), and oligos would have specific complements so that they assemble into a long strand of DNA that then encodes desired information. However, besides the desired interactions, oligos could bind in all other undesired ways. To optimize the selection of oligonucleotides that would constitute an oligolibrary, it is necessary to estimate the affinity of intended and unintended matches. In this research molecular dynamics is employed for the goal of assessing the stability of unintended and unintended matches. A structure is derived from sequences using AlphaFold3[1], and molecular dynamics using Gromacs are run to research dynamical properties of pairs of oligonucleotides from an example oligolibrary.

Keywords: molecular dynamics, DNA data storage, short oligonucleotides

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Biophysical properties of transcription initiation during embryonic development

Katarina Kajan[1*], Damir Baranašić[1]

[1]Division of Electronics, Ruđer Bošković Institute, Zagreb, Croatia
*kkajan@irb.hr

Transcription initiation is a fundamental process in embryonic development, tightly regulated by the biophysical properties of DNA, chromatin, and promoter architecture. In metazoans, the physical characteristics of regulatory elements play a crucial role in defining promoter function and transcriptional control. Promoters can be broadly classified as sharp, broad, or developmental, each associated with distinct modes of gene regulation and expression dynamics. To elucidate the biophysical and regulatory mechanisms underlying transcription initiation during early embryogenesis, we investigated promoter usage and transcription start site (TSS) distribution in two closely related avian species - the domestic chicken (G*allus gallus*) and the Japanese quail (*Coturnix japonica*). Using Cap Analysis of Gene Expression (CAGE), we precisely mapped TSSs at single-base resolution across four early embryonic stages. This comprehensive comparative analysis allowed us to identify conserved and species-specific promoter features, revealing how the physical properties of DNA and chromatin influence promoter activity and transcriptional output. Our findings provide new insights into how genes are activated following zygotic genome activation and highlight the shared and divergent transcriptional strategies that underpin early development in metazoans.

Keywords: gene regulation, transcriptional regulatory elements, Cap Analysis of Gene Expression (CAGE)

High-resolution mapping of transcription start sites using CAGE-seq

Marta Bošnjaković[1*], Damir Baranašić[1]

[1]Division of Electronics, Ruđer Bošković Institute, Zagreb, Croatia
*mbosnjak@irb.hr

Precise characterization of regulatory elements, particularly transcription start sites (TSSs) at base pair resolution, is crucial for better understanding of gene regulation. Using Cap Analysis of Gene Expression sequencing (CAGE-seq), we identified TSSs at base-pair resolution across seven adult zebrafish (*Danio rerio*) organs. This promoter level atlas strengthens the use of adult zebrafish for biomedical research, developmental biology, molecular genetics, oncology, toxicology, drug discovery, and regenerative medicine. By capturing the exact coordinates and usage of transcription start sites, CAGE-seq enables detailed reconstruction of promoter architecture and transcriptional regulation, particularly in regard to physical properties of the DNA molecule and chromatin organisation. The gained knowledge facilitates integration with biophysical models of transcription initiation, advancing our understanding of gene expression dynamic.

Keywords: Cap Analysis of Gene Expression (CAGE), transcription start sites, gene regulation

Unstructured C-terminal in α-helical antimicrobial peptides modulates their activity

Anamarija Budimir[1*], Iva Stojan[1], Sabrina Pacor[2], Lucija Krce[1], Tomislav Rončević[3], Alessandro Tossi[2], Larisa Zoranić[1]

[1]University of Split, Faculty of Science, Department of Physics, Split, 21000, Croatia
[2]University of Trieste, Department of Life Sciences, Trieste, 34127, Italy
[3]University of Split, Faculty of Science, Department of Biology, Split, 21000, Croatia
*Anamarija.Budimir@pmfst.hr

Antimicrobial peptides (AMPs) are promising therapeutics due to their broad-spectrum activity and diverse mechanisms of action. While most designed AMPs are short and fully helical, many natural helical AMPs feature unstructured C-terminal tails with underexplored functions. Although these regions lack intrinsic antimicrobial activity or membrane affinity, they may promote oligomerization and structural stability, raising the question of whether to include them in AMP design despite higher synthesis costs. Here, we investigate the helminth-derived peptide mesco-2, which exhibits potent activity against Gram-positive and Gram-negative bacteria [1]. Mesco-2 contains an N-terminal helix and a C-terminal unstructured tail with a CLGRC loop stabilized by a disulfide bridge. Using molecular dynamics simulations, biophysical assays, and microbiological analyses, we show that the isolated tail lacks membrane affinity, while removal of the disulfide bridge increases flexibility, impairs membrane insertion, and delays bacterial killing. Our findings underscore the functional importance of unstructured C-terminal regions in helical AMPs and their potential relevance for rational peptide design.

Keywords: antimicrobial peptides, antimicrobial resistance, disulfide bridge

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Biomolecules and their role in improving properties of calcium phosphates/silver nanoparticles composite coatings on the titanium surface

Suzana Inkret[1*], Ina Erceg[1], Silke Christiansen[2,3], Maja Dutour Sikirić[1]

[1]Ruđer Bošković Institute, Zagreb, Croatia
[2]Fraunhofer Institute for Ceramic Materials and Systems, IKTS, Forchheim, Germany
[3]Innovations Institute for Nanotechnology and Correlative Microscopy, INAM, Forchheim, Germany
*sinkret@irb.hr

Calcium phosphates (CaPs) combined with antimicrobial metal/metal oxide nanoparticles have emerged as a promising alternative to antibiotic therapy for implant-related infections. Additionally, incorporating biomolecules into these composites enhances their biological properties.[1,2] Therefore, calcium phosphates/silver nanoparticles composites (CaP/AgNP) with biomolecules are promising coating materials for widely used titanium implants, which lack bioactivity.

In this study, composite coatings of CaPs, AgNPs, and two biomolecules, bovine serum albumin (BSA) and chitosan (Chi), were prepared on etched titanium surfaces. The titanium surface was etched using a mixture of sulfuric and hydrochloric acids to improve surface properties and promote coating adhesion.[3] The effects of the different biomolecules on the roughness, wettability, morphology of CaPs, and overall coverage of the surface was investigated.

Etching altered surface properties such as roughness and wettability, as confirmed by AFM and contact angle measurements. Raman spectroscopy confirmed formation of apatitic CaPs. Although biomolecules didn't significantly affect composition and morphology of CaPs, in the presence of Chi better and more uniform coating was obtained. EDS confirmed uniform distribution of AgNPs.

Overall, the coatings improved surface wettability, a critical property for bone implants. Obtained results confirmed high potential of prepared coatings for biomedical applications.

Keywords: biomolecules, calcium phosphates, titanium surface

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