BOOK OF ABSTRACTS

17TH CHRISTMAS BIOPHYSICS WORKSHOP

CITY OF OGULIN, CROATIA, DECEMBER 11-12, 2023

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MONDAY, 11.12.2023.

09:00--10:00 **Arrival and registration**

10:00--10:45 **Magnetic resonance approaches** (chair: Ida Delač)

10:00	Fraser MacMillan	*Understanding Movement & Mechanism in Membrane Proteins: A Magnetic Resonance Spectroscopist's View*
10:25	Kristina Smokrović	*Capture of free radicals generated during ultrasonic/UV treatment of tomato juice*

10:45--11:15 **Coffee break**

11:15--12:55 **Nano 2 Bio** (chair: Tomislav Vuletić)

11:15	Ida Delač	*Organic molecule modifications of a 2D material - monolayer MoS2*
11:35	Martina Lihter	*2D Materials as Building Blocks for Advanced Biosensing Platforms*
11:55	Goran Zgrablić	*Exploring novel photochemical behaviour of BODIPY-phenol chromophores using time-resolved photoelectron spectroscopy*
12:15	Suzana Inkret	*Combined influence of silver nanoparticles and biomacromolecules on calcium phosphates precipitation and antibacterial activity*
12:35	Vedran Đerek	*Opto-bioelectronics in 3D*

12:55--15:00 **Lunch**

15:00--16:40 **Biopolymers modelling** (chair: Antonio Šiber)

15:00	Davide Marcato	*Phase behavior of semiflexible lattice polymers in poor solvent solution: mean-field theory and Monte Carlo simulations*
15:20	Ivan Gilardoni	*Development of integrative methods for Molecular Dynamics simulations*
15:40	Alex Chen Yi Zhang	*Stochastic Block Model for chromatin organization*
16:00	Elisa Posani	*Cryo-EM ensemble refinement with molecular dynamics enables the structural characterization of flexible RNAs*
16:20	Matteo Tajana	*Structure-function relationship of viral RNAs probed with pore translocation*

16:40--17:10 **Coffee break**

17:10--18:10 **Lipids, membranes and vesicles** (chair: Primož Ziherl)

17:10	Michael Kaltenegger	*Shape-based design of bitopic proteins as probes for membrane elastic stress energy*
17:30	James Jennings	*Design Rules for Membrane-Active Antimicrobial Lipidoids (AMLs) Uncovered by High-Throughput Screening*
17:50	Robert Vácha	*Amphipathic Helices Can Sense Both Positive and Negative Curvatures of Lipid Membranes*

18:45--20:00 **Guided walk around Ogulin**

20:00--23:00 **Dinner/Social event**

TUESDAY, 12.12.2023.

08:00--09:30 **Breakfast**

09:30—10:50 **Soft and active matter** (chair: Georg Pabst)

9:30	Matej Kanduč	*Unravelling the universal adhesion threshold of biomatter on materials with water contact angles of 65°*
9:50	Marin Šako	*Tensile strength of water with organic impurities*
10:10	Fabio Staniscia	*Curvature-dependent adsorption of surfactants in water nanodroplets: Insights from molecular dynamics*
10:30	Vita Movrin	*Active dynamics of wound closure*

10:50--11:20 **Coffee break**

11:20--12:40 **Novel modelling approaches** (chair: Cristian Micheletti)

11:20	Tanmoy Sarkar	*Vertex Model Challenges Meet Knowledge Graph Solutions*
11:40	Urban Železnik	*Topological Transformations in Graph Vertex model*
12:00	Samuel Tamagnone	*Coarse Graining by Normalizing Flows*
12:20	Vittorio Del Tatto	*Robust inference of causality in dynamical processes from the Information Imbalance of distance ranks*

12:40--14:00 **Lunch & Departure**

Magnetic resonance approaches

MONDAY, 10:00--10:45

Understanding Movement & Mechanism in Membrane Proteins: A Magnetic Resonance Spectroscopist's View

Fraser MacMillan

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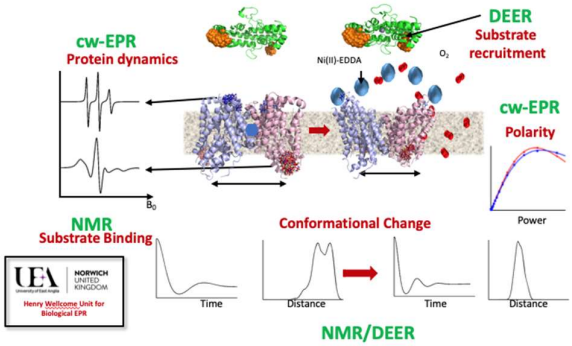
Research in the Henry Wellcome Unit for Biological EPR at UEA focuses on the architecture and functional dynamics of membrane proteins, many medically relevant with a special interest on membrane transport systems and their interaction with intra-cellular signalling pathways. There is increasing evidence that membrane proteins do not act alone, but that they are organised as nano-machineries which function through the concerted action of their individual components with high precision and specificity observed in both time and space. We seek to unravel the principles underlying the architecture and dynamics of these nano-machineries as well as their function and regulation. Our experimental approach focuses on advanced Magnetic Resonance techniques, specifically pulsed Electron Paramagnetic Resonance (EPR) – but also NMR techniques in combination with molecular biological and other biophysical methods including theoretical MD and quantum chemical approaches. Our expertise lies in the development and application of novel EPR techniques to address these key questions.



Here I will focus on method developments, which allow a shifting of the focus away from being considered purely a niche technique towards a more universal structural biological tool. I will demonstrate state-of-the-art approaches, and outline examples of where method development is ongoing to demonstrate the power of this technique to deliver key mechanistic insights into e.g., how to resolve multiple distance constraints in macromolecular ensembles, how to observe dynamic conformational changes and finally to identify the dynamic determinants of ligand binding.

Funded by The Royal Society, Wellcome Trust as EU MCSA ETN 'NeuroTrans'.

[1] J. Hall, et al. *Scientific Reports 10, 16483 (2020)*
[2] M.O. Ross, et al. *Science 364, 566-570 (2019)*
[3] S. Barber-Zucker, et al. *FEBS J 286, 2193-2215 (2019)*
[4] J. Anderson, et al. *Nature Comm 8, 358 (2017)*
[5] J. van Wonderen, et al. *Angew. Chemie, 52, 1990-1993 (2013)*
[6] T.F. Prisner, M. Rohrer & F. MacMillan *Ann.Rev.Phys.Chem. 52, 279 (2001)*

Capture of free radicals generated during ultrasonic/UV treatment of tomato juice

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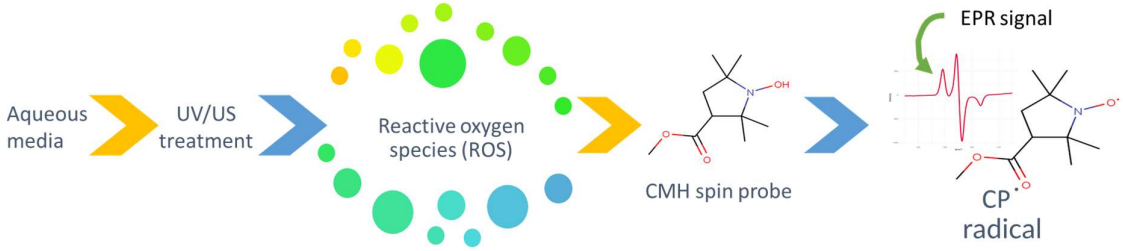
Ultrasonication is one of the emerging techniques in the field of food processing technologies aiming at the replacement of thermal treatments since they have a significant effect on the food quality and composition.
Hydroxyl and superoxide radicals are among the most common causes of damage to the cellular systems, such as DNA, proteins and lipids. Unfortunately, it is well known that they are also formed by the collapse of cavitation bubbles that are created during ultrasonic treatment. These are very reactive species, with short half-lives, making it difficult to measure their concentration directly during treatment. One of the methods to indirectly measure the amount of radicals produced is using the spin trapping method. A compound that is known to form a stable free radical species, that can be identified and quantified by EPR (electron paramagnetic resonance) spectroscopy, can be added to the system.
We used 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine hydrochloride (CMH) as a spin probe, because it reacts rapidly with reactive oxygen species (ROS).[2] The effect of ultrasound and UV treatment on tomato juice was studied, as well as the combined effect of simultaneous treatments.



[1] H. Onyeakaet al., *Food Rev. Int.,* **39**, 3663-3675 (2023).
[2] J. P. Gothamet al., *Free Radic Biol Med*., **154**: 84–94 (2020).

ACKNOWLEDGEMENTS**:** 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).

Nano 2 Bio

MONDAY, 11:15--12:55

Organic molecule modifications of a 2D material - monolayer MoS2

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Two-dimensional (2D) materials are promising candidates for the next generation of devices due to their unique electronic, optical and mechanical properties. Before their implementation in the commercial devices, there are few issues that need to be solved. Firstly, precise and controlled synthesis and methods of manipulation are necessary for the successful integration in devices, followed by the possibility of fine tuning of the properties of interest. In that regard, selected properties of 2D materials can be tuned by chemical or mechanical modulation.

We investigated the stability of CVD-synthesized 2D MoS2 samples modified with organic molecules under ambient conditions and reversibility of this modification. This follows our previous work on solvent and molecular adsorption influence on MoS2 properties [1], while here we focus on the stability of the modified samples under ambient conditions. By analysing the optical signatures of the samples using photoluminescence spectroscopy (PL), Raman spectroscopy, and surface quality using atomic force microscopy (AFM), we demonstrate that the modification of 2D MoS2 with organic molecules leads to a stable surface modification that retains its optical signature. Furthermore, we show the reversibility of the effects induced by the organic molecules, as heating the modified samples restores the original optical signatures, indicating the re-establishment of the optical properties of the pristine MoS2.

[1] A. L. Brkić et al., *Nanomaterials* **13,** 2115 (2023)

2D Materials as Building Blocks for Advanced Biosensing Platforms

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Atomically thin, two-dimensional (2D) materials possess extraordinary electronic, optical, and mechanical properties, making them highly versatile for a variety of applications ranging from (opto)electronics and (bio)sensing to catalysis and energy harvesting. The incorporation of these materials into nanofluidic devices and field-effect transistors (FET) enables the creation of next-generation biosensing devices that feature ultimate sensitivity and lower detection limits. 2D nanopore devices, which consist of a nanometer-sized hole made in thin 2D material, present a platform for label-free, real-time detection and manipulation of single molecules, and as such hold an immense potential for advancing diagnostics and studying biological processes. This presentation will focus on the nanofabrication and biosensing applications of nanopores made in molybdenum disulfide and hexagonal boron nitride [1,2], including the design of the nanopore-FET [3] device. We will also present chemical functionalization strategies [4,5] which play a pivotal role in designing novel hybrid materials and tailoring material properties to suit specific biosensing applications.

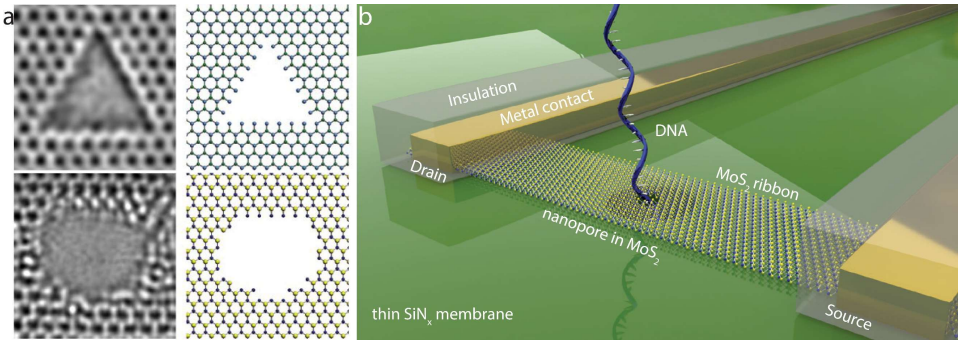

Figure 1. (a) HR-TEM images of nanopores in hexagonal boron-nitride (top) and MoS_2 (bottom). (b) The illustration of a nanopore-FET device. Figure adapted from [2] and [3].

[1] M. Graf[#], M. Lihter[#] et al., *Nat. Protoc.* **14** 1130 (2019)
[2] K. Liu[#], M. Lihter[#] et al., *Nano Lett.* **17** 4223 (2017)
[3] M. Graf, M. Lihter et al., *Nano Lett.* **19** 9075 (2019)
[4] M. Lihter et al., *ACS Appl. Nano Mater.* **4** 1076 (2021)
[5] M. Lihter et al., *Adv. Funct. Mater.* **30** 1907860 (2019)

Exploring novel photochemical behaviour of BODIPY-phenol chromophores using time-resolved photoelectron spectroscopy

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BODIPY dyes, renowned for their exceptional spectroscopic and photophysical qualities, are widely utilized in biological research for specific protein labelling. This involves attaching a BODIPY chromophore to amino acids integrated into proteins. A novel method in photolabeling proteins employs a BODIPY fluorophore that initiates an anti-Kasha photochemical reaction from a higher singlet excited state (Sn), resulting in the deamination of the BODIPY quinone methide precursor. Yet, the intricate mechanisms of this anti-Kasha photochemistry remain largely unexplored.

To shed light on this, we have probed the electronic states and ultrafast dynamics of the BODIPY-phenol chromophore using time-resolved photoelectron spectroscopy (PES). Our experimental approach involved a pump-probe scheme, where visible laser pulses excited the chromophore (pump), and XUV pulses from a High Harmonic Generation setup examined its electronic structure (probe) with a 30 fs time resolution. PES allows us to observe transient electronic structures in real time, capturing the relaxation dynamics of both ground and excited states. Critically, it offers insights into 'dark states', which are elusive to purely optical methods. Preliminary findings suggest the formation of a long-lived state (with decay time exceeding 1 ms) in the chromophore's thin film, potentially corresponding to a triplet exciton. These insights are invaluable for advancing our understanding of new photochemical processes involving organic radicals with unique functional properties in biological systems.

Combined influence of silver nanoparticles and biomacromolecules on calcium phosphates precipitation and antibacterial activity

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Calcium phosphate (CaP) composites with antimicrobial metal/metal oxide nanoparticles and/or biomacromolecules are emerging as a promising replacement for antibiotic therapy in implant-related infections and as a way of improving their biological and mechanical properties [1,2]. A promising method of preparing CaP composites with AgNPs is precipitation.

Motivated by this, in this study formation of CaPs in the presence of silver nanoparticles stabilized by citrate (cit AgNPs), and biologically active molecules, bovine serum albumin (BSA) or chitosan (Chi), was investigated. Influence of specific additive as well as their combinations was determined. In the absence of additives, CaP precipitated in two steps, as shown by potentiometric measurements. In the first step, amorphous calcium phosphate (ACP) was formed, which was transformed after 60 minutes into the mixture of poorly crystalline calcium-deficient hydroxyapatite (CaDHA) and a small quantity of octacalcium phosphate (OCP). Addition of additives inhibited ACP transformation, with Chi being the dominant inhibitor. Powder X-ray Diffraction Patterns confirmed that in the presence of cit AgNPs amount of OCP was decreased, BSA induced the formation of a small amount of another form of CaP dicalcium phosphate dihydrate (DCPD), while no Chi influence on composition of the formed precipitate was observed. SEM micrographs revealed the influence of all additives on morphology of crystalline precipitate.

The antibacterial properties of cit AgNPs and CaP / cit AgNPs composites were investigated with two bacterial strains. The results showed that cit AgNPs had the strongest inhibition of bacterial growth though only partially whereas inhibiton by CaP / cit AgNPs composites was present but to a lesser extent.

The obtained results provide new insights important for understanding CaPs formation in the presence of different additives and information of antibacterial properties of synthetized composites.

Acknowledgement. Financial support from Croatian Science Foundation, Grant HRZZ-IP-2018-01-1493 is greatly acknowledged.

[1] M. Rai et al., Biotechnol. Adv. **27,** 76–83 (2009).

[2] A. Bigi et al., J. Appl. Biomater. Funct. Mater. **15**, e313–e325 (2017).

Opto-bioelectronics in 3D

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Using electrical extracellular stimulation for single cells and neurons in a localized and wireless way is essential for developing improved therapies by stimulation of the autonomous nervous system, for guided neural tissue regeneration, neural prostheses such as artificial retinas, as well as for brain-machine interfaces. A simple and effective device which can withstand harsh in-vivo conditions and would not hurt the living organism during its operation is required for such a task.

I will present our ongoing research on using organic electrolytic photocapacitor (OEPC), a device fulfilling most of the earlier mentioned requirements. OEPC is a planar optobioelectronic device based on a *p-n* bilayer of organic semiconductor thin films deposited by vacuum-evaporation of organic pigments. Effectiveness of the OEPCs was demonstrated in-vitro by optical electrostimulation of blind retinas[1], on single cells [2], by effecting voltage-gated ion channels of the Xenopus laevis oocyte model, and by optical electro-stimulation of peripheral neurons in a rat model[3], showing stable performance of the devices in-vivo over months of duration.

I will show how 3D structuring of the OEPCs can simultaneously affect their optoelectronic, bio-electronic and mechanical properties, and thus shape their functionality. Multifold enhancement of the performance of micro-pyramid structured OEPC was measured, and the enhancement depended strongly on the micro-pyramid size and their density. The stimulation light-pulse shaping can further affect the device's bio-electronic functionality by providing transductive electrical potentials of arbitrary shape, duration and polarity to the nearby cells. Our numerical time-domain FEM model of a neuron placed in the proximity of a model OEPC has shown that shaping the electrode and the active layer size and their relative placement radically affect the stimulation threshold.

Acknowledgements
This work has been supported in part by Croatian Science Foundation under the project UIP-2019-04-1753.

[1] D. Rand, M. Jakešová , G. Lubin , I. Vėbraitė, M. David-Pur, V. Đerek, T. Cramer, N.S. Sariciftci, Y. Hanein, E.D. Głowacki, Advanced Materials, 30 (2018) 1707292
[2] M. Jakešová, M.S. Ejneby, V. Đerek, T. Schmidt, M. Gryszel, J. Brask, R. Schindl, D.T. Simon, M. Berggren, F. Elinder, E.D. Głowacki, Science advances, 5 (2019) eaav5265N.
[3] Silverå Ejneby, M., Jakešová, M., Ferrero, J.J., Migliaccio, L., Sahalianov, I., Zhao, Z., Berggren, M., Khodagholy, D., Đerek, V., Gelinas J.N., Głowacki, E.D., Nat. Biomed. Eng 6, 741–753 (2022).

Biopolymers modelling

MONDAY, 15:00--16:40

Phase behavior of semiflexible lattice polymers in poor solvent solution: mean-field theory and Monte Carlo simulations

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Predicting the phase behavior of a solution of semi-flexible polymers in poor-solvent conditions is a particularly challenging problem for several reasons. The main difficulty stems from the large number of thermodynamic and structural parameters that need to be taken into consideration. In addition to usual thermodynamic quantities such as temperature and pressure, one has to account for inter- (and intra-) chain interaction, the number of monomers in a chain, the stiffness of the fiber and the total polymer volume fraction: these all become axes of a large parameter space that is not easy to explore completely by relying only on computational tools.
With the aim of providing a guidance for navigating in such parameter space, we study a solution of self- (and mutually-) avoiding polymers with curvature energy in poor-solvent conditions on the *d*-dimensional cubic lattice ([1]). Building upon past studies on a single chain ([2]), we construct an exact field-theoretic representation of the multi-chain system and solve it within a mean-field approximation supported by Monte Carlo simulations. Our model recapitulates as particular cases several models that have been discussed in the past, in particular the Flory-Huggins theory for polymers solutions.

The same theoretical framework is currently being applied to study randomly branching polymers with annealed connectivity on the *d*-dimensional lattice, which is a topic of biological significance since RNA is known to behave as a randomly branching polymer ([3]). In particular, we're exploring the dependence of the branching probability in relation to the total monomer density, comparing mean-field results and MC simulations.

[1] D. Marcato, A. Giacometti, A. Maritan, A. Rosa, *J. Chem. Phys. 159, 154901 (2023).*

[2] S. Doniach, T. Garel, H. Orland, *J. Chem. Phys. 105, 1601 – 1608 (1996).*

[3] D. Vaupotič, A. Rosa, L. Tubiana, A. Božič, *J. Chem. Phys. 158, 234901 (2023).*

Development of integrative methods for Molecular Dynamics simulations

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Molecular Dynamics (MD) simulations play a crucial role in resolving the underlying conformational dynamics of complex biomolecules. However, their capability to reproduce and predict dynamics in agreement with experiments is limited also by the accuracy of the force-field model. This issue can be tackled by suitable integration with experimental data. To this aim, two approaches were proposed in the literature: the so-called **Ensemble Refinement** (ER) [1,2] and **Force-Field Refinement** (FFR) [3,4,5]. While the first methodology acts separately on each molecular system, lacking transferability to different systems, the second approach is based on a reasonable guess of the force-field correction terms, making the resulting corrections transferable to other systems. However, adding the same force-field correction terms to all the copies of a given molecule or residue could be over-limiting and not able to capture further relevant differences among them. In addition, the functional form of the force-field might be limited and intrinsically unable to reproduce experimental data. These two categories of methods have been so far used in a disjoint fashion. The user is thus expected to decide, based on experience, if transferable or non-transferable corrections are performing best for a given system.

In our work, a novel methodology combining ER and FFR is presented. This allows to preserve the flexibility on the resulting ensembles proper of ER while at the same time ensuring the transferability of the resulting force-field corrections to different molecules as in FFR. The efficacy of their combination is examined for a realistic case study of RNA oligomers. Within the new scheme, MD simulations are integrated with experimental data provided by nuclear-magnetic-resonance measures. By considering a sinusoidal force-field correction on α dihedral angles, we show the prediction error on validating observables to be significantly lower for the combined approach ER+FFR rather than for either ER or FFR separately applied.

[1] G. Hummer and J. Köfinger, The Journal of chemical physics 143 (2015).
[2] S. Bottaro et al., Structural bioinformatics: methods and protocols, 219–240 (2020).
[3] A. B. Norgaard et al., Biophys. J. 94, 182–192 (2008).
[4] A. Cesari et al., Journal of chemical theory and computation 15, 3425–3431 (2019).
[5] J. Köfinger and G. Hummer, The European Physical Journal B 94, 245 (2021).

Stochastic Block Model for chromatin organization

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Numerous experimental assays, such as Hi-C, ChIA-PET, or PCHiC, allow us to investigate the spatial organization of the genome within the nucleus. The data generated by these experiments can be naturally represented as a graph - or a "chromatin network" - where nodes represent genomic regions and edges represent spatial proximity between them. In this study, we propose a probabilistic generative model for random graphs, specifically based on the Stochastic Block Models (SBM) class, to describe the compartmentalization of interactions observed in Hi-C data. Moreover, in our model, the connectivity properties of the nodes are modulated by biochemical quantities, such as histone modifications and transcription factor binding. We also develop a variational algorithm to perform posterior inference on the model.

Cryo-EM ensemble refinement with molecular dynamics enables the structural characterization of flexible RNAs

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RNA molecules span a great variety of biological functions, from genetic information storage to catalysis. This is possible thanks to the highly heterogeneous conformational ensembles that these molecules can adopt [1]. The advent of the single particle cryo-electron microscopy (cryo-EM) technique enables to access large and highly flexible RNA macromolecules at near atomic-level resolution, thus allowing to fully assess their structural and functional features. Still, given that standard refinement tools assume that all the collected images are associated to a single structure, the most mobile regions are very challenging to solve.
Our aim is to tackle these problems by integration of molecular dynamics simulations and experimental density maps, using as a test case a group IIB intron ribozyme [2].
We show that a refinement done assuming a single structure leads to group II intron structural models with nucleobase base pairs that are either non-properly paired or in disagreement with the experimental data. This problem was solved by using metainference-based [3] ensemble refinement, along with ad hoc restraints to enforce correct base pairing.

[1] J.A. Doudna, T.R. Cech, Nature, 418: 222-228 (2002)
[2] D.B. Haak et al., Cell, 178: 612-623 (2019)
[3] M. Bonomi et al., Science Advances, 2: 3 (2016)

Structure-function relationship of viral RNAs probed with pore translocation

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The biological functionality of biomolecules is often directly informed by their structural organization. For instance, it is well known that the architecture of several enzymes, from kinases to hydrolases and proteases, is geared towards stabilizing the geometry of their active sites while also sustaining large scale collective motions necessary to bring the substrate to a reactive state.

By contrast, much fewer examples of structure-function relationships have been elucidated so far. A notable exception is the case of exonuclease resistant RNAs, xrRNA, a class of viral subgenomic RNAs whose numerous pseudoknots are instrumental to resist unfolding by nucleases but not by replicases and reverse transcriptases. The physical bases of this selective resistance have been recently elucidated by studies that modeled exonucleases degradation as a driven pore translocation process for the Zika xrRNA [1,2]. Despite these advances, it is still unclear whether the same properties apply more generally to viral RNAs.

Motivated by these considerations, we carried out a systematic study of the translocation response of representative viral RNAs. The study, which was conducted with using implicit-solvent atomistic simulations with a native-centric force field, helps us to shed light on previously unaddressed aspects tying structure and function of viral RNAs.

1. A. Suma, L. Coronel, G. Bussi and C. Micheletti, *Nature Commun.* **11**, 3749 (2020)
2. M. Becchi, P. Chiarantoni and C. Micheletti, *J. Phys. Chem. B* **125**, 1098-1196 (2021)

Lipids, membranes and vesicles

MONDAY, 17:10--18:10

Shape-based design of bitopic proteins as probes for membrane elastic stress energy

Michael Kaltenegger[1], Louis Percifull[2], Jona Causemann[2], Toni Radanovic[2], Birgit Grill[3], Robert Ernst[2], Gustav Oberdorfer[3], Georg Pabst[1]

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Approximately 30 % of the human proteome belongs to the group of membrane integral proteins. These hold particular significance since they are naturally located at cellular interfaces, where they rule transport and signaling processes.
The main goal of my project is to establish a system to quantify the impact of membrane-stored elastic energy on the self-association of transmembrane proteins. Therefore, we designed bitopic proteins considering their geometrical shape along the lipid bilayer normal, while matching the overall membrane thickness. The self-association of these "probes" is then to be measured by single molecular Förster resonance energy transfer microscopy (smFRET) of these probes in large unilamellar vesicles (LUVs).
We expressed the artificial proteins in E. coli. Due to the hydrophobic nature of the design, we are using a maltose binding protein (MBP) solubility tag and purified the proteins using detergent. Subsequent fluorescent labelling was carried out on the affinity column.
The initial transmembrane sequence was KKV11WA11KK. After several purification attempts with moderate success and low fluorescent labelling efficiency, we used the online ΔG prediction server of Stockholm University to maximize the interaction of the transmembrane helix with the hydrophobic core of the membrane. We mutated the TM-sequence accordingly while maintaining the overall geometry of the protein. This simultaneously improved the interaction of the proteins with detergent micelles during purification, leading to less oligomerization during purification and improving the degree of labelling.
In the first test experiments on the insertion the new protein probes into LUVs, we screened different detergent concentrations to maintain the integrity of the membrane during reconstitution. We found that the decrease in detergent, rather shrinks the vesicles, than destroying them during dialysis, while the insertion efficiency drops. Nevertheless, the modified sequence showed better interaction with the membrane, because we lost less protein during dialysis.

Design Rules for Membrane-Active Antimicrobial Lipidoids (AMLs) Uncovered by High-Throughput Screening

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The increase of antimicrobial resistance in pathogenic bacteria is a public health crisis, and compounds that target membrane lipids are one solution to overcome resistance development pathways [1]. Natural antimicrobial peptides (AMPs) and synthetic quaternary ammonium compounds (QACs) are amongst the most promising membrane-targetting candidates, but AMPs are susceptible to enzymatic degradation while QACs can be very toxic to human cells [2]. In this work, we adopt high-throughput screening approaches to study the structure-activity relationship for a new class of antimicrobial compounds: antimicrobial lipidoids (AMLs).

AMLs were synthesized containing multiple charged headgroups (≥ 2) and hydrophobic tails (≥ 4), to resemble the structure of some bacterial lipids (e.g. lipid A and cardiolipin). Upon protonation, we discovered that these multi-tail lipidoids can self-assemble into lamellar, bicontinuous cubic, and hexagonal liquid crystal phases [3], and adopt unusual molecular conformations distinct from biological lipids. By screening over 100 unique lipidoids using minimum inhibitory concentration (MIC) assays against Gram-negative and Gram-positive bacteria, we uncovered several correlations between molecular structure and antimicrobial activity. In particular, lipidoids that can adopt conical conformations, and hence have a propensity for non-lamellar phases, are amongst the compounds with highest antimicrobial activity [4]. The mechanism of membrane disruption was further confirmed by dye permeation assays, and cytotoxicity tested through hemolysis studies. Thus, this study has revealed several design rules for synthetic membrane-targeting antimicrobial compounds, and for achieving selectivity over mammalian cells.

[1] Shukla, R.; Lavore, F.; Maity, S.; Derks, M.G.N.; Jones, C.R.; Vermeulen, B.J.A.; Melcrová, A.; Morris, M.A.; Becker, L.M.; Wang, X.; et al. *Nature* ***608***, 390–396 (2022).

[2] Jennings, M.C.; Minbiole, K.P.C.; Wuest, W.M. *ACS Infect. Dis.* **1**, 288–303 (2016).

[3] Jennings, J.; Pabst, G. *Small* **19**, 2206747 (2023).

[4] Jennings, J.; Asceric, D.; Semeraro, E.; Lohner, K.; Malanovic, N.; Pabst, G. *ACS Appl. Mater. Interfaces* **15**, 40178–40190 (2023).

Amphipathic Helices Can Sense Both Positive and Negative Curvatures of Lipid Membranes

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Proteins that sense and respond to membrane curvature are essential for biological processes such as vesicle trafficking, endocytosis, and cell migration. These proteins are able to colocalize to membranes with specific curvatures at the same time, allowing them to perform their functions. Short amphipathic helices (AHs) have been shown to be able to sense positive membrane curvature, but no AH with a preference for negative membrane curvature has been discovered yet. Using a systematic computational approach, we show that the curvature sensing of peptides can be tuned by modulating their hydrophobicity. We demonstrate that this tuning can be used to convert the sensors of positive membrane curvature into the sensor of negative membrane curvature. Consistent results from both coarse-grained and atomistic simulations provide examples of AHs that preferentially localize to negatively curved membrane regions. This discovery not only deepens our understanding of biomolecular interactions with membranes but also opens avenues for designing molecules with tailored curvature sensing capabilities, which could be valuable in the development of targeted therapeutic interventions and advanced bioengineering applications.

Soft and active matter

TUESDAY, 09:30--10:50

Unraveling the universal adhesion threshold of biomatter on materials with water contact angles of 65°

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Surface phenomena, such as long-range hydrophobic attraction, adhesion of proteins and other macromolecules, as well as biofouling, are primarily controlled by a crucial material factor: the water contact angle of the substrate. Ample of experimental studies report that the minimal contact angle required for these phenomena is approximately 65°, which has been dubbed as the "Berg limit." However, despite the widespread applicability of this specific value in many different contexts, its understanding remains poor.

In this talk, I will try to clarify the underlying mechanism behind this mysterious threshold. Many of these phenomena can be effectively conceptualized as problems involving three phases: the relevant surface, water, and a generic oil-like substance representing the nonpolar components within interacting entities. The analysis demonstrates that attraction and adhesion occur when surfaces exhibit underwater oleophilic characteristics, which is when the surface prefers wetting by oil over wetting by water. Interestingly, this distinct behavior emerges at a contact angle of approximately 65°, coinciding with the Berg limit.

Tensile strength of water with organic impurities

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The stability of water is a long-standing problem in physics, studied since the 17th century [1] and continuing to be a subject of investigation today. There is a noteable discrepancy between experimental findings and theoretical predictions, as well as inconsistency in measurements across different experiments. While theory predicts that water should be remarkably stable against cavitation, experiments show quite the opposite. [1,2] In this talk, I will present our work on the conditions that lead to catastrophic cavitation events in decane and water. Additionally, I will discuss how the tensile strength of water is influenced by hydrocarbon impurities, such as oil droplets. We use a framework that combines classical nucleation theory with molecular dynamics simulations. We find that while pure bulk water is exceptionally stable against cavitation, the presence of even tiniest amounts of decane is enough to destabilize water and reduce its tensile strength to experimentally measured lower values. Using our numerical analysis, we find that a decane droplet of a radius of around 1 nm in a macroscopic volume of water is enough to destabilize the system. This is the reason why even in ultra-pure water, the measured tensile strength is significantly lower compared to theoretical predictions. We also find that the curvature correction of surface tension is important to take into account when studying cavitation, nanodroplets, or nanobubbles.

[1] Caupin, F.; Herbert, E. Cavitation in water: a review. Comptes Rendus Physique 2006, 7, 1000–1017.
[2] Caupin, F.; Stroock, A. D. The stability limit and other open questions on water at negative pressure. Liquid Polymorphism 2013, 152, 51–80.

Curvature-dependent adsorption of surfactants in water nanodroplets: Insights from molecular dynamics

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Adsorption of surfactants at curved air-water interfaces plays a major role in phenomena involving nanodroplets, such as emulsification, wetting, heterogeneous catalysis, and aerosol chemistry. Once the nanodroplet becomes small enough, its large curvature can influence the adsorption of surfactants, which has not been thoroughly investigated.
At this scope we investigate the adsorption of short-chain surfactants inside water nano-droplets using molecular dynamics simulations. We show that the curvature of the droplet interface enhances adsorption and affects other interfacial properties, such as the preferred surfactant orientation.
We relate this behavior to curvature-dependent surface tension of water, described by the Tolman length correction. We show that the influence of curvature on adsorption grows exponentially with size of the hydrophobic tail of the surfactant. Finally, we use our theoretical model to predict the behavior of larger surfactants and of larger droplets.

Active dynamics of wound closure

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Biological systems are highly robust in terms of their ability to adapt to random perturbations. These include spontaneous genetic mutations as well as damages inflicted from the environment - both are surprisingly common but only rarely fatal. We theoretically study the response of an epithelial tissue to a mechanical damage in the form of a circular wound - a region of missing cells. Our model describes a feedback loop between the generation of forces in the actomyosin and tissue mechanics, described by the vertex model. The results suggest that wound healing may be an active instability, triggered by a decreased myosin-turnover rate at the wound's periphery. We study how the myosin dynamics interplays with tissue's elastic properties to determine the ability to heal the wound.

Novel modelling approaches

TUESDAY, 11:20--12:40

Vertex Model Challenges Meet Knowledge Graph Solutions

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Vertex model (VM) shows its credibility in modeling confluent biological tissues in two dimensions. However, due to computational complexities, only a few studies have been reported since the introduction of the first three-dimensional (3D) vertex model almost two decades ago. Most of the challenges lie in handling the changes in topology during dynamic cell rearrangements, which justifies the absence of a proper algorithmic form of topological transformations so far. This issue is often viewed in the community as a complex "Engineering problem". In the talk, I will demonstrate a possible solution to this outstanding problem using a new approach called the Graph Vertex Model (GVM). GVM is founded on the concept of representing the topology of the cell network using a Knowledge Graph (KG), effectively rendering topological transformations both intuitive and mathematically well-defined. Furthermore, I will highlight the enhanced credibility and utility of the KG by introducing the GRAPh Embryo project (GRAPE). GRAPE is an online database designed to facilitate interactive analyses of an entire fly embryo, showcasing the practical applications and benefits of this approach.

Topological Transformations in Graph Vertex model

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Vertex Model (VM) shows its reliability in modeling confluent biological tissues in two dimensions (2D). However, its application in three dimensions (3D) has been constrained by computational complexities, primarily stemming from managing topological changes during cell rearrangements. In the talk, I will demonstrate how the recently introduced Graph Vertex Model (GVM) successfully addresses the challenges posed by VM. GVM achieves this by exclusively storing topological data of cell networks within a Knowledge Graph (KG). The unique data structure of KG enables cell rearrangements and divisions to be equivalent to elementary graph transformations, which are also represented by graphs. Furthermore, cell rearrangements in 3D consist of graph transformations that correspond to more fundamental T1 transitions, thereby unifying topological transitions in both 2D and 3D space-filling packings. This implies that the GVM's graph data structure may be the most natural representation for cell aggregates and tissues.

Coarse Graining by Normalizing Flows

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The problem of sampling equilibrium states from a high-dimensional and multi-modal probability distribution is ubiquitous in machine learning, inference, and physical sciences. As the dimensionality of the system under study increases and the energy barrier between metastable states gets higher, classical methods such as Molecular Dynamics may become very inefficient in generating uncorrelated samples from the desired distribution.

In recent works [1] and [2], it has been shown how invertible generative models, known as Normalizing Flows, can be trained to efficiently generate samples from high-dimensional and multi-modal distributions. In particular, in [1], a Normalizing Flow was integrated into an Adaptive Monte Carlo algorithm and used as a proposal distribution for non-local moves. In this framework, the Normalizing Flow is updated at every iteration of the Monte Carlo with the data being produced.

For large systems, such as a polymer in solution, the Adaptive Monte Carlo becomes inefficient because the Normalizing Flow fails to learn the distribution of the system in the full configurational space. We propose a new version of the Adaptive Monte Carlo algorithm in which the Normalizing Flow operates in the space of Collective Variables, which has a lower dimensionality. The new algorithm operates as follows: i) a certain number of random walkers are initialized in the modes of the distribution, ii) at each step, we either evolve the walkers classically with a local sampler or propose a new value of collective variables with the Normalizing Flow, iii) in the latter case, we move the system to the new Collective Variables using the procedure described in [3], and iv) we use the newly accepted configurations to train the Normalizing Flow.

The new algorithm is tested on a toy model consisting of a polymer immersed in Lennard Jones particles with multiple stable states for the dihedral angles.

[1] M. Gabrié, G. M. Rotskoff, and E. Vanden-Eijnden, "Adaptive Monte Carlo augmented with normalizing flows," Proceedings of the National Academy of Sciences, vol. 119, no. 10, p. e2109420119, Mar. 2022
[2] Frank Noé et al., "Boltzmann generators: Sampling equilibrium states of many-body systems with deep learning." Science 365, eaaw1147 (2019)
[3] J. P. Nilmeier, G. E. Crooks, D. D. L. Minh, and J. D. Chodera, "Nonequilibrium candidate monte carlo is an efficient tool for equilibrium simulation," Proceedings of the National Academy of Sciences, vol. 108, no. 45, pp. E1009–E1018, 2011J.

Robust inference of causality in dynamical processes from the Information Imbalance of distance ranks

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Uncovering causal relationships between time-dependent observables is a problem which goes at the heart of scientific research. However, distinguishing a cause-effect relationship from a correlation, possibly induced by a quantity which is not observed, or unknown, is still considered an open problem, especially if the effect is triggered by the simultaneous variation of a large number of variables. Nowadays, the performance of many causal detection methods degrades quickly with the number of variables that one should consider. Moreover, in real-world contexts most of the approaches lead to false-positives, namely have difficulties in recognizing the absence of causality.

In this talk, I will illustrate a new method built on the Information Imbalance measure [1], which significantly mitigates these problems. The method tests whether the predictability of a putative driven system Y can be improved by incorporating information from a potential driver system X, without explicitly modelling the underlying dynamics and without the need to compute probability densities of the dynamic variables [2].
I will illustrate how the approach can help in uncovering causal mechanisms in diverse real-world data, from electroencephalographic experiments to molecular dynamics simulations.

[1] A. Glielmo, C. Zeni, B. Cheng, G. Csányi, A. Laio, *PNAS Nexus* **1,** 2752-6542 (2022).

[2] V. Del Tatto, G. Fortunato, D. Bueti, A. Laio, arXiv:2305.10817