16^{th} Christmas Biophysics Workshop

Book of Abstracts

Hotel Brdo, Brdo pri Kranju, Slovenia
December 12–13 2022

Organizers: Urška Andrenšek, Matej Krajnc, Matej Kanduč, Anže Božič and Primož Ziherl

Welcome

The Christmas Biophysics Workshops (XBWs) are annual regional meetings of researchers from Austria, Croatia, Italy, and Slovenia who work in biophysics, soft condensed matter, and related fields. The meeting series was initiated by Rudi Podgornik and Silvia Tomić in 2006. Within a year or two, the scope and the format converged, and the workshops have established themselves as productive events where new, often unpublished results and ideas can be discussed in an informal atmosphere. This promotes scientific collaboration and exchange between the universities and institutes in the region.
The Brdo Estate is known for the renaissance Brdo Castle, well-kept gardens, pastures, forests, hunting grounds, golf course, and Lipizzaner horses, and it hosts many conferences and other events, including diplomatic meetings. We are convinced that you, too, will feel very welcome. With some luck, this year's after-dinner event will be stellar.

Urška Andrenšek
Matej Krajnc
Matej Kanduč
Anže Božič
Primož Ziherl

PROGRAM

Monday, December 12

8:00-9:30	Registration and check-in	
9:30-9:40	Opening words: Matej Kanduč	
9:40 - 11:00	**Section I: Membranes & lipid vesicles I**	Chair: Primož Ziherl
9:40	Saša Svetina: *Shapes of phospholipid vesicles containing a single embedded Piezo1 channel*	
10:05	Matej Kanduč: *Adsorption of surfactants: Molecular simulations*	
10:30	Simon Čopar: *Shaping nematic droplets with surface tension*	
11:00-11:30	Coffee break	
11:30-13:00	**Section II: Packing, topology & shape**	Chair: Cristian Micheletti
11:30	Andraž Gnidovec: *Dense packings of geodesic hard ellipses an a sphere*	
11:55	Andrea Tagliabue: *A panoramic virtual tour in the world of knotted polyelectrolytes*	
12:20	Urška Andrenšek: *Epithelial wrinkling*	
13:00-14:30	Lunch	
14:30-16:00	**Section III: Water and other liquids**	Chair: Tomislav Vuletić
14:30	Douwe Jan Bonthuis: *A glass of water with a pinch of salt*	
14:55	Marin Šako: *Tensile strength of water*	
15:20	Fabio Staniscia: *Apparent line tension induced by surface-active impurities*	
16:00-16:30	Coffee break	
16:30-18:00	**Section IV: Membranes & lipid vesicles II**	Chair: Simon Čopar
16:30	Ida Delač: *Organic solution and solute droplet deposition for the functionalization of MoS2*	
16:55	Anze Božič: *Scaling properties of RNA as a branched polymer*	
17:20	Danijela Bakarić: *Experimental and computational insight into the surface curvature of DPPS lipid bilayers*	
18:00-19:30	Dinner	
19:30	Night sky observation & wine tasting	

Tuesday, December 13

8:00-9:30	Breakfast & check-out	
9:30-10:20	**Section V: Proteins and membranes**	Chair: Ida Delač
9:30	Eva Žerovnik*: Amyloid fibrils intrinsic fluorescence and its usage for labelfree monitoring*	
9:50	Matej Kanduč: *Pressure response of polymer brushes*	
10:10	Suzana Inkret: *Influence of bovine albumine serum on formation of calcium phosphates and silver nanoparticles composites*	
10:30-11:00	Coffee break	
11:00-12:30	**Section VI: RNA & biopolymers**	Chair: Matej Kanduč
11:00	Valerio Piomponi: *Using computational methods to investigate the impact of modified nucleotides on RNA structural dynamics*	
11:25	Angelo Rosa: *Deciphering the interaction of genetic and epigenetic factors through enhanced polymer models*	
11:50	Francesco Slongo: *A QUBO model for melts of self-assembling ring polymers*	
12:20	Closing words: Primož Ziherl	
12:30	Lunch	
14:00	Departure	

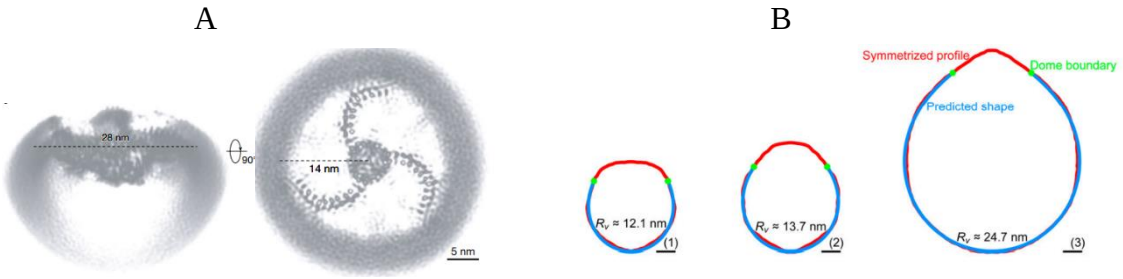
Shapes of vesicles with a single embedded Piezo1 channel

S. Svetina[1,2] and B. Božič[2]

[1]*Jožef Stefan Institute, Ljubljana, Slovenia*
[2]*Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

Piezo1, a nonspecific cation channel, is a giant homotrimer with each of its subunits involving 38 transmembrane helices (THs). Two of them participate in the formation of the trimer´s central part comprising the channel. The other 36 THs form a chain of nine segments, formed by 4 THs, which extends into the surrounding bilayer membrane. These chains, called arms, are curved and therefore have an effect on local membrane curvature. Effect is so strong that already a single in vesicle membrane embedded Piezo1 channel modifies its shape in an observable manner. Moreover, it was also observed that there is an effect of vesicle size on Piezo1 conformation [1]. It is believed that the effects of Piezo1 and the surrounding bilayer membrane on each other underlie the mechanism of how Piezo proteins transform mechanical forces acting on the membrane into physiologically important electrical or biochemical signals.

Our first attempt to theoretically model Piezo1 – membrane interaction [2], based on the results presented in ref. [1], required too many assumptions. This year came out better data [3-5] (Figures A and B). The measured vesicle shapes were also analyzed theoretically [4,5]. Here we shall describe these new results and show how in ref. [4] presented theory should be amended to understand vesicle shapes obtained at differently oriented Piezo1 channel by a single simple model.



A: CryoEM obtained shape of vesicle in which Piezo1 is oriented in vesicle membrane as in biological membranes, presented in Fig. 2f of ref. [3] (side view and top view).

B: Axial cross-sections of three vesicles with different membrane area in which Piezo1 is oriented in the membrane oppositely to biological membranes, presented in Fig. 4 of ref. [4]. R_v is the radius of sphere with membrane area of each vesicle.

[1] Y-C. Lin et al., *Nature*, **573**, 230 (2019).
[2] S. Svetina and B. Božič, *presented at 14th XBW, Gradisca d´Isoncio, Italy* (2019).
[3] X. Yang et al., *Nature*, **604**, 377 (2022).
[4] C. A. Haselwandter et al., *Proc. Nat. Acad. Sci*, **119**, e2208027119 (2022).
[5] C. A. Haselwandter et al., *Proc. Nat. Acad. Sci*, **119**, e2208034119 (2022).

Adsorption of surfactants: Molecular simulations

Matej Kanduč

Jožef Stefan Institute, Ljubljana, Slovenia

Adsorption of amphiphilic molecules to aqueous interfaces occurs in many technological and biological settings, sometimes desired (stabilization of foams), while other times not (contamination). I will discuss how atomistic computer simulations can help us elucidate molecular mechanisms of surfactant adsorption, ranging from short-chain alcohols to double-tail lipids. Small surfactants form loose monolayers and exhibit rapid exchange between the interface and bulk, which can be followed in the simulations. Surfactants with longer alkyl chains adsorb as denser monolayers and exchange on experimental timescales that are too slow to be captured in simulations, which challenges molecular modeling. The modeling of this regime requires advanced computational techniques that connect interface and bulk phases via precise determinations of their chemical potentials. Finally, double-chain surfactants (also called "lipids") do not exchange between bulk and interface both on experimental and simulation timescales.

Shaping nematic droplets with surface tension

K. Peddireddy[1], S. Čopar[2], K.V. Lee[3], I. Muševič[2,4], C. Bahr[1] and V.S.R. Jampani[4]

[1]*Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany*
[2]*Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Slovenia*
[3]*Tokyo University of Science, Tokyo, Japan*
[4]*Jožef Stefan Institute, Ljubljana, Slovenia*

Shaping liquids is a difficult task, as the flowing nature combined with surface tension tend to force the liquids into a round shape. In liquid crystals, anisotropy of the bulk material and the corresponding elastic response can induce deviation from the spherical shape, forming tactoids and generalized elliptical shapes. Electric fields can be used to further stretch and deform the resulting drops. However, as long as the surface tension dominates, the shapes will not deviate much from a spherical droplet.

I will present a system, where a combination of two surfactants – one dispersed in the aqueous host liquid, and the other in the nematic LC droplet – is used to achieve vanishing and effectively even negative surface tension. This allows for the surface of the droplet to grow freely, and the bulk elasticity is instead limiting emulsification and shaping the droplets [1].

In the nematic phase, multiple fibers of a consistent thickness start growing out of an initial droplet upon cooling. The molecular orientation at the interface is forced to be perpendicular, which induces radial configuration in a droplet and escaped radial structure in a fiber. A theoretical model can be constructed that describes the equilibrium shape of the droplet-fiber construct, and predicts its dimensions. If the system is cooled even further into the smectic phase, the fiber breaks up into a string of monodisperse droplets.

The same mechanism of forcing growth of the interface surface area can be shown to produce different results based on the phase of the liquid crystal – e.g. smectic, chiral smectic, or chiral nematic. Thus we can manipulate liquids to grow slowly and reversibly into shapes that lend themselves into further manipulation, which can be a stepping stone for further advancements in soft matter engineering.

[1] K. Peddireddy, S. Čopar, K. V. Le, I. Muševič, C. Bahr and V. S. R. Jampani, *Proc. Natl. Acad. Sci.* **118**, e2011174118 (2021).

Dense packings of geodesic hard ellipses an a sphere

A. Gnidovec[1], A. Božič[2], U. Jelerčič[3], and S. Čopar[1]

[1]*Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Slovenia*
[2]*Jožef Stefan Institute, Ljubljana, Slovenia*
[3]*Department of Chemical Engineering, Ilse Kats Institute for Nanoscale Science and Technology, Ben Gurion University of the Negev, Beer-Sheva, Israel*

Packing roblems are abundant in nature and are thoroughly researched both experimentally and in numerical simualtions. In particular, packings of anisotropic, elliptical particles emerge in models of liquid crystals, colloids, as well as in granular and jammed matter. While most of these studies deal with packings in Euclidean geometry, there are many experimental systems where anisotropically shaped particles are confined to a curved surface, such as Pickering emulsions stabilised by elliptical particles, and protein adsorbates on vesicles. In our work, we study random close packing in a two-dimensional model of hard spherical ellipses. We first present an algorithm we developed to detect overlaps between two spherical ellipses that is based on a solution of an eigenvalue problem and is essential in our packing simulations. We then show the packing results where we focus on the interplay between finite-size effects and curvature that is most prominent at smaller system sizes. We demonstrate that on a spherical surface, monodisperse ellipse packings are inherently disordered, with a non-monotonic dependence of both their packing fraction and the mean contact number on the ellipse aspect ratio, as has also been observed in packings of ellipsoids in both 2D and 3D flat space. We also point out some fundamental differences with previous Euclidean studies and discuss the effects of curvature on our results. Importantly, we show that the underlying spherical surface introduces frustration and results in disordered packing configurations even in systems of monodispersed particles, in contrast to the 2D Euclidean case of ellipse packing. This demonstrates that closed curved surfaces can be effective at introducing disorder in a system and could facilitate the study of monodispersed random packings.

[1] A. Gnidovec, A. Božič, U. Jelerčič, S. Čopar, *Proc. R. Soc. A*, **478**, 20210807 (2022).
[2] A. Gnidovec, A. Božič, S. Čopar, *Soft Matter*, **18**, 7670 (2022).

A panoramic virtual tour in the world of knotted polyelectrolytes

A. Tagliabue[1,2], M. Mella[1], and C. Micheletti[2]

[1]*Dipartimento di Scienza e Alta Teconologia, Università degli Studi dell'Insubria, Como, Italy*
[2]*Molecular and Statistical Biophysics, SISSA, Trieste, Italy*

Topological constraints, and in particular knots, are ubiquitous in both natural (e.g., DNA and proteins) and synthetic polymers, and, in the last years, the convergence of theoretical and experimental studies has provided a wide number of examples of how chain-uncrossability defines an impressive range of polymer properties. For example, the presence of knots can endow polymer chains with peculiar relaxation kinetics or scaling behavior, and markedly affect their response to mechanical stretching and elongational flows, or their absorption into porous materials. Despite these promising results, the impact of knots on the properties of polyelectrolytes (i.e., polymers that carry on themselves charged, or ionizable, groups), has not been investigated as in depth as for their neutral counterparts.
Exploiting *in silico* simulations and coarse-grained models of polyelectrolytes, we show that knots markedly modify the behavior of water-soluble ring-shaped polyelectrolyte, often in very counterintuitive ways. Though numerous examples, we demonstrate that the knot length and position along the chain, and consequently the polyelectrolyte size (e.g., its radius of gyration), is very sensitive not only to the chemical composition of the chain [1,2] but also to several solution properties, such as solvent quality and screening power, concentration and valency background salts, or pH [1,3]. This gives rise to an impressive range of conformational and dynamical properties that has no counterpart in species with a trivial topology, thus making knotted charged polymers promising candidates for the design of stimuli-responsive materials.

[1] A. Tagliabue, L. Izzo, M. Mella, *J. Phys. Chem. B*, **14**, 124 (2020).
[2] A. Tagliabue, C. Micheletti, M. Mella, *ACS Macro Lett.*, **10**, 11 (2021).
[3] A. Tagliabue, C. Micheletti, M. Mella, *Macromolecules*, just accepted.

Epithelial wrinkling

U. Andrenšek[1,2], P. Ziherl[1, 2], and M. Krajnc[2]

[1]*Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana Slovenia*
[2]*Jožef Stefan Institute, Ljubljana, Slovenia*

Buckling of epithelial tissues may be a result of different processes and properties of the cells. Forces generated within the actomyosin network and cells' surface properties can be studied by the differential-tension model, which describes tissues as tight packings of discrete cells with different surface tension of their apical, basal, and lateral sides. We study wrinkling instabilities of a two-dimensional tissue cross section. We derive continuum approximation of elasticity theory and compare it to numerical results. We compare continuum theory to elasticity theory of thin supported sheets and find that the apico-basal tension difference can act as a phantom substrate in unsupported epithelial sheets. Furthermore we investigate the change in surface pattern in tissues with fixed basal surface, which leads to cells acting as a substrate for the apical surface of the tissue.

A glass of water with a pinch of salt

Douwe Jan Bonthuis

Graz University of Technology, Graz, Austria

Understanding the collective behavior of ions at charged surfaces is of paramount importance for geological, electrochemical and biochemical processes. Ions screen the surface charge, and interfacial fields break the centro-symmetry near the surface, which can be probed using second-order nonlinear spectroscopy. We use sum-frequency generation (SFG) spectroscopy to probe the symmetry-breaking of water molecules at a charged silica surface in contact with alkaline metal chloride solutions (LiCl, NaCl, KCl, and CsCl) at various concentrations. The amplitude of the SFG response is strongly ion-specific, following the Hofmeister series Li>Na>K>Cs. Whereas variation of the second-harmonic response is typically ascribed to variation of the electrostatic surface potential, we use molecular dynamics simulations and continuum modeling to show that not the local electrostatic potential is ion-specific, but the orientational distribution of the interfacial water layer.

Tensile strength of water

Marin Šako and Matej Kanduč

Jožef Stefan Institute, Ljubljana, Slovenia

The tensile strength of water − the maximal tension that water can sustain before cavitating − has been a topic of debate in soft matter physics since the seventeenth century, and it is still researched today.Water under tension is found in many systems, from turbomachinery to biological living organisms[1,2], and as such, understanding it is important not only for scientific knowledge but also for the engineering of hydrodynamic systems.
Classical nucleation theory predicts that water is highly resistant to tension, while experiments show that it cavitates very quickly after being exposed to a few orders of magnitude lower tension. Furthermore, the measurements of tensile strength vary up to an order of magnitude among different experiments [3,4]. This discrepancy arises because cavitation never occurs in pure bulk water, as postulated by classical nucleation theory. Instead, cavitation happens in weak spots (such as preexisting gas bubbles, walls, interfaces, impurities, hydrophobic patches, etc.) that act as catalysts for nucleation [5].
I will present our theoretical approach using molecular dynamics simulations and classical nucleation theory to identify and quantify an important factor that limits the tensile strength water.

[1] A. M. Smith. Negative pressure generated by octopus suckers: a study of the tensile strength of water in nature. *Journal of Experimental Biology*, 157(1):257−271, 1991.

[2] X. Noblin, N. O. Rojas, J. Westbrook, C. Llorens, M. Argentina, and J. Dumais. The fern sporangium: a unique catapult. *Science*, 335(6074):1322−1322, 2012.

[3] F. Caupin and E. Herbert. Cavitation in water: a review. *Comptes Rendus Physique*, 7(9-10):1000−1017, 2006.

[4] F. Caupin and A.D. Stroock. The stability limit and other open questions on water at negative pressure. *Liquid Polymorphism: Advances in Chemical Physics*, 152:51−80, 2013.

[5] E. Newton Harvey, D. K. Barnes, W. D. McElroy, A. H. Whiteley, D. C. Pease, and K. W. Cooper. Bubble formation in animals. *Journal of Cellular and Comparative Physiology*, 24(1):1−22, 1944.

Apparent line tension induced by surface-active impurities

Fabio Staniscia and Matej Kanduč

Jožef Stefan Institute, Ljubljana, Slovenia

Line tension in wetting processes is of high scientific and technological relevance, but its understanding remains vague, mainly because it is difficult to determine. A widely used method to extract line tension relies on the variation of a droplet's contact angle with the droplet's size. Such an approach yields the apparent line tension, which is an effective parameter that factors in numerous contributions to the finite- size dependence, thus masking the actual line tension in terms of the excess free energy of the three-phase contact line. Based on a recent computer simulation study, it is investigated how small amounts of nonionic surfactants, such as surface-active impurities, contribute to the apparent line tension in aqueous droplets. When depositing polydisperse droplets, their different surface area-to-volume ratios can result in different final bulk concentrations of surfactants, different excess adsorptions to the interfaces, and, consequently, different contact angles. It is shown that already trace amounts of longer-chained surfactants in a pre-contaminated liquid are enough to affect measurements of the apparent line tension. The analysis quantifies to what extent "background" impurities, inevitably present in all kinds of experimental settings, limit the resolution of line tension measurements, which is crucial for avoiding data misinterpretation.

Organic solution and solute droplet deposition for the functionalization of MoS_2

A. L. Brkić[1], A. Supina[1], D. Čapeta[1], L. Ptiček[2], L. Racanè[2], I. Delač Marion[1]

[1]*Center of Excellence for Advanced Materials and Sensing Devices, Institute of Physics, Zagreb, Croatia*
[2]*University of Zagreb, Faculty of Textile Technology, Zagreb, Croatia*

Since molecular adsorption can influence properties like doping, bandgap, or optical response, the study of molecular self-assembly on 2D materials is a new and rapidly developing research field with two main goals: (I) modifying the (opto)electronic properties of 2D materials, and (II) using 2D materials as a decoupling layer to preserve the properties (e.g., magnetic or catalytic) of the adsorbed molecules. We are interested in how functionalization with covalently or non-covalently bound organic molecules alters the characteristics of 2D materials.

Drops of a specific concentration solution are the primary method of deposition of organic compounds in our current research. The first step in distinguishing the effects of the solvent from those of the organic molecules is the characterization of 2D material following exposure to different solvents.

I'll talk about our efforts to optimize the solvent concentration, deposition volume, and deposition procedure for applying this technology to water-soluble organic compounds, as well as some preliminary results.

Scaling properties of RNA as a branched polymer

D. Vaupotič[1], A. Rosa[2], L. Tubiana[3], and A. Božič[1]

[1]*Department of Theoretical Physics, Jožef Stefan Institute, Ljubljana, Slovenia*
[2]*Scuola Internazionale di Studi Avanzati (SISSA), Trieste, Italy*
[3]*Department of Physics, University of Trento, Trento, Italy*

Formation of both short- and long-range base pairs between the nucleotides of an RNA sequence gives rise to an often highly branched secondary and tertiary RNA structure. While numerous studies have demonstrated the importance of the high degree of branching of RNA structure for some of its functions—most notably in the genomes of positive-sense, single-stranded RNA (+ssRNA) viruses—the nature of the RNA branching topology remains largely unexplored. We use the theory of branched polymers to determine the scaling properties of both random RNA sequences as well as of genomes of +ssRNA viruses by mapping their secondary structures onto planar graphs. We derive scaling exponents ε and ρ, related to the topology of branching, in two different ways, not only from simple scaling relationships but also from distributions of the related topological quantities of individual RNAs. This allows us to compare the general scaling behaviour of RNA to known classes of branched polymers, and to compare the scaling behaviour of the genomes of +ssRNA viruses to random RNA sequences. In this way, we are able to elucidate the branching properties of RNA within the framework of branched polymers and simultaneously point out where this description becomes insufficient. Understanding the scaling properties of RNA related to its branching structure aims to improve our understanding of the principles which give rise to it, opening up the possibility for de novo design of RNA sequences with desired topological properties.

Experimental and computational insight into the surface curvature of DPPS lipid bilayers

D. Bakarić, L. Pašalić, and B. Pem

Division for Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia

Understanding the structure and function of inherently asymmetric eukaryotic plasma membranes is almost inevitably preceded by the research into the structural and dynamic features of single-lipid symmetric membranes [1]. Among the latter, the most common lipid membranes are those built from zwitterionic phosphatidylcholine (PC) lipids present in both membrane leaflets, while those made from the major anionic phosphatidylserine (PS) lipid dominantly found in the inner membrane leaflet are significantly less common [2]. Apart from studies focusing on multilamellar liposomes (MLV) made from 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine (DPPS) [3], the studies of corresponding unilamellar liposomes (LUV) are extremely rare.

With the aim of shedding the light on the structure and properties of LUVs made of DPPS lipids, we present calorimetric, spectroscopic and MD simulation study of DPPS in the form of LUV in a phosphate buffer (pH = 7.4), with the corresponding MLV examined as a reference. Melting of LUV in a wide temperature range (50-59 °C), associated with rather high uncertainty data level emerged from temperature-dependent UV/Vis spectra (Fig. 1) is presumably related with LUV instability and/or fluctuation of structural features on LUV surface. The signatures of carbonyl backbone obtained from FTIR data support the existence of highly curved surface of LUV, the phenomenon of which is not observed in MLV, whereas MD data unravel the contribution of interlamellar water on the surface features in MLV.

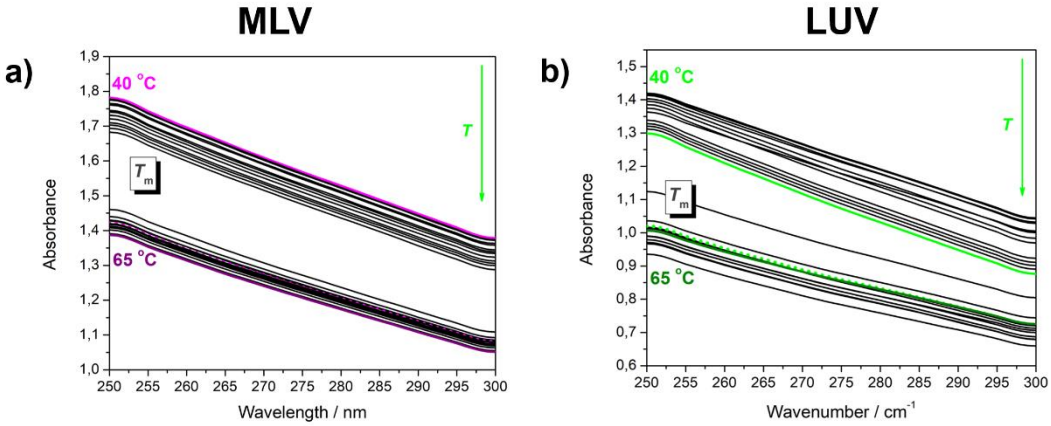


Fig. 1. Temperature-dependent UV/Vis spectra of DPPS in the form of a) MLV and b) LUV.

[1] M. Cebecauer, M. Amaro, P. Jurkiewicz, M. João Sarmento, R. Šachl, L. Cwiklik, M. Hof, *Chem. Rev.*, **118**, 11259 (2018).

[2] H. L. Scott, F. A. Heberle, J. Katsaras, F. N. Barrera, *Biophys. J.*, **116**, 15495 (2019).

[3] L. de Araújo Pimenta, E. L. Duarte, G. S. Vignoli Muniz, K. F. Mesquita Pasqualoto, M. R. de Mattos Fontes, M. T. Lamy, S. Coccuzzo Sampaio, *Sci. Rep.*, **11**, 23712 (2021).

Amyloid fibrils intrinsic fluorescence and its usage for label-free monitoring

M. Žganec[1,2], M. Škarabot[1] and E. Žerovnik[1]

[1]*Jožef Stefan Institute, Ljubljana, Slovenia*
[2]*Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Slovenia*

Amyloid fibrils share properties of nematic phases similar to liquid crystals[1,2]. Intrinsic birefringence of such regular arrays of amyloid fibrils can be applied in labelfree detection of amyloid deposits in biological samples or usage of such material in nanotechnology and biomedicine (as sensors or scaffolds)[3]. Another useful property for labelfree detection of amyloid fibrils and prefibrillar oligomers is the red shift of emission, the so called "the red edge excitation shift (REES)". This phenomenom is due to aromatic amino acid residues of proteins. However, the enhanced deep blue autofluorescence (dbAF) in amyloid fibrils and monomeric proteins unrelated to aromatic amino acids has also been observed[4,5]. Different proposals to explain this phenomena can be found, such as hydrogen bonding network in fibrils or enhanced carbonyl-based fluorescence present in any amino acid and other carbonyl-containing compounds[5]. Covalent oxidation of residues were proposed as the source of (dbAF).
We have shown, working with our amyloid forming protein (AFP) human stefin B, that fluorescence at 425 nm (upon excitation at 330 nm in steps +- 20 nm) arises from early stages of fibrillation (stage of protofibrils). Similar observation was made by some other AFPs and that could be an important detection method for initial states[6,7].

[1] Corrigan, A. M., Müller, C. & Krebs, M.R. The formation of nematic liquid crystal phases by hen lysozyme amyloid fibrils. *Journal of the American Chemical Society* **128**, 14740-14741 (2006).
[2] Bagnani, M., Azzari, P., Assenza, S. & Mezzenga, R. Six-fold director field configuration in amyloid nematic and cholesteric phases. *Scientific reports* **9**, 12654 (2019).
[3] Mankar, S., Anoop, A., Sen, S. & Maji, S.K. Nanomaterials: amyloids reflect their brighter side. *Nano Rev* **2**(2011).
[4] Chan, F.T.S., *et al.* Protein amyloids develop an intrinsic fluorescence signature during aggregation. *Analyst* **138**, 2156-2162 (2013).
[5] Niyangoda, C., Miti, T., Breydo, L., Uversky, V. & Muschol, M. Carbonyl-based blue autofluorescence of proteins and amino acids. *Plos One* **12**, e0176983-e0176983 (2017).
[6] Tikhonova, T. N., *et al.* Dissection of the deep-blue autofluorescence changes accompanying amyloid fibrillation. *Archives of Biochemistry and Biophysics* **651**, 13-20 (2018).
[7] Ziaunys, M., Sneideris, T. & Smirnovas, V. Exploring the potential of deep-blue autofluorescence for monitoring amyloid fibril formation and dissociation. *PeerJ* **7**, e7554 (2019).

Pressure response of polymer brushes

Matej Kanduč

Jožef Stefan Institute, Ljubljana

I will present our recent study on the conformational transition of nonionic brushes and semi-dilute solutions induced by hydrostatic pressure and temperature variations. Interestingly, the pressure-temperature phase diagram for the coil-to-globule transition of brushes, probed by neutron reflectometry, nearly coincides with that in semi-dilute solutions. We also show that the phase behavior can be understood and predicted with simple thermodynamic concepts employed so far for the denaturation of proteins. Full-atomistic molecular dynamics simulations provide molecular insight into pressure-responsive behavior. Combining all three approaches allows us to demonstrate that pressure-induced hydration of nonionic polymers at low pressure is universal as it is dictated by water and is polymer-independent. In contrast, the pressure-induced dehydration at high pressure is strongly polymer-specific.

Influence of BSA on formation of calcium phosphates and silver nanoparticles composites

S. Inkret[1], M. Ćurlin[2], and M. Dutour Sikirić[1]

[1]*Ruđer Bošković Institute, Zagreb, Croatia*
[2]*School of Medicine, Catholic University of Croatia, Zagreb, Croatia*

Due to their similarity with mineral component of bone tissue, calcium phosphates (CaP) attract special attention in the development of novel bone biomaterials.[1] As a way of improving their biological and mechanical properties, CaPs composites with nanomaterials and/or biomacromolecules are emerging.[2] As nanomaterials can have antimicrobial or magnetic properties, such composites can be considered truly multifunctional materials.[3] However, for such materials to be succesfully applied, the interactions between their components should be understood.

Motivated by this, in this study formation of CaPs in the presence of both nanomaterials, silver nanoparticles (AgNPs), and biologically active molecule, bovine serum albumin (BSA), was investigated. In the absence of both 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 BSA and/or differently coated AgNPs (citrate, polyvinyl pyrrolidone, and sodium bis(2-ethylhexyl) sulfosuccinate) inhibited ACP transformation, with BSA being dominant inhibitor. Powder X-ray Diffraction Patterns and Fourier Transform Infra Red Spectra confirmed that in the presence of AgNPs amount of OCP was decreased, while no BSA influence on composition of the formed precipitate was observed. TEM and SEM micrographs revealed influence of both additives on morphology of both ACP and crystalline precipitate.

Obtained results can contribute to development of low-temperature procedures for ternary composites synthesis.

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

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Using computational methods to investigate the impact of modified nucleotides on RNA structural dynamics

Valerio Piomponi, and Giovanni Bussi

Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

Post-transcriptional modifications of RNA are the object of growing interest in the community, since they have been shown to be functional in a variety of biological processes, and consist of biochemical alterations of nucleotides that can directly impact RNA structure and/or dynamics. The N6-methyladenosine (m6A) is the most prevalent chemical modification in messenger RNAs and has been observed both in coding and non-coding RNAs, showing the capability of regulating interaction of RNA with proteins. Although m6A is widely studied by the RNA community, the number of applications of molecular dynamics (MD) simulations to N6-methylated RNAs reported to date is still limited. MD is a powerful tool to access structural dynamics of RNA at the atomistic level, but the quality of the simulations strongly rely on the quality of the force fields parameters used. For these reasons, we made an effort to improve the quality of the m6A force field by fitting parameters to denaturation experiments performed on m6A-containing duplexes and NMR measurements that estimate the populations of m6A *syn/anti* isomers [1]. Our fitting strategy makes use of achemical free-energy calculations, which allows us to estimate the destabilization induced by the methylation on duplexes, by integrating along an alchemical path describing the transformation of a standard adenine into its N6-methylated version. In my talk, I will show the results of our fitting, illustrating how the refinement of 6 charges of the nucleobase plus the addition of a torsional potential allows us to significatively improve the agreement between computations and experiments. Furthermore, I will show how the alchemical computations methods we set up for m6A, plus the improved force field parameters that we derived, can be used in further studies to quantitatively estimate the impact of modifications on RNA structural dynamics and in binding affinities between RNA and proteins [2].

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Deciphering the interaction of genetic and epigenetic factors through enhanced polymer models

A. Chen Yi Zhang, A. Rosa, and G. Sanguinetti

Scuola Int. Superiore di Studi Avanzati (SISSA), Trieste, Italy

Cellular functions crucially depend on the precise regulation of gene expression. This process involves complex biochemical reactions taking place on the chromatin polymer in a tightly confined environment. Despite the availability of large data sets probing this process from multiple angles, we still lack a quantitative understanding of how biochemical processes, governed by genetic sequence affinity, interact with the 3D polymer nature of the chromatin. Here we propose a new statistical polymer model which naturally incorporates observational data about sequence-driven biochemical processes, such as the binding of transcription factor proteins, in a 3D model of chromatin structure. We introduce a new algorithm for approximate Bayesian inference and show on a case study that our model not only provides better predictions of chromatin state compared to purely data-driven approaches, but also gives a quantitative estimate of the relative importance of biochemical and polymer terms in determining chromatin state, leading to a significant revision of previous estimates. Furthermore, we show that, with no additional input from genome 3D structure data, our model can predict highly non-trivial geometrical structures in the folding of DNA within the nucleus. Our model demonstrates the importance of introducing physically realistic statistical models for predicting chromatin state from data and opens the way to a new class of approaches to interpreting epigenomic data.

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A QUBO model for melts of self-assembling ring polymers

F. Slongo[1], P. Hauke[2], P. Faccioli[3] and C. Micheletti[1]

[1]*SISSA, Trieste, Italy*
[2]*Physics Department of Trento University and INO-CNR BEC Center, Trento, Italy*
[3]*Physics Department of Trento University and INFN-TIFPA, Trento, Italy*

Sampling polymer melts remains a paradigmatically hard problem in computational physics, despite the various ingenious Monte Carlo and Molecular strategies that have been developed so far. As a matter of fact, achieving an efficient and unbiased sampling of densely packed polymers is hard even in the minimalistic case of crossable polymers on a lattice. Here we tackle the problem from a novel perspective, namely by using a quadratic unconstrained binary optimization (QUBO) model to generate systems of self-assembling ring polymers on arbitrary lattices (regular or not). The QUBO model naturally lends to imposing various physical constraints that would otherwise be difficult to handle with conventional MC and MD schemes. These constraints include fixing the packing density (lattice filling fraction), contact energy, and bending energy (curvature) of the system. This facile handling of multiple physical constraints enables the study of properties not addressed before, as we demonstrate by computing the overall entanglement properties of self-assembling rings. Finally, the model is amenable to being implemented on quantum machines that, in the case of D-Wave quantum annealers can speed up sampling by orders of magnitudes.

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Membrane curvature stress-mediated self-aggregation of proteins

(postponed till next workshop)

M. Kaltenegger[1], B. Grill[2], L. Percifull[3], D. Glück[1], H. Siboni[4], R. Ernst[3], G. Oberdorfer[2], G. Pabst[1]

[1]*Institute of Molecular Biosciences, University of Graz, Graz, Austria*
[2]*Institute of Biochemistry, Graz University of Technology, Graz, Austria*
[3]*Saarland University, Center of Molecualar Signalling, Homburg, Germany*
[4]*Institute of Chemistry, University of Graz, Graz, Austria*

Membrane embedded proteins play an important role in cell signaling- and transport mechanisms. The correct folding and therefore functionality of these complexes is in part ruled by the aggregation of the transmembrane domains in the lipid membrane.
In general, the physical effects promoting or inhibiting aggregation of peptides in cellular membranes can be subdivided into two properties of the lipid membrane: On one hand hydrophobic matching and on the other hand the internal membrane curvature stress. In both cases not only the lipid composition of the membrane, but also the protein's geometrical properties determine the overall interaction potential.
The focus of my project is directed to the influence of the elastic curvature stress of the lipid bilayer. The goal is to investigate and quantify the interdependencies of lipid environments and transmembrane peptide (TMP) shape. Therefore, we will measure single- molecule Förster resonance energy transfer (smFRET) on peptides of varying shapes in lipid vesicles of differing molecular composition. The smFRET experiments of the proteliposomes will be done via total internal reflection fluorescence microscopy, which requires tethering of the liposomes. The integrity of the immobilized liposomes is checked via atomic force microscopy. These measurements will also give insights into the bending rigidity of the lipid bilayer. [1]
Additionally, we will investigate the effects of the TM-peptides on the lipid bilayer structure, using small angle X-ray scattering.
The first TMP to investigate is of asymmetric shape, in particular it is composed of a valin- & an alanine- stretch. The helical structure was predicted by the group of Gustav Oberdorfer. We were able to successfully express and purify the peptide in an expression system developed in the laboratory of Robert Ernst using a maltose binding protein solubility tag. [2] After reconstitution into small unilamellar vesicles, we conducted first FRET- measurements. Subsequently we will proceed with single molecular experiments.

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Design rules for membrane-active antimicrobial lipidoids (AMLs) uncovered by high-throughput screening

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J. Jennings, D. Asceric, N. Malanovic, E. Semeraro, and G. Pabst

Institute of Molecular Biosciences, University of Graz, Graz, Austria

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 developement 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). Synthetic "lipidoids" containing multiple charged headgroups (≥ 2) and hydrophobic tails (≥ 4) resemble the structure of some bacterial lipids (e.g. lipid A and cardiolipin). Upon protonation, these multi-tail lipidoids can self-assemble into lamellar, bicontinuous cubic, and hexagonal liquid crystal phases.[3] To form non-lamellar phases, lipidoids adopt unusual disc-like conformations, in which the headgroup spans the hydrophilic domain and tails point in all directions. Screening 117 structurally-distinct lipidoids using minimum inhibitory concentration (MIC) assays against Gram negative and positive bacteria uncovers correlations between molecular structure and antimicrobial activity. Surprisingly, lipidoids that can adopt disc conformations in the active methylated state, and hence have a propensity for non-lamellar phases, are the compounds with highest antimicrobial activity. Furthermore, the molecules with the highest selectivity for bacteria over mammalian cells were predominantly comprised of headgroups from natural polyamines (spermine and spermidine); molecules with a range of functions in prokaryotic and eukaryotic cells. Thus, this study has revealed several design rules for synthetic membrane-targetting antimicrobial compounds.

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Asymmetric distribution of aminophospholipids stiffens fluid membranes

(postponed till next workshop)

M.P.K Frewein[1,2], P. Piller[1], E. F. Semeraro[1], O. Czakkel[2], Y. Gerelli[3], L. Porcar[2], and G. Pabst[1]

[1] Institute of Molecular Biosciences, University of Graz, 8010 Graz, Austria
[2] Institut Laue-Langevin, 38042 Grenoble, France
[3]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy

Cellular envelopes contain a large number of lipid species that distribute asymmetrically between the two leaflets of the bilayer. For example, mammalian plasma membranes are composed of an outer leaflet enriched in cholinephospholipids, while the majority of the aminophospholipids are confined to the inner leaflet. Membrane asymmetry can theoretically lead to distinct effects regarding its elastic behavior, either due to lipid specific properties (e.g. size, shape), or simply lipid over/under-crowding of a given leaflet. Corresponding experimental data are scarce. This prompted us to determine the structure and bending rigidity of minimal plasma membrane mimics composed of milk sphingomyelin (MSM), palmitoyl oleoyl phosphatidylcholine (POPC), palmitoyl oleoyl phosphatidylethanolamine (POPE), and palmitoyl oleoyl phosphatidylserine (POPS) using small-angle neutron/X-ray scattering and neutron spin echo spectroscopy. Most strikingly, we observed an anomalous stiffening of freely-floating fluid vesicles (size ~ 100 nm) in case of inner leaflets composed of POPE/POPS mixtures and outer leaflets enriched in POPC. That is their bending rigidities did not only exceed those of their scrambled (symmetric) analogs, but also those of symmetric vesicles mimicking either their inner and outer leaflets. Addition MSM in the outer leaflet partially alleviated this effect, possibly due to hydrocarbon chain interdigitation. To reconcile our findings, we speculate that lipid shape and charge asymmetry may increase the weight of short-wavelength undulatory modes in membranes by inducing a coupling at distances smaller than the membrane thickness.

Allosteric modulation of integral protein activity by differential stress in asymmetric membranes

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P. Piller[1], E.F. Semeraro[1], G.N. Rechberger[2], S. Keller[1], and G. Pabst[1]

[1]Biophysics, Institute of Molecular Bioscience (IMB), NAWI Graz, University of Graz, Graz, Austria
[2]Biochemistry, Institute of Molecular Bioscience (IMB), NAWI Graz, University of Graz, Graz, Austria

We reasoned that membrane-protein activity can mechanically couple to membrane asymmetry, a hallmark of all cellular envelopes. In particular, we hypothesized that outer membrane phospholipase A (OmpLA) is highly susceptible to the lateral pressure differences that build up between such asymmetric membrane leaflets. Reconstituting OmpLA into synthetic, chemically well-defined phospholipid bilayers exhibiting different lateral pressure profiles, we indeed found a significant decrease in the enzyme's hydrolysis rate with increasing membrane asymmetry. No such effects were observed in symmetric mixtures of the same lipids, thus confirming a dominant role of membrane asymmetry rather than hydrophobic mismatch. Developing a simple allosteric model within the lateral pressure framework, we found a potential inhibition of the enzyme due the differential stress in asymmetric bilayers, in good overall agreement with our experimental data.

Lactoferricins impair the cytosolic membrane of *Escherichia coli* within a few seconds and accumulate inside the cell

(postponed till next workshop)

E. F. Semeraro[1,2], L. Marx[1,2], J. Mandl[1,2], I. Letofsky-Papst[3,4], C. Mayrhofer[4], M. P. K. Frewein[1,2,5], H. L. Scott[6,7], S. Prévost[5], H. Bergler[1,2], K. Lohner[1,2], G. Pabst[1,2]

[1]*University of Graz, Institute of Molecular Biosciences, NAWI Graz, Graz, Austria*
[2]*Field of Excellence BioHealth, University of Graz, Graz, Austria*
[3]*Institute of Electron Microscopy and Nanoanalysis, TU Graz, Graz, Austria*
[4]*Center for Electron Microscopy, Graz, Austria*
[5]*Institut Laue-Langevin, Grenoble, France*
[6]*Center of Enviromental Biotechnology, University of Tennesse, TN, Knoxville, USA*
[7]*Shull Wollan Center, Oak Ridge National Laboratory, TN, Oak Ridge, USA*

Novel antibiotics based on membrane active antimicrobial peptides (AMPs) are promising candidates for defending the spread of diseases caused by multi-resistant pathogenic bacteria. Notwithstanding the number of works that explore the relationship between AMP activity and membrane architecture, the full mechanism that leads to cell death is currently not clear. This prompted us to investigate the mode-of-action of lactoferricin derivatives on both live *E. coli* and biological lipid-membrane mimics.
In particular, we explored AMP partitioning in bacteria and lipid-only vesicles revisiting susceptibility and leakage assays, respectively, and zeta-potential and Trp-fluorescence spectroscopy [1,2]. The structural rearrangement in vesicles and bacteria upon mixing with AMPs was probed by transmission electron microscopy and small-angle neutron and X-ray scattering [3]. The latter permitted to access the kinetics in live cells with an unprecedented time (milliseconds) and length (nanometre to sub-micrometre) scales.
To name but a few, results suggest that these AMPs quickly partition into the lipid membranes, simultaneously translocating into the intracellular space (~3 s) and causing weak leakage. Strikingly, membrane remodeling in both membrane mimics and live cells is significant but only incidental to bacterial killing.

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