11th Christmas Biophysics Workshop (XBW2016)

Dec 12/13 2016
St. Nikolai im Sausal, Austria

Book of Abstracts

Organizers:

- Barbara Eicher
- Lisa Marx
- Michal Belička
- Georg Pabst (Chair)

Workshop Homepage: http://xmas-biophysics-workshop2016.uni-graz.at/en/

Program

Monday, December 12th

09:50-10:00 **Opening word**

Section I: Membranes

Chairman: Georg Pabst

10:00-10:20	*Gerhard Kahl*	**Spontaneous Assembly of a Hybrid Crystal-Liquid Phase in a System of Inverse Patchy Colloids**
10:20-10:40	*Lukas Schrangl*	**T-Cell Antigen Recognition on Lipid Bilayers**
10:40-11:00	*Susan Šegota*	**The Microcalorimetry and Force Spectroscopy Studies of Myricetin and Myricitrin Interactions with Model and Neuronal Cell Membranes**
11:00-11:20	*Barbara Eicher*	**Transbilayer Coupling Mechanism of Asymmetric Lipid Bilayers**
11:20-11:40	*Michal Belička*	**Fine Structure of Coexisting Microscopic and Nanoscopic Lipid Domains**
11:40-12:00	*Primož Ziherl*	**Role of Inverse-Cone-Shape Lipids in Temperature-Controlled Self-Reproduction of Binary Vesicles**

12:00-14:00 **Lunch**

Section II: Biopolymers

Chairman: Rudi Podgornik

14:00-14:20	*Angelo Rosa*	**Chromosome Organization and the Physics of Crumpled Polymers**
14:20-14:40	*Lucia Coronel*	**Knotting in Semiflexible Ring Polymers**
14:40-15:00	*Antonio Suma*	**Pore Translocation of DNA Chains With Physical Knots**
15:00-15:20	*Mattia Marenda*	**Sorting Ring Polymers by Knot Type with Modulated Nanochannels**
15:20-15:40	*Anže Lošdorfer Božič*	**Context Folding of Mammalian Mitochondrial tRNA Sequences**

15:40-16:20 **Coffee break**

Section III: Proteins

Chairman: Antonio Šiber

16:20-16:40	*Gerhard J. Schütz*	**Direct PIP_2 Binding Mediates Stable Oligomer-Formation of the Serotonin Transporter**
16:40-17:00	*Eva Žerovnik*	**Co-Chaperoning by Amyloid-Forming Proteins**
17:00-17:20	*Nataša Adžić*	**The Influence of Multivalent Ions on Protein-Protein Interaction**

17:20-17:40 *Viktoria Motsch* **DNA Origami Platform for Protein Interaction Analysis**

18:00-19:30 **Dinner**
20:00- **Social event: Wine tasting**

Tuesday, December 13th

07:30-09:20 **Breakfast**

Section IV: Cells

Chairman: Tomislav Vuletič

09:20-09:40 *Saša Svetina* **A Model for the Effect of Actin Filament Cross-Linking Proteins on the Elastic Behavior of Actin Comet Tail**
09:40-10:00 *Tjaša Švelc Kebe* **Model of the Abrupted Skeleton at Large Deformations of the Red Blood Cell Aspirated Into Micropipette**
10:00-10:20 *Biljana Stojković* **Using Optical Tweezers for Measureíng Visco-Elastic Properties of Biological Fluids and Cells**

10:20-11:00 **Coffee break**

Section V: Cells and New Techniques

Chairman: Primož Ziherl

11:00-11:20 *Maja Novak* **The Shape of K-Fibers reveals the existence of Torques at the Spindle Poles**
11:20-11:40 *Janez Rozman* **Mechanical Model of Epithelial Sheet Growth During Fruit Fly Egg Chamber Development**
11:40-12:00 *Mihovil Jurdana* **NanoporeArray: Ion-Beam Patterned Nanopore Arrays in Polymer Supported 2D Materials**

12:00-12:10 **Closing word**

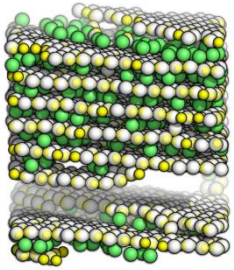
12:10-14:00 **Lunch**

14:00- **Departure**

SPONTANEOUS ASSEMBLY OF A HYBRID CRYSTAL-LIQUID PHASE IN A SYSTEM OF INVERSE PATCHY COLLOIDS

Gerhard Kahl[1], Silvano Ferrari[1], Emanuela Bianchi[1]
[1] *Institut für Theoretische Physik, TU Wien, Austria.*

Materials with well-defined architectures are heavily sought after in view of their diverse technological applications. Among the desired target architectures, lamellar phases stand out for their exceptional mechanical and optical features. Here we show that charged colloids, decorated on their poles by two oppositely charged regions possess the unusual ability to spontaneously assemble in different morphologies of (semi-)ordered, layered particle arrangements which maintain their structural stability over a surprisingly large temperature range. This remarkable capacity is related to a characteristic bonding mechanism: stable *intra-layer* bonds guarantee the formation of planar aggregates, while strong *inter-layer* bonds favor the stacking of the emerging planar assemblies. These two types of bonds together are responsible for the self-healing processes occurring during the spontaneous assembly. The resulting phases are characterized by parallel, densely packed, particle layers connected by a relatively small number of intra-layer particles. We investigate the properties of the (semi-)ordered phases in terms of static and dynamic correlation functions, focusing in particular on a novel hybrid crystal-liquid phase that prevails at intermediate temperatures where the inter-layer particles form a mobile, fluid phase.



Simulation snapshot of a hybrid crystal-liquid particle configuration [1].

[1] S. Ferrari, E. Bianchi, and G. Kahl, *Nanoscale* (in press).

T CELL ANTIGEN RECOGNITION ON LIPID BILAYERS

Lukas Schrangl[1], Gerhard J. Schütz[1]
[1]*Institute of Applied Physics, TU Wien, Wiedner Haupstraße 8-10, 1040 Vienna, Austria.*

The interaction between T cells and antigen presenting cells (APCs) is fundamental to the adaptive immune system. T cells screen the surfaces of APCs for pathogenic peptides in order to trigger an immunological response if necessary. The pivotal interaction in this process takes place between the T cell receptor (TCR) and peptide-presenting MHC (pMHC) complexes. It enables the T cell to detect even very subtle differences in the antigenic peptides while at the same time only very low densities of agonistic pMHCs are required to cause T cell signaling. One way to investigate the precise mechanisms behind this exceptional sensitivity and specificity is to substitute the APC with a supported lipid bilayer (SLB) containing fluorescently labeled proteins of interest and to image the interface between T cells and the SLB utilizing TIRF microscopy.

With this system, using well-chosen lipids and proteins, we explore a range of aspects of the T cell—APC interaction, such as interaction times between the TCR and pMHC, immobilization of proteins, and mechanical forces.

THE MICROCALORIMETRY AND FORCE SPECTROSCOPY STUDIES OF MYRICETIN AND MYRICITRIN INTERACTIONS WITH MODEL AND NEURONAL CELL MEMBRANES

S. Šegota[1], I. Crnolatac[2], V. Čadež[1], M. Jazvinšćak Jembrek[3], Maja Dutour Sikirić[1], and Darija Domazet Jurašin[1]

[1] *Division of Physical Chemistry Ruđer Bošković Institute, Zagreb, Croatia.*

[2] *Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia.*

[3] *Division of Molecular Medicine, Ruđer Bošković Institute, Zagreb, Croatia.*

Flavonoids, polyphenolic biomolecules with antioxidative activity, have recently emerged as potential novel therapeutics for neurodegenerative diseases. In order to gain more insight in the mechanisms of their protective activity, interactions of model flavonoids, myricetin [1] and myricitrin [2], with model and neuronal cell membrane have been investigated by combination of microcalorimetry and force spectroscopy before and after membranes exposure to the oxidative stress.

Model membranes were made of a lipid mixture of unsaturated phosphatidylcholine, sphinogomyelin and cholesterol (PC/SM/Chol), while P19 neurons were chosen to work with in order to test their ability to inhibit lipid peroxidation in model membranes and neurons *in vitro*.

By combining AFM imaging data, force spectroscopy (FS), and microcalorimetry, local altered nanomechanical properties and structural reorganization of membrane as well as the changes within the cytoplasm, cell nucleus, and particularly cytoskeleton components induced by peroxidation were recorded.

Obtained results pointed that the time needed to complete the oxidative reaction might be considered as the measure of flavonoid protective power. The nanomechanics (elasticity), surface topography (roughness) of model lipid membranes and P19 neurons as well as the thermodynamic and kinetic data that result from the oxidative damage are quantified for the first time. This highlights the potential of combining AFM, FS and microcalorimetry in elucidating the mechanism of such a complex interactions.

[1] Choi Seon-Min *et al.*, *Chonnam. Med. J.* **50** 45 (2014)

[2] J. G. Cho *et al.*, *J. Agric. Food. Chem.* **61** 10354 (2013)

TRANSBILAYER COUPLING MECHANISM OF ASYMMETRIC LIPID BILAYERS

B. Eicher[1,2], D. Marquardt[1,2], F. Heberle[3], J. Katsaras[3] and G. Pabst[1,2]
[1] *Institute of Molecular Biosciences, Biophysics Division, University of Graz, Austria.*
[2] *BioTechMed-Graz, Graz, Austria.*
[3] *Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA.*

Mammalian plasma membranes display an asymmetric lipid distribution along the bilayer. Membrane asymmetry is thought to affect also structural properties like bilayer thickness and thickness of the single leaflets. Of recent, we developed protocols allowing the preparation and characterization of stress-free, free-floating asymmetric vesicles (aLUVs) via a cyclodextrin mediated exchange [1]. Quantification of bilayer composition and the degree of asymmetry enables the determination of transverse structural parameters such as area per lipid and the thickness of the single leaflets. Structural parameters were determined via small- and wide-angle x-ray scattering (SWAXS) and small-angle neutron scattering (SANS). First results have shown that there is no hydrocarbon chain correlation in the wide angle regime of DPPC/POPC aLUVs, which is consistent with an earlier observed partial fluidization of gel DPPC through coupling to a fluid POPC leaflet [1]. In contrast, POPE/POPC aLUVs revealed a WAXS-peak typical of hydrocarbons packed in a 2D hexagonal lattice. Due to the coupling with POPC the area per lipid increased slightly, however, the underlying energetic contributions are insufficient for breaking up the hydrocarbon bond-stabilized PEs.

This work is supported by the Austrian Science Fund FWF, Project No.P27083-B20 (to G.P.).

[1] Heberle, F. A., Marquardt, D., Doktorova, M., Geier, B., Standaert, R. F., Heftberger, P., Kollmitzer, B., Nickels, J. D., Dick, R. A., Feigenson, G. W. *et al.*, *Langmuir*, **32** 5195 (2016).

FINE STRUCTURE OF COEXISTING MICROSCOPIC AND NANOSCOPIC LIPID DOMAINS

Michal Belička[1,2], Anna Weitzer[1,2], Georg Pabst[1,2]
[1] *Insitute of Molecular Biosciences, University of Graz, Graz, Austria.*
[2] *BioTechMed-Graz, Graz, Austria.*

Coexisting liquid-disordered (Ld) and liquid-ordered (Lo) domains in fully hydrated multilamellar lipid vesicles (MLVs) were investigated using small-angle X-ray scattering (SAXS). In particular, we focused on DOPC/DSPC/Cholesterol (0.46 : 0.3 : 0.24), exhibiting micron-sized lipid domains and POPC/DSPC/Cholesterol (0.39 : 0.39 : 0.22), containing nanoscopic domains. Large domains exhibited long-range out-of-plane positional correlations of like domains, consistent with previous reports. In contrast, such correlations were absent in nanoscopic domains. Advancing a global analysis of the *in situ* data allowed us to gain deep insight into structural and elastic properties of the coexisting domains, including the partitioning of cholesterol in each domain. In agreement with a previous report we found that the thickness mismatch between ordered and disordered domains decreased for nanoscopic domains. At the same time we found the lipid packing mismatch to be decreased for nano-domains, mainly by liquid-disordered domains becoming more densely packed when decreasing their size.

ROLE OF INVERSE-CONE-SHAPE LIPIDS IN TEMPERATURE-CONTROLLED SELF-REPRODUCTION OF BINARY VESICLES

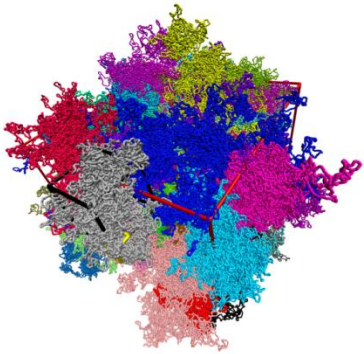
T. Jimbo[1], Y. Sakuma[1], N. Urakami[2], P. Ziherl[3], and M. Imai[1]
[1] *Tohoku University, Sendai, Japan.*
[2] *Yamaguchi University, Yamaguchi, Japan.*
[3] *Jožef Stefan Institute and University of Ljubljana, Ljubljana, Slovenia.*

We investigate a temperature-driven recursive division of binary DLPE-DPPC giant unilamellar vesicles (GUVs). During the heating step of the heating-cooling cycle, the spherical mother vesicle deforms to a budded limiting shape using up the excess area produced by the chain melting of the lipids and then splits off into two daughter vesicles. Upon cooling, the daughter vesicle opens a pore and recovers the spherical shape of the mother vesicle. During each cycle, vesicle deformation is monitored by a fast confocal microscope and the images are analyzed to obtain the time evolution of reduced volume and reduced monolayer area difference as the key geometric parameters that quantify vesicle shape. By interpreting the deformation pathway using the area-difference elasticity theory, we conclude that vesicle division relies on (1) a tiny asymmetric distribution of DLPE within the bilayer, which controls the observed deformation from the sphere to the budded shape; and (2) redistribution of DLPE during the deformation-division stage, which ensures that the process is recursive. The spontaneous coupling between membrane curvature and PE lipid distribution is responsible for the observed recursive division of GUVs. These results shed light on the mechanisms of vesicle self-reproduction.

CHROMOSOME ORGANIZATION AND THE PHYSICS OF CRUMPLED POLYMERS

Angelo Rosa[1], Ralf Everaers[2], Ana-Maria Florescu[1]
[1]*SISSA - Scuola Internazionale Superiore di Studi Avanzati, Via Bonomea 265, 34136 Trieste, Italy.*
[2]*Univ Lyon, ENS de Lyon, Univ Claude Bernard Lyon 1, CNRS, Laboratoire de Physique and Centre Blaise Pascal, F-69342 Lyon, France.*

The link between chromosome organization (in space and time) and such major events like gene expression and regulation constitutes the object of an intense experimental effort. Yet, our comprehension of this link is still largely incomplete. Recently, physically-based polymer models for chromosomes supplemented by massive computer simulations have shown to provide a remarkable accurate description of chromosome behavior under various conditions and over wide ranges of length- and time-scales.



In this talk, I will discuss the 'special' analogy between chromosome conformations and the Physics of crumpled polymers in entangled solutions. In particular, I will show how this analogy is at the basis of a quantitative explanation for the experimentally observed generic behavior of chromosomes in cell nuclei during interphase, and I will describe a fast computational scheme for building model conformations of large chromosomes with different degrees of resolution.

KNOTTING IN SEMIFLEXIBLE RING POLYMERS

L. Coronel[1], C. Micheletti[1], E. Orlandini[2]
[1]*SISSA, International School of Advanced Studies, via Bonomea 265, I-34136 Trieste, Italy.*
[2]*IDipartimento di Fisica and Sezione INFN, Universita' di Padova, I-35131 Padova, Italy.*

The ubiquity of knots in biopolymers and their implications for molecular processes have been actively studied in recent years. One key aspect, that has remained largely unexplored is the interplay between chain stiffness and knotting probability. We studied this interplay using a Monte Carlo sampling strategy for self-avoiding rings of beads with various bending rigidities. The results suggest that a weak bending rigidity is a promoter, and not a suppressor, of knotting. Next, we investigate how stiffness affects the metric properties of the rings, and thus provide a connection and an explanation for the observed knotting behaviour.

PORE TRANSLOCATION OF DNA CHAINS WITH PHYSICAL KNOTS

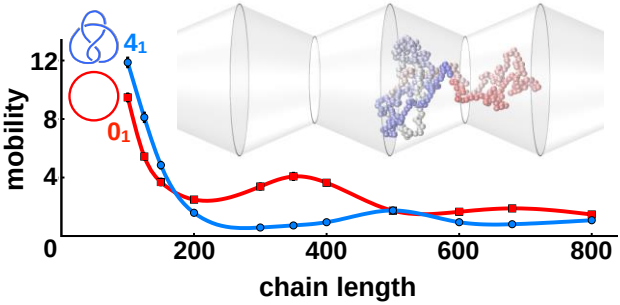
A. Suma[1], A. Rosa[1], C. Micheletti[1]
[1]*SISSA, International School for Advanced Studies, via Bonomea 265, I-34136 Trieste, Italy.*

Long biopolymers have a large probability of being knotted. These form of self-entanglement can arise spontaneously both in the context of nucleic acids and proteins. Here we focus on the impact of such knots during the translocation through narrow pores, as they can affect both biological processes and experimental setups in general. With the aid of coarse-grained molecular dynamic simulations, we study systematically the pore translocation of flexible chains tied in different knot types, including composite ones. We find a rich phenomenology as a function of knot complexity and driving force and show that it can be rationalized within a simple theoretical framework, which can also be used as a guide for experimental designs. We will also report on preliminary results for more detailed models of systems that have been recently characterized experimentally.

SORTING RING POLYMERS BY KNOT TYPE WITH MODULATED NANOCHANNELS

M. Marenda[1], E. Orlandini[2] and C. Micheletti[1]
[1]*SISSA, International School for Advanced Studies, via Bonomea 265, I-34136 Trieste.*
[2]*Dipartimento di Fisica e Astronomia "Galileo Galilei", sezione CNISM, Universit\`a degli Studi di Padova, via Marzolo 8, I-35131 Padova, Italy.*

In this theoretical study we discuss a novel method for sorting ring polymers according to their topological, knotted state. The proposed approach harnesses the rich dynamical behaviour of polymers confined inside spatially-modulated nanochannels. The longitudinal mobility of the rings is shown to have two key properties that are ideally suited for knot sorting. First, at fixed topology, the mobility has an intriguing oscillatory dependence on chain length. Second, the mobility ranking of different knot types is inverted upon increasing the chain length. We show that this complex interplay of channel geometry, chain length and topology can be rationalised within a simple theoretical framework based on Fick-Jacobs's diffusive theory. The results and the interpretative scheme ought to be useful for designing microfluidic devices with optimal topological sorting capabilities.



CONTEXT FOLDING OF MAMMALIAN MITOCHONDRIAL TRNA SEQUENCES

A. Lošdorfer Božič[1] and L. Tubiana[2]
[1] *Department of Theoretical Physics, Jožef Stefan Institute, Ljubljana, Slovenia*.
[2] *Computational Physics, Faculty of Physics, University of Vienna, Vienna, Austria*.

In the usual context of RNA folding, RNA sequences assume a secondary structure through the pairing of their own bases – the RNA sequence therefore determines its fold. However, in certain cases this assumption can prove to be over-simplistic. Mitochondrial tRNAs provide a good example, since, even though they take on a well-defined structure individually, they are originally part of a much longer polycistronic transcript stemming from the mitochondrial genome. Consequently, it is highly possible that before cleavage, the sequence of the mithocondrial tRNAs can be paired with the surrounding (context) sequence, thus changing their folding. As the structure of the tRNAs is thought to play a significant role in the cleavage of the polycistronic transcript (the "tRNA punctuation model"), it is important to determine the influence of the context sequence on their structure. We therefore investigate computationally the secondary structure folding of mammalian mitochondrial tRNAs in the presence of increasingly larger context sequences. By comparing the folded tRNAs to their predicted native structures, we show how the inclusion of context sequences can disrupt the native fold. We also point out some conserved elements of certain tRNAs that remain correctly folded even when the context sequence spans several hundred nucleotides.

DIRECT PIP_2 BINDING MEDIATES STABLE OLIGOMER-FORMATION OF THE SEROTONIN TRANSPORTER

Andreas Anderluh[1], Tina Hofmaier[2], Enrico Klotzsch[3], Oliver Kudlacek[2], Thomas Stockner[2], Harald H. Sitte[2], Gerhard J. Schütz[1]

[1] Institute of Applied Physics, TU Wien, Wiedner Hauptstrasse 8-10, 1040 Vienna, Austria.
[2] Center for Physiology and Pharmacology, Institute of Pharmacology, Medical University Vienna, Währingerstrasse 13a, A-1090 Vienna, Austria.
[3] EMBL Australia Node in Single Molecule Science, School of Medical Sciences, ARC Centre of Excellence in Advanced Molecular Imaging, University of New South Wales, Sydney, NSW, Australia.

The human serotonin transporter (hSERT) mediates uptake of serotonin from the synaptic cleft and thereby terminates serotonergic signaling. We have previously found by single-molecule microscopy that SERT forms stable higher-order oligomers of differing stoichiometry at the plasma membrane of living cells [1]. Here, we unraveled an important mechanism in the oligomerization process. Protomer assembly to SERT oligomers is equilibrated at the endoplasmic reticulum (ER) membrane, which is reflected by rapid exchange of subunits between different oligomers, and by a concentration dependence of the degree of oligomerization. After trafficking to the plasma membrane, however, the SERT stoichiometry is fixed. Stabilization of the oligomeric SERT complexes is mediated by the direct binding to phosphoinositide phosphatidylinositol-4,5-biphosphate (PIP2). The observed spatial decoupling of oligomer formation from the site of oligomer operation provides cells with the ability to define protein quaternary structures independent of protein density at the cell surface.

[1] Anderluh, A. *et al.*, Single molecule analysis reveals coexistence of stable serotonin transporter monomers and oligomers in the live cell plasma membrane. *J. Biol. Chem.* **289**, 4387 (2014).

CO-CHAPERONING BY AMYLOID-FORMING PROTEINS

E. Žerovnik[1,2,3]

[1] *Department of Biochemistry and Molecular and Structural Biology, Jožef Stefan Institute and*
[2] *Jožef Stefan International Postgraduate School, both, Jamova 39, 1000 Ljubljana, Slovenia*
[3] *CipKeBip - Center of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Jamova 39, 1000 Ljubljana, Slovenia*

The correlation between Alzheimer´s disease (AD) and the occurrence of extracellular amyloid plaques remains controversial. Aβ pre-fibrillar oligomers that precede the formation of proto-fibrils and amyloid fibrils have been shown to be the most toxic[1]. However, chaperones a-like Aβ-crystallin[2], clusterinBα and heat shock protein (Hsp)B8, which all bind Aβ peptide and influence its aggregation are often part of the extracellular Aβ plaques. Not only the specialized chaperone proteins inhibit amyloid fibril formation, other such proteins, termed "amateur chaperones"[3] also bind amyloid forming peptides and thus prevent their aggregation, among them are cystatins[4-5].

[1] Cleary, J.P., *et al.*, Natural oligomers of the amyloid-protein specifically disrupt cognitive function. *Nature Neuroscience* **8**, 79 (2005).
[2] Shammas, S.L., *et al.*, Binding of the Molecular Chaperone alpha B-Crystallin to A beta Amyloid Fibrils Inhibits Fibril Elongation. *Biophys. J.* **101**, 1681 (2011).
[3] Wilhelmus, M.M., de Waal, R.M. & Verbeek, M.M. Heat shock proteins and amateur chaperones in amyloid-Beta accumulation and clearance in Alzheimer's disease. *Mol. Neurobiol.* **35**, 203 (2007).
[4] Sastre, M., *et al.*, Binding of cystatin C to Alzheimer's amyloid beta inhibits in vitro amyloid fibril formation. *Neurobiol. Aging* **25**, 1033 (2004).
[5] Skerget, K., *et al.*, Interaction between oligomers of stefin B and amyloid-beta in vitro and in cells. *J. Biol. Chem.* **285**, 3201 (2010).

THE INFLUENCE OF MULTIVALENT IONS ON PROTEIN-PROTEIN INTERACTION

N. Adžić[1], R. Podgornik[1,2]
[1] *Jožef Stefan Institute, Ljubljana, Slovenia.*
[2] *Faculty of Mathematics and Physics, University of Ljubljana, Slovenia.*

One of the biggest discoveries in the colloidal science is certainly the exotic phenomenon of like-charge attraction arising between same-charged macromolecules in a multivalent ion solution. Although it was firstly observed in simulation back in 1984 and soon after confirmed in experiments, the proper theoretic explanation came two decades later, when the so-called field-theoretic approach to Coulomb fluids was developed. Its advantage is in providing a consistent description of weakly charged systems, as well as the systems where the strong ion correlations dominate. Nevertheless, it is restricted to macromolecules bearing fixed surface charge and fails to describe systems dependent on acid/base equilibrium, such as proteins. In those systems, where the charge of macromolecule is regulated by the changes in local solution conditions, another exotic phenomenon appears i.e. the attraction at isoelectric point, known as the Kirkwood-Shumaker interaction (KS). KS interaction obeys different scaling laws than van der Waals interaction, ubiquitous between electroneutral bodies. Here we present a theory that brings these two phenomena together. Namely, we studied a model system with dissociable sites uniformly distributed across two plane-parallel surfaces in a mixture of monovalent and polyvalent salt. We generalized the existing field-theoretic approach to properly include charge regulation phenomenon in its formalism and derived the general form of KS interaction, valid also in the regime where the original result fails.

DNA ORIGAMI PLATFORM FOR PROTEIN INTERACTION ANALYSIS

V. Motsch[1], G. J. Schütz[1] and E. Sevcsik[1]
[1] *Institute of Applied Physics, TU Wien, Wiedner Haupstr. 8-10, 1040 Vienna, Austria*

The nanoscale spatial distribution of membrane-bound ligands is thought to play an important role in membrane receptor-mediated signaling. In particular, the formation of protein complexes in the immunological synapse, in the contact area of a T cell and an antigen-presenting cell (APC), plays a key role in the initiation of the immune response. Despite extensive studies, the quantitative molecular details of complex formation, as well as the kinetics are still poorly understood.

Here, we use DNA origami nanostructures of 50 nm x 50 nm x 2 nm featuring up to 44 engineered capture sites for a target protein at the top side at well-definded positions. The number of capture sites, their functionalization, their orientation and their distance can be adjusted at will. At the bottom side (opposite of the protein capture sites), the employed DNA rectangles can be functionalized with cholesterol moieties which serve as membrane anchors. The DNA constructs adhere to a lipid bilayer mediated by the cholesterol anchors and serve as mobile platforms for protein recruitment.

Here, DNA origami are decorated with recombinant and site-specifically labeled T cell receptor (TCR)β-reactive single chain antibody fragment (scFV) and embedded on planar glass-supported lipid bilayers, which harbors costimulatory molecules and adhesion proteins. This system is used to closely imitate the APC, allowing to re-organize the TCR in living T cells according to the pattern given by the scFv template on the origami. We use this system to address and answer central questions in T cell immunology: the role of TCR clustering in T cell activation, the unknown composition of protein complexes in the T cell plasma membrane as well as the mechanisms of their cohesion.

A MODEL FOR THE EFFECT OF ACTIN FILAMENT CROSS-LINKING PROTEINS ON THE ELASTIC BEHAVIOR OF ACTIN COMET TAIL

S. Svetina[1, 2]
[1] *Institute of Biophysics, Faculty of Medicine, University of Ljubljana and*
[2] *Jožef Stefan Institute, Ljubljana, Slovenia*

Actin comet tails are branched networks of actin filaments that grow on surfaces containing an activator of Arp2/3 complex, with the latter needed to initiate the polymerization of actin monomers. They cause the propulsion of objects such as bacterial pathogen *Listeria monocytogenes*, endosomes, and also correspondingly functionalized plastic beads. The minimal molecular composition of the surrounding medium needed for the propulsion to occur has been identified. It is also known that the velocity of thus propelled objects depends on physical characteristics of the system, notably on the elastic modulus of the tail and its dependence on the concentration of actin filaments cross-linking proteins. Here we shall shortly reveal some basic structural features of the actin comet tail. A simple model of its elastic properties will then be introduced based on the assumption that the amount of bound cross-linking molecules depends on actin filament density. The predicted dependence of the tail´s elastic modulus on the concentration of cross-linkers will be shown to be in accord with the observation of its enhancement induced by fascin. The obtained results will be used to comment on the force-velocity relationship in the actin-based mechanism for cell motility.

MODEL OF THE RUPTURED SKELETON AT LARGE DEFORMATIONS OF THE RED BLOOD CELL ASPIRATED INTO MICROPIPETTE

Tjaša Švelc Kebe[1], Saša Svetina[2,3]

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

Mechanical response to the deformation of the red blood cell (RBC) is governed by its membrane which is a composite of an incompressible two-dimensional liquid – lipid bilayer, and of an underlaid two-dimensional elastic network – membrane skeleton. At the mechanical stress the deformed shape of the RBC is defined by its bilayer whereas the skeleton responds by being redistributed in the plane of the membrane. For a given deformation of the bilayer, the lateral redistribution of the skeleton and its minimal energy can be obtained with a model of the membrane skeleton which is based on the elastic properties of its main component – spectrin, where spectrin mesh is considered as a triangulated network of simple elastic bonds with Hooks potential. With the help of this model small deformations of the RBC with micropipette are well described [1]. In our recent studies we are investigating larger micropipette deformations of the RBC. Here we shall report the results on how membrane skeleton redistributes when it is under such conditions ruptured.

[1] Svetina S., Kokot G., Švelc Kebe T., Žekš B., Waugh R.E. A novel strain energy relationship for red blood cell membrane skeleton based on spectrin stiffness and its application to micropipette deformation. *Biomech. Model. Mechanobiol.* **15**, 745 (2016).

USING OPTICAL TWEEZERS FOR MEASURING VISCO-ELASTIC PROPERTIES OF BIOLOGICAL FLUIDS AND CELLS

B. Stojković[1], S. Sretenovic[2], Birgit Teuble[4], I. Dogsa[2], Š. Zemljic-Jokhadar[1], I. Poberaj[3], D. Stopar[2], Eva Robbelg[4] Jure Derganc[1], Mirjana Liovic[5]
[1] *Medical Faculty, Institute of Biophysics, University of Ljubljana, Ljubljana, Slovenia, biljana.stojkovic@mf.uni-lj.si*
[2] *Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia, david.stopar@bf.uni-lj.si*
[3] *Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Slovenia, igor.poberaj@fmf.uni-lj.si*
[4] *University of Graz, Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, 8010 Graz, Austria, eva.roblegg@uni-graz.at*
[5] *Medical Faculty, Institute of Biochemistry, University of Ljubljana, Ljubljana, Slovenia, mirjana.liovic@mf.uni-lj.si*

We described accurate microrheological measurements of various homogeneous and inhomogeneous biological samples, and showed that optical tweezers are powerful tool to study viscoelastic properties of those systems. First, we analyzed mixtures of DNA and levan (a non-ionic, homogeneous, very soluble fructan polymer), which are important components of extracellular matrix of *Bacillus subtilis* biofilms. We addressed phase separation and formation of levan aggregates at critical DNA concentration. With optical tweezers technique, we performed microrheological measurements and obtained rheological parameters both within and outside the levan aggregates. Microrheology measurements give comparable results to the bulk rheology measurements in homogenous samples, but results are significantly different in heterogeneous mixtures. The results obtained highlight the importance of microrheology in the study of microbial biofilms and importance of DNA in the formation of biofilms. [1] The same type of experiments were used to obtain viscosity and elastic modulus of human whole saliva, which revealed novel information on saliva structure and biological role of this fluid in oral cavity. Finally, we also used optical tweezers to measure visco-elastic properties of living cells. In our experiments we analysed keratinocytes, i.e. cells from human skin, and showed qualitative differences between normal and mutant cells, related to a certain type of genetic skin disease.

[1] Stojković B., Sretenovic S., Dogsa I., Poberaj, I., Stopar, D. Viscoelastic Properties of Levan-DNA Mixtures Important in Microbial Biofilm Formation as Determined by Micro-and Macrorheology. *Biophys. J.* **108**, 758 (2015).

THE SHAPE OF K-FIBERS REVEALS THE EXISTENCE OF TORQUES AT THE SPINDLE POLES

Maja Novak[1,2], Bruno Polak[2], Zvonimir Boban[1], Iva M. Tolić[2], Nenad Pavin[1]
[1] *Faculty of Science, University of Zagreb, Zagreb, Croatia*
[2] *Ruđer Bošković Institute, Zagreb, Croatia*

During cell division, the mitotic spindle made of microtubules drives segregation of the genetic material into two nascent cells. Bundles of microtubules known as k-fibers pull on kinetochores, protein complexes on the chromosomes. Recently, by investigating bundles of microtubules at the outer part of the spindle, we have found that bridging microtubules, which link sister k-fibers, attain a C-shape and balance the forces at the kinetochores (Kajtez *et al*, *Nat Commun* 2016). However, it is unknown what forces and torques are present in the inner part of the spindle. To answer this question, we have developed a theoretical model, where sister k-fibers are represented as an elastic slender rod shaped by forces and torques generated at the spindle poles. We found that k-fibers attain a general helical shape, whose projection on a plane can be identified as C-, S- and M- shape. By live-cell imaging experiments, we observed these three characteristic shapes, indicating a helical shape of k-fibers and consequently torques in the direction of the major axis. In addition, we found that helical shapes can exist under both tension and compression. We conclude that torques, as well as forces at the spindle poles determine the shape of mitotic spindle.

FLUIDIZATION OF TISSUES BY CELL-LEVEL ACTIVE TRANSFORMATIONS

M. Krajnc[1], S. Dasgupta[2], P. Ziherl[1,3], and J. Prost[2,4]
[1]*Jožef Stefan Institute, Ljubljana, Slovenia.*
[2]*Mechanobiology Institute, National University of Singapore, Singapore.*
[3]*Faculty of mathematics and physics, University of Ljubljana, Slovenia.*
[4]*Institut Curie, Paris, France.*

The order-disorder transition in epithelial tissues is often associated with cell properties such as cell-cell adhesion strength and cortical tension, but it also depends on cell rearrangements caused by, e.g., mitosis. We propose a mechanism for tissue fluidization based on active topological transformations which promote cell rearrangement including neighbor exchange (T1 transitions), extrusion, and division even if they do not lower the energy. Within a three-dimensional vertex model, we study how the stationary structure of a single-cell-thick epithelium depends on the parameters that control the activity within the tissue. We also investigate the dynamical response of the tissue to in-plane shear deformation, which allows us to quantify its shear modulus and viscosity. The proposed mechanism may be important for the understanding of cell flow and rearrangement during epithelial morphogenesis, for example in embryonic development.

MECHANICAL MODEL OF EPITHELIAL SHEET GROWTH DURING FRUIT FLY EGG CHAMBER DEVELOPMENT

J. Rozman[1], S. Y. Shvartsman[2], J. I. Alsous[2], A. Kosmrlj[2]
1 Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Slovenia
2 Princeton University, Princeton, NJ, USA

In the early stages of egg chamber development in *Drosophila*, the oocyte and 15 nurse cells get wrapped by a monolayer of approximately 60 epithelial cells. In order to accommodate the subsequent growth of the oocyte and nurse cells, epithelial cells undergo several rounds of division [1]. Here, we present a theoretically study of this process, where the oocyte and nurse cells are represented as a growing spherical container, which is wrapped by a sheet of polygonal epithelial cells. The mechanics and rearrangement of epithelial cells is modelled with the vertex-based model [2,3] and epithelial cells divide once their size increases beyond certain threshold [4]. We find that the geometry and topology of epithelial cells (distributions of cell sizes and number of neighbours) reflect not only their mechanical properties, but also the cell-level rules guiding division and post-division growth. Therefore, the comparison with the geometry and topology of epithelial cells obtained from experimental images, may provide some cues for the molecular mechanisms of growth regulation in epithelial sheets.

[1] S. Horne-Badovinac and D. Bilder, Mass Transit: epithelial morphogenesis in the Drosophila egg chamber, *Dev. Dynam.* **232**, 559 (2005)
[2] A. G. Fletcher, M. Osterfield, R. E. Baker and S. Y. Shvartsman, Vertex Models of Epithelial Morphogenesis, *Biophys. J.* **106**, 2291 (2014)
[3] X. Du, M. Osterfield and S. Y Shvartsman, Computational analysis of three-dimensional epithelial morphogenesis using vertex models, *Phys. Biol.* **11**, 066007 (2014)
[4] A. Puliafito, L. Hufnagel, P. Neveua, S. Streichan, A. Sigal, D. K. Fygenson and B. I. Shraiman, Collective and single cell behavior in epithelial contact inhibition, *PNAS* **109**, 739 (2012)

NANOPOREARRAY: ION-BEAM PATTERNED NANOPORE ARRAYS IN POLYMER SUPPORTED 2D MATERIALS

Mihovil Jurdana[1], Davor Čapeta[2], Marko Kralj[1], Tomislav Vuletić[1]
[1]*Institute of Physics; Bijenička cesta 46, Zagreb, Croatia*
[2]*Faculty of Science; Physics Department, University of Zagreb; Bijenička cesta 32, Zagreb, Croatia*

A concept of making ion track-etch pores will be presented. Ion track etching is a method where holes are "drilled" in a polymer film with ion irradiation and subsequent etching to controllably create monodisperse high-aspect ratio approach channels to 2D material (graphene, MoS_2...) embedded (sandwiched) within the polymer film. Embedding 2D material in polymer also serves as a support to reduce mechanical strain on the monoatomic layer. A nanopore subsequently made in the 2D material exposed in the bottom of the channel can then be used in single (macro)molecule translocation experiments. These are performed with the technological application as the primary motive - for physical DNA sequencing by measuring the drop in ionic current that flows through the 2D material. Ion track-etch holes can be patterned as an array using an ion microprobe in a similar way an electron beam is employed in e-beam lithography. This approach offers a possibility of making microfluidic device with multiple nanopores arranged in an array, while avoiding silicon technology. Current progress and further development is to be presented.