8th Christmas Biophysics Workshop

Book of Abstracts

Dobrna, December 16-17 2013

organized by Jožef Stefan Institute

edited by Matej Krajnc and Primož Ziherl

Foreword

The Christmas Biophysics Workshops organized since 2006 have evolved into a small but focused annual meeting providing a regional forum for biophysicists from Austria, Croatia, Italy, and Slovenia. It appears that these meetings are especially important because of the numerous collaborations among researchers from the four countries, many of which have been initiated at the Workshops. We have all appreciated the rather informal but productive style of the past meetings held in Zagreb, Bled, Donja Stubica, Leibnitz, Ptuj, Varaždin, and Riegersburg, and we are hopeful that you will find the Dobrna Workshop equally enjoyable.

M. Krajnc and P. Ziherl

Program

Monday December 16 2013

9.00-10.00 Arrival and Registration

10.00-11.20 Membranes, Vesicles and Cells I (chair: Rudolf Podgornik)

10.00 Andreas Ebner: *Investigation of the morphological changes and molecular function of cells, membranes and proteins by single molecule sensing AFM*
10.20 Peter Heftberger: *The evolution of a high resolution global SAXS data analysis for multilamellar lipid vesicles*
10.40 Mojca Mally: *Partitioning of oleic acid into phosphatidylcholine membranes is amplified by strain*
11.00 Benjamin Kollmitzer: *Protein partitioning in liquid-ordered (Lo) / liquid-disordered (Ld) domains depends on lipid composition and protein shape*

11.20-11.50 Coffee Break

11.50-12.50 Tissues (chair: Tomislav Vuletić)

11.50 Karoline Mühlbacher: *Analyzing wear debris of artificial joints using aerosol-physics technology*
12.10 Damir Vurnek: *Morphogenesis and ageing of MDCK epithelial tissues depend on substrate elasticity*
12.30 Matej Krajnc: *Continuum theory of finite-thickness tissues*
12.40 Nick Štorgel: *Mechanics of epithelial tissues with basement membrane*

13.00-15.00 Lunch

15.00-16.40 Macromolecules (chair: Cristian Micheletti)

15.00 Ida Delač Marion: *SAXS study of a binary mixtures of DNA and hyaluronic acid*
15.20 Nataša Adžić: *Kirkwood-Shumaker interaction*
15.40 Galja Pletikapić: *Force spectroscopy of marine biopolymers assembled into gel network*
16.00 Michael Leitner: *Technical improvements in bio AFM techniques*
16.20 Eva Žerovnik: *Amyloid forming proteins: in vitro and ex vivo studies*

16.40-17.10 Coffee Break

17.10-18.20 Membranes, Vesicles and Cells II (chair: Georg Pabst)

17.10 Jure Derganc: *The influence of protein crowding on membrane bending*
17.30 Bojan Božič: *Shape characteristics of red blood cells in capillary occlusions*
17.50 Bor Kavčič: *Limiting shapes of confined lipid vesicles*
18.00 Saša Svetina*: A possible physical basis for the endocytotic neck constriction*

19.00-21.00 Dinner

21.00- Social Event

Tuesday December 17 2013

8.00-9.00 Breakfast

9.00-10.40 Soft Matter and Computational Biophysics (chair: Antonio Šiber)

9.00 Staš Bevc: *Multiscale modeling and simulation of biomolecular systems*
9.20 Marco Di Stefano: *Colocalization of coregulated genes: a steered molecular dynamics approach*
9.40 Daniel Svenšek: *Tensorial conservation law for nematic polymers*
10.00 Gerhard Kahl: *Ultrasoft, cluster-forming particles exposed to pressure and temperature*
10.20 Labrini Athanasopoulou: *Packing of soft nanocolloids*
10.30 Primož Ziherl: *Mosaic two-lengthscale quasicrystals*

10.40-11.10 Coffee Break

11.10-12.50 Viruses (chair: Andreas Ebner)

11.10 Anže Lošdorfer Božič: *Multivalent ion effects on electrostatic stability of virus-like particles and encapsidated charged droplets*
11.30 Sanjin Marion: *Modeling DNA in viral capsids: Liquid crystal with bending*
11.50 Guido Polles: *Mechanical and assembly units of viral capsids identified via quasi-rigid domain decomposition*
12.10 Antonio Šiber: *Non-toroidal conformations of DNA condensed in spherical confinement*
12.30 Luca Tubiana: *Synonymous mutations affect physical properties of viral RNA genomes*

13.00-14.00 Lunch

Departure

Abstracts

Investigation of the morphological changes and molecular function of cells, membranes and proteins by single molecule sensing AFM

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Atomic Force Microscopy (AFM) has developed to an important tool in life sciences. It allows determining the surface roughness of soft biological samples like cells and membranes at near physiological conditions, label-free, and with a lateral resolution in the nanometer range. In addition, upgrading the usually inert AFM tip by tethering biological molecules to the tip allows gaining more information. Topography and Recognition Imaging [1] (TREC) combines tapping mode imaging with a biosensing AFM tip [2] and allows to simultaneously map the surface topography as well as to localize specific binding partners. A second technique, also based on the use of molecular sensing bio-functionalized AFM tips, is Molecular Recognition Force Spectroscopy [3-6] (MRFS). There a biosensing tip is repeatedly approached and withdrawn from a surface containing the corresponding binding partner of interest. Within every force distance cycle a ligand-receptor complex can be formed followed by forced rupturing. By varying the pulling velocities the complex energy landscape can be explored giving insights into kinetic and energetic aspects of the interaction [3-6].

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[5] M. Rangl, A. Ebner, J. Yamada, C. Rankl, R. Tampé, H. J. Gruber, and P. Hinterdorfer, *Angew. Chem. Int. Edit.* **125**, 10546 (2013).

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The evolution of a high resolution global SAXS data analysis for multilamellar lipid vesicles

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The spatial organization of lipids in biological membranes plays an important role in diverse cellular processes. Of particular interest are membrane rafts which are considered to enable cellular signaling and transport. Such membrane rafts are currently mimicked by liquid ordered (Lo) domains, observed in several lipid-only mixtures. The physical properties of such Lo domains and the coexisting liquid disordered (Ld) phase are presently not well known. We developed a global small-angle x-ray scattering data analysis for multilamellar vesicles that allows to determine membrane structural parameters and bending fluctuations of coexisting lipid domains. Different scattering length density profiles were used for modeling the lipid bilayers with increasing details and complexity. The technique was applied to different ternary and quarternary phospholipid mixtures. We determined membrane thickness as well as bending elasticity for Lo and Ld phases as a function of lipid composition and temperature.

Partitioning of oleic acid into phosphatidylcholine membranes is amplified by strain

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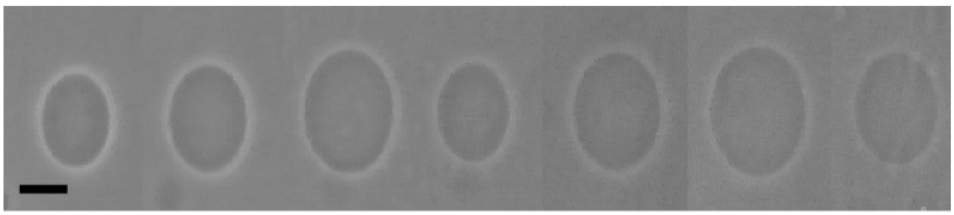
Partitioning of fatty acids into phospholipid membranes is studied on giant unilamellar vesicles (GUVs) utilizing phase-contrast microscopy. Using a micropipette, an individual GUV is transferred from a vesicle suspension in a mixed glucose/sucrose solution into an isomolar glycerol solution with a small amount of oleic acid added. Oleic acid molecules intercalate into the phospholipid membrane and thus increase the membrane area, while glycerol permeates into the vesicle interior and thus via osmotic inflation causes an increase of the vesicle volume. The conditions are chosen at which a vesicle swells as a sphere. At sufficiently low oleic acid concentrations, when the critical membrane strain is reached, the membrane bursts and part of vesicle content is ejected, upon which the membrane reseals and the swelling commences again. Vesicle's radius before and after the burst is determined at different concentrations of oleic acid in suspension. The results of our experiments show that the oleic acid partitioning increases when the membrane strain is increased. The observed behavior is interpreted on the basis of a tension-dependent intercalation of oleic acid into the membrane.



30 s 183 s 540 s 541 s 1025 s 1753 s 1754 s

Protein partitioning in liquid-ordered (Lo) / liquid-disordered (Ld) domains depends on lipid composition and protein shape

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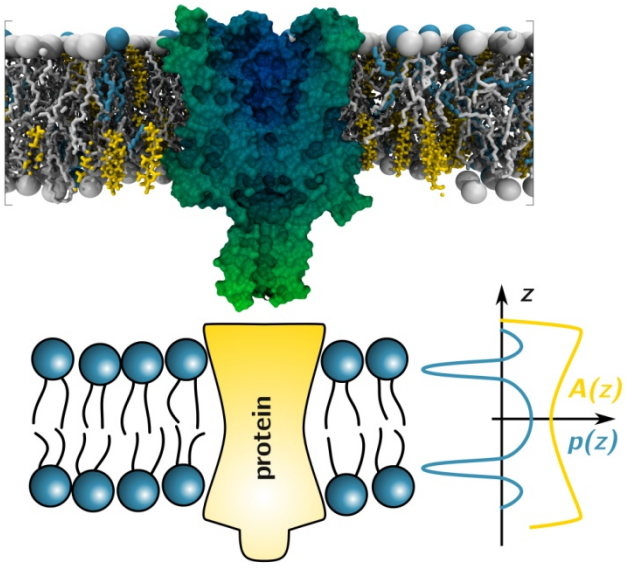
The lack of transmembrane proteins partitioned in the current lipid-only models for membrane rafts, i.e. Lo phases, calls for close scrutiny of raft mimetics. Using small angle X-ray scattering and molecular dynamic simulations, we determined structural and elastic parameters (spontaneous curvature, bending rigidity, Gaussian curvature modulus) for coexisting Lo/Ld domains in ternary mixtures of dioleoylphosphatidylcholine/|dipalmitoylphosphatidylcholine/|cholesterol (DOPC/|DPPC/|Chol) and dioleoylphosphatidylcholine/|distearoylphosphatidylcholine/|cholesterol (DOPC/|DSPC/|Chol) [1,2]. Substituting these values into theoretical calculations yields the energy penalty upon insertion of transmembrane proteins into Lo and Ld phases, and consequently the preferred partitioning in one of these domains. We discuss our findings for different geometric protein shapes.



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Analyzing wear debris of artificial joints using aerosol-physics technology

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Aseptic loosening is responsible for up to 44% revisions of knee and up to 70% of hip arthroplasties, whereas wear is a common cause. The characterization of wear particles in terms of number and size is essential for the study of their biological effects on cells and indicate possible revision surgery of artificial joints. The purpose of this study was to develop an efficient automated diagnostical method for number size distribution of wear debris. The presented electro spray setup, an automated counting method commonly applied in aerosol physics, mass spectrometry, was successfully used and validated for the first time to characterize wear debris from total joint replacement. The advantages of this diagnostic method over the conventional methods are its time and financial efficiency, low requirement of testing fluid (e.g. synovial fluid) and its suitability for testing of different materials.

Morphogenesis and ageing of MDCK epithelial tissues depend on substrate elasticity

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Morphogenesis of epithelial tissues is the key to understanding tissue development and regeneration, or tumor growth. It is believed to be dominated by intercellular interactions, and hence, independent of substrate rigidity. However, here we show that different regimes of growth occur on soft and hard substrates. Substrates with a rigidity higher than 5 kPa promote radially growing clusters, which in early stages expand exponentially with a persistently low density of cells. When the cluster grows sufficiently large its area increases linearly in time. During that period, a bulk tissue of higher density forms in the center of the cluster, whereas the edge remains at a constant low density, independently of the cluster size. On 1 kPa substrates the cells initially form small multilayered droplets that, if sufficiently large, nucleate a very dense and well structured monolayer in its center. These clusters expand to macroscopic sizes by adopting irregular shapes, while maintaining the initial monolayer morphology. In both cases, tissues age, the signature of which are (i) an inhomogeneous density, and (ii) nuclei that deform strongly due to the substrate sensitive restructuring of the actin cortex. Furthermore, dome-like and tubule-like structures are found on hard substrates, while soft substrates promote cell extrusion.

Continuum theory of finite-thickness tissues

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We derive the continuum theory of 2D finite-thickness tissues. Besides bending and stretching elasticity, energy functional contains a term describing the coupling between local curvature and local thickness. The microscopic origin of this theory is in the discrete model of simple epithelial tissues, where each cell carries a surface energy associated with cortex and interfacial tension as well as cell-cell adhesion [1]. We use this theory to qualitatively model the equilibrium shapes of corrugated simple epithelia [2] as well as to calculate the optimal shapes of 2D vesicles with finite thickness [3].

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Mechanics of epithelial tissues with basement membrane

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Morphogenesis of epithelial tissues is a fundamental biological process in the development of all living creature yet it is still not entirely understood. We propose a simple mechanical model of a single-layered epithelium consisting of incompressible cells associated with cortex and interfacial tension as well as cell-cell adhesion. The basal, the lateral, and the apical cell faces are each characterized by a specific effective surface tension. To make a model more realistic, cells are attached to a basement membrane, adding an additional bending elasticity energy ter proportional to the curvature squared. We explore the periodic minimal-energy configurations of an infinite 2D epithelium, observing the transition between corrugated and flat shapes. As the bending modulus of basemant membrane is increased, the corrugated epithelium is stretched and eventually replaced by the flat state. Finally, using the proposed continuum version of the discrete model, we analyze the shapes, explaining the coupling between epithelial curvature and thickness, as well as observed symmetry breaking caused by basement membrane.

SAXS study of a binary mixtures of DNA and hyaluronic acid

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In a living cell a multitude of biomacromolecules create a crowded, strongly interacting environment in which variety of cellular mechanisms take place. Biomacromolecules in question are mainly polyelectrolytes (PE), *i.e.* they dissociate in polar solvents (*e.g.* water) into polyions and small counterions. The effects of the long-range electrostatic interaction render these environments more complex than those provided by neutral polymers [1]. The interaction between charged polyion chains leads these systems to spatially arrange themselves in a multitude of ways which strongly depend on the PE and added salt concentration, PE rigidity, or valence of counterions [2]. In the so-called semidilute regime the mesh-like arrangement can be described with a single parameter which is de Gennes correlation length ξ, also known as the mesh size [3].

We investigated binary mixtures [4] of two salt-free biopolyelectrolytes, DNA and hyaluronic acid (HA) with small angle x-ray scattering (SAXS). In a previous study [5], we have shown by SAXS that the semidilute DNA and HA can be simply described, due to the relative rigidity of these macromolecules, with the mesh size given by $\xi = (bn)^{-1/2}$, where b and n are monomer size and number concentration, respectively. Scattering intensity of DNA features a PE correlation peak at a wave vector q^* which directly relates to this mesh size as $\xi = 2\pi/q^* \approx (bn)^{-1/2}$, while for HA this feature is lacking. Thus, when studying DNA+HA mixtures, SAXS measurements show only the peak at a q^* defined by the density of DNA mesh. Interestingly, SAXS showed DNA mesh to have higher densities than expected from a nominal concentration of DNA. This would be possible if DNA+HA mixtures were microphase separated. We confirmed by polarizing microscopy that this separation occurs, contrary to some theoretical expectations for PEs [4]. These subphases should be in an osmotic pressure equilibrium but our data additionaly indicate that HA osmotic pressure scales simply with monomer concentration as for DNA i.e., $\Pi \sim c^{9/8}$ [6,7]. Description of DNA and HA subphase osmotic pressures *via* separate counterion and polyion contributions [1] does not seem to explain the data.

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Kirkwood-Shumaker interaction

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In order to find the exact form of the Kirkwood-Shumaker interaction [1], describing perturbatively the electrostatic interaction between two proteins in aqueous solution, we have studied two macroscopic surfaces with proton adsorption sites, immersed in a counterion-only ionic solution. Field-theoretic representation of the grand canonical partition function was derived and evaluated at saddle point approximation, giving the mean-field free energy leading to repulsive interaction pressure. Gaussian fluctuations around the saddle point then give the lowest order correction. We calculated this correction analytically using path integral for a harmonic oscillator with time-depended frequency. We obtained the first order free energy correction consisting of the surface self-energy and the interaction free energy. Taking the proper limits, the interaction part of the free energy reduces to the zero-frequency van der Waals term, but also gives the correct Kirkwood-Shumaker result. Our general form of the interaction free energy gives attractive, long-ranged, monopolar surface charge fluctuation interactions which depend on the pH of the solution. This result shows that the model introduced is a good starting point for investigating the electrostatic interactions in protein physics.

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Force spectroscopy of marine biopolymers assembled into gel network

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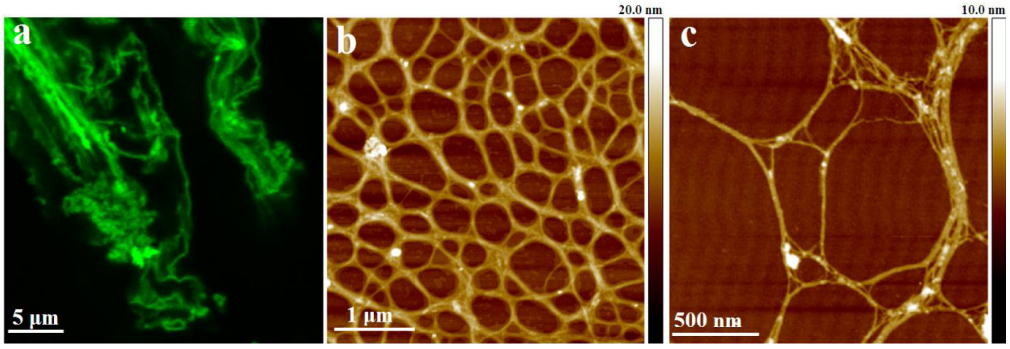
Marine gel biopolymers were recently visualized at the molecular level using atomic force microscopy (AFM) to reveal fine fibril-forming networks with low to high degrees of cross-linking (Figure 1). Due to the inherent complexity and heterogeneity of the marine gel phase it is difficult to isolate the physical forces in the biopolymer network assemblies. In this work, we used force spectroscopy to quantify the intra- and intermolecular forces within the marine gel network. Combining force measurements with AFM imaging allowed us to identify the microscopic origins of distinct mechanical responses. At the single fibril level we uncovered force-extension curves that resemble those of individual polysaccharide fibrils. They exhibit entropic elasticity followed by extensions associated with chair-to-boat transitions specific to the type of polysaccharide at high forces. Surprisingly, a low degree of cross-linking led to sawtooth patterns that are for the first time attributed to the unraveling of polysaccharide entanglements. At a high degree of cross-linking, we observed force plateaus that arise from the unzipping of helical bundles. Finally, the complex 3D network structure gave rise to force staircases of increasing height that may correspond to the hierarchical peeling of fibrils away from the junction zones. In addition, we showed that these diverse mechanical responses also arise in reconstituted polysaccharide gels, which highlights their dominant role in the mechanical architecture of marine gels.



Marine gel fibrils at micro- and nanometer scales: (a) a polysaccharide network of marine gel aggregate imaged in seawater by confocal microscopy after FITC-Concavalin A staining; (b, c) high resolution AFM images of marine gel fibrils with different degrees of cross-linking. AFM images were acquired in tapping mode in air using mica as a substrate.

Technical improvements in bio AFM techniques

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Since its invention in the middle of the 1980´s the atomic force microscope has developed to one of the key techniques in nanoscience. Especially in life science the main benefit is the ability to take images of biological samples under aqueous, near physiological conditions at nanometer resolution. Enhancement of the cantilever to a molecular biosensor yields to techniques which allow the detection of forces in the piconewton range [1]. The combination of recognition and topographical measurements (TREC) has been developed to determine receptor distributions on surfaces [2].
Nevertheless there are several limitations for a much wider use of these techniques in applied life science. Topographical imaging is time consuming, molecular recognition force spectroscopy (MRFS) and TREC suffer from complex cantilever functionalization and also the handling limits these techniques to experts in basic science.
But exchanging the conventional cantilevers to smaller, faster and more sensitive ones increases the imaging speed by a factor of ten and the sensitivity by a factor of five [3]. Using DNA building blocks in combination with DNA aptamers to functionalize cantilevers for MRFS and TREC simplifies the complex standard functionalization to simple incubation steps [4]. To improve the general tip handling and stability, active cantilevers for electronic, instead of optical readout have been developed. The newest generation of active cantilevers can be used for bio imaging in liquid and are nearly as sensitive as commercial bio cantilevers [5].

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Amyloid forming proteins: *in vitro* and *ex vivo* studies

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I will present our work on a model protein, a simple globular protein of 98 amino acids, humans stefin B. It served as a useful model protein thus far for folding [1] and aggregation [2] studies. The protein is very well characterized structurally [3], whereas its physiological role apart from cathepsins inhibition, is still being researched. Among other alternative functions it was shown to reduce oxidative stress, to act as an amyloid-beta-binding protein [4] and to be involved in innate immunity. It is not reported to cause any amyloid disease, however, its gene is mutated in an inherited progressive myoclonus epilepsy (EPM1), which also is a neurodegenerative condition. In the first part, in vitro studies of morphology and the mechanism of amyloid fibril formation by this protein will be shown. As well, how it interacts with lipid membranes in oligomer specific manner, resembling pore forming toxins (reviewed in Ref. [5]). In the second part, cellular studies will be shown, where stefin B mutants prone to form aggregates were over expressed in cells. It proved that the aggregates cause an increase in oxidative stress and lead ultimately to cell death in the form of necrosis. The toxicity of the aggregates was not correlated with their abundance but rather with their other properties. So, more scattered and smaller aggregates of the mutants which retain the protein fold were found more toxic than the bigger perinuclear aggresome-like aggregates. These latter are a way for the cell to sequester the protein aggregates and degrade them by autophagy. The presentation will end with the open, hot questions we want to address in the future studies.

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The influence of protein crowding on membrane bending

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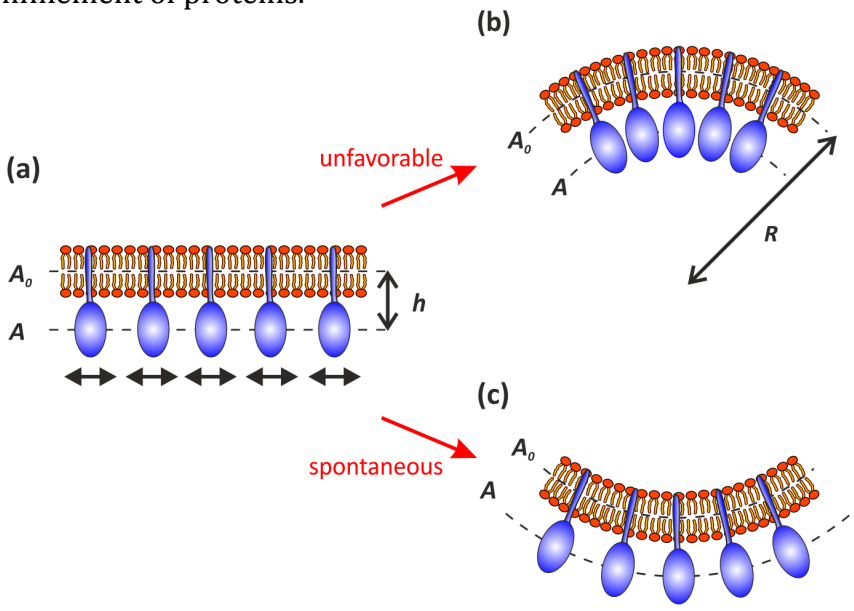
A recent study of COPII vesicle formation in yeast indicated that crowding of membrane proteins can have an important effect on membrane remodeling in physiological processes [1]. Here we present a theoretical analysis of how the crowding of membrane proteins influences the membrane bending [2]. The proteins diffusing in the membrane are modeled as a hard-sphere gas exhibiting a lateral pressure that increases non-linearly with the protein density (Figure a). Consequently, if the proteins have large extra-membrane domains on only one side of the membrane, the membrane spontaneously bends away from the protein mass (Figure c). In contrast, bending the membrane towards the protein mass is energetically unfavorable because it involves compression of the protein gas (Figure b). We show how the crowding effect depends on the lateral confinement of proteins.



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Shape characteristics of red blood cells in capillary occlusions

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The shape transformations of red blood cells stuck in capillary narrowings where the maximum deformations occur are analyzed. The membrane skeleton deformations are described within the effective network model and the continuum elastic model, whereas the area-difference elasticity model is applied to describe the phospholipid bilayer. A minimization of the total energy is performed to determine cell shapes in a stopped flow. The shapes are calculated by a triangulated representation of the membrane surface. They are asymmetric, characterized by a single invagination, which decreases with decreasing radii of the narrowing and vanishes at its critical radius. The largest stretching deformations of the skeleton are at the ends of the elongated shape and remarkable shear deformations appear around the invagination. The membrane's mechanical energy increases with the decreasing radius of the narrowing, predominantly due to the deformation of membrane skeleton. The increase in the shear energy is significantly larger than any other energy contribution within both models. The pressure differences needed for the penetration into the narrowing are strongly coupled with the membrane's mechanical energy. Their values were found to be of the order of ten pascals. Both models correspond well.

Limiting shapes of confined lipid vesicles

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We propose a theoretical model of lipid vesicles confined to a spherical cavity in limit of tight confinement. We derive the ADE energy of several limiting shapes composed of planar and quadric surfaces and we calculate their phase diagram. The phase diagram is compared to the numerically obtained results and the limiting shapes of free vesicles, and the limiting shapes are classified according to their symmetry and the number of compartments.

A possible physical basis for the endocytotic neck constriction

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The neck which connects in the endocytosis formed bud to the parent membrane has initially in general a finite radius. For the membrane to reform so that the nascent vesicle can be pinched off, it has to be constricted. In cells, neck constriction is believed to be controlled by protein complexes. However, some of the mechanisms that have been proposed to explain neck constriction have also an analogy in the behavior of simple lipid vesicles. Vesicles can be studied by applying rigorous approaches which is useful because the exact knowledge about membrane shape transformations can reveal some fine details of studied phenomena which due to the complexity of cellular processes might be otherwise overlooked. Here we examine, in a simple lipid vesicle, neck constriction by applying radial forces on the bud equator. We fix the volume and spontaneous curvature at values giving a stable axially symmetric shape in the absence of external forces. This shape consists of two sphere-like regions, one large and the other small (the bud) connected by a narrow neck. This vesicle is now deformed by a rigid ring of a fixed radius dilating the bud along its equator. The corresponding vesicle shape is determined by solving the shape equation with the appropriate boundary conditions. Our principal result is that the neck radius decreases monotonically with increasing ring radius, attaining the value zero with a finite radial force on the bud equator. It can be concluded from this model that it is possible to narrow the neck not only by forces that constrict its radius directly but also by remote forces reshaping the vesicle. These results provide a new perspective on the role played by actin polymerization in the process of vesicle formation.

Multiscale modeling and simulation of biomolecular systems

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Biomolecular systems are very challenging systems for computer simulation due to their complexity and related number of degrees of freedom. Despite the increasing computer power there are still many problems that are beyond the possibilities of current and near future computers. Multiscale modeling techniques have become increasingly important to bridge the vast span of spatial and temporal scales in these systems. Here, we present recent results of our multiscale simulations of biomolecular systems, e.g., salt solutions [1], DNA, etc.

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Colocalization of coregulated genes: A steered molecular dynamics approach

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The observation that the concerted activity of certain groups of genes significantly correlates with their proximity in the nucleus [1] poses the general question of whether it is at all feasible to bring close together the numerous coregulated gene pairs on a chromosome. We tackled this problem by using steered molecular dynamics simulations of a coarse-grained (30nm) model for the gene-rich human chromosome 19. We enforced the colocalization of ~ 1500 pairs of genes, whose expression patterns over ~ 20000 microarray experiments are significantly correlated (coregulation). Remarkably, we showed that most ($\sim 82\%$) of the target gene pairings can be accomplished. This was found to depend on: (i) the low degree of entanglement in chromatin fibers, and (ii) the large number of cliques (genes coregulated all together as groups) in the gene coregulatory network. Finally, chromosome organization from the steering procedure was shown to be arranged in spatial macro-domains, similar to those inferred from recent HiC experiments. These findings indicate that gene coregulation and colocalization are largely compatible, and that our computational strategy can be further applied to draft the spatial organization of chromosomes in other eukaryotes [2].

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Tensorial conservation law for nematic polymers

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We derive the "conservation law" for nematic polymers in tensorial form valid for quadrupolar orientational order [1], in contradistinction to the conservation law in the case of polar orientational order [2,3]. Due to microscopic differences in the coupling between the orientational field deformations and the density variations for polar and quadrupolar order, we find that the respective order parameters satisfy fundamentally distinct constraints. Being necessarily scalar in its form, the tensorial conservation law is obtained straightforwardly from the gradients of the polymer nematic tensor field and connects the spatial variation of this tensor field with density variations. We analyze the differences between the polar and the tensorial forms of the conservation law, present some explicit orientational fields that satisfy the tensorial constraint, and discuss the role of singular "hairpins," which do not affect the local quadrupolar order of polymer nematics, but nevertheless influence its gradients.

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Ultrasoft, cluster-forming particles exposed to pressure and temperature

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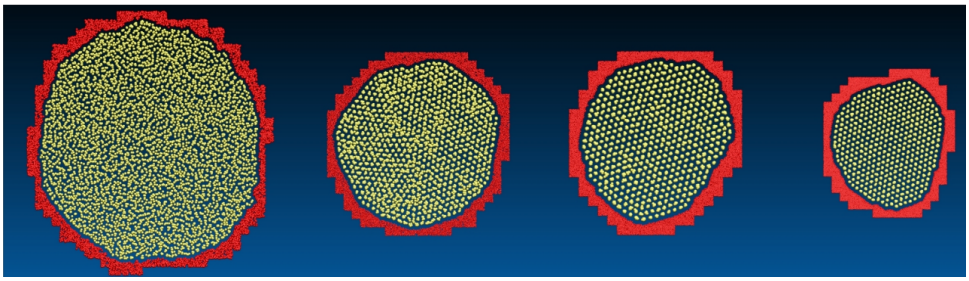
The formation of stable clusters by ultrasoft repulsive particles has received much attention in the field of soft matter physics during recent years [1]. One of the most intriguing features of ordered cluster phases is the density independence of the lattice constant: the cluster occupation increases linearly with the density, inducing thereby a density-independent value for the lattice constant. So far, this behaviour has been verified via *NVT* simulations carried out at isolated density-values. Under a continuous increase of the density, as, for instance, induced by an external, variable pressure the system is expected to respond by dissolving a sufficient amount of clusters and reassigning the liberated particles to other clusters. We have studied the effect of a pressure- and temperature-bath on a system of ultrasoft, repulsive, cluster-forming particles. The pressure and the temperature are tuned via a combined thermo-barostat, formed by ideal gas particles which interact with the ultrasoft colloids via an inverse power law [2]. We have studied the two-dimensional case where the system undergoes with increasing pressure a transition from a fluid to a hexagonal lattice. Once the system as reached the ordered cluster phase an increase of the pressure causes particles with the highest thermal agitation to abandon their host cluster and to start a trajectory through the crystal, looking for a new hosting cluster. Clusters which have lost a sufficient amount of particles will melt, the liberated particles will rearrange in existing clusters and volume will be freed for the clusters to rearrange. The use of molecular dynamics simulations allows to investigate this process in detail, to trace the trajectories of the individual particles and to study the temperature distribution within the system.



Snapshots during a compression process of a two-dimensional system of ultrasoft, cluster-forming particles. Pressure increases from left to right, transforming the fluid to the hexagonal ordered phase.

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Packing of soft nanocolloids

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Hard nanoparticles such as colloids form close-packed structures (e.g. FCC) at high densities. It appears that this is not the case for soft colloids such as dendrimers and star brush polymers that at high densities form open lattices, such as BCC and the A15 phase [1,2]. In our work we try to explain this behavior by modeling the soft nanoparticles as soft spheres that interact via elastic interactions [3]. Using the theory of elasticity to describe the behavior of a soft sphere system at high density and at zero temperature we construct the phase diagram of packing at different densities for different values of the Poisson's ratio. The contact problem is solved numerically using the finite element analysis [4].

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Mosaic two-lengthscale quasicrystals

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Over the past decade quasicrystalline order was reported in many soft-matter systems including dendritic micelles, star and tetrablock terpolymer melts, and diblock copolymer and surfactant micelles, establishing soft quasicrystals (QCs) as an integral part of the field. The existence of QCs in fuzzy macromolecular micelles suggests that they must be induced by a generic mechanism rather by specific chemistry. Indeed, micellar softness has been postulated and shown to lead to QC order. We comprehensively explore this link by studying two-dimensional hard disks decorated with square-shoulder repulsion mimicking, e.g., the soft alkyl shell around the aromatic core in dendritic micelles. We find a family of QCs with 10-, 12-, 18-, and 24-fold bond orientational order which originate from mosaics of equilateral and isosceles triangles formed by particles arranged core-to-core and shoulder-to-shoulder. The pair interaction responsible for the novel phases emphasises the role of local packing geometry in quasicrystallinity in soft matter, complementing the principles leading to QCs in hard tetrahedra. Based on simple interparticle potentials, quasicrystalline mosaics may well find their way to diverse applications ranging from improved image reproduction to advanced photonic materials.

Multivalent ion effects on electrostatic stability of virus-like particles and encapsidated charged droplets

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We investigate the electrostatic stability of charged virus-like nanoparticles and encapsidated charged droplets immersed in a neutralizing asymmetric electrolyte background. The charged droplet and the encapsidating particle are modeled as permeable, charged spheres/shells, and the theoretical description within the "dressed multivalent ion approximation" is compared with implicit Monte-Carlo simulations. Phase diagrams involving electrostatic pressure exhibit positive and negative values, corresponding to outward and inward facing forces on the particles, respectively. This provides an explanation for the high sensitivity of viral capsid stability and self-assembly on the ionic environment. The counter-intuitive effects of multivalent counterions also include the increased stability of a charged droplet with large charge density, increased stability in the case when the droplet is encapsidated by a shell of charge density of the same sign as the charged droplet, as well as the possibility to dispense altogether with the encapsidating shell, its confining effect being taken over by the multivalent ions.

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Modeling DNA in viral capsids: Liquid crystal with bending

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The problem of the encapsidation and subsequent ejection of DNA in bacteriophages is still an unsolved problem. It is known that, depending on both the length of the encapsidated genome as well as the physicochemical environment, the viral DNA undergoes several phase transitions [1]. There are currently two predominant models of the DNA packaging and ejection in bacteriophages [2]: the continuum mechanics model and the hydrodynamic model. Both models do not take into account the phase changes seen in the conformation of DNA in partially filled capsids – as the capsid is emptied DNA undergoes a series of transitions from a densely packed hexagonal state trough a liquid crystal and ending in an isotropic state before the last of the DNA is ejected. The problem of the DNA organization in the presence of monovalent and divalent counterions at low packing densities is approached using the Onsager approach to lyotropic polymer liquid crystals [3]. We obtain the free energy of the encapsidated genome in confinement with added contributions from DNA bending. Implications for DNA ejection in bacteriophages are discussed.

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Mechanical and assembly units of viral capsids identified via quasi-rigid domain decomposition

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Key steps in a viral life-cycle, such as self-assembly of a protective protein container or in some cases also subsequent maturation events, are governed by the interplay of physico-chemical mechanisms involving various spatial and temporal scales. These salient aspects of a viral life cycle are hence well described and rationalised from a mesoscopic perspective. Accordingly, various experimental and computational efforts have been directed towards identifying the fundamental building blocks that are instrumental for the mechanical response, or constitute the assembly units, of a few specific viral shells. Motivated by these earlier studies we introduce and apply a general and efficient computational scheme for identifying the stable domains of a given viral capsid. The method is based on elastic network models and quasi-rigid domain decomposition. It is first applied to a heterogeneous set of well-characterized viruses (CCMV, MS2, STNV, STMV) for which the known mechanical or assembly domains are correctly identified. The validated method is next applied to other viral particles such as L-A, Pariacoto and polyoma viruses, whose fundamental functional domains are still unknown or debated and for which we formulate verifiable predictions.

Non-toroidal conformations of DNA condensed in spherical confinement

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When condensed in free space, long DNA typically assumes a shape of a toroid. The geometry of the toroid, its inner and outer radii and the shape of its cross-section can be understood from a phenomenological model, describing condensation as an interplay between the (unfavorable) surface energy of the toroid and the (unfavorable) bending energy of the DNA strands in it – the only favorable contribution to the free energy is the volume term, requiring that the DNA strands be next to each other [1]. When condensed in confinement, e.g. in virus capsids, it is known that the, sufficiently short, DNA also assumes toroidal conformations, but the free energy balance is in that case additionally complicated by the adsorption energy (DNA-capsid interaction) and by the capsid confinement [2]. It has been proposed in the literature that the, sufficiently long DNA, may condense in conformations which are non-toroidal, i.e. which do not have the cylindrical axis of symmetry [3]. Nevertheless, such propositions were never tested in a suitable model, explaining the free energies of all the conformations that can be envisioned. I will show a generalization of the previously proposed models [1] to account for non-toroidal conformations of DNA condensed in spherical confinement. Such conformations may occur in viruses when they are completely filled. The model that I will present reproduces conformations that were previously predicted [3], but also several intriguing conformations that were never predicted in the context of viruses.

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Synonymous mutations affect physical properties of viral RNA genomes

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Recent studies of RNA viruses showed that their genomes fold in compact structures; this compactness has been argued to arise from an evolutionary pressure to facilitate the virus assembly process. However, up to now, explicit studies on evolutionary fitness of RNA viruses ignored such physical characteristics of the virion assembly to focus on the properties and viability of viral proteins and how mutations affect them. Implicit in those studies lies the assumption that synonymous mutations, which do not affect amino-acid sequences, are evolutionarily neutral. Here we show that such assumption is untenable, as synonymous mutations, performed both at fixed chemical composition or fixed dinucleotides frequencies, completely erase the characteristic compactness of viral RNAs, making them physically indistinguishable from random RNAs.