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Bending Stiffness and Curvature Coupling of Ternary Lipid Mixtures

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There exists a wide range of curvature gradients within and between cellular organelles. Differences between membrane morphologies play important roles in cell homeostasis, for example, in the sorting and trafficking of membrane components, as well as in controlling the activities of membrane associated proteins. To better understand the mechanisms by which curvature regulates cellular functions, here, we investigate membrane curvature coupling to membrane composition and mechanical properties.

We find that bending stiffness depends on membrane curvature of micro-scale homogeneous ternary lipid mixtures. Curvature gradients were generated by lipid tethers with controllable radius pulled from giant vesicles, and bending stiffness was obtained from tether radius and membrane tension measurements. As curvature increases, bending energy overcomes mixing entropy such that highly flexible lipid groups are sorted into the tube from the flat membrane. The sorting is enhanced as composition approaches the neighborhood of the mixing-demixing critical point, through two trajectories: parallel and perpendicular to the phase boundary. An expression that predicts bending stiffness to be a quadratic function of curvature in ternary mixture is derived, from which curvature sorting efficiency is obtained. We then interpret the sorting efficiency to be the ratio of a driving force for and a resistance to sorting. In addition, we estimate the bending stiffness of ternary mixtures at zero curvature, finding consistency with our measurements from the micropipette aspiration method.

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Electric Fields and Giant Vesicles

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Electric fields are omnipresent in our world, and are relevant not only from a physics point of view. Indeed, they also play a crucial role in several biological mechanisms occurring in living organisms, and they can turn out to be useful and easy-to-use tools to alter or to measure various biomaterials properties [1].

Provided they have the appropriate duration and amplitude, electric pulses can induce transient permeabilization of cell membranes. This phenomenon, called electropermeabilization or electroporation, sets the basis of several medical applications such as electrochemotherapy and electrogenetherapy [2]. Although its increasing popularity as a therapeutic compound delivery method, the underlying mechanisms of electroporation are far from being fully understood. In order to get a better insight at the process on the molecular level in an electropermeabilized membrane, our teams focus on the effects of electric pulses on giant unilamellar vesicles (GUVs).

We will present several results on the behavior of giant liposomes exposed to permeabilizing electric pulses. First, we found that electropermeabilization is associated with lipid loss and decrease of the vesicle size [3]. Then, we showed that it is possible to efficiently load GUVs with high molecular mass plasmid DNA whose transfer in living cells still remains problematical [4]. Finally, we used electric pulses as a simple tool to porate giant vesicles, and developed a novel method for measuring the edge tension of lipid membranes [5].

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Rna-Lipid Interaction At the Air Liquid Interface

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There is accumulating evidence of substantial amounts of phospholipids in the cell nuclei[1], although the function of these lipids is still not fully understood. It has been shown that the chromatin complex composed of DNA, RNA and proteins also includes phospholipids, and that RNA co-localize with these[2]. Although the RNA-phospholipid interactions may have important implications to biological function, in gene therapy and in medicine, very little work has been dedicated to the characterization of RNA interaction with phospholipids. The objective of this work is to investigate the adsorption behavior of short single stranded 10 bases long RNA ($ssRNA_{10}$) molecules (similar to miRNA) to lipid monolayers at the air-water interface as well as to study how the presence of RNA affect the domain formation in the monolayers using fluorescence microscopy. Monolayer studies have shown adsorption of $ssRNA_{10}$ to monolayers consisting of zwitterionic DPPC as well as to monolayers consisting of cationic DODAB. The adsorption behavior of these very short nucleic acids differ significantly from the adsorption process for longer nucleic acids as for example a 2000 base pairs long ds DNA ($dsDNA_{2000}$) which has been used as a reference system[3]. Viewed by fluorescence microscopy, the presence of ssRNA10 is observed to alter the characteristic domain shape of DPPC monolayers in the plateau region (coexistence LE/LC phase) and induces foam like structures on the monolayer surface. The presence of $ssRNA_{10}$significantly changes the compression isotherm of both DPPC and DODAB monolayers.

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Intracellular Calcium Mediated Stiffness of Red Blood Cells Is Reversed By Hypoxic Pre-Incubation With Nitrite Ions

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Normal red blood cells (RBCs) need to be highly deformable to pass through microcapillaries in order to deliver oxygen. It has been reported that an intracellular increase of calcium ions in RBCs causes them to undergo oxidative stress. Our earlier studies suggested that in hypoxia, nitrite ions react with deoxyhemoglobin (Hb(II)) to produce stable bioactive NO intermediates and Hb(II)NO that interact with the membrane and can potentially release NO and/or react with membrane protein thiols. We hypothesize that calcium-induced oxidative stress will decrease RBC deformability and thereby increase stiffness of RBCs, which will be inhibited by the production of bioactive NO in the RBCs in hypoxia. In this study we have used a newly available microfluidic ektacytometer to measure RBC-deformability in human blood expressed as the elongation index (EI, normal values 0.31-0.35) at a shear stress of 3Pa. We observed that EI of RBCs decreased to about 50% in 30minutes at 37^{o}C when A23187 ionophore-mediated calcium ions (as low as 0.01mM) enter cells. However, when RBCs are pre-incubated with a 10:1 heme:nitrite molar ratio of nitrite ions in hypoxia prior to ionophore-mediated calcium ion entry, the decrease in EI of RBCs is inhibited. Support for NO bioactivity is provided by the observation of similar results with RBCs pre-incubated with nitroprusside, a NO-donor (at 40μM concentration). This suggests that NO released from nitrite-reacted RBCs in hypoxia blocks intracellular calcium rise-mediated decrease in RBC deformability. Experiments are underway to determine the mechanism of this protective effect of NO.

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Investigation of Dynamics of Molecules in Supported Phospholipid Bilayers By Single Molecule Trajectories in Combination With Spot Size Analysis

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The dynamics of molecules in supported phospholipid bilayers are studied by a newly developed single molecule trajectory (SMT) analysis method.

Our proposed method combines two SMT analysis methods: the mean square displacement analysis (MSD) and the spot size analysis (SSA). While both methods aim to obtain diffusion coefficients for a SMT, their combination allows the investigation of underlying physical processes in a given trajectory.

Our proposed analysis method simultaneously compares the step size for a given SMT with its spot size within each frame, allowing in principle to resolve two diffusion processes:

In a *continuous diffusion* model the step size is less than or equal to the 2D-Gaussian fitted spot size resulting in overlapping spot sizes within a trajectory. The underlying physical nature of this diffusion behavior is based on Brownian motion.

In contrast, *hopping diffusion* is defined by a smaller spot size compared to the step size for a given trajectory. The underlying physical nature of this process is trapping events caused by heterogeneities in the environment of a SMT.

The ability to distinguish between these two diffusion behaviors allows the detection of heterogeneities even within a short SMT with high statistical

accuracy. Additionally, phenomena such as splitting events (multiple spots) within a trajectory in a single frame are naturally analyzed by our proposed method.
The model system chosen to investigate these diffusion behaviors are on glass coverslip supported phospholipid bilayers (DLPC, POPC, DMPC and DPPC). Sub-nM solutions of an amphiphilic cationic carbocynanine dye (DiI) with varying hydrophobic chain lengths are equilibrated and movies of diffusing single molecules are acquired at the lipid-water interface by TIRF-microscopy.

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Micropatterned Model Membranes Composed of Polymerized and Fluid Lipid Bilayers
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Substrate supported planar lipid bilayers (SPBs) are versatile models of the biological membrane on solid substrates. We have developed a methodology for generating SPBs composed of polymeric and fluid phospholipid bilayers by using a photo-polymerizable diacetylene phospholipid (DiynePC).(1) Polymeric bilayers could be generated with micro-patterns by the conventional photolithography, and the degree of polymerization could be controlled by modulating UV irradiation doses.(2)(3) After removing non-reacted monomers, fluid lipid membranes could be integrated with polymeric bilayers. The presence of preformed polymeric bilayer domains enhanced the incorporation of fluid bilayer membranes into the voids between them.(4) We could also immobilize biological membranes (sarcoplasmic reticulum (SR) membranes from rabbit skeletal muscle) by utilizing mixtures of SR membrane with a short chain phospholipid, 1,2-hexyanoyl-*sn*-glycero-3-phosphocholine (DHPC). These results clearly suggest the possibilities to reconstitute biological membranes on solid substrates for analyzing their properties in a structurally well-defined platform. In the present paper, I discuss on the unique features of the micropatterned composite model membranes and our recent approaches to construct more complex model biological membranes based on them.
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Self-Assembly in Phospholipid DNA - Protein Mixtures With Applications To Complex Formation in Cationic Liposome-Chromatin Systems
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We prepared complexes between cationic liposomes (CL) from a mixture of the neutral (DOPC) and the cationic (DOTAP) lipids with either nucleosome core particles (NCP) or chromatin arrays, prepared by in vitro over-expression. Microscopy showed the presence of distinct globules. Fluorescence labelling of the histone protein, DNA and lipid components showed complete co-localization under many conditions at the optical length scale, while separation of the histones from the DNA and lipids was sometimes found.
Cryo-EM confirmed array aggregates with excess of positively charged lipid forming ordered complexes of multilamellar lipids. For complexes with lower cationic charge (50% DOTAP and 50% neutral DOPC), there is an indication of less order. In most Cryo-EM samples the complexes seems to have a ''subunit size'' on the order of 100-200 nm, consistent with DLS data.
Synchrotron SAXS measurements were performed. The X-ray diffraction pattern demonstrates the lamellar Bragg peaks corresponding to an inter-lamellar separation of about 6-8 nm and sometimes presence of an in-plane DNA-DNA peak. This confirms a remarkably interesting phase behaviour for the system of DNA/protein (NCP or chromatin) with lipids. The SAXS and Cryo-TEM data clearly shows the formation of multilamellar aggregates with DNA sandwiched in between. This means that the DNA and the protein histone-octamer complex of the NCP and the chromatin arrays have dissociated. Hence, the question arises where the histone proteins are located, given the demonstrated co-localization at the length scale of the multilamellar complexes (a few hundred nm). One hypothesis is that the histone proteins are partially embedded in the bilayer and partially between the DNA domains. Alternatively, a few 50-100 nm size DNA-lipid complexes cluster, with histones associated in between, a type of associative phase separation.

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Entropy Driven Structures and Interactions of Lipid Based Self Assemblies
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At present there is a surge of interest in biophysical research in elucidating collective interactions between cellular proteins, membranes and associated biomolecules leading to supramolecular structures, with the ultimate goal of relating structure to function. We present x-ray scattering data, osmotic stress experiments, cryo-electron microscopy, and optical imaging data, in self assembled systems of charged lipid bilayers and lipid-peptide complexes, which reveal unexpected structures and intermolecular forces not predicted by current electrostatic theories of charged systems. Those structures are reversible and are entropy driven due to the soft nature of the membrane interfaces. We show how membrane composition, charge density, spontaneous curvature, membrane bending rigidity and temperature control the structures and forces in those systems.

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Frustrated Phase Transformations in Supported, Interdigitating Lipid Bilayers
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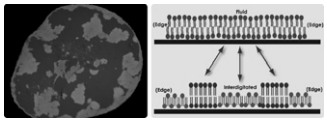
In free bilayers, the fluid to gel main phase transition of a monofluorinated phospholipid (F- DPPC) transforms a disordered fluid bilayer into a fully-interdigitated monolayer consisting of ordered acyl tails. This transformation results in an increase in molecular area and decrease in bilayer thickness. We show that when confined in patches near a solid surface, this reorganiza- tion proceeds under constraints of planar topography and total surface area. One consequence of these constraints is to limit the complete formation of the energetically-favored, interdigi- tated gel phase. The non-interdigitated lipids experience enhanced lateral tension, due to the expansion of the growing interdigitated phase within the constant area. The corresponding rise in equilibrium transition temperatures produces supercooled lipids that vitrify when cooled further. Ultimately, this frustrated phase change reflects a coupling between dynamics and thermodynamics, and gives rise to an unusual phase coexistence characterized by the presence of two qualitatively different gel phases.



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Effect of Smooth Bacterial Lipopolysaccharide on the Behavior of DPPC Films
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The airspaces are lined with a DPPC-rich film called pulmonary surfactant, named for its ability to maintain normal respiratory mechanics by reducing surface tension at the air/liquid interface. Inhaled airborne particles containing smooth bacterial lipopolysaccharide (s-LPS) might incorporate into the surfactant monolayer. In this study, we evaluated the effect of s-LPS on the behavior of DPPC films by using epifluorescence microscopy combined with a surface balance. In addition, we investigated whether LPS effects could be counteracted by surfactant protein A (SP-A), which is a LPS binding protein, with the peculiarity that this protein is associated to the surfactant monolayer. Thus SP-A is in the initial defence barrier against inhaled airborne particles. Our data show that s-LPS injected in the aqueous subphase penetrated into DPPC films to form mixed DPPC/s-LPS monolayers. Low amounts of s-LPS fluidized the DPPC monolayer, as demonstrated by fluorescence microscopy and changes in the compressibility modulus. This promoted early collapse and prevented the attainment of high surface pressures. The interaction of SP-A with DPPC/s-LPS film further fluidized the monolayers and facilitated the extraction of s-LPS at surface pressures where SP-A was expelled from the mixed films, suggesting that SP-A is an LPS scavenger. A better understanding of the biophysical properties of lung surfactant monolayer and its susceptibility to LPS inhibition is important for the development of new surfactant formulations for respiratory diseases.

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Membrane Curvature Modeling and Lipid Organization in Supported Lipid Bilayers
Matthew I. Hoopes, Roland Faller, Marjorie L. Longo.