408-Pos

Bending Stiffness and Curvature Coupling of Ternary Lipid Mixtures

Aiwei Tian, Benjamin Capraro, Cinzia Esposito, Tobias Baumgart.
Upenn, Philadelphia, PA, USA.

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.

409-Pos

Electric Fields and Giant Vesicles

Thomas Portet[1,2], Rumiana Dimova[3], David S. Dean[2], Marie-Pierre Rols[1].
[1]IPBS, Toulouse, France, [2]LPT, Toulouse, France, [3]MPIKG, Potsdam, Germany.

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].

We acknowledge financial support from IUF and AFM.

[1] Dimova *et al*, Soft Matter, 3:817, 2007; 5:3201, 2009.
[2] Escoffre *et al*, European Biophysics Journal, 36:973, 2007.
[3] Portet *et al*, Biophysical Journal, 96:4109, 2009.
[4] Portet *et al*, in preparation.
[5] Portet and Dimova, submitted to Biophysical Journal.

410-Pos

Rna-Lipid Interaction At the Air Liquid Interface

Agnes Michanek, Tommy Nylander, Emma Sparr.
Physical chemistry, Lund University Getingevägen 60 221 00, Lund, Sweden.

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.

1 Hunt, J. Cell Biochem.,97,244 (2006)
2 Micheli, Et al. FEBS Lett.,431,443 (1998)
3 Cardenas, Et al. J Coll. Interf. Sci., 286(1): 166-175 (2005).

411-Pos

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

Joy G. Mohanty[1], Viachaslau Barodka[2], Dan E. Berkowitz[2], Joseph M. Rifkind[1].
[1]National Inst. on Aging, Baltimore, MD, USA, [2]Johns Hopkins University School of Medicine, Baltimore, MD, USA.

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.

(This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging and Johns Hopkins University School of Medicine)

412-Pos

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

Moussa Barhoum[1], Karl-Heinz Gericke[2], Joel M. Harris[1].
[1]Department of Chemistry, University of Utah, Salt Lake City, UT, USA, [2]Department of Physical and Theoretical Chemistry, Technische Universität Braunschweig, Braunschweig, Germany.

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