predicted to be 1/3 [Gomez et al., Saeki et al.] or 1/4 [Yanagisawa et al.]. The power law exponent has been measured previously for liquid domains in vesicle membranes as 0.15 [Saeki et al.] and 2/3 [Yanagisawa et al.]. Here we present an independent measurement of the power law exponent.

Gomez, J., Sagues, F., & Reigada, R. (2008). Use of an enhanced bulk diffusion-based algorithm for phase separation of ternary mixture. The Journal of Chemical Physics , 184115-1-184115-9.

Saeki, D., Hamada, T., & Yoshikawa, K. (2006). Domain-Growth Kinetics in a Cell-Sized Liposome. Journal of the Physical Society of Japan, 013602-1-013602-3.

Yanagisawa, M., Imai, M., Masui, T., Komura, S., & Ohta, T. (2007). Growth Dynamics of Domains in Ternary Fluid Vesicles. Biophysical Journal , 115-125.

403-Pos

Molecular Interactions in Phase Separation of DOPC/DSPC/cholesterol Ternary Mixtures

Mohammad Alwarawrah, Jian Dai, Juyang Huang.
Texas Tech University, Lubbock, TX, USA.

With the aim of investigating molecular interactions between lipids involved in lipid-raft formation, the experimental phase diagram of a DOPC/DSPC/cholesterol ternary system was simulated using Monte Carlo simulation. Both pairwise (Ising-like) and multibody interactions were used to simulate the phase boundary of liquid-ordered phase and liquid-disordered (Lo-Ld) phase coexistence regions. The ''Composition Histogram Method'' (CHM) was specifically developed to quickly determine the compositions of coexisting phases as well as the thermodynamic tie-lines. The simulation demonstrated that the phase boundaries produced by pairwise (Ising-like) interactions alone generally do not agree with the experimental phase boundary. A much better fit for the experimental phase boundary was obtained by including a ''domain edge energy'' term, which is expressed in a form ofssmultibody interaction. Our result shows that the ''domain edge energy'' is essential for creating phase separation in lipid raft mixtures. The magnitude of this interaction energy determines the location of the critical point, the shape the phase boundary, and the size distribution of lipid domains in lipid raft mixtures. Any experimental condition that alters the domain edge energy, could significantly change the shape and location of the Lo-Ld phase boundary.

404-Pos

Molecular Dynamics Simulations of Ceramide Flip-Flop and Desorption in Lipid Rafts

W.F. Drew Bennett, D. Peter Tieleman.
University of Calgary, Calgary, AB, Canada.

Ceramides are important signaling lipids, involved in processes such as apoptosis and cell differentiation. Upon stimulus acid sphingomyelinase hydrolyses sphingomyelin into ceramide on the extracellular leaflet. How ceramide generated on the extracellular leaflet induces an intracellular response remains unknown. Ceramide has been shown to associate and stabilize lipid rafts, which could create a signaling platform. We have undertaken molecular dynamics computer simulations (MD) of ceramide in both putative raft and non-raft bilayers. Using umbrella sampling we determined free energy profiles for moving ceramide and cholesterol from water to the center of raft and non-raft bilayers. The free energy barrier for ceramide flip-flop is 49 kJ/mol and 62 kJ/mol in the non-raft and raft bilayers. From these barriers, we estimate the rate of ceramide flip-flop is 0.3 s-1 and 0.003 s-1 in the non-raft and raft bilayers. The free energy for desorption can be equated to the excess chemical potential of ceramide in the bilayer compared to water. By comparing the chemical potentials, we can infer the relative affinity of ceramide and cholesterol for the raft and non-raft bilayers. Cholesterol has a large affinity for the raft bilayer compared to the non-raft bilayer, while ceramide has only a slight preference for the raft bilayer. These results provide a thermodynamic molecular-level description of the interactions of ceramide with lipid rafts, and the rate of translocation.

405-Pos

Dna Lipoplexes: Prediction of Phase Architectures Using Cg Simulations and Experimental Validation

Josephine Corsi[1], Oscar Ces[2], Syma Khalid[1], George S. Attard[1].
[1]University of Southampton, Southampton, United Kingdom, [2]Imperial College London, London, United Kingdom.

DNA lipoplexes are important mediators of transfection that offer a safer, although less efficient alternative to their viral counterparts. Thus improving the efficacy of DNA lipoplexes is essential for their exploitation in nanomedicine. Experimentally, it has been shown that the architecture of DNA lipoplexes is linked to their biological efficacy. Therefore the ability to predict the architectures of compositions of DNA lipoplexes would be highly desirable. However, prediction of the phase behaviour of such systems is difficult, largely owing to a complex interplay of intermolecular forces. Molecular dynamics simulations provide a potential strategy for predicting phase behaviour, but traditional, atomistic methods are not applicable to large DNA-lipid systems. Here, we present coarse-grained simulations of the lyotropic phase transitions of DNA lipoplexes as a function of lipid composition and water content. Our coarse-grained model of DNA uses a ~ 4 to 1 mapping of atoms to particles and is compatible with existing coarse-grained models of biomolecules. With the appropriate balance of water content and lipid composition, we are able to capture the transition from the originally lamellar phase to the inverse hexagonal phase. Our simulation results show an inverse hexagonal phase with a calculated d-spacing of 6.2 nm for a DOPE-DNA system. Together with the disorder of the hexagonal phase, this d-spacing increases with increasing cationic lipid (DOTAP) content, in agreement with experimental data obtained by SAXS and polarizing light microscopy. Our simulations have provide insights into the rearrangements that occur to effect the transition to the inverse hexagonal phase; this level of detail is difficult to obtain using experimental methods alone. Furthermore, our simulations have highlighted the increasingly important role of coarse-grained simulation methods for the design of novel DNA lipoplexes and applications in synthetic biology, in general.

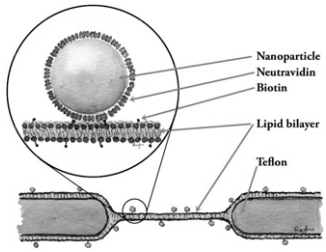
406-Pos

Microrheology of Freestanding Lipid Bilayers

Chris Harland, Miranda Bradley, Raghuveer Parthasarathy.
University of Oregon, Eugene, OR, USA.

The macroscopic material properties of cellular membranes, determined by the composition and interactions of their constituent lipids, are important factors in the structure and function of all living cells. Fluidity is a key material property of membranes, yet the underlying lipid bilayer viscosity and other rheological parameters remain poorly quantified.

We adopt recently developed microrheological methods to study multiple composite freestanding ''black'' lipid membranes. Using high speed video particle tracking, we monitor dynamics of membrane-anchored nano- and micro-particles across a range of temperatures that span bilayer phase transitions. Two particle spatial correlation functions and the complex shear modulus are extracted from such measurements and provide information about fundamental membrane material properties. We find striking and previously unreported signatures of viscoelasticity in these lipid bilayers whose properties are sensitive to the bilayers' temperature dependent liquid ordered to liquid disordered phase transitions.



407-Pos

Coping With the Cold: Effect of Hibernation on Pulmonary Surfactant in the Thirteen-Lined Ground Squirrel

Fred Possmayer[1], Lynda McCaig[1], Li-Juan Yao[1], Lin Zhao[1], James Staples[1], Sandra Orgeig[2,3], Ruud A. Veldhuizen[1].
[1]University of Western Ontario, London, ON, Canada, [2]University of South Australia, Adelaide, ON, Canada, [3]University of South Australia, Adelaide, Australia.

Pulmonary surfactant, a mixture of phospholipids, cholesterol, and proteins, stabilizes the lung by reducing surface tension at the air-water interface of the alveoli. We hypothesized that lowering body temperature to approximately 5°C during hibernation would require compositional changes in surfactant lipids and perhaps proteins in order to maintain lung function. Large aggregate (LA) fractions were obtained by centrifuging lung lavage at 40,000 g for 15 min, with small aggregates (SA) remaining in the supernatant. Because hibernating animals have lower body masses, surfactant levels per animal were compared. Hibernation resulted in an increase in total surfactant due to increased LA with little change in SA. Cholesterol at 8 wt% PL was not altered. Hibernation was accompanied by a small (~15%) decrease in disaturated phosphatidylcholine and phosphatidylglycerol. Decreases were observed in the mRNAs for the surfactant proteins (SP-). Western analysis revealed levels of all SP-s decreased to approximately 10% (SP-A), 50% (SP-B), 90% (SP-C), and 50% (SP-D) of warm active levels. We speculate that the changes in surfactant LA levels might reflect the much lower breathing rate during hibernation. Furthermore, the compositional changes could arise, in part, from a slowing of metabolism. The decrease in disaturated phospholipid levels could lead to enhanced adsorption due to increased fluidity, but this suggestion will have to be confirmed experimentally.

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