specialized for motion toward either the (+) or (-) end of actin filaments. Previous work has shown that directionality of recombinant myosins may be altered via the genetic insertion [1] or removal [2,3,4] of structural motifs that redirect the lever arm. We have challenged our understanding of myosin structure and function by constructing novel myosin motors that can reversibly switch their direction of motion in response to an external signal. Our general strategy relies on controlling the effective length of lever arms by triggering helix-coil transitions. In one successful design using [Ca++] as the control signal, we have built myosin VI variants with chimeric lever arms composed of an alpha-actinin fragment [5] fused to two or more calmodulin-binding IQ repeats. In vitro motility assays show that the engineered motors reverse directionality in response to physiological levels of [Ca++], as expected.

[1] Tsiavaliaris, G., Fujita-Becker, S. and Manstein, D. J. (2004). *Nature*, **427**, 558-561.
[2] Bryant, Z., Altman, D. and Spudich, J. A. (2007). *Prot. Natl Acad. Sci. USA,***104**, 772-777.
[3] Park, H., Li, A., Chen, L., Houdusse, A., Selvin, P. R. and Sweeney, H. L. (2007). *Proc. Natl Acad. Sci. USA*, **104**, 778-783.
[4] Liao, J., Elting, M. W., Delp, S. L., Spudich, J. A. and Bryant, Z. (2009). *J. Mol. Biol*, **392**, 862-867.
[5] Anson, M., Greeves, M. A., Kurzawa, S. E. and Manstein, D. J. (1996). *EMBO J.***15**, 6069-6074.

Platform AB: Membrane Physical Chemistry I

1195-Plat
Quantification of the Nanomechanical Stablility of Multicomponent Lipid Bilayers
Shan Zou.
National Research Council Canada, Ottawa, ON, Canada.
Quantification of the mechanical stability of lipid bilayers is important in establishing the composition-structure-property relations, and shed light on understanding functions of biological membranes. We report a direct correlation of the self-organized structures exhibited in phase-segregated supported lipid bilayers consisting of dioleoylphosphatidylcholine/egg sphingomyelin/cholesterol (DEC) in the absence and presence of ceramide (DEC-Ceramide) with their nanomechanical properties using AFM imaging and high-resolution force mapping. Direct incorporation of ceramide into phase-segregated supported lipid bilayers formed ceramide-enriched domains, where the height topography was found to be imaging setpoint dependent. In contrast, liquid ordered domains in both DEC and DEC-Ceramide presented similar heights regardless of AFM imaging settings. Owing to its capability for simultaneous determination of the topology and interaction forces, AFM-based force mapping was used in our study to directly correlate the structures and mechanical responses of different coexisting phases. We also designed an experiment to directly probe and quantify the nanomechanical stability and rigidity of the ceramide-enriched platforms that play a distinctive role in a variety of cellular processes. Our force mapping results have demonstrated that the ceramide-enriched domains require both methyl β-cyclodextrin (MbCD) and chloroform treatments to weaken their highly ordered organization, suggesting a lipid packing different from typical gel states. Our results also show the expulsion of cholesterol from the sphingolipid/cholesterol- enriched domains as a result of ceramide incorporation. This work provides quantitative information on the nanomechanical stability and rigidity of coexisting phase-segregated lipid bilayers with the presence of ceramide-enriched platforms, indicating that generation of ceramide in cells drastically alters the structural organization and the mechanical property of biological membranes.

1196-Plat
Simulations of Lipid Bilayer Domain Formation: Effects of Steroid Structure and Asymmetry
Jason D. Perlmutter, Jonathan N. Sachs.
University of Minnesota - Twin Cities, Minneapolis, MN, USA.
There is a growing amount of evidence that laterally segregated domains of lipids are an integral part of biological membrane structure and function. Using Coarse Grain and Atomistic Molecular Dynamics Simulations we investigate the role of steroid structure and asymmetry in domain formation. Cholesterol, an essential component of animal membranes, appears to be well suited for ordering neighboring lipid chains and promoting domain formation. We demonstrate that alterations to the steroid headgroup hydrophobicity trigger a conversion from domain promoting to domain inhibiting. Those steroids which inhibit domain formation are observed to be less stable in the typical, upright orientation, and instead insert into the bilayer hydrophobic core and reside in an orientation perpendicular to the bilayer normal axis. A second set of simulations are used to address the role of bilayer asymmetry in domain formation. Cellular membranes are thought to be asymmetric, containing different lipid compositions on opposing leaflets, with the outer leaflet capable of domain formation and the inner leaflet uniformly disordered. These simulations suggest how domain formation is affected by an opposing uniformly disordered leaflet.

1197-Plat
Probing Structure and Dynamics of Lipid Microdomains with Tagged Proteins and Lipids: A Hybrid Particle-Continuum Simulation Approach
Jun Fan[1], Maria Sammalkorpi[1], Mikko P. Haataja[1,2].
[1]Department of Mechanical and Aerospace Engineering, Pinrceton University, Princeton, NJ, USA, [2]Princeton Institute for the Science and Technology of Materials, Program in Applied and Computational Mathematics, Princeton University, Princeton, NJ, USA.
Lipid rafts are functional microdomains in the cell membrane. They have been implicated in many important cellular processes such as signal transduction, protein sorting and viral entry. At th is point, our understanding of the collective dynamics of lipids and lipid clusters in vivo is rather limited. To this end, by employing a hybrid particle-continuum approach, we simulate the coupled dynamics of diffusing probe particles (both proteins and lipids) and the evolving membrane composition. Importantly, we demonstrate that the structure and dynamics of lipid microdomains can be extracted from the fluctuating dynamics of the probe particles. These results suggest novel experimental ways of exploring raft dynamics.

1198-Plat
Cholesterol-Rich Fluid Membranes Solubilize Ceramide Gel Domains. Implications for the Organization of Mammalian Membranes
Bruno M. Castro[1], Liana C. Silva[1,2], Rodrigo F.M. de Almeida[3], Manuel Prieto[1].
[1]CQFM and IN, Lisboa, Portugal, [2]Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel, [3]CQB, FCUL, Lisboa, Portugal.
A uniquely sensitive method for ceramide-domain detection allowed us to study in detail cholesterol-ceramide interactions in lipid bilayers with low (physiological) ceramide (Cer) concentrations, and ranging from low or no cholesterol (Chol) (a situation similar to intracellular membranes, such as endoplasmic reticulum) to high Chol, (similar to mammalian plasma membrane). Fluorescence spectroscopy and microscopy experiments were conducted showing that for low Chol amounts Cer segregates into gel domains that disappear upon increasing Chol levels. This was observed in raft (sphingomyelin/Chol-containing) and non-raft (sphingomyelin-absent) membranes, *i.e.* mimicking different types of cell membranes. Chol-Cer interactions have been described mainly as raft sphingomyelin-dependent. In this work, sphingomyelin independence is demonstrated. Moreover, we show that Cer-rich domains re-appear when either Chol is converted by cholesterol oxidase to cholestenone, or temperature is decreased. The inability of cholestenone-rich membranes to dissolve Cer-gel domains shows that the cholesterol ordering and packing properties are fundamental to the mixing process. Cer solubility is dependent on the average gel-fluid transition temperature of the remaining membrane lipids, and is higher in Chol-rich fluid membranes than in Chol-poor ones. We also show that the solubility of Chol in Cer domains is low. The results are rationalized by a ternary phospholipid/ ceramide/ cholesterol phase diagram, providing the framework for a better understanding of biochemical phenomena modulated by Chol-Cer interactions such as cholesterol oxidase activity, lipoprotein metabolism and lipid targeting in cancer therapy. It also suggests that the lipid compositions of different organelles are such that ceramide gel domains are not formed, unless a stress or pathological situation occurs.
Further details in Castro, BM, Silva, LC, Fedorov, A, de Almeida, RFM, and Prieto, M (2009). *J. Biol. Chem.***284** (5), 22978-22987.
FCT (Portugal) is acknowledged for research grants and scholarships.

1199-Plat
Texture of Membrane Gel Domains
Uffe Bernchou, Jonathan Brewer, Henrik Midtiby, John H. Ipsen, Luis A. Bagatolli, **Adam C. Simonsen**.
MEMPHYS, University of Southern Denmark, Odense M, Denmark.
In this work[1] we investigate the texture of gel (g) domains in supported binary lipid membranes. Lateral organization of lipid bilayer membranes is a topic of fundamental and biological importance. Whereas questions related to the size and composition of fluid domains are well studied, the possibility of texture in condensed solid/gel domains has received limited attention. Gel domains are expected to be prominent in skin membranes and in ceramide domains

during apoptosis. When using polarized light for two-photon excitation of the lipid probe Laurdan, the emission intensity is highly sensitive to the angle between the polarization and the tilt orientation of lipid acyl chains. By imaging the intensity variations as a function of the polarization angle, we map the lateral variations of the lipid tilt within domains. Results reveal that gel domains are composed of distinct subdomains with different lipid tilt directions. Vortex structures centered at the domain core can be observed. Texture patterns of the same type have historically been associated with the presence of hexatic order in monolayers. The hexatic phase is an intermediate phase between the crystal and fluid states, having short range positional order and long range orientation order. The present results provide some support for the notion that hexatic order may persist in bilayers. Using the generalized polarization (GP) function of Laurdan, we demonstrate that although gel domains have heterogeneous texture, the membrane phase state is uniform within domains.

[1] U. Bernchou et. al. 'Texture of Lipid Bilayer Domains'. J. Am. Chem. Soc., 2009. DOI: 10.1021/ja903375m

1200-Plat

Understanding the Behavior of Nanometer-Size Lipid Domains in Model Membranes: A Small Angle Neutron Scattering Study

Sumit Garg[1], Lionel Porcar[2], Paul Butler[3], Ursula Perez-Salas[4].
[1]Argonne National Lab, Argonne, IL, USA, [2]Institut Laue-Langevin, Grenoble, France, [3]National Institute of Standards and Technology, Gaithersburg, MD, USA, [4]University of Illinois at Chicago, Chicago, IL, USA.

Lipid-lipid phase separation is important in understanding the behavior of the biological membrane. Such phenomenon has been studied extensively in model lipid membranes using microscopy techniques where domains are found to be microns in size. In actual biological membranes, however, domains are smaller, and microscopy techniques are unable to detect them. A hypothesis to explain these small domains is that the cytoskeleton generates boundaries to compartmentalize the membrane into small sub-membrane regions with access to only small amounts of lipids in the lifespan of lipid domains. Therefore, to be able to correlate studies of model membranes to the actual plasma membrane, there is a need to characterize lipid domains in a system where they cannot grow more than few nanometers in size. To achieve such a goal, we use small Unilemellar Vesicles (ULVs) made of 1:1 and 3:7 ratios of DPPC (deuterated-DPPC) and DLPC respectively for which phase separation in large vesicles has been observed. Using small vesicles with varying sizes (diameters from 30nm to 400nm) not only provides a means to control curvature, but also limits the amount of available lipids for domain growth. Small Angle Neutron Scattering was used to characterize the size, density and average composition of the domains, which appeared as the temperature was lowered below Tm, the melting temperature of the system. The scattering curves were fitted using a pair-correlation method in order to extract the "local structure" of the vesicles. The results interestingly suggest that the nanometer domains in these systems do not coalesce to form a single stable domain as observed in giant vesicles. Overall, thiswork provides insight into the behavior of nano-meter size lipid-lipid phase separation as a function of composition, temperature, vesicle-size and curvature.

1201-Plat

Direct Imaging of the Structure of Lipid Rafts by Atomic Force Microscopy

Khizar H. Sheikh.
University College Dublin, Dublin, Ireland.

According to the fluid mosaic model, lipid bilayers have been thought of as two-dimensional homogenous mixtures of lipids, embedded with membrane proteins[1]. This model has recently been extended by the lipid raft model in which biologically functional structural lipid domains, rich in sphingolipids and cholesterol.[2]

Previously, a low-noise AFM system[3] was developed within the our group, capable of imaging the surface structure of lipid bilayers in aqueous buffer with Angstrom resolution.[4,5] We now extend this work to the structure of the these lipid raft components at Angstrom resolution and discover a subtle organisation of the lipids headgroups of the molecules in these structures, stabilised by a combination of salt bridges and hydrogen bonds.

References

1. S.J. Singer, G.L. Nicolson, *The fluid mosaic model of the structure of cell membranes*. *Science,* **175**, 720-31 (1972).
2. K. Simons, E. Ikonen, *Functional rafts in cell membranes*, Nature,**387,** 569-72 (2007)
3. T. Fukuma and S.P. Jarvis, *Development of liquid-environment frequency modulation atomic force microscope with low noise deflection sensor for cantilevers of various dimensions*, Review of Scientific Instruments, **77**, 043701 (2006).
4. T. Fukuma, M. J. Higgins and S. P. Jarvis, *Direct imaging of individual intrinsic hydration layers on lipid bilayers at Ångstrom resolution*, Biophysical Journal, **92**, 3603-3609 (2007).
5. T. Fukuma, M. J. Higgins and S. P. Jarvis, *Direct imaging of lipid-ion network formation under physiological conditions by frequency modulation atomic force microscopy*, Physical Review Letters, **98**, 106101 (2007).

1202-Plat

High Pressure Static and Time-Resolved X-Ray Studies of Inverse Phases in Cholesterol / Lipid Mixtures

Arwen I.I. Tyler, Gemma C. Shearman, Nicholas J. Brooks, Richard H. Templer, Oscar Ces, Robert V. Law, John M. Seddon.
Department of Chemistry and Chemical Biology Centre, Imperial College London, London, United Kingdom.

Non-bilayer phases are thought to be of considerable biological relevance. Whenever there is a topological change in the membrane, corresponding to events such as membrane fusion, non-bilayer structures are assumed to be adopted locally. Several complex three-dimensional lyotropic liquid crystal phases are already known, such as the bicontinuous cubic phases, but for many years only a single example was found - a cubic phase of spacegroup Fd3m - of a structure based upon a complex close packing of inverse micelles. We have recently reported the discovery (1) of a novel lyotropic liquid crystal phase, of spacegroup, $P6_3/mmc$, whose structure is based upon a hexagonal close packing of identical quasi-spherical inverse micelles.

Although a plethora of equilibrium phase diagrams have been published, there is a scarcity of knowledge regarding the kinetics and mechanisms of lyotropic phase transitions. If we are to further our knowledge of events such as membrane fusion then a comprehensive understanding of the processes governing phase transitions, the type of intermediates formed and the mechanism by which a transition occurs are vital.

A superb technique for monitoring and initiating the structural evolution of such systems, in the millisecond regime, is time resolved X-ray diffraction, using pressure as the trigger mechanism. We have employed this technique to investigate lamellar - non-lamellar ($P6_3/mmc$ phase) transition kinetics in cholesterol/ phospholipid/ diacylglycerol model membrane systems. Equilibrium pressure - temperature composition diagrams have been constructed, allowing us to choose appropriate pressure-jump parameters (temperature, initial and final pressures) for the kinetic studies.

(1) G. C. Shearman, A. I. I. Tyler, N. J. Brooks, R. H. Templer, O. Ces, R. V. Law, J. M. Seddon, *J. Am. Chem. Soc.* **131**, 1678 (2009)