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.

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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.

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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.

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