

bilayers. These probably transient microstructures indicate that the micellization of detergent super-saturated vesicles occurs via a series of three stages: (i) bending of detergent-rich monolayers into curved thread-like cover of the perimeter of the holes, (ii) formation of thread-like micelles attached to the vesicles due to the line tension of the holes and (iii) detachment of the (most stable) mixed micelles from the vesicles. All the available data (spectroscopic, microscopic and calorimetric) are consistent with this mechanistic model.

168-Plat

Measuring Lipid Bilayer Bending Energy in a Dual-Beam Optical Trap

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While cell membrane bending is central to many physiological processes, techniques for measuring the bending energy of lipid bilayers have reported widely divergent results. Here, we show that a dual-beam optical trap (DBOT) can be used to apply finely controlled tensions to lipid bilayer membranes in a giant unilamellar vesicle (GUV) format. Optical force from the trap stretches the GUV; video microscopy is used to measure the change in membrane area during stretching. As laser power is increased, the surface area of the GUV also increases. Laser power is translated to membrane tension using a ray optics approach. The resulting tension-area relationship is fit to a model of membrane mechanics to yield a bending modulus.

The entire DBOT system is integrated with a microfluidic flow channel in such a manner as to facilitate the high-throughput analysis of large populations of GUVs. Performing such an analysis, we have shown that the presence of cholesterol has no effect on the bending modulus of bilayers made from the unsaturated lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). Both pure POPC bilayers and bilayers consisting of 80% POPC, 20% cholesterol have a bending modulus around 8 kT.

This technique is a promising route to detailed, accurate data relating lipid bilayer composition to membrane mechanical properties.

169-Plat

DNA-Based Patterning of Tethered Membrane Patches

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Solid-supported lipid bilayers are useful model systems for mimicking cellular membranes; however, the interaction of the bilayer with the surface can disrupt the function of integral membrane proteins. As a result, many groups have introduced tethered lipid bilayers, which retain the proximity to the surface, enabling surface-sensitive techniques, but physically distance the bilayer from the surface. We have recently developed a method for spatially separating a lipid bilayer from a solid support using DNA lipids (Chung and Boxer et al., *J. Struct. Biol.*, 2009). In this system, a DNA strand is covalently attached to a silane-modified glass slide or SiO₂ wafer. The complementary DNA strand conjugated to a lipid moiety is inserted into giant unilamellar vesicles (GUVs), and the DNA-modified GUVs hybridize to the strands on the surface, inducing flattening and rupture of the GUV to a planar tethered lipid bilayer. However, the location of the patch is random, determined by where the DNA-GUV initially binds with its complement. To allow greater versatility and control, we sought a way to pattern tethered membrane patches. We present a method for creating spatially distinct tethered membrane patches on a glass slide using microarray printing. Surface-reactive DNA sequences are spotted onto the slide, incubated to covalently link the DNA to the surface, and DNA-GUVs patches are formed selectively on the printed DNA. Different DNA sequences can be printed on the same slide, creating a unique handle on each GUV patch. This handle enables the creation of patches of different lipid compositions, dyes, and/or DNA-lipid sequences in adjacent but distinct areas, and the control over the placement of the tethered lipid bilayer potentially allows interfacing with devices. This approach would also enable rapid screening of different patches in protein binding assays and as targets for membrane fusion.

170-Plat

Sleeping Bubbles: Effects of Volatile Anesthetics in the Lateral Structure of Giant Unilamellar Vesicles

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Volatile anesthetics have been widely used for more than 170 years. However, the mechanisms underlying the effects of anesthetics on membrane dynamics and structure are still under debate. Herein we study this problem for first time using fluorescence confocal microscopy experiments of giant Unilamellar Vesicles (GUVs). This approach allows obtaining spatially resolved information on membrane structure at a microscopic level.

GUVs were prepared using DLPC/DPPC 3:7 mol. This mixture showed a characteristic gel (Lbeta)/liquid disordered (Lalpha) phase coexistence at room temperature, with line-shape domains (Lbeta) of variable width depending on the temperature[1]. The volatile anesthetic used was Sevoflurane. The administration strategies were two: high concentration with reduced exposure time; or low concentration (clinically relevant) with long term exposition.

A dramatic alteration of the phase coexistence was observed, with a marked effect on the morphology of the gel phase domains. For the experiments with high anesthetic concentration, the boundaries of the domains became diffuse with an increment of the domain's perimeter/area ratio. When low anesthetic concentrations were used, a complete loss of the domains structure was observed with appearance of small circular shaped domains.

Sevoflurane dramatically affected the lateral structure of the studied membranes, suggesting that similar mechanisms may occur in biological relevant membranes. Particularly, at low concentration of the anesthetic our results show some structural characteristic to that observed for cholesterol in canonical raft ternary lipid mixtures (formation of round domains). More research is underway to better understand the mechanisms underlying the sevoflurane effects on membranes.

[1] L.A. Bagatolli and E. Gratton. 2000, *Biophys J.* 78:290-305.

171-Plat

The Influence of Noble Gases in Protein-Free Membranes and the Pressure Reversal Effect

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The knowledge gathered so far reports a surprising variety of different chemical substances to induce anesthesia. The questions about how the noble gases modify the excitability of nerve cells and even how such excitability can be recovered under hyperbaric pressure remain open [1]. In the literature, one finds competing theories relating anesthesia to their effect on lipid membranes or on their effect on proteins, but the origin of the anesthetic effect is still not understood. Currently, the fashion of the biological mechanisms make us resort to proteins, however, in anesthesia, we must not neglect that anesthetics produce a melting point depression in pure lipid systems [2]. Furthermore, the pressure reversal in the effect produced by some anesthetics is well-known in animals, so that it seems hardly explainable regarding the protein receptors theory. Whereby, the intrinsic physical properties of the noble gases may give us hints to understand the general anesthesia mechanism. In the present work we show, for the first time, calorimetric results of the melting point depression phenomenon in protein-free membranes induced by noble gases, followed by a reversal effect of such depression with hydrostatic pressure. We finally correlate the electric polarizability of noble gases with the shift in the melting transition of the lipid membranes. Our results, in a pure lipid system, concur with other findings to underwrite the idea that anesthesia does not need a specific binding site in a protein and allow us to speculate that anesthesia only depends on the ability of certain atom or molecule to solubilized in lipids increasing the disorder of the membrane.

References

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172-Plat

Restructuring of Membrane Bilayers due to Osmotic Pressure: Deuterium Solid-State NMR Study

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Lipid membrane composition and biophysical properties have substantial influences on cellular functions. Studies of environmental effects on membrane bilayers are a prerequisite for understanding membrane protein functions. Experimental measures of structural parameters like cross-sectional area/lipid of membrane bilayers are vital for molecular dynamics simulations [1,2]. We