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Intrinsic Heterogeneity in Liposome Suspensions Caused by the Dynamic Spontaneous Formation of Hydrophobic Active Sites in Lipid Membranes
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The spontaneous, dynamic formation of hydrophobic active sites in lipid bilayer membranes is studied and characterized. It is shown that the rates of formation and consumption of these active sites control several important properties of liposomes, including their affinity for hydrophobic surfaces, the rate by which they spontaneously release encapsulated molecules, their fusion, their resistance to surfactants, their promptness to rupture, etc. The adhesion and spreading of liposomes onto hydrophobic polystyrene nanoparticles, the spontaneous leakage of an encapsulated fluorescent dye, the liposome-surfactant association isotherm and the vesicles resistance to rupture on hydrophilic and hydrophobic flat surfaces were monitored for different liposome compositions employing Cryo-TEM, DLS, QCM-D, turbidity and fluorescence measurements. It was observed that an apparently homogeneous, monodisperse liposome suspension behaves as if composed by two different populations: an active, fast leaking population that presents enhanced affinity for surfactants and hydrophobic substrates, as well as a marked tendency to rupture and spread on hydrophilic surfaces; and an inactive, slow leaking population showing significantly less activity in those processes. The results suggest that the proportion of liposomes in each population changes over time until a dynamic equilibrium is reached. It is shown that this phenomenom can lead to irreproducibility in, e.g., spontaneous leakage experiments, as extruded liposomes leak much faster just after preparation than 24 hours afterwards. These findings account for discrepancies in several experimental results reported in the literature. Further, understanding the differences between active and inactive populations may help to design liposomes with an excess of the desired population.

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2D Lipid Bilayer Viscosity Measured by Observing Shear Flow Transmission
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Few methods for measuring the two-dimensional viscosity of a liquid-phase lipid bilayer exist, although this quantity is of interest to many researchers. Previous estimates have been made by observing diffusion rates of liquid domains in a membrane (1), and by observing lifetimes of dynamic domain fluctuations (2). Recent simulation work (3) suggests a third method for obtaining this useful quantity. A hemispherical vesicle attached to a surface and surrounded by a shear flow experiences corresponding flows in the membrane surface, and bulk flow is induced inside the vesicle (4). The membrane viscosity may be calculated by carefully observing the drop in shear transmission from outside the vesicle to inside. Here we use the shear transmission method to find the viscosity for membranes with various lipid compositions and to validate predicted flow patterns. The composition of the vesicle is chosen to contain gel-liquid coexistence, and small gel domains are used as markers for observing the flow velocity of the liquid membrane.
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Line Tension in Lipid Monolayers with Liquid-Liquid Phase Coexistence
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When two phases coexist in a membrane, there is an energy penalty for the generation on the 1D-border between each phase, which is measured trough a parameter called ''line tension''. In analogy to surface tension, line tension (λ) is a consequence of the different energetic of molecules in the border of a 2D domain. Its influence becomes noticeable when a domain is driven out of the more stable shape, since λ induces the restoration of its original shape.
The line tension has been determined in monolayers and bilayers using several techniques. In this work we present a novel manner of determining λ in monolayers with liquid/liquid coexistence that overcomes some of the drawbacks of the previously described techniques. Using this technique, we determined the line tension of binary mixtures of different lipids and a molecule similar to cholesterol but less oxidable. We analyze the effect of the hydrocarbon chain length and of the polar head-group. The results indicate that an increment from 12 to 16 carbons in the hydrocarbon chain does not influence the value of λ. On the contrary, the nature of the polar head-group has an important influence on λ; PE presents high λ values while PG presents low values. PS and PC show intermediate values. The results are discussed in relation to the chemical structure of the lipids, their interaction with cholesterol and the mechanical properties of the lipid membranes that they form.

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Methods for Measuring Lipid Bilayer Viscosity
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The two-dimensional fluidity of lipid bilayers is crucial to biological function, as it enables the motion of membrane macromolecules. Yet, despite the importance of membrane fluidity, lipid bilayer viscosity remains poorly quantified. We address this with two complimentary experimental approaches, neither of which have previously been applied to lipid bilayers. The first, two-point microrheology, involves examining the correlated motion of thermally excited tracer particles. The second involves measuring the rotational diffusion coefficient of membrane bound tracer particles. We describe the implementation of these methods.

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Model Membranes with Coexisting Liquid Phases using Nontraditional Lipid Compositions
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Model membranes containing ternary mixtures of a lipid with a high melting temperature (Tm), a low Tm, and a sterol phase separate into two coexisting liquid phases over a range of temperatures and compositions. The two phases are termed liquid-ordered (Lo) and liquid-disordered (Ld). In mammalian cell membranes, it is generally assumed that that the high Tm lipid is a sphingolipid, that the low Tm lipid is unsaturated, and that the sterol is cholesterol. Furthermore, it is assumed that the Lo phase is thicker than the Ld phase. Our group has previously explored cases in which phase separation successfully occurs in membranes for which the sterol is not cholesterol (Beattie et al., 2005) or the high-Tm lipid is not a sphingolipid (Veatch & Keller, 2005). Here we present transition temperatures for successful miscibility phase separation in two additional nontraditional lipid combinations. In the first case the low-Tm lipid is not unsaturated. In the second, we predict that the Ld phase is thicker than the Lo phase. Our intent is to better understand which structural properties lead to phase separation.

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Incorporation of a Potassium Channel into a Suspended Lipid Bilayer Platform
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Membrane proteins are notoriously difficult to study. While supported lipid bilayers offer stability and allow the application of surface measurement techniques, integral membrane proteins are often not fully functional when close to a solid surface. We have developed a membrane interferometer which allows free standing membranes suspended above an atomically flat silicon surface to be studied by fluorescence interference measurements (Prasad V. Ganesan and Steven G. Boxer, *PNAS*, 2009,106, 5627-5632). In this platform, a lipid bilayer is suspended across a micron-sized well, allowing the use of Variable Incidence Angle Fluorescence Interference Contrast Microscopy (VIA-FLIC; Caroline Ajo-Franklin, Prasad V. Ganesan, and Steven G. Boxer, *Biophys. J.*, 2005, 89, 2759-2769). The interferometry measurements in VIA-FLIC can be used to determine the height of fluorescent dyes relative to the mirror with an axial resolution of a few nanometers. We have expressed and purified a voltage-gated potassium channel, KvAP, labeled it with a fluorescent dye, and reconstituted it into synthetic lipid vesicles. Here, we report on progress towards incorporating KvAP into the membrane interferometer, which