associated with severe pathologies such as the neonatal respiratory distress syndrome (NRDS) or Acute RDS in adults. Treatment of babies suffering or at risk of NRDS consists in an intratraqueal application of a dense aqueous suspension of exogenous surfactant. Widespread application of exogenous surfactant therapies in adults is still under development, in part due to limited availability of clinical surfactant and because it is an invasive therapy which requires the intubation of the patient. The fact that surfactant must be stored at 4°C to preserve its functional properties, causes some difficulties during transport and storage, mainly in developing countries where these therapies are strongly needed to treat premature newborns. In this context, lyophilisation is contemplated as a way to keep surfactant properties. This work assesses the effect of lyophilization and later reconstitution on the interfacial properties of the lateral structure of porcine native lung surfactant films.

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Transient Effect of Calcium Influx on PIP2 Clusters and Cholesterol-Stabilized Nano-Domains in the Inner Plasma Membrane Leaflet of Intact Cells

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When calcium ion channels open, local calcium levels are estimated to reach up to 500uM, which may be sufficient to affect negatively charged lipids, such as phosphatidylinositol 4,5-bisphosphate (PIP2), in the inner leaflet of the plasma membrane (PM). The PM is a complex lipid protein mixture in which at least two mechanisms create lateral order: interactions between the acyl-chains of the lipids, stabilized by Cholesterol, lead to transient submicroscopic nano-domains, and lipid head-group interactions of charged lipids with divalent ions may cluster lipids. Here, we study the influence of calcium on the formation of PIP_2 clusters and cholesterol-stabilized-nano-domains in intact cells. We study these domains in intact cells over time and upon calcium channel activation by analyzing the diffusion of GFP-tagged inner-leaflet membrane proteins. Using bimFCS, we measure diffusion on multiple length scales simultaneously to derive domains information. To study the formation of PIP2 clusters we use GFP-PHPLCdelta to mark PIP2, and as marker for cholesterol-stabilized-nano-domains we use Lck-mGFP and full-length Lyn-mGFP. We observe that opening TRPV1 channels leads to a transient rise in calcium imaged with GCaMP5G, knocks GFP-PHPLCdelta off from the PM, and increases the interaction between Lck-mGFP and cholesterol-domains. Within 2-4 minutes, the interaction between the Lck-mGFP and cholesterol-domains decreases to baseline as the cell down-regulates the intracellular calcium level. Using ionophore to clamp the calcium level at fixed values, we determine thresholds for these effects. To control for large scale signaling, we image membrane cytoskeleton using mCherry-alpha-actinin. These results suggest a concentration dependence of calcium-induced PIP2 clusters and cholesterol-stabilized-nano-domains in PM.

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Cholesterol Transbilayer Distribution in Mammalian Cells: Mechanisms and Functions

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Phospholipids and proteins in the plasma membrane (PM) bilayer are well established to be both laterally and transversely asymmetric. However, cholesterol transbilayer distribution within PM remains inconclusive. A fluorescent sterol, dehydroergosterol (DHE), is well-documented to primarily reside in the inner leaflet of PM (~80%). It remains unclear if native cholesterol shares this surprising asymmetry.

The purpose of this study is to first determine cholesterol transbilayer distribution in PM. We developed a protocol that is capable of analyzing cholesterol in a leaflet-specific manner using β-cyclodextrin (βCD). In symmetric large unilamellar vesicles (LUVs), we found that cholesterol flip-flop is rapid at 37°C, leading to 100% extraction/exchange by βCD. However, at 0°C, βCD is only able to remove exactly 50% cholesterol, indicating a complete inhibition of cholesterol flip-flop. We then applied this protocol to erythrocytes and found that only 20-25% cholesterol is accessible by βCD at 0°C, although 100% is accessible at 37°C. Therefore, most cholesterol resides in the inner leaflet of PM in mammalian cells.

We then investigated the role of phospholipid transbilayer asymmetry on cholesterol asymmetry. We found that, only in the asymmetric LUVs with long chain (22 carbon) sphingomyelin in the outer leaflet, could we observe cholesterol enrichment in the inner leaflet. Similar experiments with short chain (16 carbon) sphingomyelin and phosphatidylcholine (16:0/18:1) failed to influence cholesterol distribution.

We therefore conclude that, like DHE, cholesterol is enriched in the inner leaflet of PM and that this asymmetry is regulated by the phospholipid asymmetry and, more specifically, by long chain sphingolipids. We suggest that the current lipid raft model may need to be revised to reflect this cholesterol asymmetry.

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Phosphatidylinositol Patches in a Reconstituted Lipid Membrane and its Dynamics

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Amoeboid locomotion is one of the fundamental modes of cell motion that accompanies dynamic deformation of plasma membrane. From yeast to human cell, localized activation of small GTPase and phosphatidylinositol signaling initiates breaking of intracellular symmetry and are thought to play critical roles in generating polarized membrane extension and retraction. Although it is generally believed that some forms of reaction-diffusion driven mechanisms underlie these ''symmetry breaking'' events, the physicochemical basis of its onset, i.e. autonomous nucleation events of the localized signaling patches and actin polymerization remains unclear. Here, we report on the behavior observed in our ''two-dimensional reconstituted-system'' consisting of a cytosolic extract and a solid-supported lipid membrane. We tested whether the solid-supported lipid membrane in a microchamber containing cytosol extracted by *Dictyostelium discoideum* provides an appropriate microenvironment for localized signaling by observing translocation of PH-domain protein and PTEN which were tagged with RFP and GFP respectively. Signal intensities were analyzed by a confocal laser scanning fluorescence and bright field microscopy. We found that both the PH-domain protein and PTEN attached to the membrane composed of both lipid extract and 2- or 3- component lipid mixture. In the case of lipid extract membrane, inhomogeneous domain formation of micrometer scale was observed. We examined the effect of several inhibitors related phosphatidylinositol signaling and found different patterning of the lipid membranes. We will discuss how the current system may be employed to address the origin of self-organized PIP2/PIP3 patches during amoeboid deformation.

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Asymmetry Determines the Effect of Ceramides on Model Membranes. In Natural Membranes Too?

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Ceramides can dramatically influence the lateral organization of biological membranes. In particular, ceramide-induced alterations of protein-lipid domains can be involved in several cellular processes, ranging from senescence to immune response. In this context, an important role is played by the length of the fatty acid bound to the sphingosine moiety. Asymmetric, heterogeneous ceramides, with more than 20 or less than 16 carbon atoms in the fatty acyl chain, in fact exert diverging effects in vivo if compared to their symmetric counterparts. In this work, we investigated the role of ceramide asymmetry and heterogeneity in model membranes showing raft-like phase separation, using a combination of fluorescence imaging, atomic force microscopy, fluorescence correlation spectroscopy and differential scanning calorimetry. We show that ceramide produced enzymatically from natural mixtures of sphingomyelin can dramatically alter the mixing behaviour of proteins and lipids in the membrane, inducing a homogenization of the bilayer. Furthermore, we characterized the physical properties of coexisting lipid phases at equilibrium in membranes with varying ceramide content, emphasizing the differences between symmetric-homogeneous and asymmetric-heterogeneous ceramides. While symmetric ceramides always produce enhanced order, asymmetric ceramides display a more complex behavior similar to that of cholesterol. Our results might help contribute to a more precise understanding of the rearrangements induced by different kinds of ceramide generation in cellular membranes.

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Curved Fluid Membranes Behave Laterally as an Effective Viscoelastic Medium

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The lateral mobility of membrane inclusions is essential in biological processes involving membrane-bound macromolecules, which often take place in highly curved geometries such as membrane tubes or small organelles. Probe mobility is assisted by lateral fluidity, which is thought to be purely viscous for lipid