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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.

439-Pos Board B194

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

Weixiang Jin, Heng Huang, Arnd Pralle.
Physics, University at Buffalo, Buffalo, NY, USA.

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

Kevin Courtney[1,2], Xiaohui Zha[1,2].
[1]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]University of Ottawa, Ottawa, ON, Canada.

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

Kei Takahashi[1], Nao Shimada[1], Taro Toyota[2], Satoshi Sawai[2,3].
[1]Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan, [2]Graduate School of Arts and Sciences, Research Center for Complex Systems Biology, University of Tokyo, Tokyo, Japan, [3]PRESTO, Japan Science and Technology Agency, Tokyo, Japan.

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.

442-Pos Board B197

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

Dolores C. Carrer[1,2], Eva Kummer[2], Grzegorz Chwastek[3], Salvatore Chiantia[2], Petra Schwille[3].
[1]Instituto Ferreyra - INIMEC (CONICET-UNC), Cordoba, Argentina, [2]Biotec-TU Dresden, Dresden, Germany, [3]MPI for Biochemistry, Munich, Germany.

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.

443-Pos Board B198

Curved Fluid Membranes Behave Laterally as an Effective Viscoelastic Medium

Mohammad Rahimi[1], **Marino Arroyo**[2].
[1]Princeton University, Princeton, NJ, USA, [2]Universitat Politecnica de Catalunya-BarcelonaTech, Barcelona, Spain.

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

bilayers and synthetic systems such as polymersomes. In previous theoretical studies, the hydrodynamical mobility is estimated assuming fixed membrane geometry. However, fluid membranes are very flexible out-of-plane. By accounting for the deformability of the membrane and in the presence of curvature, we show that the lateral motion of an inclusion produces a normal force, which results in a nonuniform membrane deformation. Such a deformation mobilizes bending elasticity, produces extra lateral viscous and elastic forces, and results in an effective lateral viscoelastic behavior. The coupling between lateral and out-of-plane mechanics is mediated by the interfacial hydrodynamics and curvature. We analyze the frequency and curvature dependent rheology of flexible fluid membranes, and interpret it with a simple four-element model, which provides a background for microrheological experiments.
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Prediction of Blood-Brain Barrier Permeability from Molecular Dynamics Simulations
Timothy S. Carpenter, Edmond Y. Lau, Daniel A. Kirshner, Felice C. Lightstone.
BBTD, Lawrence Livermore Natl Lab, Livermore, CA, USA.
The blood-brain barrier (BBB) is formed by endothelial cells that line brain capillaries. Specialized tight junctions between these cells form a selective barrier between the brain and the rest of the body. A major problem in drug design is the ability of the compound to cross the BBB. Neuroactive drugs are required to cross the BBB to function. Conversely, drugs that target other parts of the body should not cross the BBB in order to avoid possible psychotropic side effects. The task of predicting BBB permeability of new compounds is thus of great importance. Two gold-standard experimental measures of BBB permeability are logBB (concentration of drug in the brain divided by concentration in the blood) and logPS (the permeability-surface area product). Both are time-consuming and expensive to determine, and while logPS is considered more informative, it is lower-throughput, and more resource-intensive. We make computational predictions of these two parameters for a sample of 13 relatively small compounds. Atomistic molecular dynamics (MD) simulations measure the potential of mean force (PMF) for these compounds through a 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) bilayer - a system often used as a simple BBB mimetic. Additionally, 1D position-dependent diffusion rates are calculated from the MD trajectories. These diffusion rates are combined with the free energy landscape to calculate permeabilities for each sample compound. The relative values of these permeabilities were compared to logBB and logPS. Our computational predictions correlate remarkably well with experimental values of both logBB ($R2 = 0.92$) and logPS ($R2 = 0.95$). Thus, we demonstrate that this approach may have the potential to relatively inexpensively and quickly give reliable, quantitative predictions of BBB permeability.
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445-Pos Board B200
Asymmetric Supported Lipid Bilayer Formed via Methyl-β-Cyclodextrin Mediated Lipid Exchange: a Membrane Model System to Study Phase Separation and Transbilayer Lipid Movement
Ilaria Visco.
MPI for Biochemistry, Martinsried, Germany.
Supported lipid bilayers (SLBs) are broadly used as a membrane model system and commonly produced by vesicle fusion (VF). Despite its advantages, VF does not allow the controlled formation of asymmetric bilayers that mimic the asymmetry in lipid composition normally found in biological systems. Here we present a simple, quick and versatile method to produce stable SLBs with a desired asymmetric lipid composition. We apply a methyl-β-cyclodextrin (MβCD) mediated lipid exchange method to SLBs formed by VF to enrich the distal leaflet of the bilayer with sphingomyelin (SM). Bilayer asymmetry is assessed by fluorescence correlation spectroscopy (FCS), measuring lipid mobility separately in each leaflet. To check the compatibility of the method with the most common protein reconstitution approaches, we also report the production of asymmetric SLBs (aSLBs) in presence of a membrane protein, reconstituted both via direct protein insertion (i.e. directional insertion) and proteoliposomes fusion.
aSLBs are a suitable membrane model system to study phase separation and transbilayer lipid movement of raft-mimicking lipid mixtures. Although their phase behavior was extensively investigated, the effect of compositional asymmetry on phase separation remains unclear. To address this question, we compared SLBs and aSLBs with the same overall lipid composition. The observed differences in terms of phase separation provide further experimental evidence that the transversal lipid distribution affect the overall lipid miscibility and allow to temporally investigate leaflet mixing (i.e. lipid flip-flop). Additionally, cholesterol distribution between cytosolic and extracellular side of the plasma membrane is of great importance in determining protein-membrane interaction and the formation of raft-like domains in vivo. Therefore, we use a combined FCS and mass spectrometric approach to investigate cholesterol distribution between inner and outer leaflet of SM-containing aSLBs.

446-Pos Board B201
Non-Equilibrium Phase Behaviour in Giant Lipid Vesicles Following Very Rapid Temperature Changes
Lucia Parolini, Will R. Fletcher, Pietro Cicuta.
University of Cambridge, Cambridge, United Kingdom.
Artificial lipid bilayers are useful models of biological membranes, appealing for applicative purposes, and an interesting example of quasi-2D liquid systems on which to investigate new physics. It is well known that ternary-component lipid membranes, depending on the relevant thermodynamic parameters (composition, temperature and pressure), can show phase coexistence between two liquid phases, L_o and L_d, that can be imaged by fluorescence microscopy. We have performed experiments observing both equilibrium and non-equilibrium morphology of lipid phases. Specifically we have investigated a new dynamical regime in which we follow the diffusive mixing of miscible phases, and observe pattern formation out of equilibrium. This has been possible thanks to the development of a method to induce rapid temperature changes, by infrared irradiation. In this fashion, a temperature change can be imposed faster (in about 1s) than the diffusive time over relevant lengthscales (several 10s).
Our observations are that the line tension rapidly vanishes upon heating the system above the miscibility transition temperature. After a few seconds, the spectrum of the interface fluctuations becomes very different from the equilibrium capillary waves, growing in amplitude and losing the characteristic $1/\text{wavevector}^2$ equilibrium form. The interface from then blurs out, resembling fractal growth fronts, until the phases are fully mixed.
The same fast temperature change allows us to very rapidly cool the system. This extends previous measurements by other groups, giving insight into processes that take place over the first few seconds of phase separation.
Investigating these non-equilibrium conditions is relevant because biological membranes in living systems are not in a steady state: they are continually subject to rapid changes in composition, temperature and curvature, and their response is known to couple into a variety of biochemical processes by mediating protein binding and interactions.

447-Pos Board B202
Probing Simultaneously Membrane Dynamics and Protein Activity in Suspended Bilayers in a Microfluidic Format
Verena C. Stimberg, Johan G. Bomer Hans, L. de Boer, Albert van den Berg, Séverine Le Gac.
BIOS, Lab on a Chip Group, University of Twente, Enschede, Netherlands.
Membrane dynamics affect the structure and function of ion channels, a point that deserves more attention while studying membrane proteins. One important factor in the local lipidic environment of the ion channels, is the membrane fluidity which is directly connected to the free diffusion and packing of the phospholipids. Typically, FRAP is utilized to investigate this parameter in simplified cell membrane models, and preferably supported bilayer lipid membranes (BLMs), which are more stable than their free-standing counterparts. However, supported membranes are less suitable for the incorporation of ion channels and electrophysiological monitoring of their activity than suspended ones.
Here, we propose a novel approach for simultaneous FRAP and electrophysiological measurements on suspended BLMs in a microfluidic format. Our platform is suitable for high-resolution imaging providing the bottom substrate is thinned down to < 200 μm, and BLM stability typically exceeds 2 h (Stimberg et al., Small 2013). Gramicidin, whose characteristics (channel activity, lifetime and conductance) are influenced by changes in the lipid environment, is chosen as a model protein, and it is incorporated at a fixed peptide:lipid ratio ($1{:}1.5\text{x}10^7$) in BLMs supplemented with 1% mol NBD-PE. So far, simultaneous FRAP and electrophysiological measurements have been successfully recorded in both DPhPC and L-α-PC BLMs. A ~10 fold increase in gramicidin channel activity (channels/minute) is measured in L-α-PC BLMs compared to DPhPC BLMs, which correlates with an expected lower packing density. Currently, we are optimizing our FRAP protocol to assess lipid diffusion and relate it to the gramicidin activity. In a next step, we will study L-α-PC BLMs with various amounts of cholesterol, using the herein proposed strategy.