investigation and screening of ICs and has been investigated with several model ion channels.
The electrical properties and stability of lipid bilayers suspended across glass micropipettes compared natural and polymerized lipids. Breakdown voltage, capacitance, and conductance of the several pure and mixed polymerizable/non-polymerizable lipid bilayers were determined using a patch clamp apparatus.
Using, polymerizable phospholipids, we have synthesized membranes with markedly enhanced lifetimes from ca. 3 hours to upwards of 3 weeks. These poly(lipid) bilayers have been used to monitor IC activity of alpha-hemolysin for ca. 1week before loss of alpha-hemolysin. However, poly(lipid) bilayers are rigid and do not support the function of ICs that require membrane fluidity, To address this limitation, binary bilayers composed of poly(lipids) and non-polymerizable lipids were investigated. The resulting mixed bilayers demonstrate markedly enhanced long term stability compared to non-polymerized bilayers and facility IC studies. Alamethicin, a model for voltage-gated ion channels, was shown to be non-functional when reconstituted into homogeneous poly(lipid) bilayers, whereas reconstitution in to mixed bilayers revealed alamethicin activity that and enhanced membrane stability.
A functional, truncated form of the K_{ATP} channel complex, 6xHis-EGFP-Kir6.2d26, was chosen as a model ligand-gated IC, expressed and purified from yeast. Long-term goals are to reconstitute the truncated version of K_{ATP} channels into polymerizable and non-polymerizable bilayers using varying strategies into biomimetic sensing platforms screening ligands and drug candidates for activity.

1495-Pos
Solid-Supported Bilayer Lipid Membranes from Lipid Mixtures: Structure and Composition
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Biological membranes are of overarching importance for all aspects of cell structure and function in living organisms. Planar tethered bilayer lipid membranes (tBLMs) are synthetic membrane models stabilized by the proximity of a solid substrate that enhances its long-time stability by orders of magnitude.[1,2] A nanometer-thin hydration layer between the bilayer and the substrate ensures that the biomimetic lipid membrane remains fluid with in-plane lipid dynamics similar to that in vesicles.[3] In this work we establish tBLMs composed of binary and ternary lipid mixtures as more complex, and hence more realistic, membrane models. Such membranes may be used for studies of protein-membrane interactions.[4] Biophysical properties of mixed tBLMs vary significantly with bilayer composition. We report a structural and compositional characterization by neutron reflectometry of tBLMs that comprise various lipid compositions including cholesterol. With specific deuteration of selected bilayer components, such studies enable the determination of volume fractions of individual lipid species in the asymmetric tBLM. A new composition-space model was developed to interpret neutron reflectivity data of such systems. This model enables for the first time to extract more detailed information about the bilayer leaflet proximal to the substrate and lets us explore in more detail the distribution of lipid components across the bilayer. Such a detailed structural and compositional assessment is the prerequisite for more detailed studies of the association of amyloid-beta oligomer particles with membranes in studies on the origin of Alzheimer's disease.[4]
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1496-Pos
Condensing and Fluidizing Effects of Gangliosides on Various Phospholipid Films
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In model membrane mixtures that mimic lipid raft compositions, the more ordered domains are enriched in the ganglioside, G_{M1}, which contains four neutral sugars and a negatively charged sialic acid. To understand the organization and partitioning of G_{M1} in cell membranes, the outer leaflet of the cell membrane was modeled using Langmuir monolayers of DPPC and varying concentrations of G_{M1}. At low biologically relevant concentrations, G_{M1} condenses the DPPC monolayer while at higher concentrations, it fluidizes, with a switch-over point between the two behaviors at a ratio of 3:1 DPPC:G_{M1}. Atomic force microscopy performed on deposited monolayers indicated that G_{M1} is located in nanoscale clusters within the condensed DPPC domains. The total surface area of these nanosize domains is larger than that attributable to G_{M1} molecules alone, suggesting the regions are due to G_{M1} and DPPC packing preferentially in condensed complexes due to variations in molecular geometry.
To further study this effect, geometry of the phospholipid was varied. The zwitterionic lipid, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), with its smaller headgroup cross-sectional area compared to DPPC, was combined with various ratios of G_{M1}. Additivity plots constructed for the mixtures to show deviations from ideal mixing indicated a 3:2 DMPE:G_{M1} ratio was most condensed compared to the individual components. Molecular geometry of the phospholipid headgroup plays a role in the condensation effect of G_{M1} on neighboring phospholipids. Additional experiments on certain monolayer mixtures of 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE) and G_{M1}, components that are each fluid in pure monolayers, showed formation of condensed domains. This indicates the condensation effect of G_{M1} is strong enough to induce biologically relevant ordering phase transitions. Results will also be shown from experiments combining phospholipids containing negatively charged phosphatidylglycerol headgroups with G_{M1} to show the effect of electrostatic repulsion on the induced condensation.

1497-Pos
Determining the Water Content of Lipid Membranes by Neutron Diffraction
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Knowledge of the structure of fluid lipid bilayers is essential for understanding complex biological phenomena in cellular membranes. Water, in particular, is an important component of the membrane since membrane proteins anchor and function in cellular membranes through interactions with water and lipid polar headgroups. Here we present an experimental method to determine directly the content of water in a membrane, at thermodynamic equilibrium with its environment, which does not require knowledge of the density of water. Neutron diffraction and specific lipid deuteration is employed to determine the number of waters in a unit cell (lipid alone or lipid/peptide and lipid/cholesterol mixtures) of oriented lipid multilayers hydrated from water vapor phase, under various humidity conditions. Having determined the number of deuterium atoms per lipid by Mass Spectroscopy, the number of water molecules per lipid can be determined with high precision by neutron diffraction, using the content of deuterium in the sample as a calibration measure. The number of water molecules per unit cell will be presented for a few lipid types (phosphocholines or charged-headgroup lipids), and compared with results obtained by other methods. The extent to which the water held in a membrane is altered by the presence of cholesterol or a voltage-sensing trans-membrane peptide will be demonstrated.

1498-Pos
Observation of Intermediates in Lamellar to Cubic Phase Transformations of Lipid Nanoparticles
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Self-assembled lipid systems have recently come to prominence in medical applications as potential carriers for a range of bioactive agents (1); these include medical imaging, therapeutic compounds. One of their primary advantages is versatility as they can be adapted to different agents and target sites. During the preparation of cubic phase lipid nanoparticulate dispersions, we observed the presence of new intermediates during the transition from the fluid lamellar lyotropic phase to the cubic phase. This phase transition is regarded as a model for membrane-fusion processes(2). Many organelles demonstrate highly ordered cubic membrane structures. Determining the mechanistic origins of such lipid organelle complexity has been elusive.
We increased the lifetime of very short-lived non-equilibrium intermediate structures by the use of steric stabilizer in the dispersions. These structures were characterized using synchrotron small-angle X-ray scattering and

cryo-transmission electron microscopy. We visualised the intermediate initial bilayer contacts and stalk formation, followed by pore development, pore evolution into 2D hexagonally packed lattices, and finally creation of 3D bicontinuous cubic structures (3).
In a biological context, the experimental corroboration of transitional lipid self-assembly structures furthers the understanding of organelle morphogenesis and maturation.
The ability to manipulate intermediate structures in nanoparticulate dispersions of self-assembled structures may provide a unique system for encapsulation and controlled release of bioactives. The capability to control intermediate transformations may also permit the development of flexible growth media for applications such as in-cubo integral membrane protein crystallization or liquid crystal templating of nanostructured materials.
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1499-Pos
Native Pulmonary Surfactant Membranes in Mice Show Coexistence of Two Different Phases in Bilayers and Monolayers: When the Lipid Composition can Predict the Structural Phase Segregations
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Pulmonary surfactant is a surface active material composed both of lipids (aprox. 90% by weight) and proteins (aprox. 10%) produced by type II pneumocyte cells in the alveoli. This tension-active material forms a unique air-liquid interface at the alveolar cell surface that reduces surface tension close to 0 mN/m and maintains lung volumes and alveolar homeostasis at the end of the expiration. There are four pulmonary surfactant proteins (SP-A, -B, -C and -D). SP-A and -D have an important role in the immunological response against pathogens. The particular lipid composition of the lung surfactant suggests that surfactant mono- and bi-layer-based structures could exhibit lateral phase segregation at physiological temperatures. This work shows that in native pulmonary surfactant membranes a close lipid compositional study is crucial to understand the structure and biophysical function of these complex mixtures. Observing Giant Unilamellar Vesicles under conventional and two-photon excitation microscopy allow us to characterize and quantify the coexistence of two fluid-like phases in the wild-type (wt) native pulmonary surfactant membranes and a gel/fluid-like segregation pattern in the Knocked-out protein D (KOD) membranes. The atomic force microscopy studies of supported Langmuir-Blodgett bilayers and monolayers at different surface pressure show the same phase pattern before the collapse surface pressure of native pulmonary surfactant (~40mN/m). Above this surface pressure different protruded structures can be observed arising from the more fluid phases. A closer look at the lipid composition reveals a higher content of saturated phospholipid species in the KOD native pulmonary surfactant membranes. This last finding explain the coexistence phenomena observed and allow us to conclude that the pulmonary surfactant segregation pattern could be predicted by an accurate lipid compositional study.

Membrane Receptors & Signal Transduction I

1500-Pos

WITHDRAWN.

1501-Pos
Quantitative GPCR Assay Using Time-Resolved Fret
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Quantitative study of membrane proteins presents considerable technical challenges. Here, we report a time-resolved fluorescence resonance energy transfer (TR-FRET) assay to quantify functional Chemokine (C-C motif) Receptor 5 (CCR5). CCR5 is a seven-transmembrane helical G protein-coupled receptor (GPCR) expressed primarily on immune cells. It is of significant interest due to its role as the co-receptor of R5-tropic HIV-1. The TR-FRET assay exploits energy transfer between a long-lived europium cryptate donor fluorophore and an appropriate acceptor. We have developed a homogeneous sandwich-type immunoassay with labeled antibodies against a conformationally-sensitive epitope on the extracellular domain of CCR5 and an engineered C-terminal 1D4-mAb epitope. The assay yields a quantitative FRET signal corresponding to the total amount receptor. To quantitate ''functional'' receptor, a labeled anti-HA (hemagglutinin) antibody against an engineered N-terminal HA epitope is used in conjunction with labeled MIP-1a, a chemokine ligand for CCR5. We are also interested in quantifying the degree of chemical labeling of unnatural amino acids incorporated into expressed GPCRs by amber codon suppression technology. The Staudinger-Bertozzi ligation links a commercially-available FLAG-phosphine reagent to p-L-azidophenylalanine. A europium-labeled anti-FLAG antibody can then be used in another version of the sandwich assay. The resulting FRET signals are directly proportional to amount of total, functional, and labeled receptor, but must be calibrated precisely to extract an absolute concentration. Calibration is accomplished by measuring binding of a fluorescent derivative of a small-molecule CCR5 antagonist. The assay is highly specific due to the long lifetime of the europium donor, and nanomolar concentrations of receptor are detectable. This GPCR assay technology can be used to optimize CCR5 reconstitution conditions and can be readily extended to other members of the chemokine receptor family.

1502-Pos
Effects of Sensory Rhodopsin II Complexation with its Cognate Transducer HtrII on the Local Environment of Internal Water Molecules
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Recent studies indicate that internal water molecules play a critical role in membrane protein function. Here we report evidence that the local environment of one or more internal water molecules in sensory rhodopsin II (SRII) is altered by interaction with its cognate transducer HtrII. The SRII-HtrII complex mediates blue-light repellent phototaxis in halophilic archaea, using a signaling pathway similar to that in bacterial chemotaxis. We studied the photocycle of SRII and a SRII-HtrII fusion complex from *Natronobacterium pharaonis* using low-temperature static and room temperature time-resolved Fourier transform infrared (FTIR) difference spectroscopy. When cooled to 80 K, and illuminated the protein is trapped in its K state. A shift of 2 cm^{-1} between SRII (3626 cm^{-1}) and SRII-HtrII (3628 cm^{-1}) is found for a negative OH stretching band assigned to an internal water molecule, most likely located near the active Schiff base. In contrast, the OH stretching band for this water in the K state appears at the same frequency (3619 cm^{-1}) for both the free and complexed receptor. Similar shifts are observed upon hydration with H_2O^{18} shifted to a lower frequency confirming these bands arise from the OH stretching mode of water. Data are also presented on the effects of lipid environment on structural changes of internal water molecules and the receptor-transducer interactions. This work was supported by National Institutes of Health Grants
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1503-Pos
Exploring the Mechanics and Energetics of Epidermal Growth Factor Receptor Activation
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The activation of epidermal growth factor receptor (EGFR) is a complex molecular process. To date, because of technical limitations in dealing with a full-length receptor construct, our understanding of this process comes from structural and biochemical studies of isolated fragments of the receptor. Here we seek detailed molecular insight into the activation process of EGFR in the context a full-length receptor construct. To be able to handle computationally such a large protein (~1000 amino acids) we have developed ELNEDIN: a coarse-grained modeling approach that can describe reliably the dynamics and interactions of proteins. We have built full-length models of human EGFR in the active (extended) and inactive (tethered) states. The models include explicit representations of the lipid membrane and aqueous environments. The conformational space of the tethered and extended state at equilibrium was sampled extensively in the microsecond time scale using a combination of classical molecular dynamics and enhanced sampling techniques. Using these enhanced sampling techniques we have also generated paths that describe the transition from the tethered to the extended state, i.e. the activation of the receptor. These paths have yielded a clear molecular picture of the sequence of conformational changes that lead to activation of EGFR. This picture is remarkably consistent with that derived from experimental approaches, but it also provides new insights into the activation process. Notably, it shows how the conformational changes that occur on the extracellular side of the membrane affect the structure dynamics of the intracellular components of the receptor. Finally, the free-energy surface associated the activation was obtained