MβCD. Significant large-scale self-clustering of IgE-FcεRI occurs upon cross-linking with multivalent antigen, and we describe analytical methods to correct for multiple gold binding and quantify clustering in these stimulated cells. Our correlation function particle distribution approach is likely to have wide applicability in nanoscale image analysis.

1490-Pos

Design of a Biologically Relevant Supported Bilayer Platform for the Study of Membrane Active Peptides

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Membrane active peptides represent a class of soluble proteins that interact and disrupt the plasma membrane. Examples of these include antimicrobial peptides, cancer therapeutics, and cell-penetrating peptides. These peptides are amphipathic and, in a concentration dependent manner, can self assemble to destabilize the lipid bilayer. These peptides are rich in positively charged lysine and arginine residues and thus have a strong preference for negatively charged bilayers. In order to study the insertion mechanism and kinetics of these peptides, we have designed a negatively charged, supported bilayer platform on silicon. The negative charge serves to electrostatically drive peptides to bind to the lipid bilayer interface. Furthermore, this platform is electrically addressable through electrochemical impedance spectroscopy, which yields bilayer resistance, thickness, and structural heterogeneity data. This platform consists of an asymmetrical bilayer with 10 mol% negatively charged POPS, cholesterol, and POPC in the upper leaflet and DPhPC lipids in the lower leaflet, all supported by a PEG cushion on a silicon wafer. Resistances up to $2*10^4$ Oημ cm^2 and capacitances of 0.8 υF cm^{-2} have been measured for the platform. The high resistance allows for high accuracy in the detection of the activity of membrane active peptides of interest.

1491-Pos

***Staphylococcus aureus* Enriched in Ordered Lipids Present Resistance Towards the Antibacterial Agent sPLA$_2$-IIA: An Unusual Mechanism to Survive**

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Bacterial membranes present solid-ordered/liquid-disordered (*so/ld*) cooperative melting event close to physiological temperature. The cellular advantage of this thermotropic melting event is yet to be determined. We show that this thermal behavior provides resistance towards a membrane active antibacterial agent.

Phospholipase A_2 type IIA (sPLA$_2$-IIA) is a hydrolytic enzyme which presents antibacterial properties towards Gram positive bacteria. The enzyme has higher activity in *ld* phase compared to *so* phase in anionic membranes. We show that the lipid phase behavior of Staphylococcus aureus (*S.aureus*) membranes as measured by FTIR modulates sPLA$_2$-IIA by inducing a sharp drop in activity below the melting temperature of the membrane (centered at 15.3°C).

The effects of sPLA$_2$-IIA treatment on cell viability are also investigated. While above the main melting event viability drops to 20% of the initial CFU after treatment, below the main melting event cell viability only drops to 60% under the same treatment. This strongly suggests that cells in the solid-ordered phase are better adapted to survive the enzymatic insult.

These results led us to explore if a subpopulation of *S.aureus* enriched in ordered lipids can be selected after repeated treatment with sPLA$_2$-IIA at 37°C. After selecting for resistance at 37°C we measured growth curves, membrane order, and cell viability as a function of treatment temperature. The results suggest that at 37°C there is a bacterial subpopulation with increased membrane rigidity that insures survival of the colony to an insult by sPLA$_2$-IIA. This subpopulation also presents a longer latency period which can be explained by the increased presence in ordered lipids, which are known to inhibit cell division. Even if the growth conditions are not optimal, the presence of this subpopulation ensures survival from the antibacterial insult.

1492-Pos

Lifetime of Hyaluronan Containing Tethers Obeys a Generalized Bell Model

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Hyaluronan (HA), an unbranched non-sulfated glycosaminoglycan, is an important component of the extracellular and pericellular matrix of various cell types. It has key roles in many biological processes such as wound healing, angiogenesis, embryonic development, tumor progression and invasion. HA is especially abundant around tumor cells in malignant gliomas where it is associated with high invasivity and a poor prognosis. However it is unknown how malignancy is correlated with the biomechanical properties of the cellular glycocalyx and the lifetime of the chemical bonds formed by HA with its ligands. Here we introduce a method applicable to the study of biophysical properties of cellular glycocalyx through tether extraction. Specifically, we reveal the extent of the cellular ECM of a glioma cell line (HB), we demonstrate that tethers formed through non specific binding can be pulled from the cellular glycocalyx and by using a magnetic tweezers we determine the lifetime of these tethers. To calculate lifetime we simultaneously extract multiple tethers under constant force using paramagnetic beads as force transducer. We demonstrate that the stochastic lifetimes of these tethers and thus the bonds they are associated with are exponentially distributed and can be parametrized by a generalized Bell model. We determine the maximum likelihood estimates of the relevant parameters, such as force-free dissociation constant and reactive compliance. We test the consistency of our approach using computer simulations. This method could be employed in the development of therapies which interfere with HA organization and HA-receptor binding.

1493-Pos

Analytical Derivation of Thermodynamic Properties of Bolalipid Membrabe

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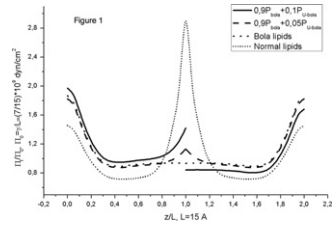
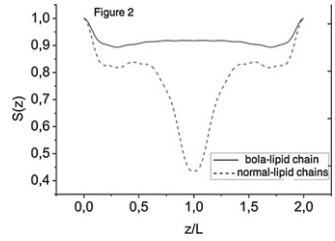
A model of bilayer lipid membrane with bola-lipids is studied. The bola-lipid is modeled by linking tails of the hydrophobic chains in the opposite monolayers within bilayer. We use for analytical derivations a flexible string model of hydrocarbon chain (Mukhin, Baoukina 2005) with modified condition at the linked chains ends. Calculated lateral pressure profiles are asymmetrical due to different concentrations of the U-shaped bolalipids in the opposite monolayers, Fig. 1, and orientational order parameters for linked and regular chains differ significantly at the monolayers interface, Fig. 2.





1494-Pos

Highly Stable Poly(Lipid) Bilayers for Long-Term Ion Channel Recordings

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Long-term ion channel (IC) screening using cell-based assays is currently limited by throughput and cell to cell variability. ICs isolated and reconstituted into suspended lipid membranes offer an isolated view into IC structure and function, but IC recordings are limited by the short lifetime of the bilayer. Polymerizable lipids (poly(lipids)) offers one potential strategy for long-term

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