3457-Pos
Ceramide Gel Domain Formation in a Phospholipid Bilayer: The Impact of Ceramide Acyl Chain
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Nowadays, it is recognize the critical role of Ceramide (Cer) in signalling pathways that leads to cell proliferation, apoptosis, growth and inflammation. It is believed that the biological action of Cer depends largelly on its biophysical properties, e.g. the formation of Cer platforms in cell membranes was suggested to lead to the activation of apoptotic signaling pathways. Moreover, it has been proposed that Cer containing different fatty acids have distinct impact upon cell physiology. Regarding these observations, the biophysical behaviour of long and saturated (C16:0, C18:0) and very long and unsaturated (C24:1) acyl chain Cer in a fluid membrane (palmitoyloleoylphosphatidylcholine (POPC)) was studied and compared.
The application of i) fluorescent spectroscopy and ii) confocal fluorescence microscopy showed that Cer acyl chain is the limiting factor on Cer/fluid phospholipd interaction. For instance, at room temperature (24oC) at least 10% of C24:1 Cer are needed to observe lateral segregation, in contrast only 4% of C16:0 and C18:0 Cer are required for microdomains formation. From confocal fluorescent microscopy complex tubular structure, known as cochleate, were only observed for bilayers composed of small amounts of C24:1 Cer (20-30%). Moreover the used of fluorescence correlation spectroscopy for studding N-rhodamine-dipalmitoylphosphatidylethanolamine diffusion in giant unilamellar vesicles (Cer/POPC), revealed a higher capacity of C16:0 and C18:0 for ordering fluid areas surrounding Cer platforms. Altogether, these observations support the suggestion that each Cer are involved in distinct cellular processes and membrane physical properties are a key mechanism for cell metabolism regulation.

3458-Pos
Probing Cholesterol Dependent Lipid Properties Using Molecular Dynamics Simulations of BODIPY-PC in Explicit DMPC and DPPC Membranes
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Dunn and co-workers have used polarized total internal reflection fluorescence microscopy to measure the tilt angle of a single molecule (BODIPY-PC) relative to the membrane normal to characterize the membrane properties. Recently, they found that increasing quantities of cholesterol in monolayer and bilayer membrane systems leads to a much smaller tilt angle of the BODIPY-PC molecule. To better understand the influence of cholesterol on membrane properties and its relation to BODIPY-PC tilting, we have performed molecular dynamics (MD) simulations of BODIPY-PC in explicit dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphocholine (DMPC) monolayer and bilayer membrane systems with systematically increasing concentrations of cholesterol (0, 5, 10, 15, 20, 33, 50%). After 4 ns of equilibration, each system has been simulated for another 16 ns. Preliminary results indicate that the BODIPY-PC tilt angle decreases as a function of increasing cholesterol concentration until 10% cholesterol after which there appears to be cholesterol saturation and the rigidifying affect ceases. Other points of interest include confirming the applicability of monolayer data for bilayer systems and explaining any quantifiable differences between the DPPC and DMPC membrane systems. We will present the orientation (azimuthal and rotational angle distribution) of BODIPY-PC, the BODIPY-PC tilting energetics, various membrane properties, and the spatial distribution of the cholesterol as a function of cholesterol concentration.

3459-Pos
Dynamics of Phase Separation in Lipid Membranes
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We present a continuum method to simulate the large-scale dynamics of multicomponent model membranes, as observed in various recent fluorescence microscopy experiments. Our model includes both thermal fluctuations and the quasi-two-dimensional hydrodynamics appropriate to a membrane immersed in an outside fluid, which are crucially important for the simulation of membrane domain flickering. Using this coarse-grained scheme allows us to simulate length scales up to tens of microns, and time scales of seconds. We apply our model to phase separation, domain fluctuations, and diffusion of domains in lipid systems, and show agreement between our simulations, experiments, and theories for known limiting cases.

3460-Pos
Molecular Dynamics Simulations of In-Plane Density Fluctuations in Phospholipid Bilayers
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At room temperature many lipids form the bilayer structure of biological membranes, which resembles liquid crystals. The structural features of the fluid lipid bilayer are well-known, but many details of the dynamic behavior of biological membranes still remain to be understood.
Molecular motions in liquids can be monitored by the intermediate scattering function, F(q,t). In membranes it probes the propagation and decay of in-plane density flutuations at wave vector q. An attractive property of the intermediate scattering function is that it can equally well be determined from scattering experiments as from molecular dynamics simulations, opening the possibility of direct comparison between experimental and simulation data. Density fluctuations in lipid bilayers can stay correlated for hundreds of nanoseconds which implies that in contrast to simple liquids, an exponential decay of F(q,t) as suggested by a purely single diffusive model, does not describe how fluid membranes behave. Microsecond molecular dynamics simulations on thousands of lipids, both atomistic and coarse-grained, has been used to explore F(q,t). The atomistic simulations span membrane patches of tens of nanometers while the coarse-grained simulations, including almost 100 000 lipids, reach the low micrometer domain. The main purpose was to establish a dispersion relation for the density fluctuations, i.e. a relation between the wave vector and the decay rate. Different model functions are compared to find the dispersion relation that best fits the simulation data. The important question of how material properties (bending modulus and area compressibility) are related to the molecular structure of the complex liquid is adressed in the context of the different models.

3461-Pos
Age Dependent Deformability Changes of RBCS Evaluated Using a Cyclically Reversing Shear Flow Generator
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In this study, using a cyclically reversing shear flow generator we studied deformability of blood cells separated into young and old RBCs. We investigated the relationships between aging of RBCs and its deformability sheared by a cyclically reversing flow.
To study the effect of aging of cells on their mechanical properties, RBCs were separated by centrifugation according to their density. The sampled RBCs were then mixed with Dextran solution to prepare 0.15% Hematocrit RBC suspension.
The RBC suspension was placed between the two parallel glass plates separated by a gap distance of 30μm under a microscope; with the bottom plate stationary, the upper plate made a reciprocal motion by cam-motor machinery (the amplitude and frequency of 1.25mm and 2, 4, and 6 Hz, respectively) creating a quasi-Couette flow of RBC suspension with the peak shear stress of 37.46 Pa. The images of RBCs were magnified by a ×40 objective lens combined with a×1.6 middle lens, and recorded by a high speed video camera. Subsequently, the RBCs images have gone through averaging, digitization, and then morphological analysis of their properties in terms of the major (L) and minor (W) axes of the projected two dimensional figures, yielding the deformation index of L/W.
The L/W of both groups of RBCs followed closely the shear stress changes, exhibiting asymmetrical response patterns consisting of fast deformation or stretching and slow recovery phase. The mean L/W of young RBCs was 1.096 ± 0.0079 times that of the old ones ($p=0.0003$).
Our preliminary study indicated more rigid membrane properties in the old RBCs as compared with the young cells at three different shearing frequencies.

3462-Pos
Biomimetic Metallic Electrodes for Intracellular Electrical Measurements
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Electrical interfaces represent a powerful approach for interrogating or perturbing biological systems, with applications in neural prostheses, the regulation of artificial neuronal networks and arrayed patch clamp diagnostics. A key step towards achieving this interface is the development of inorganic nanostructures that can specifically and non-destructively incorporate into biological membranes. Here, we report the development of nanoscale metallic posts that mimic transmembrane proteins, allowing their spontaneous insertion into lipid membranes. These electrode posts were formed by metal evaporation with

a nanoscale gold band that can be functionalized with alkanethiols to be hydrophobic. We describe nanoscale electrical measurements with these post-electrodes on cells and demonstrate Giga-ohm seal formation at the electrode-membrane interface. Moreover, we use coarse-grained molecular dynamics simulations to elucidate the mechanism and structures of electrode-membrane interface formation.

3463-Pos

Characterizing the Structure and Dynamics of Nanodisc Lipid Bilayers of Different Compositions

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One of the key roles of the cellular membrane is the regulation and activation of membrane-anchoring proteins. The lipid composition of the membrane and the ionic content of the immediate solution significantly modify structural properties of the bilayer surface. Nanodiscs are lipoprotein particles of precisely controlled size and composition that proved to be valuable in experimental studies of protein-membrane interactions, for example in studying membrane binding and activation of blood coagulation factors. The enzymatic activity of several coagulation factors is regulated by their binding to anioic regions of the cellular membrane. Nanodiscs consist of a patch of lipid bilayer encircled in a belt-like fashion by a pair of amphipathic helical membrane scaffold proteins (MSP) and can serve as a membrane model. The structure and dynamics of lipid molecules in Nanodiscs are highly relevant to their physicochemical properties, and to the mode of interaction between Nanodiscs and membrane-anchoring proteins and peptides. We employ molecular dynamics simulations to investigate these aspects in solvated Nanodiscs. Extended simulations (on the order of 10s of nanoseconds) with Nanodiscs including anionic phosphatidylserine (POPS), zwitterionic phospatidylcholine (POPC), or POPS/POPC binary mixtures provide for detailed analysis of structural changes that occur due to lipid-lipid and lipid-ion interactions. The methodology supplies us with the atomic level description sufficient to investigate whether MSP influences the boundary lipids. Simulations revealed stable particles of consistent geometry. Presence of divalent cations Ca2+ shows its coordination with lipid head groups and modulates their orientation in the membrane bilayer, thus, preparing the stage for interaction with proteins.

3464-Pos

Inter-Membrane Adhesion Mediated by Mobile Linkers: Effect of Receptor Shortage

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Cell adhesion is a complex process essential for life. It is mediated by specific binding between cell surface proteins that eventually cluster and form supramolecular structures. However, the initial steps of cell adhesion, where physical forces can be expected to dominate over active processes, are barely understood.

Aiming at a rigorous analysis of the physical effects induced by membrane adhesion we developed a simplified passive model system. It consisted of a giant unilamellar vesicle (GUV) adhering via specific biotin-neutravidin interactions to a supported lipid bilayer (SLB). Receptors and ligands diffused freely within the plane of the respective membrane. A new microscopy set-up was developed enabling simultaneous imaging by reflection interference contrast microscopy (RICM) and fluorescence microscopy as well as determination of molecular diffusion by continuous photobleaching.

At high receptor concentrations we found GUV adhesion changed SLB fluidity as well as receptor mobility and distribution. The adhering membrane caused homogeneous accumulation and immobilization of the initially mobile receptors. Due to the introduction of these obstacles in the SLB its fluidity decreased significantly as well. Friction to the tightly bound GUV membrane furthermore enhanced the reduction of SLB fluidity.

At low receptor concentrations a characteristic ring-like accumulation pattern emerged. Due to the low efficiency of receptor diffusion at large distances receptors were accumulated only at the edge of the adhering GUV. In addition, GUV adhesion was found to be incomplete. The balance between the loss of translational entropy of the receptors and the gain in Gibbs'free enthalpy by receptor-ligand binding determined the final adhesion state.

The present results suggest that in addition to employing different receptor/ligand pairs, cells may regulate cell-cell adhesion by careful control of the receptor surface concentration.

3465-Pos

Collective Membrane Dynamics under Osmotic Stress

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Phospholipid membranes are highly dynamic, ordered structures that involve molecular motions of phospholipids together with collective fluctuations of the bilayer [1]. Membrane structural dynamics on these length scales are sensitive to changes in properties such as temperature, pressure, and chemical potential. Structural deformation accompanying the removal of water from the membrane is well characterized, yet perturbation of membrane dynamics under osmotic stress conditions has not been studied. Here we show that membrane dynamics as revealed by ^{2}H NMR relaxation measurements are sensitive to osmotic stress. Specifically, we measured the segmental order parameters (S_{CD}) and ^{2}H spin-lattice relaxation rates (R_{1Z}) over a broad range of hydration levels. Empirical correlations of acyl chain S_{CD} and R_{1Z} profiles follow a theoretically predicted square-law functional dependence. However, for a given acyl position R_{1Z} is essentially independent of S_{CD} as the hydration water is varied. This is expected if the correlation length of the collective and segmental fluctuations remains unperturbed. The fast segmental fluctuations are decoupled from larger amplitude lipid motions within the osmotically stressed membrane. This result contrasts with studies involving cholesterol, where variations of S_{CD} on the order of those observed in the osmotic stress experiment lead to significant reductions in R_{1Z} rates [2]. In this case, interaction with cholesterol couples local segmental dynamics to collective viscoelastic modes. These results show that the relation of S_{CD} to R_{1Z} is a characteristic marker of lipid matrix composition and collective lipid interactions. Furthermore, our results highlight intrinsic differences in the sensitivity of membrane dynamics, as may be encountered for peripheral protein-membrane interactions and integral membrane-lipid interactions. [1] M.F. Brown and S.I. Chan, *Encyclopedia of Nuclear Magnetic Resonance*, Wiley, New York 1996, 871-885. [2] G.V. Martinez *et al.* (2004) *Langmuir* **20**,1043-1046.

3466-Pos

Differential Effect of Isoflurane on the Anisotropy of Diphenylhexatriene and its Cationic Trimethylamine Analog

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The mechanism of action of volatile general anesthetics has not yet been resolved. Recent developments in the understanding of lipid physics, including the discovery of microdomains and computing the lateral pressure profile in simulations, suggest a need to revisit possible indirect effects of lipids on ion channels during anesthesia. We present strong experimental evidence that volatile general anesthetics localize at the headgroup region of a lipid bilayer and therefore increase lateral membrane pressure near the bilayer surface. Theoretically, this increase in pressure in the head group region, in conjunction with a decrease in pressure in the tail group region, may induce conformational changes in ion channels to produce the characteristic effects of volatile general anesthetics. To examine this idea, the anisotropy of fluorophores localized in either the head (trimethylamino-diphenylhexatriene, TMA-DPH) or tail group regions (DPH) of small unilamellar vesicles of dipalmitoylphosphatidylcholine was assessed. These measurements were repeated at multiple temperatures between 20 and 55 °C in the presence or absence of various concentrations of the anesthetic isoflurane. In treated samples, the main phase transition (41.5 °C) was shifted down by 2 to 10 °C depending on the concentration of isoflurane (3.8-13.0 mM). Melting reduced anisotropy by 0.1 (TMA-DPH) or 0.2 (DPH) units. Interestingly, isoflurane caused opposite changes in anisotropy of the two probes in the liquid crystalline phase: DPH anisotropy decreased by ~0.02 units whereas TMA-DPH anisotropy increased by the same magnitude. This observation suggests that isoflurane partitions into the headgroup region of the bilayer where it increases lateral pressure, while reducing it in the tail region.

3467-Pos

Deformation of Vesicles Controlled by Local Spontaneous Curvatures of Membrane

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The metabolic pathways are fundamental processes to maintain the life, which is supported by the cell membrane deformations such as, membrane adhesion, fusion and pore formation. In the present living organisms, the membrane