Membrane Dynamics & Bilayer Probes

365-Pos Effects of Surface Charge Density of Lipid Membranes on the Pore Formation Induced by Magainin 2: the Single GUV Method Study

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Board B198

Interactions of antimicrobial peptides with lipid membranes have been investigated using a suspension of small liposomes, and their details remain unclear. Recently we have proposed a novel method, the single GUV method; we observe and measure physical properties of single GUVs, and analyze these results over many single GUVs statistically, which will provide much new information that cannot be obtained by the conventional LUV suspension method [e. g.,1]. Using the single GUV method, we have succeeded in revealing elementary processes of the pore formation in lipid membranes induced by magainin 2 [2]. In this report, to elucidate the mechanism of the magainin 2-induced pore formation, we investigated the effect of surface charge density of membranes on the pore formation.

To change the surface charge density, we controlled negatively charged DOPG concentration in DOPG/DOPC membrane from 30 to 60 mol%. We found that, in all kinds of GUVs, magainin 2 induced a rapid leakage of calcein from single GUVs, showing that magainin 2 formed pores in the membrane. For GUVs with the same charge density, the fraction of leaked GUV, P_{LS}, increased with time, and P_{LS} at a fixed time increased with magainin 2 concentration. The magainin 2 concentration at $P_{LS} = 0.5$ at a fixed time increased with a decrease in the surface charge density, indicating that higher concentrations of magainin 2 in a buffer were required to induce the pore formation in GUVs with lower surface charge density. Using the Gouy-Chapman theory, we obtained magainin 2 concentration in the membrane interface. On the basis of these results, we discuss the mechanism of the magainin 2-induced pore formation.

References

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366-Pos Diffusion on Ruffled Membrane Surfaces

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Board B199

We present a position Langevin equation for overdamped particle motion on rough two-dimensional surfaces. A Brownian dynamics algorithm is suggested to evolve this equation numerically, allowing for the prediction of effective (projected) diffusion coefficients over corrugated surfaces. In the case of static surface roughness, we find that a simple area-scaling prediction for the projected diffusion coefficient leads to seemingly quantitative agreement with numerical results. To study the effect of dynamic surface evolution on the diffusive process, we consider particle diffusion over both thermally and actively fluctuating elastic membranes. Thermal fluctuation has the effect of increasing the effective diffusivity toward a well-known limiting annealed-surface value. We argue that protein motion over cell surfaces spans a variety of physical regimes, making it impossible to identify a single approximation scheme appropriate to all measurements of interest.

367-Pos Phase Transition Behavior And Lipid Interactions In Myelin Sheath

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Board B200

Myelin is a stacked membrane structure that allows for fast, efficient conduction of nerve impulses. It has 8 kinds of lipid molecules on two alternating bilayers and proteins such as Myelin Basic Protein (MBP) which has an important role in maintaining myelin structure. The compact bilayer organization of healthy myelin is believed to require a well-defined range of lipid and protein composition, and bilayer-bilayer interaction. Even though we know that multiple sclerosis (MS) is a morphological transformation involving loss of adhesion between myelin lamellae and sometimes formation of myelin vesicle, the mechanism and causes of demyelination are still under investigation. We have used fluorescence microscopy, Langmuir isotherms, and the Langmuir-Blodgett technique to investigate how lipid composition of myelin lipid affects the phase transition behavior and structure of myelin monolayers and bilayers depending on lateral pressure, temperature, and pH. We currently study the topographic changes and the interactions of two symmetrical myelin bilayers in the absence and presence of MBP isoforms using Atomic Force Microscopy (AFM), and the Surface Force Apparatus (SFA) techniques. The acute experimental allergic encephalomyelitis (EAE) in the common marmoset which is a highly relevant model of MS was used as a comparison.

368-Pos Biphenyl Phospholipid Bicelles: A Model Membrane That Remains Macroscopically Oriented Outside Magnetic Fields During Several Days

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Board B201

A phosphatidylcholine lipid (PC) containing a biphenyl group in one of its acyl chains (1-Tetradecanoyl-2-(4-(4-Biphenyl)Butanoyl)-sn-glycero-3-PC, TBBPC) was successfully synthesized with high yield. Water mixtures of TBBPC with a short chain C6 lipid, dicaproylPC (DCPC), lead to bicelle systems formation. Freeze-fracture electron microscopy evidenced the presence of flat bilayered discs of 800 Å diameters for adequate composition, hydration and temperature conditions. Due to the presence of the biphenyl group, which confers to the molecule a positive magnetic anisotropy, the discs align with their normal, n, parallel to the magnetic field B0, as directly detected by 31P, 14N, 2H solid state NMR and also using Small Angle X-rays Scattering after annealing in the field. Temperature-composition and temperature-hydration diagrams were established. Domains where discs of TBBPC/DCPC align with their normal parallel to the field were compared to chain saturated lipid bicelles made of DMPC(dimyristoylPC)/DCPC, which orient with their normal perpendicular to B0 (Raffard, et al. Langmuir, 2000, *16*:7655–7662). TBBPC/DCPC bicelles exist on a narrow range of long vs. short chain lipids ratios (3%) but over a large temperature span around room temperature (10–75°C), whereas DMPC/DCPC bicelles exhibit the reverse situation, i.e., large compositional range (22%) and narrow temperature span (25–45°C) (Loudet et al. Biophys J, 2007, *92*:3949–3959). The two types of bicelles present orienting properties up to 95% dilution. Of great interest, the biphenyl bicelles once oriented by magnetic fields stay macroscopically aligned, outside the field, for days. This opens an entire field for studies of molecules embedded in membranes.

369-Pos Determination of the Phase Boundaries for DOPC:DPPC: Cholesterol Mixtures

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Board B202

^{2}H NMR (Nuclear Magnetic Resonance) spectroscopy provides a quantitative means of measuring molecular order in solid and liquid crystalline lipid phases. Ternary lipid mixtures of DOPC (dioleoylphosphatidylcholine): DPPC (dipalmitoylphosphatidylcholine): cholesterol, when fully hydrated, can form liquid disordered (l_d), liquid ordered (l_o) and gel (g) phases. The liquid disordered phase and the gel phase are commonly observed in bilayers composed only of phospholipids, whereas the liquid ordered phase is observed for some systems that have high cholesterol content. Lipid rafts found in detergent extracts of biological membranes are rich in long-chain saturated lipids and cholesterol, and are thought to be in the liquid ordered phase. Depending on the temperature and sample composition of a ternary mixture of DOPC:DPPC:cholesterol, we can observe any one of the three single phases, or two or three phases in coexistence, using ^{2}H NMR (with chain perdeuteriated DPPC, DPPC-d_{62}). Using 17 different sample compositions, we have mapped out the part of the ternary phase diagram that includes the two and three phase coexistence regions. We will describe both the experimental results and our methods of quantitatively determining the phase boundaries, and present a partial ternary phase diagram for this system. Some results using confocal fluorescence microscopy to visualize domains in GUVs (giant unilamellar vesicles) composed of the same ternary mixture will be compared to the ^{2}H NMR data.

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370-Pos Exploration of the Regulatory Role of Cholesterol Domains in the Interactions of Beta Amyloid Peptide with Multicomponent Lipid Bilayers

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Board B203

Beta amyloid (betaA) is a 39 to 43 residue peptide generated by a proteolytic cleavage of a large transmembrane amyloid precursor protein in neuronal membranes. The misfolding and self-aggregation of this peptide, as well as its interactions with neuronal membranes, have been linked to the early onset of pathogenesis of Alzheimer disease. At present, the role of lateral organization of cholesterol, cholesterol domains with regular or superlattice-like distribution in particular, on the interactions of betaA (1–40) with the lipid bilayer is still unclear. In this study, compositionally homogeneous ternary liposomes consisting of phosphatidylserine, phosphatidylcholine and cholesterol were employed as model neuronal membranes. Using a fluorescent analog of cholesterol, dehydroergosterol (DHE), as a membrane probe, the site-specific pertubation of the lipid bilayer in the presence of monomeric and oligomeric betaA was examined as a function of cholesterol content using fluorescence anisotropy of DHE and FRET (peptide-to-DHE) techniques. Biphasic behavior in the anisotropy and FRET parameters were observed at certain critical cholesterol-to-lipid mole ratios, particularly at 0.40. Regular distribution of cholesterol in lipid bilayers has been implicated at those critical cholesterol contents by previous studies, e.g., Cannon et al., J. Phys. Chem.*110*: 6339 (2006). Atomistic molecular dynamics simulations and circular dichlorism were also used to further explore the structural conformation of the peptide with cholesterol containing bilayers. Our results indicate that the interaction of betaA with lipid bilayer may be regulated by the presence of ordered cholesterol domains with regular distribution at predictable cholesterol contents.

371-Pos Bax-Endophilin B1 Oligomers Trigger Membrane Activity

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Endophilin B proteins belong to a subfamily of endophilins, which are cytoplasmic proteins containing N-BAR domain and a C-terminal SH3 domain. They are involved in regulation of membrane dynamics. In particular, endophilin B1 was shown to play a major role in the maintenance of mitochondrial morphology. It was also shown to interact with the proapoptotic protein Bax and to target mitochondria during apoptosis. Recently, in a yeast two-hybrid screening, B1 was found to associate with a novel protein, endophilin B2. Although B2 is likely to be involved in B1 targeting of the mitochondrial outer membrane (MOM) in apoptosis, little is known about the mechanisms of interactions of these proteins.

In this study we used confocal fluorescence microscopy to determine the interactions of B1 and B2 with giant unilamellar vesicles (GUVs), as a model for understanding possible effects of these proteins on MOM. We found that B2 induces massive shape transition of GUVs prepared with DOPC. This change is manifested by an overall reduction of the size of the liposomes, generation of small vesicles, and liposome fission and fusion. Interestingly, under the same experimental conditions, B1 alone does not exert a similar effect. However, after pre-incubation with Bax, B1 causes extensive vesiculation of the GUVs. We applied fluorescence correlation spectroscopy to probe the interactions between B1, B2 and Bax. We found that with Bax the diffusion time of the B1 increased by almost 4 fold and that this effect was enhanced further at acidic pH. In contrast, there were little interactions between B2 and Bax. These results demonstrate differences between the two endophilins B in their interactions with Bax and lipid membranes. We interpret this finding as Bax-induced aggregation/oligomerization of B1 and speculate about possible mechanisms of B1 and B2 interaction with lipid membranes.

372-Pos Combining FCS and NSOM Enables Analysis of Single-Molecule Dynamics in Sub-Diffraction Limited Volumes

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Board B205

Fluorescence Correlation Spectroscopy (FCS) enables the study of the dynamics of single molecules, e.g. local concentrations, molecular brightness, diffusion coefficients, reaction rates. The prerequisite of FCS is to have only a few fluorescent molecules in the detection volume in order to generate significant fluorescence fluctuations due to single molecule dynamics. As a result the common FCS setup based on confocal microscopy requires pico- to nanomolar concentrations of fluorophore. In biological systems concentrations are more often at a micromolar level. Here we propose a combination of FCS with near-field scanning optical microscopy (NSOM) to overcome this limitation.

Our NSOM system is based on an optical-fiber probe as a source of excitation light. One end of the fiber is tapered into a very sharp tip and the sample is illuminated through a small aperture at the end of the tip. The size of the aperture determines the size of the excitation volume. We can routinely make probes with an aperture diameter down to 50nm. That makes the NSOM excitation volume roughly an order of magnitude smaller than the confocal microscopes' volume.

Here we present the first demonstration of FCS-NSOM. We successfully applied the FCS-NSOM to measure lateral lipid diffusion in mica supported lipid bilayers. Our results highlight the potential of FCS-NSOM applications in single molecule dynamics studies at physiological concentrations in living systems.

373-Pos Characterization of Changes in Membrane Viscosity with the Molecular Rotor FCVJ

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Board B206

Membrane viscosity is a key parameter in cell physiology, cell function, and cell signaling. To examine changes in membrane viscosity, several methods have been developed, the most popular ones being fluorescence recovery after photobleaching (FRAP) and fluorescence anisotropy. Recent interest in a group of viscosity sensitive fluorophores, termed molecular rotors, led to the development of (2-carboxy-2-cyanovinyl)-julolidine farnesyl ester (FCVJ), a molecular rotor that partitions into lipid membranes. The purpose of this study was to examine the fluorescence characteristics of FCVJ in model membranes exposed to various drugs of known influence on membrane viscosity. We show that alcohols, DMSO, and cyclohexane decreased membrane viscosity as evidenced by decreased FCVJ steady-state emission. Conversely, cholesterol and nimesulide increased membrane viscosity as evidenced by increased FCVJ emission. The calculated change in viscosity from changes in fluorescence of FCVJ correlated well with FRAP measurements. In the case of alcohols, the decrease of FCVJ emission depended strongly on the chain length and concentration of the alcohol. Since molecular rotors, including FCVJ, allow for straightforward imaging of viscosity with high spatial resolution and fast response time, this study indicates that FCVJ may be useful to monitor viscosity changes in cell membranes quantitatively.

374-Pos Lateral Diffusion of Model Peptides in Artificial Membrane Systems

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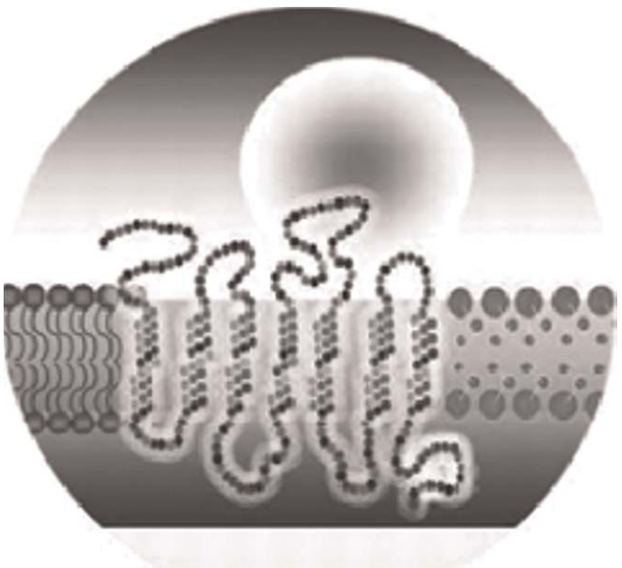
Board B207

The protein-lipid interactions in biological membranes are an interesting field in ongoing research. The importance of targeted dynamics of specific proteins in membrane architectures has been observed, but the control mechanisms and boundary conditions of these movements are still not elucidated.

We present a model system of planar lipid membranes, which allows for systematic analysis of a fluorescently labelled model peptide as probing moiety to understand the influence of synthetic components, mimicking the membrane structure, such as amphiphilic block - copolymers.



375-Pos Configuration-Induced Phase Change at Bilayer Edges

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Board B208

Defects and discontinuities are rarely encountered in supramolecular microphases which form via self-assembly of lipids in water. Rather these amphiphiles adopt a variety of closed shapes and configurations in dilute aqueous solutions determined by their spontaneous curvature or packing parameter. In heterogeneous membranes, however, compositional undulations and interactions with proteins often stabilize topographic defects and pores. Such defects also occur when fluid phospholipids organized as supported bilayers encounter obstacles and diffusion barriers. Monitoring the temperature-dependent fluorescent preference for lipid packing using phase-sensitive lipid dyes, we show that a structurally dense, gel-like phase emerges near these topographic discontinuities in supported lipid bilayers. Furthermore, temperature-programmed Fluorescent Recovery After Photobleaching measurements and Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy of edge-enriched bilayer patterns provide direct evidence for higher effective transition temperature of a multi-edged bilayer generated by UV patterning. Together with the postulated hemimiceller edge structure, these results point to configuration-induced phase change near edges in otherwise homogeneous lipid microphase.

376-Pos Zipper Dynamics Of Merging Lipid Nanotubes

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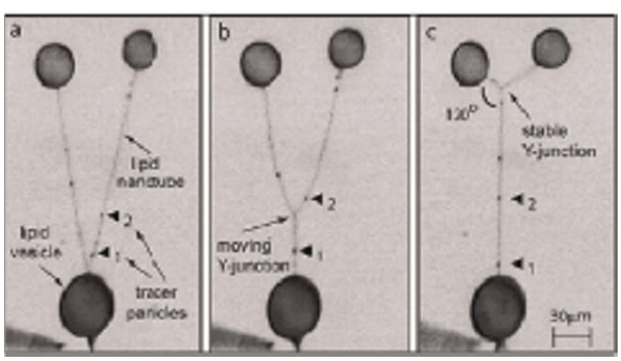
Board B209

Membrane tubulovesicular complexes in biological cells are often connected through three-way junctions. We study the dynamics of such junctions by using a nanotube-vesicle network made of continuous lipid bilayer as a model system. Lipid nanotubes (radius 50–120 nm) are suspended between surface-adhered lipid vesicles (radius 10–30 μm), figure a. When two nanotubes connected to the same vesicle merge, they form a three-way junction, *i.e.* a Y-junction, figure b. Such a junction is unstable and spontaneously moves towards a state with minimum free energy. In the final state the nanotubes are straight and meet at the angles of 120° at the Y-junction, figure c. By using fluorescing membrane aggregates on lipid nanotubes as tracer particles, we investigate the mechanism of nanotube merging and propagation of the Y-junction. From the experimental observations and theoretical modeling we conclude that Y-junction moves in a zipper-like manner. Zipper dynamics implies that lipids from the two merging nanotube branches are flowing through the Y-junction, and reside on the third nanotube branch, thereby extending it. These results are important for understanding shape transitions in cell membrane networks, as well as the trafficking phenomena within and between biological cells.



377-Pos Alterations of Membrane Domain Dynamics in Bone Stem Cells of Mice with Allelic Gene Differences

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Board B210

The plasma membrane surface of bone marrow stromal cells (BMSC) consists of dynamic heterogeneous micro domains; these domains are a critical component of signal transduction. The flask shaped invaginations of the membrane surface known as caveolae, typically range from 50 – 350 nm in diameter and are enriched with sphingolipids, cholesterol, and caveolin proteins. Besides signal transduction these domains are involved with endocytosis and cell differentiation. Current knockout studies of caveolin-1 protein in mice show an overall size difference of bones including increases of both cortical and trabecular bone. However, the molecular mechanism leading to this phenotype is poorly understood.

In our study we characterized the dynamics of caveolae of differentiating BMSCs and the role it takes part in during bone formation. The membrane domain dynamics of differentiating bone marrow stromal cells were studied from three different mice models. Two of the mice models are congenic strains carrying genetic allele differences from Chromosomes 6 and 1 of C3H/HeJ placed on the C57BL/6 (B6) strain, designated B6.C3H-1–12 (1–12) and B6. C3H-6T (6T). The 1–12 and 6T congenics have increased and decreased peak bone mass compared with B6 controls. Utilizing atomic force microscopy (AFM) and Family of Image Correlation Spectroscopy (FICS), we measured the topography and dynamics of the membrane surface of the B6, 6T, and 1–12 BMSCs. Fractal analysis was performed and revealed similar fractal data, while the perimeter was significantly different. The membrane domain dynamics of differentiating BMSCs of three different allelic genetic mice models different from each other. These results suggest that changes in the membrane domain dynamics of BMSCs may be related to differences in peak bone mass characteristics of the congenic strains.

378-Pos Diffusion Of Lipids In Stacked Supported Bilayers

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Board B211

Proteins embedded in the cellular plasma membrane fulfill a large variety of important functions for the life of the cell, such as the transport of molecules or signalization across the membrane. Many of these functions involve the encounter of several partners at the cell surface and are thus closely related to their diffusive properties. In order to unravel the complex dynamics and organization of membrane constituents, it is useful to investigate them in a well-characterized medium. Here, we analyzed diffusion in an original system of *double* supported bilayers, designed in order to avoid the interaction, present in single supported bilayers, of the extra-membranous parts of proteins with the underlying glass coverslip. We first focused on the characterization of this purely lipidic system, enabling us in the future to incorporate membrane proteins in it.

We have finalized a protocol of successive formation of bilayers stacked on a glass coverslip by fusion of liposomes. The formation kinetics and the stability of each bilayer (up to 4) have first been analyzed by Quartz Crystal Microbalance (QCM) as a function of the ionic strength of the buffer. Consecutively, by introducing a fluorescent lipid analog in the upper supported bilayer, we have performed fluorescence recovery after photo-bleaching (FRAP) experiments to measure its diffusion coefficient D. The observed increase of D with the number of stacked membranes suggests a decrease of the interactions between successive bilayers, in agreement with QCM experiments. We propose a theoretical model, taking into account the thermal fluctuations of the membranes, that accounts for the different experimental observations.

379-Pos Domain Formation of POPC/DPPC Binary System Investigated by Fluorescence Resonance Energy Transfer and Monte Carlo Simulations

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Board B212

We measured the fluorescence resonance energy transfer (FRET) between two common membrane fluorescent probes NBD-DOPE, and Lissamine-Rhodamine (LRH)-DOPE in large unilamellar POPC vesicles. Due to a large overlap between the emission of NBD-DOPE and the absorption of LRH-DOPE they form an excellent FRET pair. Characterization of the two probes was accomplished by a calibration of the peak ratio to the energy transfer efficiency. The probes were then used to characterize biophysical properties of the binary system POPC, and DPPC. Both NBD-DOPE and LRH-DOPE prefer to partition into liquid POPC domains. A third probe, NBD-DPPC, however, prefers to partition into solid DPPC domains. Comparing the energy transfers for POPC/DPPC vesicles containing NBD-DOPE and LRH-DOPE, with POPC/DOPC vesicles containing NBD-DPPC and LRH-DOPE clearly demonstrated that domains are formed in the POPC/DPPC

binary system. The decrease in FRET efficiency is a consequence of phase separation. Using a combination of FRET and Monte Carlo simulations we were able to verify domain formation, and estimate lipid-lipid interactions in the binary system.

This research was supported in part by grant CC6246 form Research Corporation.

380-Pos Molecular Dynamics Simulation of Styryl-Type Voltage-Sensitive Dyes in Biomembranes: Location, Tilt and Binding

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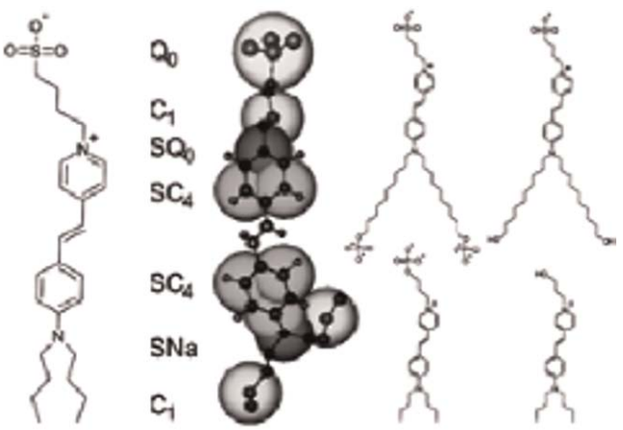
Board B213

Voltage-sensitive dyes are used as fast optical probes for membrane potential in neurons. We present a Molecular Dynamics study on the interaction of selected dyes with a lipid membrane.

We constructed an all-atom (GROMOS 53A6 force field) and a coarse-grained representation (MARTINI[1] force field) of the dye Di-4-ASPBS. In a DPPC membrane, the dye behaved similarly at both levels of detail; the dye localization and chromophore tilt agreed with experimental data.

Based on this validated coarse-grained model of Di-4-ASPBS, we constructed topologies of recently developed dyes which can be enzymatically induced to bind to membranes[2,3]. Their free energy of membrane binding, obtained by umbrella sampling, was in reasonable agreement with experiment. In contrast to traditional QSAR methods, this method reproduced the different impacts of polar groups on binding depending on their position in the dye. While all-atom methods should provide more accurate data, the coarse-grained methodology allows rapid screening of a large number of compounds to estimate binding free energies.



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381-Pos Fluorescence Correlation Spectroscopy Measurement of Anomalous Diffusion and Crowding of Lipid-bound Proteins

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Board B214

In cell membranes, proteins and lipids diffuse in a highly heterogeneous landscape. Aggregates and dense domains of proteins or lipids can modify the path of diffusing molecules, giving rise to anomalous transport. We study two-dimensional diffusion in membranes that are heterogeneous due to protein crowding. Using fluorescence correlation spectroscopy (FCS), we measure the diffusion of the protein avidin bound to biotinylated lipids in a supported bilayer. The density of avidin is controlled by varying the concentration of the lipid anchors. A clear distinction between anomalous and normal diffusion can be achieved with long measurement times (~200s) and analysis of the mean squared displacement (MSD) of the diffusing protein. This experimental approach offers an alternative to standard methods of fitting autocorrelated FCS data to probe the dynamic arrangement of molecules in heterogeneous membranes. Both anomalous and normal diffusion are observed over a range of protein area densities, from dilute to highly crowded regimes. When we observe anomalous diffusion, as the membrane becomes more crowded, protein diffusion becomes more anomalous. When normal diffusion is observed, the protein diffusion constant does not change significantly despite protein crowding. In this case, our observations suggest that normal diffusing lipids in the underlying bilayer direct the movement of the bound protein. A mechanism is suggested by which cell membrane-associated molecules remain mobile in crowded environments due to the stable diffusion of lipids.

382-Pos Dielectric Spectroscopy: Noninvasive And Fast Method For Measuring Changes In The Membrane Potential

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This poster presents a noninvasive and fast method, dielectric spectroscopy, to measure changes in the membrane potential of live cell suspension, in particular to E. coli. This technique can be applied virtually to any cell suspension, regardless of size or shape and is tested against the traditional one-using voltage sensitive dyes.

Precise measurements of the dielectric permittivity ε and conductivity σ of live cells suspensions require prior elimination of the polarization errors. Polarization errors are caused by the ionic content of a buffer, and they affect the total impedance in the low frequency interval. We hereby present our approach of polarization removal in low frequency limit by fitting both real and imaginary

experimental curves with an ideal impedance Z=d/iε*S, where e*=e+1/iws. In these formulas, e and s represent the fitting parameters; a higher weight is given to each of them for the high frequency domain (3kHz-10kHz) where polarization effects were proven negligible. Measurements were performed in a low electric field (1V/cm) and 40Hz-10kHz frequency domain. Different buffers are measured, such as HEPES, DMEM with different KCl concentrations.

Changes in the membrane potential of E. coli are triggered in a few ways such as adding different KCl concentration or ionophores. Those changes are measured using dielectric spectroscopy and voltage sensitive dyes and the results are presented side by side.

383-Pos Controlling the Three-Dimensional Structure of Lipid Bilayers: Effect of Lipid Composition and Environment

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Board B216

Biological membranes are two-dimensional fluids capable of occupying three-dimensional space and their ability to adopt various degrees of curvature is essential to cellular function (e.g. vesicle fission/fusion, membrane-protein interactions). To study the role of lipids in defining membrane shape, curved, 3-D structures have been formed in supported bilayers composed of phosphatidic acid and phosphatidylcholine. Using epi-fluorescence microscopy, the effects of lipid composition, osmotic pressure, and screening environment can be examined. We will present results which further detail the role that lipid composition plays in the formation and stability of these structures. In cells, local lipid composition can be changed rapidly by the action of enzymes, thus we have also investigated methods for altering the composition of a bilayer after it has been formed on the solid support.

384-Pos Experimental And Monte Carlo Investigations Of Nystatin Channel Current Decay And Sterol Mosaics In Mixed Lipid/Ergosterol Domains At Moderate Ergosterol Mol Fraction

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Multiple nystatin (nys) monomers form ion channels in membranes rich in ergosterol (erg). At certain mol fractions ($\xi_{erg} = C_r$) erg molecules form regular structures (superlattices) occupying particular acyl chain sites in a lipid bilayer. In our model the nys channels form on the boundaries of domains containing superlattices (Ergosterol Superlattice Domains - ESLDs). Our Monte Carlo (MC) simulations show that mosaics of ESLDs, with structures strongly dependent on ξ_{erg} , form in bilayers for all ξ_{erg} studied. When vesicles containing nys and erg fuse with sterol-free bilayers, characteristic spike changes in membrane conductance are observed, resulting from nys/erg channels transported to the bilayer in the fusion. Our experimental studies were of the decay of membrane conductance resulting from channel breakup as erg diffuses from ESLDs into the erg-free bilayer. The dynamics of channel breakup are dependent on ξ_{erg} through the mosaic structure and diffusion rates from individual ESLDs. We compared results of MC simulations of bilayer conductance with experiment to establish interaction energies for the MC Hamiltonian. We then conducted MC simulations to produce ESLD mosaics formed on bilayers for $0.2 < \xi_{erg} < 0.4$. Our results reproduce characteristic increases in sterol clustering, which have been experimentally observed by others[1].

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385-Pos Mapping the Activity of Phospholipase A_2 within the POPC-Sphingomyelin-Cholesterol Phase Diagram

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POPC/sphingomyelin/cholesterol mixtures provide a physiologically relevant model of the outer leaflet of the plasma membrane. It has been well established that this particular ternary system can form liquid-ordered (*lo*), liquid-disordered (*ld*), and solid-ordered (*so*) lipid phases, and that the ternary phase diagram presents compositional regimes showing coexistence of two or three of these phases. Lipid domain formation is expected within these regions due to phase separation. Phospholipase A_2 (PLA_2) catalyzes the ester bond at the *sn-2* position in glycerofosfolipids, and plays a key role in several physiologically relevant functions, which involve membrane structural modifications, and the formation of bioactive molecules. Recent studies have shown that PLA2 presents preferential hydrolysis for fluid phase lipids in the presence of lipid domains, and that increased lipid packing inhibits the hydrolytic event. It is therefore relevant to investigate under what lipid phase and domain conditions is PLA2 activity expected. In this work we have determined the phase coexistence regions in the POPC/sphingomyelin/cholesterol system by Laurdan GP spectroscopy, and mapped the activity of the enzyme along the different tie-lines of phase coexistence. We observe high activity in the liquid-disordered regime, with decreasing activity as we move away from the POPC vertex. We determine the compositional limits for enzyme activity, and relate this information to known plasma membrane compositions, in order to predict under what conditions is PLA_2 activity expected.

386-Pos Direct Observation of the Flip-Flop of Oleic Acid and Growth of Single GUVs of Phosphatidylcholine Membranes

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So far all the studies on the transport of long chain fatty acids in biomembrane have been conducted using a suspension of many LUVs. Recently, using the single GUV method, we have succeeded in obtaining the rate constants of elementary processes such as the antimicrobial peptide-induced pore formation [1]. In this study, we investigated the transport of oleic acid (OA) in DOPC membrane in a physiological ion concentration using the single GUV method. Low concentrations (e.g., 10 μM) of OA solution were continuously added in the vicinity of single spherical DOPC-GUVs through a micropipet. During the interaction of the OA solution with a single GUV, its radius increased with time without changing its shape, and finally it reached an equilibrium value. The analysis of the growth of the single DOPC-GUVs clearly shows that OA entered into the external monolayer of the GUV from aqueous solution, and then the flip-flop of OA occurred from the external to the internal monolayers, and finally OA transferred from the internal monolayer of the GUV into the aqueous solution inside the GUV. This is the first direct observation of the growth of single spherical GUVs, and the flip-flop of FA through the lipid membrane. We analyzed this result quantitatively, and obtained several important constants such as a partition coefficient of OA from aqueous solution into the membrane. On the other hand, a little higher concentrations of OA solution induced several shape changes into single spherical GUVs, indicating that the rate-determining step was the flip-flop of OA in the membrane, but in contrast in the low OA concentrations it was the insertion of OA from the outside aqueous solution.

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387-Pos Amphiphile-Induced Changes in Curvature and Elasticity and Their Roles in Ion Channel Modulation

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Poly-unsaturated fatty acids (PUFAs) alter the function of many membrane proteins; monounsatured fatty acids generally are inert. Using gramicidin A (gA) channels of varying lengths as force tranducers, we have demonstrated that at pH 7 PUFAs decrease the bilayer stiffness, consistent with an amphiphile-induced increase in elasticity but not with the expected negative-going change in intrinsic curvature. 1-palmitoyl-2-docosahexaenoyl-phosphatidylcholine, with a poly-unsaturated DHA chain, forms softer bilayers than DOPC, indicating that changes in elasticity dominate over changes in curvature. The monounsaturated oleic acid (OA) does not alter bilayer properties (as monitored using gA channels), and the neutral DHA-methyl ester has reduced effects on gA channel function. To further understand how PUFAs and other amphiphiles may alter curvature- and elasticity-dependent contributions to the bilayer deformation energy, we explored the role of the acyl-chain headgroup charge and size. We altered the fatty acid protonation state by titration; docosahexaenoic acid (DHA) is more potent (than at pH 7) at pH 9 and is inert at pH 4. At pH 9, OA increases gA channel lifetime and appearance rates similar to DHA. The positively charged oleylamine (OAm), which has a small head-group and therefore should be a potent negative-curvature promoter, decreases channel lifetime. These changes in gA channel function do not depend on hydrophobic mismatch, indicating a curvature-dominated effect. Furthermore, the negatively charged fatty acids increase channel current by increasing the local negative charge whereas the positively charged OAm has no effect. These results suggest that PUFAs alter bilayer stiffness by accumulating in the vicinity of the channel to increase the local elasticity. Partitioning into the perturbed bilayer depends on the headgroup charge and size (volume); PUFAs act locally whereas OAm acts more globally, altering the intrinsic curvature.

388-Pos Line Tension At Lipid Phase Boundaries Drives Formation of Membrane Vesicles in Living Cells

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In artificial membranes composed of phospholipids, cholesterol and sphingolipids in the proportions found in biological membranes, the lipid phases readily segregate into μm sized liquid-ordered (L_o) and liquid-disordered (L_d) domains. However, in living cells, phase separation is either absent or the size of L_o phase domains is on the nanometer scale. Here, we provide evidence that large-scale lipid phase separation can occur in living cells and destabilize the membrane leading to vesicle formation. In living unperturbed cells, stained with the L_d marker 1,1′-dioctadecyl-3,3-3′3′-tetramethylindocarbocyanine perchlorate (DiI-C_{18}) and the L_o marker isothiocyanate labelled cholera toxin B subunit which binds specifically to ganglioside GM_1, a heterogeneous phase distribution is observed. On the apical portion of the plasma membrane, we observe exocytic vesicles composed predominantly of DiI-C_{18} in contrast to the basal portion, where both markers overlap across focal adhesions. Cholesterol depletion with methyl-β-cyclodextrin (MβCD) promotes the phase coalescence and L_o/L_d phase separation and stimulates L_d vesicle formation. This is accompanied by a detachment of the subcortical cytoskeleton from the membrane. Based on these data we describe the energetic requirements for membrane bending, which suggests that vesicle formation can be driven by the line

tension arising from the L_o/L_d boundary. Thus, the presence of large-scale L_o/L_d separation causes line tension of sufficient magnitude to overcome the energetic cost of bending from a flat to a cap-like L_d phase budding vesicle.

389-Pos Changes in membrane water content after Cholesterol removal revealed by Laurdan Generalized Polarization Microscopy

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The cell membrane is composed by lipids, proteins, and carbohydrates. The amphipathic nature of lipid molecules allows them to pack tightly while maintaining a high degree of lateral mobility. In addition, lipids are organized into microdomains (e.g., rafts) that may provide important boundaries that organize integral membrane proteins. Cholesterol is a small lipid molecule that nestles among the hydrophobic tails of the phospholipids in the interior of the membrane. It is know to make the membrane more fluid and at the same time it is indicated as the main constituent of lipid rafts. The decrease in fluidity of the membrane after removing cholesterol from the membranes is normally used as an indication for the involvement of lipid raft in the process under study. In artificial systems depending on composition of the bilayer, the presence of cholesterol may increase or decrease their fluidity (measured as water content). In this report, we show that this observation is also valid in natural membranes. We used Laurdan Generalized Polarization Microscopy to measure water content in the membrane of several cell types before and after removing cholesterol using methyl-β-cyclodextrins and reconstituted HDL particles. Our results indicate that removal of cholesterol from the cellular membrane produce either increase or decrease in fluidity depending on the membrane composition.

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390-Pos Dynamical Acyl Chain Mismatch And Elasticity Of Polyunsaturated Bilayers Revealed By ^{2}H NMR Relaxation

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Distinct physical properties of polyunsaturated lipid bilayers [1] have been shown to influence membrane function, as in the case of G protein coupled receptors (GPCRs). These properties include elastic curvature stress of the membrane coupled to the activation of the receptor protein, and subsequent signal transduction. To provide information on coupling between polyunsaturation and membrane spontaneous curvature, we present order and relaxation parameters for a homologous series of mixed-chain polyunsaturated lipids having a deuterium-labeled palmitoyl chain at the *sn*-1 position. Analysis of solid-state ^{2}H NMR data for the homologous series of (n:0)(22:6)PC lipids in terms of a square-law dependence between relaxation rates and order parameters suggests a pronounced effect of acyl chain mismatch. Deviations from a putative square-law dependence are discovered as acyl mismatch between the saturated *sn*-1 and the -3 22:6 chain increases. Data also suggest that collective fluctuations of lipid bilayers are the major contribution to the relaxation mechanism [2]. Moreover, chain mismatch becomes more pronounced as temperature is decreased. The intercepts of R_{1Z} vs. $S_{CD}{}^2$ curves for the upper and lower *sn*-1 chain are consistent with order of magnitude differences between correlation times along the acyl chains. Acyl chain mismatch has direct consequences on bilayer elasticity and spontaneous curvature and is manifested as a displacement of acyl chain mass along the membrane normal [1]. The distribution of lateral pressures [3] within mixed saturated-polyunsaturated lipid bilayers creates bending moments capable of shifting free energies of membrane receptors and affecting biomembrane function.

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391-Pos Generalized Polarization and Fluorescence Lifetime Imaging Analyses of Laurdan Labeled Supported Lipid Bilayers

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Board B224

Laurdan generalized polarization (GP) has been used extensively to characterize lipid domain formation in giant unilamellar vesicles (GUV). In this work we show novel applications of GP measurements for analyzing domain formation in supported lipid bilayers. The supported lipid bilayers are made by means of spin-coating the lipids onto a mica substrate. The GP values of the supported lipid membrane are found to be lower then those found in measurements of similar GUVs. This is due to photo selection and the different symmetry of the two systems influencing the intensity of the emitted light from the different domains.

To circumvent the problems due to photo selection we use fluorescence life time imaging. The fluorescence lifetime of laurdan depends on the environment in which it is and has a characteristic

fluorescence lifetime in the different lipid domains. The lifetime is independent of the intensity of the emitted light from the different domains and is therefore not effected by the photo selection and the different symmetry of the two systems. It is therefore possible to directly compare lifetime data obtained from GUVs and supported lipid bilayers and use this data to calibrate the GP measurements.

This method enables combination of GP measurements with surface sensitive measurement techniques such as AFM.

392-Pos Visco-elastic Membrane Tethers extracted from Escherichia coli by Optical Tweezers

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Tethers were created between a living *Escherichia coli* bacterium and a bead by unspecifically attaching the bead to the outer membrane and pulling it away using optical tweezers. Upon release the bead returned to the bacterium thus showing the existence of an elastic tether between the bead and the bacterium. These tethers can be tens of microns long, several times the bacterial length. Using mutants expressing different parts of the outer membrane structure, we have shown that an intact core lipopolysaccharide is a necessary condition for tether formation, regardless of whether the beads were uncoated polystyrene or beads coated with lectin. A physical characterization of the tethers has been performed yielding visco-elastic tether force-extension relationships: For first pull tethers a spring constant of 10–12 pN/^{1}m describes the tether visco-elasticity, for subsequent pulls the spring constant decreases to 6–7 pN/^{1}m, and typical relaxation timescales of hundreds of seconds are observed. Studies of tether stability in the presence of proteases, lipases, and amylases lead us to propose that the extracted tether is primarily composed of the asymmetric lipopolysaccharide containing bilayer of the outer membrane. This unspecific tethered attachment mechanism could be important in the initiation of bacterial adhesion.

393-Pos A Complete Phase Diagram For Palmitoylsphingomyelin-cholesterol Liposomes

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The purpose of this study was to create a cholesterol/temperature phase diagram for palmitoylsphingomyelin (PSM) liposomes. Cholesterol concentrations were varied from 0 to 40 mole percent. The diagram was constructed with data from differential scanning calorimetry (DSC) experiments and steady state emission and/or anisotropy measurements using laurdan, diphenylhexatriene, or merocyanine 540. Laurdan was particularly useful because both spectral and anisotropy information could be gathered simultaneously. This concurrent data acquisition allowed changes in membrane order detected by emission spectra to be compared directly to changes in membrane fluidity derived from anisotropy data. The behavior observed with the PSM liposomes was evaluated in the context of analogous results obtained previously for dipamitoylphosphatydilcholine (DPPC) liposomes. In general, both systems displayed similar dependencies on temperature and cholesterol concentration. For example, phase diagrams displayed solid ordered, liquid disordered and liquid ordered phase regions in both cases, as expected. Temperature dependence within the liquid ordered phase region that appears to reflect changes in lipid spacing was detected by merocyanine 540 in both types of liposomes. Nevertheless, some important differences between PSM and DPPC were also noticed. For example, the PSM phase diagram lacked the pretransition characteristic of DPPC. Furthermore, merocyanine 540 binding to PSM liposomes was attenuated compared to that observed with DPPC. These differences may reflect interactions among phospholipid heads unique to PSM.

394-Pos Cholesterol gradient in biological membranes

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It is known that the levels of cholesterol vary greatly among cellular organelles. In cultured fibroblasts, the plasma membrane exhibits the highest levels of cholesterol. Estimated percentage of cellular cholesterol in the plasma membrane ranges from 40–90 %. In the plasma membrane, 30 mol % of total lipids are estimated to be cholesterol. It is suggested that cholesterol is not evenly distributed within a membrane bilayer. However, the concentration difference of cholesterol within biological membranes has not been reported.

Filipin and υ-toxin are well established cholesterol markers. Published results indicate these two probes recognize different membrane concentration of cholesterol. Our results of atomic force microscopy employing sphingomyelin/dioleoylphosphatidylcholine/cholesterol ternary system revealed that filipin preferentially partitions to sphingomyelin and cholesterol-rich domains whereas υ-toxin binds to the membrane when cholesterol is above miscibility transition. Thus, in model membranes, the toxin selectively binds to the uniform phase where the lipid domains are collapsed. These results suggest that the binding of υ-toxin to biological membranes indicates the presence of uniform phase where cholesterol is above miscibility transition. We used non-toxic derivative of υ-toxin, BCυ to examine cellular distribution of cholesterol. BCυ weakly labeled the whole cell surface of trypsinized HeLa cells. In contrast, during spreading, BCυ strongly labeled cell edge and the particular structure which co-localized with actin ring. These results suggest that

cholesterol is not uniformly distributed in the plasma membrane during cell spreading. Although filipin and lysenin, which recognizes sphingomyelin cluster, labeled cell edge, these probes did not colocalize with actin ring. Low concentration of methyl-β-cyclodextrin treatment inhibited BCυ labeling without affecting filipin labeling. This treatment was accompanied by remodeling of F-actin and phosphorylation of Src and EGF receptors. These results suggest that the lipid domain recognized by BCυ plays an important role in Src/EGF receptor-dependent actin reorganization.

395-Pos The Bilayer-dependent Changes In Gramicidin Channel Gating Can Be Described By A Linear Free Energy Relationship

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Gramicidin channels are formed by trans-bilayer dimerization of a monomer from each monolayer in a lipid bilayer. The monomer-dimer reaction represents a simple molecular reaction with a well-understood spatial reaction coordinate. Channel formation involves a local bilayer deformation - and changes in the bilayer material properties affect the activation energy for monomer association and dissociation, as well as the free energy difference of channel formation (DGact, a, DGact, d and DG, respectively). Changes in these parameters may be determined from the measured changes in channel appearance rate, lifetime, and the number of conducting channels. We examined how the changes in DGact, a, DGact, d and DG are related when the bilayer material properties are altered (by adsorption of small water-soluble amphiphiles or by changes in lipid head group interactions) - as well as when the channel length is altered. In all cases the changes in DGact, a, DGact, d and DG are linearly related (obey a linearly free energy relation). These results indicate that the effects of the changes in the bilayer properties can be described by changes in a single parameter summarizing the bilayer material properties. They further provide a well-defined example of a "membrane protein", where non-specific effects of changes in bilayer composition on one functional transition quantitatively predict the effects on another (indeed several).

396-Pos Measurement of Line Tension in GUVs and Monolayers Using Flicker Spectroscopy

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Board B229

Biological membrane compositional heterogeneity is an area of particular interest driven in large part by the question of how functional heterogeneities are regulated and to what extent they are important in living cells. One important defining characteristic that exists between distinct phases of matter is a phase boundary associated with a tension; for systems which are roughly two-dimensional in nature, this tension is referred to as line tension. The magnitude of line tension affects the stability and the extent of positional fluctuations of the phase boundary, and its modulation can influence the size of the domain. Membrane domains may play a key role in cell functions including trans-membrane signaling and trafficking.

We have implemented and improved the technique of flicker spectroscopy to perform Fourier mode power analysis of the in-plane fluctuations of lipid domain boundaries for both monolayer and bilayer systems. From such an analysis, we are able to determine an average line tension, as well as deviations from capillary wave theory as, for example, are seen in lipid monolayers where dipolar interactions modulate mode powers. By studying giant unilamellar vesicles obtained from simple lipid mixtures, we are able to carefully control membrane compositions and measure the resultant changes in line tension. These efforts are directed towards identifying potential biological components that might function in modulating line tension in membranes of biological cells.

397-Pos Effect of Cholesterol on the (−)-Epigallocatechin Gallate-Induced Burst of PC-GUVs

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Board B230

Tea catechins such as (−)-epigallocatechin gallate (EGCg) have been considered to have antibacterial activity and antioxidant. Several investigations indicated that lipid membranes are one of the targets of catechins for their activities. However, the detail interaction of catechins with lipid membranes remained unclear. In our previous report [1], using the single GUV method, we found that low concentrations of EGCg induced a rapid leakage of a fluorescent probe, calcein, from the inside of a single egg PC-GUV, which changed into a small lump of lipids after the leakage. The strong correlation between the leakage and the burst of GUVs indicated that the leakage of calcein occurred as a result of the burst of the GUV. In contrast, human cells have an activity to protect the EGCg-induced bursting of cells. Thereby it is important to elucidate a factor of the protection. In this report, we investigated the effect of cholesterol (chol) on the EGC-induced bursting of GUVs.

At first, we investigated effect of EGCg on DOPC-GUVs. Low concentrations of EGCg ($\geq$ 30 μM) induced bursting of GUVs, which is similar to that of egg PC-GUVs. On the other hand, much higher concentrations of EGCg were required for the burst of DOPC/chol (6/4; molar ratio)-GUV; at a concentration $<$ 100 μM, no bursting was observed, and the fraction of burst GUV was 0.5 at 500 μM EGCg (cf. for DOPC-GUV, 50 μM EGCg). We also determined the partition coefficient of EGCg from aqueous solution into membranes, K_p. K_p for DOPC/chol(6/4) membranes ($= 4 \times 10^4$) was larger than that for DOPC membranes ($= 1 \times 10^4$). These results indicate that cholesterol increases the stability of PC-GUV against the binding of EGCg to the membrane.

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398-Pos The Effect of Propoxyciane HCl on the Physical Properties of Neuronal Membranes

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Fluorescent probe techniques were used to evaluate the effect of propoxyciane·HCl on the physical properties (transbilayer asymmetric lateral mobility, annular lipid fluidity and protein distribution) of synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. An experimental procedure was used based on selective quenching of 1,3-di(1-pyrenyl)propane (Py-3-Py) by trinitrophenyl groups, and radiationless energy transfer from the tryptophans of membrane proteins to Py-3-Py. Propoxyciane·HCl increased the bulk lateral mobility, and annular lipid fluidity in SPMV lipid bilayers, and had a greater fluidizing effect on the inner monolayer than the outer monolayer. The magnitude of increasing effect on annular lipid fluidity in SPMV lipid bilayer induced by propoxyciane·HCl was significantly far greater than magnitude of increasing effect of the drug on the lateral mobility of bulk SPMV lipid bilayer. It also caused membrane proteins to cluster. These effects of propoxyciane·HCl on neuronal membranes may be responsible for some, though not all, of the local anesthetic actions of propoxyciane·HCl.

399-Pos

No Abstract

400-Pos Imaging of Fatty Acid Transport in Living Cells by Two-photon Confocal Microcsopy

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The mechanism of fatty acid (FA) transport across biological membranes is an intensely debated subject. Elucidating the molecular mechanism(s) of FA transport has key implications for diabetes, cardiovascular disease and obesity. In this study, live cell imaging was performed with two-photon laser scanning confocal microscopy to monitor FA transport across the cell membrane of living cells (HEK and 3T3L-1). Excitation with longer wavelengths in two-photon microscopy reduces phototoxicity to live cells and causes negligible out-of-plane photobleaching. Two different probes in the same cells (HEK) were used for monitoring simultaneously different events of FA transport. The probe FPE was added to cells and is restricted to the external leaflet of the cell membrane, which allowed monitoring FA adsorption due to charge sensitivity of the FPE molecule. The pH-sensitive dye SNARF-5F was confined to the cytosol to monitor protons released following passive diffusion (flip-flop) of the FA. Images were captured before and after the addition of oleic acid (18:1) complexed or uncomplexed with cyclodextrin (CD). We observed a decrease in fluorescence intensity of both probes following the addition of FA-CD complex, in accord with predictions of the flip-flop model. Sequential images were also captured at a rate of 1frame/26 sec following addition of FA-CD in HEK cells loaded with FPE. Furthermore, FA complexed with CD application was not accompanied by any detectable changes in cell membrane morphology. Control experiments with addition of an equal amount of CD showed no decrease in fluorescence intensity. These results indicate that FA incorporates into the outer membrane leaflet and moves into the cytosol by a mechanism which resulted in a lower intracellular pH, thus providing visual evidence of a flipflop mechanism of FA entry into cells.

401-Pos Using Gramicidin Channels To Test Whether Molecules That Reverse Cholesterol Accumulation In Niemann-Pick Disease Alter Bilayer Properties

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Board B233

Niemann-Pick disease type C (NPC) is an autosomal recessive genetic disorder manifested by abnormal accumulation of unesterified cholesterol and other lipids. In a previous study (Pipalia et al., J Lipid Res. 2006 *47*:284–301) some of us screened combinatorially synthesized chemical libraries to identify compounds that partially reversed the cholesterol accumulation in NPC cells. Several such compounds were identified; they reduced the cellular cholesterol accumulation at concentrations ranging between 0.1 and 10 μM. Because the active compounds are hydrophobic, we examined whether they might alter lipid bilayer material properties (lipid bilayer stiffness) using gramicidin A (gA) channels of different lengths as probes. Some of the active compounds indeed decreased lipid bilayer stiffness, which raises the question whether they may exert some of their effects on NPC cells by a bilayer-mediated mechanism. Because these compounds had a greater effect on the shorter gA channels we conclude that they increase the lipid bilayer elasticity, as would be expected for reversibly adsorbing amphiphiles. Other active compounds had no bilayer-modifying effects at the highest concentration tested (10 μM), which would indicate that these compounds exert their effects solely by altering the function of proteins involved in cellular cholesterol turnover. These compounds

therefore would be suitable lead compounds for the development of drugs against the cholesterol accumulation in Niemann-Pick disease type C

402-Pos Diffusion-Controlled Reaction Kinetics In The Trap Hierarchy Model

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A diffusion-controlled reaction in the presence of a finite hierarchy of traps is modeled [Saxton, Biophys J, in press]. A biological interpretation of the trap hierarchy was proposed earlier [Saxton, Biophys J *92* (2007) 1178]. The model is applicable to 2D diffusion in the plasma membrane and 3D diffusion in the cytoplasm and nucleus, so Monte Carlo calculations are carried out for the triangular, square, and cubic lattices. The reaction is described by the mean capture time, defined as the first passage time for a random walker at a random initial site to reach an immobile target site in the presence of the hierarchy of traps. The mean capture time is expressed as the product of three factors: the analytical expression of Montroll for the mean capture time in a system with a single target and no binding sites; an exact expression for the mean escape time from the set of traps; and a correction factor for the number of targets present. The correction factor, obtained from Monte Carlo calculations, is between one and two. It is shown that trapping may contribute significantly to noise in reaction rates.

(Supported by NIH grant GM038133)

403-Pos Low pH-Induced Transformation of Bilayer Membrane into Bicontinuous Cubic Phase in Dioleoylphosphatidylserine/Monoolein Membranes

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Board B235

Cubic biomembranes, nonbilayer membranes with connections in three-dimensional space that have a cubic symmetry, have been observed in various cells. Interconversion between the bilayer liquid-crystalline (L_α) phase and cubic phases attracted much attention in terms of both biological and physicochemical aspects. Recently, we have found that electrostatic interaction due to surface charges is an important factor for phase transitions between the L_α and cubic phases [1]. In cells, a decrease in pH from neutral to low pH is widely used for structural changes of biomembranes. Thereby, we can expect that in cells a transformation between cubic phases and bilayer membranes would occur by the decrease in pH. In this report, we examine the possibility of inducing a phase transition between the L_α and cubic phases through a change in pH. For this purpose, we investigated the effect of pH on the phase and structure of dioleoylphosphatidylserine (DOPS)/monoolein (MO) membranes using small-angle X-ray scattering. Multilamellar vesicles (MLVs) of DOPS/MO membranes in the L_α phase were formed at neutral pH. A decrease in pH (< 3.0) of the MLV suspension rapidly induced a transition from the L_α to cubic (Q^{224}) phase (within 1 h). This phase transition was reversible; a subsequent increase in pH (neutral) of the suspension of membranes transformed the cubic phase into the original L_α phase. Further, we found that a decrease in pH transformed large unilamellar vesicles of DOPS/MO membranes into the cubic phase under similar conditions. This finding is the first demonstration that a change in pH can induce a rapid reversible phase transition between the L_α and cubic phases of lipid membranes.

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404-Pos Membrane Protein Function and Lipid Intrinsic Curvature - Why Many Water-Soluble Amphiphiles Do Not Follow The Rules

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Membrane protein function is regulated by the molecular composition of the cell membrane lipid bilayer. Often the effects of changes in bilayer lipid composition on membrane protein function correlate with the associated changes in lipid intrinsic (or monolayer spontaneous) curvature. Several descriptors have been proposed for the underlying mechanism(s), e.g., changes in; bilayer curvature stress; curvature strain; lateral pressure profile, etc. Though couched in different terms, these descriptors all relate the regulation of protein function to changes in the energetic cost of monolayer bending. Nevertheless, many water-soluble amphiphiles that cause either negative-going or positive-going changes in curvature have similar effects on the function of voltage-dependent sodium channels, GABAA receptors and other membrane proteins - as well as on gramicidin channels. These results (which likely are due to changes in the bilayer elastic moduli, caused by reversible bilayer adsorption of such compounds) demonstrate the difficulty of unimodal attempts to explain the regulation of membrane protein function by changes in bilayer composition. The use of gA channels as molecular force transducers provides a powerful tool to indentify whether the major effects of a change in lipid composition, or adsorption of an amphiphile, are due to changes in lipid curvature vs. the bilayer elastic moduli. We demonstrate that such gramicidin-based measurements provide for predictions of the effects of a number of different of pharmaceutical compounds (e.g., 2,3-Butanedione monoxime, capsaicin, capsazepine, diazepam, genistein, pentobarbital, curcumin and phloretin), lipids (e.g., cholesterol, fatty acids

and lysolipids) and other amphipaths (e.g., Triton X, beta-octylglucoside and other surfactants) on membrane protein function.

405-Pos Order Changes in Cell Membrane as Biophysical Mechanisms of the Cellular Effects induced by 2.45 GHZ Electromagnetic Field

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We choose simple phenomena and biological models in the attempt to dissociate the specific microwave interaction with the substrate from the non-specific thermal effect. Two phenomena were chosen to analyse the specific interaction mechanisms.

1/The changes of *lipid packing* in *membrane bilayer* potentially induced by microwaves and formally reflected in a fluorescent marker mobility and in critical events accompanying the phase transition of the lipid bilayer. 2/The change in *generalized membrane polarizability* induced by microwave application which must be reflected in fluorescent emission of Laurdan - a fluorescent dye, sensitive to polarization of hydrophilic environment within the lipid bilayer.

Our experiments provide the evidence that in simple bilayer systems as well as in a living cell membrane there is a change in local order of lipid packing and polar molecules orientation induced by microwave irradiation. These changes are consistent with many reported cellular effects of microwaves and provide a meaningful biophysical mechanism for them.

406-Pos Giant Unilamellar Vesicles Electroformed From Native Membranes And Organic Lipid Mixtures At Physiological Conditions

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In the last years giant unilamellar vesicles (GUVs) have become objects of intense scrutiny by chemists, biologists, and physicists who are interested in the many aspects of biological membranes. In particular, this "cell size" model system allows *direct visualization* of particular membrane-related phenomena at the level of single vesicles using fluorescence microscopy-related techniques. However, this model system lacks two relevant features with respect to biological membranes:

(i) the conventional preparation of GUVs currently requires very low salt concentration, thus precluding experimentation under physiological conditions, and
(ii) the model system lacks membrane compositional asymmetry.

Here we show that GUVs can be prepared using a new protocol based on the electroformation method either from native membranes or organic lipid mixtures at *physiological ionic strength* (1). Additionally, for the GUVs composed of native membranes we show that membrane proteins and glycosphingolipids preserve their natural orientation after electroformation. We anticipate our result to be important in order to revisit a vast variety of findings performed with GUVs under low or no salt conditions. These studies, that include results on artificial cell assembly, membrane mechanical properties, lipid domain formation, partition of membrane proteins into lipid domains, DNA-lipid interactions and activity of interfacial enzymes, are likely to be affected by the amount of salt present in the solution.

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407-Pos Fundamental Investigations of the Dynamics and Mechanics of Vesicle Membranes

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The cell membrane region is a complex entity that mediates many chemical and biochemical pathways by a variety of mechanisms. It is increasingly being recognized that the dynamics and mechanics of the cell membrane region play critical roles in the membrane mediated processes. In particular the response of the cell membrane to external stimuli, namely mechanotransduction is an important phenomenon to understand. We have employed vesicles derived from phosphatidylcholines and phosphatidylserines as cell models to discern the dynamics and mechanics of the membrane region. Our studies to date have focused on

1. Phase transition in the bilayer region of vesicles derived from single lipids such as DMPC and DMPS; these have been accomplished by steady state fluorescence anisotropy, time correlated fluorescence anisotropy, and dithionite reduction kinetics of fluorescent NBD molecules incorporated in the polar and nonpolar regions of the vesicle membrane region. Comparison between single vesicle and vesicle ensembles have been made.

2. Characterization of line tension, by forming transient pores in cell membranes; these pores have been generated by optoporation of vesicles derived from various lipids and line tensions calculated from the rate of pore closing. Results reveal interesting differences between the vesicles that are a function of hydrocarbon chain length and phase behavior of the membrane region.
3. Deformation and fusion of vesicles induced by a weak optical force generated from a focused laser beam; these studies provide information on the membrane bending and curvature.

Current studies are also focused on the inclusion of mechanosensitive proteins such as gramicidin in the vesicle bilayer region to gain further insights into mechanotransduction. Significant results of these studies will be presented.

Membrane Transporters & Exchangers

408-Pos An EPR Spectroscopy Analysis of the Magnesium Transport System CorA in the Closed-State

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In bacteria, magnesium uptake is primarily mediated by the CorA family of membrane proteins of which the ortholog from *Thermotoga maritima* has been recently crystallized, revealing an unprecedented fold. Using a cystein mutagenesis scan approach, we carried out mobility, solvent and O_2 accessibility measurements on 102 positions within the CorA sequence. The scan starts from residue 246, located at the very beginning of the stalk helice connecting the cytoplasmic domain to the transmembrane domain, and ends-up at the C-terminal residue after TM2. Each mutant was individually spin-labelled and reconstituted into liposomes in the presence of a saturating concentration of Magnesium. Solvent accessibility data were mapped on the crystal structure of CorA and the structure was relaxed to fully satisfy the EPR constrains using an *in silico* pseudo-atom approach based on a modified CHARMM force-field, method detailed by P. Sompornpisut *et al.*

We also provide molecular constrains to build the connecting loop between TM1 and TM2 (the most conserved motif), absent in all the crystal structures. Our modeling procedure uses an *ab-initio* step (using Rosetta), with a second step were models are later constrained to satisfy EPR derived data. Co-(III) hexamine, a structural analog of the fully hydrated Mg^{2+} has been showed to inhibit in vivo CorA mediated Mg^{2+} accumulation, a property that has been interpreted as CorA and related transport systems must initially bind a hydrated cation. Based on that observation, we have built our model so that the extracellular loops can accommodate an ion of the size of a hydrated Mg^{2+}.

Together, these results provide structural and dynamic insights on CorA embedded in a native environment; this model will serve our on going study on conformational changes associated with gating.

409-Pos Determining The Osmotic Permeability Coefficient Of Single Transporters And Channels In Plasma Membranes Of Epithelial Cells Via Fluorescence Correlation Spectroscopy (FCS)

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Osmotic water flow dilutes the solution it enters. Since the effect is maximal in stagnant water layers close to epithelia, the actual osmolyte concentration which acts as a driving force is unknown. As a result, the epithelial water permeability is usually underestimated. Calculation of the turnover rate per transport protein is additionally hampered by uncertainties in determining transport protein concentration in plasma membranes. We applied fluorescence correlation spectroscopy (FCS) to carry out spatially resolved measurements of dye dilution in the aqueous solution close to MDCK monolayers which stably expressed AQP1. A mathematical model was developed to derive the velocity of water flow and osmolyte concentrations in the immediate vicinity of the epithelial from the time-dependent dye distribution. The validity of the approach was confirmed by steady state measurements of osmotic water permeability which were undertaken by scanning ion sensitive microlectrodes. By moving the observation volume of the confocal microscope to the plasma membrane, the transport protein concentration was determined by FCS. For this purpose, we tagged the human sodium glucose co-transporter (hSGLT1) to EGFP. The osmotic permeability coefficient of single hSGLT1 molecules was determined using this procedure in combination with aqueous dye dilution measurements.

410-Pos Characterization of the Proteolytic Cleavage of the hCTR1 Copper Transporter that Occurs in the Absence Of O-glycosylation at Ser27

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The human copper transporter hCTR1 is a homotrimeric plasma membrane protein. The hCTR1 protein contains an extracellular amino-terminus of 65 amino acids that contains N-linked polysaccharides at Asn15. We showed that hCTR1 also contains O-linked polysaccharides at Thr27. In the absence of O-glycosylation at