suffer from two significant difficulties: integral membrane proteins inserted in the lipid bilayer encounter non-physiological interactions with the support and the substrate-proximal leaflet is not accessible to modifications. Nanoporous substrates provide a good method of overcoming these problems. In this study, nanoporous xerogel structures were used to support two-phase lipid bilayers made of 1,2-Distearoyl-Glycero-3-Phosphocholine (DSPC) and 1,2-Dioleoyl-Glycero-3-Phosphocholine (DOPC) in 1:2 mole ratio. Domain formation on xerogel surfaces was achieved. The surfaces of the xerogels and lipid bilayers supported by xerogels were characterized in terms of roughness by using AFM. The domain sizes were measured by using the epifluorescence microscopy images. The lateral mobility of the lipids was confirmed by Fluorescence Recovery After Photobleaching (FRAP) method and the diffusion coefficients were calculated by using Fluorescence Correlation Spectroscopy (FCS). The results were compared to mica supported lipid bilayers. We observed that the lipid bilayer followed the surface topography of the xerogel under contact mode imaging conditions. Both the average size of the domains and the standard deviation on xerogel surfaces were found to be larger compared to mica surfaces. Future work will explore several materials chemistries to make xerogel supports for lipid layer assembly.

1185-Pos Photo-induced phases in the Lipid Bilayer

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Board B160

The self-assembly of biological amphiphiles has proved a fascinating topic in recent years, the hollow cylindrical lipid tubule morphology being of particular interest due to its potential relevance to intercellular transporting channels and applicability to controlled-release systems, chemical micro-reactors and nano-conduits. Co-existence of the liquid-ordered and liquid-disordered phases in the lipid bilayer has recently been observed in biologically-relevant three-component giant unilamellar vesicles. We have generated stable, photo-induced micron-scale phase separation in lipid tubules formed from ternary lipid mixtures, inducing a new bilayer disc structure. This investigation not only aids in our understanding of lipid sorting phenomena in cell membranes (suggesting a mechanism for bilayer disc formation in retinal rod-cells), but is also a fascinating route to the generation of new, functional structures.

Membrane Structure - II

1186-Pos Condensing And Fluidizing Effects Of Ganglioside G_{M1} On Phospholipid Films

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In model membrane mixtures that mimic lipid raft compositions, the ganglioside, G_{M1}, which contains four neutral sugars and a negatively charged sialic acid, is enriched in the more ordered domains. In an effort to understand the organization and partitioning of G_{M1} in cell membranes, we have modeled the outer leaflet of the cell membrane using Langmuir monolayers of 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC) and added varying concentrations of G_{M1}. At low biologically relevant concentrations, G_{M1} has a condensing effect on the DPPC monolayer while at higher concentrations, it acts to fluidize, with a switch-over point between the two behaviors at a ratio of 3:1 DPPC:G_{M1}. To examine phase morphology and organization, the monolayers were transferred onto solid substrates and imaged with atomic force microscopy. At concentrations below the switch-over point, G_{M1} is located in nanoscale clusters within the condensed DPPC domains, as shown by a height difference attributed to the bulky sugar groups. The total surface area of these nanosize domains is larger than that attributable to G_{M1} molecules alone, suggesting the higher regions are due to G_{M1} and DPPC packing preferentially in condensed geometric complexes. At higher concentrations, G_{M1} is also located with DPPC in the more fluid phase. X-ray grazing incidence diffraction and reflectivity measurements characterized the molecular packing and structure of pure and mixed monolayers, showing quantitatively how the presence of G_{M1} both condenses and alters the molecular ordering of the phospholipid film. To pinpoint the structural portion of G_{M1} that gives rise to the condensing effect, parallel experiments have been performed with structurally related gangliosides, ceramide, and a PEGylated lipid series. Our results indicate that the bulky sugar headgroup of G_{M1} is responsible for the significant effects on phase behavior and organization of the surrounding lipid molecules.

1187-Pos Perfluorocarbon Compounds Are Chemically Inert But Able To Induce Unusual Vesicle Aggregation And Alter Membrane Packing

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Board B163

Because of their unique physicochemical characteristics, perfluorocarbons (PFCs) have multiple biomedical applications, especially as oxygen transporting agents. Despite the high clinical interest in the development of PFC applications, there is a lack of a fundamental understanding about the interactions of PFCs with cell membranes. Here, large unilamellar vesicles made of POPC and cholesterol (molar ratio: 7:3) were used in conjunction with spectroscopic

methods to explore the effects of PFCs on lipid packing and vesicle stability. Lipid packing was monitored using the environmentally sensitive fluorescent probe LAURDAN. Dynamic light scattering was used to measure vesicle size. Lipid mixing and internal content mixing were monitored by two fluorescent assays. Four PFCs were studied in an attempt to correlate their vapor pressure (VP, ranging from 9.6-294 mmHg) with the effects produced on the vesicles. The results demonstrate that, after LUVs being exposed to PFCs, membrane structure is altered significantly and slowly over a time period $>$ 120 hrs. In the first 2 hrs, PFCs induce a tighter lipid packing, especially for the PFCs with low VP. Afterwards, the lipid packing becomes loose, in conjunction with an increase in vesicle size. The vesicle size increase is due to vesicle aggregation and possibly vesicle fusion. These effects are strongly correlated to the vapor pressure of PFCs. PFCs with a higher VP induce a larger increase in vesicle size. PFCs with a lower vapor pressure produce a tighter lipid packing prior to vesicle aggregation. These findings may give insights into the molecular interactions of PFCs with membrane lipids. (PDOH and AHA).

1188-Pos Interactions Between Non-Steroidal Anti-Inflammatory Drugs And Lipid Membranes-Molecular Dynamics And Neutron Scattering Studies

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Board B164

Non-steroidal anti-inflammatory drugs(NSAIDs) are the most widely prescribed drugs worldwide[1] for their pain, fever and inflammation reducing action. Chronic usage of NSAIDs, however, leads to gastrointestinal(GI) toxicity. The mechanism by which NSAIDs cause GI toxicity remains unclear and recent clinical evidences strongly point that the cause could be due to direct interactions between NSAIDs and phospholipid membranes. However, the molecular details of such interactions are not fully elucidated. Also, NSAIDs pre-associated with phospholipid vesicles are purported to be safer and therapeutically more effective alternatives to the unmodified ones.

Our initial studies on the partitioning of two most common NSAIDs(Aspirin and Ibuprofen), by experiments and simulations, clearly indicate the role played by the structure of the drug in their interaction with the lipid membrane. Motivated by those results, we systematically performed molecular dynamics(MD) simulations of lipid membranes with various NSAIDs that are of different size, structure and pKa values. Our MD results suggest high partition coefficients for these NSAIDs in bilayer membrane as compared to water and strong thinning effect on the bilayer in the presence of NSAIDs. Also, our recent neutron scattering studies(small angle neutron scattering and neutron reflectivity) on DMPC-Ibuprofen systems indicate that the drug affects both the $\sim$5 nm thick bilayer as well as the overall $\sim$ 100 nm diameter vesicle, indicating that NSAIDs affect lipid vesicles on various length scales. In this talk, we will discuss the structural perturbations to lipid membranes due to NSAIDs at clinically relevant molar ratios to lipid molecules and other aspects like drug diffusion mechanism, size, charge state and hydrophobicity and their implications on the use of lipid vesicles as drug delivery vehicles for NSAIDs.

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1189-Pos 1-Alkanols and Membranes: A Story of Attraction

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Although 1-alkanols have long been known to act as penetration enhancers and anesthetics, the mode of operation is not yet understood. In this study, long-time molecular dynamics simulations have been performed to investigate the effect of 1-alkanols of various carbon chain lengths onto the structure and dynamics of dimyristoylphosphatidylcholine bilayers [1]. The simulations were complemented by microcalorimetry, continuous bleaching and filmbalance experiments. In the simulations, all investigated 1-alkanols assembled inside the lipid bilayer within tens of nanoseconds. Their hydroxyl groups bound preferentially to the lipid carbonyl group and the hydrocarbon chains stretched into the hydrophobic core of the bilayer. Both, molecular dynamics simulations and experiments showed that all 1-alkanols drastically affected the bilayer properties. Insertion of long-chain 1-alkanols decreased the area per lipid while increasing the thickness of the bilayer and the order of the lipids. The bilayer elasticity was reduced and the diffusive motion of the lipids within the bilayer plane was suppressed. On the other hand, integration of ethanol into the bilayer enlarged the area per lipid. The bilayer became softer and lipid diffusion was enhanced. The influence of the 1-alkanols on the lateral pressure profiles across the bilayer was investigated and pressure reversal of anesthesia was tested by applying an external pressure.

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1190-Pos Critical Fluctuations in Plasma Membrane Vesicles

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Board B166

Micron-scale critical fluctuations are observed in giant plasma membrane vesicles (GPMVs) that are blebbed directly from living RBL mast cells and other cell types. These plasma membrane vesicles contain a large variety of lipids and proteins found in the plasma membranes of intact cells, and have been shown to phase separate into liquid-ordered and liquid-disordered phases at low temperatures. Critical fluctuations are distinguished from phase separated domains because they are dynamic and non-circular, their average size depends on temperature, and they occur within a narrow temperature range that is slightly above the large-scale miscibility transition temperature. In GPMVs, micron-scale critical fluctuations are observed at temperatures in the range of ∼10–30°C when cells are pre-labeled with fluorescent lipid probes or proteins. We have characterized how domain size depends on temperature, and this analysis predicts that ∼50 nm-sized domains are likely present in GPMV membranes at 37°C. Importantly, fluctuations can be modulated by membrane treatments such as lipid modification and cross-linking of membrane components. Our observations of robust critical fluctuations in GPMVs suggest the intriguing possibility that the composition of mammalian plasma membranes is tuned to reside near a miscibility critical point, and that critical fluctuations persist to physiological temperatures. Potential relevance to lipid rafts and receptor mediated cell signaling will be discussed.

1191-Pos Size And Shape Differences Between Proteins Can Create Fluid Phases In Crowded Membranes

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Board B167

Static and dynamic domains of like-proteins are often observed within biological membranes. It is often assumed that such domains are formed because of weak-electrostatic attractions between these proteins or, in absence of such forces, the existence of lipid rafts. However, it is well known in colloidal science that entropy alone is sufficient to produce ordered states of non-interacting hard bodies, as well as bulk or microphase separation through what is known as depletion-induced attraction or macromolecular crowding. Besides, colloidal bodies may also experience elastic interactions resulting from curvature mismatch. Here we present coarse grained modeling results of one of the best described biological membranes: the photosynthetic membrane of the purple bacterium *Rhodobacter sphaeroides.* Our model does not include any lipids or attractive forces between the proteins. Nevertheless, results show both static and dynamic domain formation solely due to crowding and geometry differences between the proteins. Furthermore, we show that the size differences can be tuned in order to create fluid phases and that shape differences can cause membranes to curve or bud. Effectively, depletion interactions between the proteins in densely packed membranes results in the partitioning into fluid and ordered domains which are of key importance in photosynthesis, where both close packing and diffusion are a functional necessity.

1192-Pos Field Driven Transitions In Bilayers Of Phospholipids At Electrode Surfaces

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Board B168

We will describe electrochemical, neutron reflectivity, infrared reflection absorption spectroscopy (IRRAS) and scanning probe microscopy (STM and AFM) studies of the structure of thin organic films formed by phospholipids deposited onto a metal electrode at the metal-solution interface. Specifically, we will describe how phospholipid molecules aggregate to form monolayer or bilayer films. What the stability of these films is in the presence of electric fields that are comparable in magnitude to the fields acting on biological membranes. How these fields affect ordering of molecules within the membrane and how they cause a phase transition from the liquid crystalline to the gel state. We will also show how the electric field affects the stability of mixed bilayers composed of phospholipids and cholesterol, incorporation of peptides into the bilayer.

The metal electrode surface, covered by a film of surfactants or phospholipids, can be charged and electric fields on the order of 108V/m can be applied to these supported films. These fields have comparable magnitude to the fields acting on biological membranes. The field may be conveniently used to manipulate organic molecules within the monolayer and the bilayer membrane. By turning a knob on the control instrument one can force phase transitions in the film of organic molecules or force them to disperse or to aggregate at the surface. We use electrochemical techniques to control the physical state of the film while the spectroscopic, surface imaging and neutron scattering techniques are employed to study conformational changes of organic molecules and their ordering within the membrane.

1193-Pos Multiscale Modeling of Structure and Phase Behavior in Supported and Unsupported Bilayers

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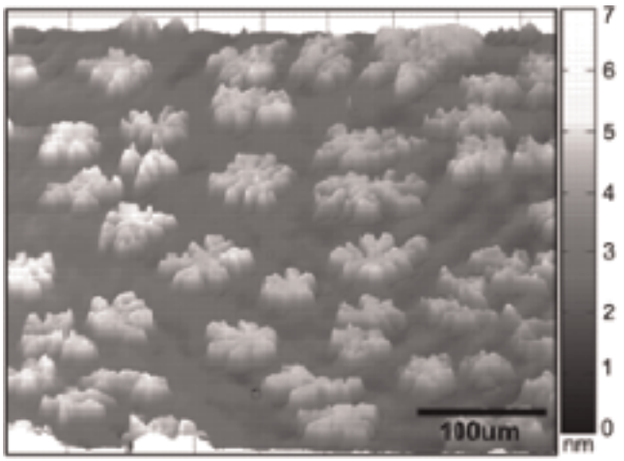
Board B169

The study of lipid structure and phase behavior at the nano scale length is of importance due to implications in understanding the role of the lipids in biochemical membrane processes. We performed a variety of simulations in homogeneous and heterogeneous membrane systems to elucidate such behaviors. Our simulations demonstrate that various coarse grained simulation models can predict different aspects of lipid phase separation and describe the change of the system under the influences of hydrophilic and hydrophobic

support. The simulations are performed using models at different length scales ranging from the all atom scale to a scale where lipids are modeled by only three interaction sites. We are able to follow transformations, such as lipids phase transitions. These phase transitions are determined by analyzing parameters like area per lipid head group, the deuterium order parameter and dynamic properties. Phase diagrams of DLPC-DSPC and DPPC-DPPE mixtures are reproduced consistent with experiments. Additionally, we characterize individual lipid molecules using rotational correlation functions to classify different dynamic populations and we study the stability of artificially designed patterns. We study the influence of a support on the systems. We discuss the changes of the system phase behavior as well as differences between the two leaflets as induced by the support.



1194-Pos Direct Visualization of Thermally-Induced Phase Separation in Supported Phospholipid Bilayers Using Imaging Ellipsometry

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Using imaging ellipsometry, we study thermally-induced phase separation dynamics in supported phospholipid bilayers. Specifically, we image the domain formation process following the cooling from a high temperature homogeneous phase of a binary, lipid bilayer comprising two highly dissimilar lipids, 1,2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC) and galactosylceramide (GalCer) respectively for three different compsositions 10:90, 20:80, and 35:65 of GalCer:DLPC mixtures. For all compositions, large-area real time imaging using ellipsometry reveal two key morphological transformations:

1. a gradual tearing due to the changes in the molecular area of GalCer (condensation effect) and
2. the emergence of a phase-separated domain morphology reflecting the liquid-solid phase transition of the GalCer within the fluid DOPC environment.

The final room temperature morphology of the bilayer depends strongly on the cooling rate, composition, and thermal history of the samples suggesting the importance of diffusional dynamics during equilibration. Our results illustrate the use of imaging ellipsometry as a useful method for in-situ, label-free, non-contact, and large-area imaging of dynamics in interfacial fluids.

1195-Pos Lateral Organization and Non-Equilibrium Transport in Plasma Membranes

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Lateral organization and spatial heterogeneities ("lipid rafts") in the lipid composition of plasma membranes are believed to be important components of many cellular processes, including the control of transmembrane signal transduction, membrane trafficking, and cytoskeletal composition. Here we propose a generic mechanism for the formation of finite-sized lipid raft domains in the plasma membrane. Specifically, we argue that the competition between phase separation in an immiscible lipid system and non-equilibrium cellular lipid transport processes naturally leads to the formation of such domains. In our treatment, the non-equilibrium transport processes are associated with both vesicular and non-vesicular lipid trafficking events. Analytical results and numerical simulations of a physically-based continuum model reveal that the raft size distribution is broad and the average raft size is strongly dependent on the rates of cellular and interlayer lipid transport processes. Specifically, with regard to the average domain size, increasing (decreasing) the recycling rate tends to decrease (increase) it. Furthermore, we predict that a sufficiently rapid recycling is required to produce finite-sized raft domains with a relatively broad size distribution; we also expect the corresponding raft domain lifetime distribution to be broad, offering a possible explanation for the wide range of lifetimes reported in the literature. In addition, we demonstrate that local interactions with immobile membrane proteins can spatially localize the rafts and lead to further clustering. Finally, we discuss the effects of mobile membrane proteins and membrane compartmentalization on raft formation and stability.

1196-Pos Optical Determination of the Lamellar-Hexagonal Phase Transition Kinetics of DEPE

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Board B172

Determining shifts in the L_α-H_{II} phase transition temperature of DEPE (dielaidoyl phosphatidylethanolamine) due to various additives has been a frequent topic of investigation. It is also well known that this phase transition temperature is strongly rate dependent, yet a systematic study of this dependence has yet to be published. We utilize the technique of laser scattering to study the rate dependence of the phase transition of DEPE. Using the modeling techniques already developed for DOPE (dioleyol phosphatidylethanolamine (Toombes et al. 2002, Biophys. J. *82*:2504–2510), our preliminary data indicates an equilibrium phase transition temperature of T_o=61.0 ± 0.5°C. The hysteresis, or the difference between transition temperatures seen on cooling and heating at the same rate, exhibits a power law dependence on the rate with an exponent of 0.3.

1197-Pos Raft-Like Binary and Ternary Lipid Membranes Investigated by Solid-State ^{2}H NMR Spectroscopy

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Much interest has been focussed recently on sphingomyelin as an essential component of a variety of biological membranes. Evidence suggests that this lipid specifically interacts with cholesterol which may foster the formation of raft domains in the lipid bilayer. Using solid-state ^{2}H NMR spectroscopy, we investigated the effect of varying concentrations of cholesterol in binary and ternary mixtures composed of N-palmitoylsphingomyelin (PSM), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and cholesterol, both in macroscopically aligned as well as in unoriented multilamellar bilayers. The hydrocarbon chains of PSM or POPC were ^{2}H labelled which enabled us to investigate the distribution and the order profiles of the individual lipid components in the mixtures[1]. Two liquid-crystalline domains were found in distinct regions of the ternary phase diagram. A mean torque potential model [2] was employed for the analysis of the ^{2}H NMR spectra. By calculating the average hydrocarbon thickness, area per lipid, and structural parameters such as chain extension and thermal expansion coefficients, we were able to further characterize the structural properties of these domains. The data imply that phase separation takes place in ternary PSM/POPC/cholesterol mixtures over a broad temperature range, but vanishes at cholesterol concentrations equal to or higher than a molar ratio of $X_C = 0.33$. We also measured R_{1Z} relaxation rates, which provided information on mechanical properties of the ternary lipid bilayers on a mesoscopic length scale [4] to further characterize the raft-like lipid domains.

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1198-Pos Do Giant Unilamellar Vesicles Composed Of Binary Lipid Mixtures Obey The Lever Rule?: A Quantitative Microscopy Imaging Approach

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3D image deconvolution and segmentation procedures were applied to Confocal Laser Scanning Microscopy image stacks of Giant Unilamellar Vesicles displaying gel/fluid phase coexistence. From reconstructed 3D surface meshes that were refined by active surface models, the fraction of gel and fluid phase areas were calculated. The fractions were compared to values predicted from the lever rule which can be derived from ideal and regular solution theory. The data show that the domain area fractions match the theoretically predicted values well and indicate that the GUVs display lipid phases in thermodynamic equilibrium. Our new approach allows calculation of domain area ratios offering for first time a quantitative method to analyze the thermodynamics aspects of lipid phase coexistence using Giant Unilamellar vesicles/fluorescence microscopy.

1199-Pos Membrane Tension Can be Used to Isolate Rafts

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Board B175

Rafts are small and stable in artificial bilayer membranes containing biologically relevant levels of cholesterol and sphingomyelin. But if lateral tension is applied to these membranes, the small, nanoscopic rafts rapidly merge to form micron-sized domains. Similar phenomena take place if lateral tension is applied to plasma membranes of cultured cells. Fluorescent microscopy shows that the raft marker GM1 is initially dispersed, but rapidly clusters into micron-sized patches as soon as mechanical tension is generated in a cell membrane. We have used tension-induced merger of domains to isolate and biochemically identify cellular rafts. Plasma membranes were subjected to tension and fractionated using centrifugation on sucrose density gradient. One fraction shows enrichment of the raft-

associated markers sphingomyelin, the GPI-linked enzyme alkaline phosphatase and gangliosides; it is depleted in the non-raft proteins Na/K-ATPase and alkaline phosphodiesterase. Cholesterol distributed uniformly across the fractions obtained from centrifugation. This contrasts with the common assumption that rafts are particularly rich in cholesterol. This discrepancy may occur because our procedure for raft isolation is performed at physiological temperature and does not employ detergents or other chemical agents to treat cell membranes. We propose that our approach is useful for isolation of bona fide cellular rafts.

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1200-Pos Dynamic Morphologies and Coalescence of Lipid Raft Domains

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Board B176

Lipid membranes form the generic chemical barriers of the cell and are an integral regulator of the flow of materials and information between the cell and the external environment. These processes are not only controlled by the membrane and its complement of proteins, but also depend on an interplay between lipid composition and morphology. For example, viral uptake, plasma membrane tension regulation, the formation of caveolae, and lateral organization of membrane proteins in lipid rafts all require the creation and control of groups of lipids which adopt specific morphologies. Using a model ternary mixture of lipids and cholesterol we explore the interplay between the formation of lipid domains and the resulting dynamic morphologies. In particular, we focus on the 'dimpled' and 'budded' domain morphologies with relevance to the above mentioned biological processes. In the case of dimpled domains, we quantitatively measure repulsive interactions that create short-ranged order and significantly affect coalescence kinetics. These interactions lead to a stable distribution of domain sizes and the maintenance of lateral heterogeneity on a relatively short length-scale (as compared to a giant unilamellar vesicle) and long timescale (as compared to diffusion-limited coalescence). We also observe size-selective budding transitions that are quantitatively explained with a two-state model. Finally, we use these observations to build a qualitative map of the set of possible kinetic pathways for lipid organization and the resulting morphology in a phase-separated membrane.

1201-Pos Triton X-100 Does NOT Induce the Formation of Liquid Ordered (Lipid Raft) Domains

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Board B177

Immiscibility of unsaturated lipids with mixtures of sphingolipids and cholesterol can lead to the formation of co-existing liquid disordered (Ld) and liquid ordered (Lo) phases. It has been postulated that the formation of such co-existing domains has an important role in cell membrane structure and function. The detergent Triton X-100, does not readily solubilize Lo domains, but does solubilize Ld domains, and is commonly used to obtain detergent resistant membranes (DRM) from cells. The relationship between DRM and pre-existing Lo domains in cells is complex. It has been proposed by some investigators that Triton may induce Lo/Ld phase separation. In order to examine this question a fluorescence quenching approach was used to study the effect of Triton on the amount of Lo and Ld domains in lipid bilayers by measuring the thermal stability of ordered domains in liposomes composed of 1:1:1 (mol:mol) sphingomyelin /1-palmitoyl-2-oleoyl-phosphatidylcholine/cholesterol both in the presence and absence of Triton. These liposomes were found to form coexisting ordered and disordered domains at low temperature, but at high temperatures the ordered domains melted and the lipids formed more homogeneous bilayers. The thermal stability of the ordered domains, and thus the amount of ordered domains present, was not affected by the presence of Triton over a range of Triton concentrations below and above its critical micelle concentration. Although the relationship of detergent-resistant membranes from cells to lipid raft formation remains complex, these results show that Triton does not induce the formation of ordered domains, and to a significant degree alleviate the concern that DRM derived from cells originate from detergent-induced domain formation.

1202-Pos Domain Characterization Of Isotopic Dmpc Mixtures: Understanding The Mechanisms Of Lipid Raft Formation

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We investigate domain formation in an isotopic binary lipid mixture in which the lipids are essentially identical: same head group, acyl chain length and degree of saturation. Domain formation is possible because the degree and localization of the deuterium labeling in lipids shifts the characteristic temperature, Tm, at which a phase change from solid ordered (SO) to liquid disorder (LD) occurs. The Tm shift between a deuterated species and its hydrogenated counter part can be of several degrees (Tm shift ~ -5C in fully deuterated DMPC). In studying mixtures of hydrogenated and deuterated DMPC by small angle neutron scattering, where the neutron scattering length density contrast is large, the question to address is: do we observe domain formation as in other SO/LD systems? At a temperature above the Tm for both DMPC species ideal mixing is expected. As the temperature is lowered, the hydrogenated species should start to form the SO phase, separating from the deuterated species, which should be in the LD phase. At temperatures below the Tm of the deuterated species re-mixing may be observed. Recently acquired data will be discussed regarding the size, morphology and kinetics of lipid heterogeneities driven by the difference in their Tm only. Because both lipids are similar in size and packing we should be able to suppress any possible line tension effects found in other

saturated/unsaturated lipid mixtures where a more complicated mechanism is certainly involved.

Raft formation has been directly associated with the presence of cholesterol (or other sterols) in lipid mixtures having LD/SO domains. We will present the effect cholesterol has on SO/LD domains of isotopic mixtures of hydrogenated/deuterated DMPC. The pure system: deuterated DMPC and cholesterol, will serve as comparison to published work.

1203-Pos Steady State Distribution And Dynamics Of GPI-anchored Protein-nanoclusters In Cell Membranes Is Modulated By Cortical Actin Activity

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Board B179

Many lipid-tethered proteins at the cell surface, including glycosylphosphatidylinositol-anchored proteins (GPI-APs) and the Ras family of GTPases, are organized as nanoscale clusters and monomers. A significant feature of this organisation is that the ratio of clusters to monomers is independent of concentration an apparent violation of mass action. We present a quantitative study of the spatial distribution and dynamics of GPI-AP nanoclusters in the steady state. High resolution spatial maps of Fluorescence Resonance Energy Transfer (FRET) between fluorescently-labeled GPI-APs in the flat regions of the cell surface reveal a clustering of islets of enhanced FRET, corresponding to optically resolvable regions enriched in nanoclusters. To study the dynamics of clustering in the steady state, we monitor FRET recovery following photobleaching in a confocal volume at temperatures 37-15C; we find that while monomeric species are mobile, nanoclusters are immobile throughout this temperature range. The interconversion between nanoclusters and monomers is non-Arrhenius with a sharp change at 24C, and exhibits extensive spatial heterogeneity. While perturbations of cholesterol levels affect both spatial distribution and dynamics of GPI-AP nanoclusters in a systematic way, we find that it also affects cortical actin (CA) distribution. Direct perturbations of CA affects the construction, spatial organisation and dynamics of nanoclusters, while its recovery restores the original steady state. We monitor the activity of CA at different temperatures and find a sharp crossover at 24C. We provide evidence that the dynamics of interconversion is mediated by the active remodeling of CA. These results support an active composite model for the cell surface, regulated by the CA cytoskeleton.

1204-Pos Distinct Microdomain Localization Of Recombinant GPI-anchored GFPs Analyzed By Micropatterning Studies In Vivo

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Board B180

Partitioning of proteins in lipid rafts - cholesterol and sphingolipid enriched plasma membrane microdomains - is important for many signal transduction and protein sorting events. Different molecules have been implicated with lipid rafts, among them glycosylphosphatidyl-inositol (GPI)-anchored proteins.

Due to their preferential localization in microdomains, GPI-anchored GFP chimeras have been used to analyse dynamics of rafts and raft-associated proteins. It has been shown that not only naturally GPI-anchored proteins are organized in different microdomains, but also recombinant GPI-anchored GFPs differing in their GPI signal sequence confer distinct localization in plasma membrane microdomains.

Whereas most studies analyzing the distribution of lipid raft molecules have been performed by the use of biochemical tools (i.e. analysis of detergent resistant membranes - DRMs), we took advantage of our previously developed in vivo μ-patterning technique, which is capable of studying protein-protein interaction and protein co-localization, and examined a potential distinct microdomain localization of different GPI-anchored GFPs in the T24 cell line.

Indeed our results revealed that raft localization of GPI-anchored GFPs depends on the nature of this post-translational modification: the degree of co-localization of recombinant GPI-modified GFPs with different lipid raft proteins (i.e. Lck, Fyn and CD59 which have been co-purified with DRMs) varied remarkably.

Cholesterol depletion induced by the treatment of T24 cells with exogenous Cholesterol Oxidase decreased the observed co-localization drastically.

Results presented here confirm former biochemical data and unequivocally show that distinct localization of GPI-anchored proteins in micro-domains depends on the identity of the GPI-anchor per se.

1205-Pos Neutron Diffraction Studies On The Structural Organisation Of A Stratum Corneum Lipid Model Membrane

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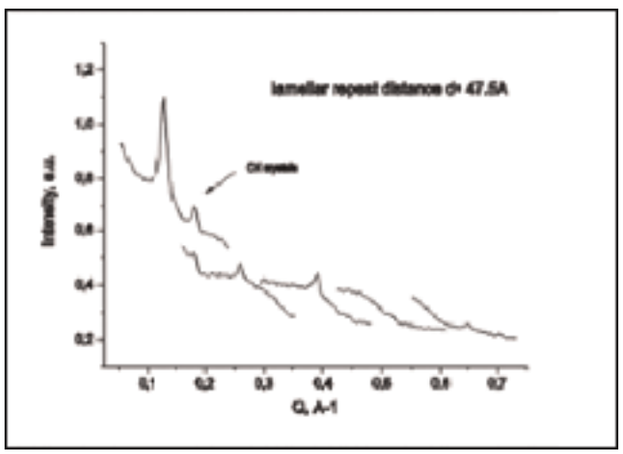
The stratum corneum (SC) represents the outermost layer of the mammalian skin and exhibits the main penetration barrier. The SC intercellular matrix is mainly formed by ceramides (CER), cholesterol (CHOL) and free fatty acids (FFA). It is essential to obtain detailed knowledge about the molecular arrangement of each lipid species in the SC for a deeper understanding of the SC barrier properties and for a rational design of dermal drug delivery systems.

The use of synthetic lipids in SC lipid model matrices for neutron diffraction studies has been shown to be an appropriate tool to characterise the internal membrane nanostructure. A system composed of CER[EOS]/ CER[AP]/ CHOL and behenic acid was chosen as a good representative of the SC lipid matrix as the main constituents were present and reasonable neutron diffraction pattern was achieved (see Fig. 1), Based on this data the neutron scattering length density profile across the membrane, the influence of temperature and humidity on the lamellar structure were calculated. After sufficient characterisation it is planned to use this model membrane as a SC lipid mimic in drug diffusion experiments applying the FTIT-ATR technique.



1206-Pos Deuterium NMR Study Of The Effect Of Ergosterol On POPE Membranes

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We have studied the effect of ergosterol on the physical properties of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) multibilayers using deuterium nuclear magnetic resonance (^{2}H NMR). In the ^{2}H NMR experiments the sn-1 chain was perdeuterated and NMR spectra were taken as a function of temperature and ergosterol concentration. The order of POPE-d31 membranes, measured through M_1, decreases progressively with erg in the gel phase all the way to 65 mol%, while it increases with erg only up to 10 mol% in the liquid-crystalline phase. A significant difference in the ability of ergosterol to disorder the gel-phase and to order the liquid-phase POPE membranes is observed. This finding differs from those observed in POPE/chol and other lipid/sterol systems. Furthermore, the temperature-composition diagram determined from NMR data will be presented, and compared with POPE/chol. The lc-H_{II} transition temperatures of POPE/erg and POPE/chol display opposite sterol dependence at high-sterol concentration range.

1207-Pos Effect of Cholesterol and Ergosterol on the Compressibility and Volume Fluctuations of Phospholipid-Sterol Bilayers in the Critical Point Region - A Molecular Acoustic and Calorimetric Study

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Although sterol-phospholipid interactions have been in the focus of interest for many years now, a complete thermodynamic profile of these systems is still missing. To contribute to a better understanding of the thermodynamic functions of these systems, we determined isothermal compressibility coefficient data for dipalmitoylphosphocholine (DPPC) and DPPC containing cholesterol and ergosterol vesicles by means of molecular acoustics (ultrasound velocimetry, densimetry) and differential scanning and pressure perturbation calorimetric techniques. A particular focus was on the influence of the differential structural properties of the two sterols on the thermodynamic properties of lipid bilayers, and on the nature of the critical point region of phospholipid-sterols systems by determining thermodynamic fluctuation parameters. Contrary to significant changes in conformational and dynamical properties of the DPPC-sterol membranes, no marked differences were found in the various thermodynamic properties studied, including the adiabatic (β_S^{lipid}) and isothermal (β_T^{lipid}) compressibility as well as the volume fluctuations. Differences in β_T^{lipid} and β_S^{lipid} become dramatic in the gel-fluid transition region only, due to a significant degree of slow relaxational processes in the μs time range in the transition region. Our data show no evidence for the existence of a typical critical point phenomenon in the concentration and temperature range where a critical point in the DPPC-sterol phase diagram is expected to appear. Hence, on a macroscopic level, it seems more appropriate to describe the sterol-phospholipid binary mixtures in the liquid-ordered/liquid-disordered coexistence region as a phase region consisting essentially of small nanodomains, only. Such small-domain dimensions with a series of particular properties, such as increased line energy, spontaneous curvature and limited lifetime, seem to be also typical of raft-like domains in cell membranes.

1208-Pos Cholesterol Interactions With Fluid Phase Phospholipids: Effect On Ordered Domain Formation

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Cholesterol and sphingomyelin are known to co-localize in cell membranes where they are believed to form sphingolipid-cholesterol-rich domains that are important for a number of cellular processes. Such domains have been observed in model membrane studies of bilayers consisting of low-Tm phospholipids, high-Tm phospholipids and cholesterol. Model membrane studies have shown that cholesterol interacts more favorably with saturated sphingomyelins than with chain matched glycerophospholipids. In this study we observed that also unsaturated oleoyl-sphingomyelin (OSM) associates more strongly with cholesterol than unsaturated POPC. This is seen as an increased 3H-cholesterol partitioning into OSM bilayers from beta-cyclodextrin as compared to POPC bilayers. Here we used fluorescence spectroscopy to study how the increased fluid phase affinity of cholesterol is reflected in the lateral organization of phospholipid/cholesterol bilayers. DPH anisotropy measurements on binary cholesterol/POPC or cholesterol/OSM samples showed that the formation of ordered sterol-rich domains required higher cholesterol concentrations in OSM bilayers than in POPC bilayers, indicating that cholesterol is better dissolved in OSM than in POPC. Using similar approaches we will report how the affinity of the fluid phase for cholesterol influences the formation of sterol-rich domains in ternary systems, including a saturated gel phase lipid.

1209-Pos Phosphonate Oxygen-modified Synthetic Sphingomyelins - Properties And Interactions With Cholesterol In Monolayers And Bilayer Membranes

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To examine the effect of the oxygen connecting the phosphate group to the sphingosine backbone (phosphonate oxygen) in sphingomyelins (SMs); we have characterized the membrane properties and interactions with cholesterol for three synthetically modified SM-analogues in multilamellar vesicles and monolayers. The SM-analogues were compared to palmitoyl-SM (PSM). The analogues have their phosphonate oxygen replaced with an S-atom (S-SM), a NH-group (NH-SM) or a CH2-group (CH2-SM) [1, 2, 3]. The DPH-anisotropy showed 2 °C lower Tms for NH-SM and CH2-SM and a 4.5 °C higher Tm for S-SM as compared to PSM. The anisotropy in the liquid-disordered phase was higher for S-SM as compared to the other compounds; these results show that an S-linked phosphocholine head-group stabilized intermolecular interactions in pure bilayer membranes. Adding cholesterol to the vesicles (20 and 30 mol%) decreased the transition indicating good miscibility with cholesterol for all the analogues. Laurdan anisotropy indicated 1–3 °C lower Tms for all compounds as compared to DPH anisotropy. When Laurdan was used to probe the hydration of the interface, S-SM and NH-SM vesicles seemed to be less hydrated. Fluorescence quenching-studies with cholestatrienol (vesicle composition; POPC/SM/cholesterol/cholestatrienol, (60:30:9:1)) showed that all compounds formed SM/sterol-rich domains. The S-SM/sterol and PSM/sterol domains displayed similar thermostability, whereas the NH-SM/sterol and CH2-SM/sterol domains were slightly less thermostable. In conclusion, these analogues help us better understand the importance of the phosphocholine linkage to SM properties in bilayers and on interactions with cholesterol.

This work was supported by grants from the Academy of Finland, the Sigrid Juselius Foundation, the Waldemar von Frenckell Foundation and the Åbo Akademi Foundation.

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1210-Pos Mean Field based Langevin Dynamics Sipulation of POPC-Cholesterol Bilayers

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Conventional atomistic simulation methods such as Molecular Dynamics (MD) and Monte Carlo (MC) cannot provide detailed information on structural or dynamical properties over time and length scales of biological interest. As a first step in bridging the gap between atomistic simulations and thermodynamic modeling, we have developed a two-dimensional coarse-grained self-consistent theoretical approach, which we apply in this poster to a mixed Cholesterol- POPC lipid bilayers. In this poster we describe the extension of our earlier Mean-Field- Langevin Dynamics model to POPC bilayers, with non-equivalent chains. As in our prior work, parameters for the coarse-grained model will be taken from MD simulation structural data and a MD-generated library of chain conformations will be used for all configuration sums in the Mean Field component. From careful analysis of the distributions of chain order we are able to locate coexisting regions of different order within the bilayer, and we use these to outline a "phase diagram" for the POPC-cholesterol mixture. We present results of the simulations and comparisons with experimental data and with modeling data for DPPC-cholesterol mixtures.

Supported by NIH Roadmap for Medical Research Grant to the NCDBN (PN2 EY016570)

1211-Pos

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WITHDRAWN

1212-Pos Comparative Calorimetric and Spectroscopic Studies of the Effects of Cholesterol and Epicholesterol on the Thermotropic Phase Behavior and Organization of Dipalmitoylphosphatidylcholine Bilayer Membranes

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EChol is an epimer of Chol in which the axially oriented hydroxyl group of C3 of Chol is replaced by an equatorially oriented hydroxyl group, resulting in a different orientation of the hydroxyl group relative to sterol fused ring system. Our DSC studies indicate that the incorporation of EChol is initially more effective than Chol in reducing the enthalpies of both the sharp and broad components of the main phase transition of DPPC bilayers. However, at higher EChol concentrations, EChol becomes less effective than Chol in reducing the enthalpy and cooperativity of the main phase transition, such that at sterol concentrations of 50 mol %, EChol does not completely abolish the cooperative hydrocarbon chain-melting phase transition as does Chol. However, EChol does not form a calorimetrically detectable crystallite phase at higher sterol concentrations, suggesting that EChol, unlike Chol, may form dimers or lower order aggregates. Our FTIR spectroscopic studies suggest that EChol incorporation produces comparably organized gel and liquid-crystalline DPPC bilayers as does Chol, but which are characterized by increased hydrogen bonding in the glycerol backbone region. These and other results indicate that monomeric EChol is less miscible in DPPC bilayers than is Chol at higher sterol concentrations, but perturbs their organization to a greater extent at lower sterol concentrations, probably due primarily to the larger effective cross-sectional area of the EChol molecule. Nevertheless, EChol does appear to produce a lamellar liquid-ordered phase in DPPC bilayers, although this phase may not be as tightly packed as in comparable Chol/DPPC mixtures.

1213-Pos Classification of Cholesterol Regulation Distributions

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Recently, significant progresses have been made in understanding the molecular mechanisms as well as the detection methods for cholesterol regular distributions (superlattices). Monte Carlo simulations using multibody interactions have successfully generated 6 cholesterol regular distribution patterns, which are located at cholesterol mole fractions of 0.154, 0.25, 0.40, 0.50, 0.571 and 0.667. In addition, free energy calculation predicted that cholesterol chemical potential must have a jump accompanying the formation of each cholesterol regular distribution. These 6 predicted jumps have been recently verified by a high-resolution cholesterol oxidase activity assay in POPC and DOPC lipid bilayers at 37 °C. Interestingly, cholesterol chemical potential does not show jumps at other cholesterol mole fractions such as 0.20 and 0.33, at which cholesterol superlattices have also been predicted based on geometry symmetry. However, our fluorescence measurements in binary mixtures of DOPC/cholesterol and POPC/cholesterol do show abrupt changes at some lipid compositions where cholesterol chemical potential does not have a jump.

These findings suggest an interesting possibility that there are two classes of regular distributions in lipid membranes: one (type-A) is always associated with a jump in chemical potential and the other (type-B) does not. Although the driving forces and the local lipid packing of the type-A regular distributions are well-understood, much remains unknown about the type-B regular distributions.

1214-Pos Crac Motif Peptide Of The HIV-1 gp41 Protein Thins SOPC Membranes And Interacts With Cholesterol

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This study uses low-angle (LAXS) and wide-angle (WAXS) x-ray synchrotron scattering, volume measurements and thin layer chromatography to determine structure and interactions of SOPC, SOPC/cholesterol mixtures, SOPC/peptide and SOPC/cholesterol/peptide mixtures. The peptides are N-*acetyl*-LWYIK-amide (LWYIK), which is the naturally-occurring CRAC motif segment in the pretransmembrane region of the gp41 protein of HIV-1, and N-*acetyl*-IWYIK-amide (IWYIK) which is an unnatural isomer, used as a control. We find that the area of fully hydrated SOPC at 30 °C is 67.0 ± 0.9 Å^2, SOPC/IWYIK (9:1 mole ratio) is 75.7 Å^2 and SOPC/LWYIK (9:1) is 75.8 Å^2. This increase in area with peptide is accompanied by a thinning of the SOPC bilayer of $\sim$3 Å by both peptides. The molecular volume of SOPC is 1311.3 Å^3 at 30 °C and the partial molecular volume of cholesterol in SOPC/cholesterol mixtures is 616 Å^3. Fitting to a model of the bilayer suggests that

LWYIK's average position is slightly closer to the bilayer center than IWYIK's, but both peptides are near the phosphate electron density. Both peptides increase the wide-angle spacing d_{cc} of SOPC without cholesterol, whereas with 50% cholesterol LWYIK still increases d_{cc} but IWYIK decreases d_{cc}. The LWYIK peptide is more hydrophobic than IWYIK, and this difference in hydrophobicity dominates the entire lipid bilayer, as observed by TLC. These results suggest that LWYIK penetrates deeper into the SOPC membrane than does IWYIK. Both peptides counteract the chain ordering effect of cholesterol to roughly the same degree, and both decrease K_C, the bending modulus, thus increasing the SOPC membrane fluidity. Both peptides nucleate crystals of cholesterol, but the LWYIK-induced crystals are weaker and dissolve more easily.

1215-Pos Role of Cholesterol Concentration in Cholesterol: DPPC Packing Patterns in Lipid Monolayers

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Current concept of plasma membrane organization presumes existence of cholesterol-enriched microdomains, which are involved in a wide variety of cellular processes. Despite of crucial role of cholesterol in a cell proliferation, theoretical models of its location within the other membrane lipids are controversial due to the lack of experimental evidence. In this work we examine the role of cholesterol in a lipid monolayer organization using Langmuir isotherms, epifluorescence microscopy, synchrotron X-ray reflectivity (XR), and grazing incident-angle X-ray diffraction (GIXD) techniques. Lipid monolayers consisted either of the pure cholesterol or DPPC and cholesterol:DPPC mixtures with various cholesterol concentrations (from 0.15 to 0.85 cholesterol:DPPC molar ratio). XR results demonstrate that cholesterol tends to stay in hydrocarbon tails region of DPPC shielded with head groups from aqueous environment for every concentration of cholesterol that is in a good agreement with the *umbrella* model. Increase in cholesterol content of the binary mixture promotes thinning of the lipid monolayer. GIXD reveals short-range crystalline order in the cholesterol:DPPC mixture (0.85 molar ratio) distinctly different from that of the pure lipids. Epifluorescence images of the cholesterol:DPPC mixture (0.15 molar ratio) indicate the dominance of DPPC packing in the lipid monolayer mixture.

1216-Pos The Umbrella Effect And Lipid Headgroup Packing In Cholesterol-Containing Lipid Bilayers Studied By Molecular Dynamics Simulation

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The orientation and packing of phospholipid headgroups in POPE, POPC, DOPC, and sphingomyelin bilayers with and without cholesterols were studied using Molecular Dynamics simulations. The distances from cholesterol's hydroxyl oxygen (O6) to the nitrogen (N4) and to phosphorus (P8) atoms of the nearest-neighbor phospholipids were computed. The distance probability distributions showed that lipids with large headgroup (such as PC or sphingomyelin) have a clear tendency to extend their headgroups toward cholesterol in all four bilayer systems we studied. The results directly support the Umbrella Model, which suggests that nonpolar cholesterol relies on the neighboring polar lipid headgroup coverage to avoid the unfavorable free energy of cholesterol contact with water. Also, the PN vectors of lipid headgroups form extensive, short-range, dynamic anti-parallel pairs in all four lipid bilayers. The driving force is likely due to the electrostatic interactions between headgroup dipoles. However, the presence of cholesterol seems to disrupt the formation of anti-parallel headgroup dipole pairs, which indicates that covering cholesterol by neighboring lipid headgroups has a higher priority than minimizing electrostatic interaction energy.

1217-Pos Correlation of Photoreceptor Membrane Physical Properties with Altered Sterol and Acyl Chain Composition in a Rat Model of Smith-Lemli-Opitz Syndrome

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Smith-Lemli-Opitz syndrome (SLOS) is a human genetic disorder caused by the enzymatically defective conversion of 7-dehydrocholesterol (7DHC) to cholesterol (Chol). An animal model of this disease has been developed by treating rats with AY9944, an inhibitor of the enzyme that is aberrant in SLOS. The retinal lipidome is globally altered in the SLOS rat model, with the most profound changes being loss of PC, PE and PS species containing docosahexaenoic acid (DHA, 22:6n3). 7DHC/Chol ratios in retinas of AY9944-treated rats are >5:1 by three months of treatment, but total sterol levels remain unchanged, relative to those of control retinas (whjch contain virtually no 7DHC). Rod outer segments (ROS) were purified from three-month old control and AY9944-treated rats. Acyl chain packing and dynamics in these membranes were assessed using fluorescence lifetime and dynamic depolarization of 1,6-diphenyl-1,3,5-hexatriene (DPH). DPH fluorescence lifetimes in ROS from control and AY9944-treated rats were essentially identical, indicating similar water permeability and headgroup packing. DPH fluorescence anisotropy decays were analyzed empirically and in terms of the Brownian rotational diffusion model, and yielded similar results in terms of DPH

orientational order and rotational motion. DPH rotational motion was more rapid and DPH orientational order was reduced in ROS from AY9944-treated animals, relative to controls, indicating greater acyl chain order and, hence, reduced ROS membrane fluidity in the SLOS rat model. The observed retinal lipidome changes predictably correlate with membrane physical properties that are known to negatively impact visual phototransduction kinetics and efficiency, consistent with the reported retinal electrophysiological defects found both in SLOS patients and in AY9944-treated rats.

1218-Pos Cholesterol Effect on Phase Behavior in Ternary Lipid Membranes: X-ray Diffraction and AFM

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There is growing evidence that lipid membranes are not uniform, but contain lipid microdomains or "rafts", which are enriched in cholesterol, saturated long-chained lipids, and particular proteins. The effects of cholesterol on lipid ordering and phase separation in lipid-raft-containing model membrane systems have been investigated by synchrotron x-ray diffraction, Atomic Force Microscope (AFM) and Differential Scanning Calorimeter (DSC). We have measured bilayer d-spacings in two ternary lipid mixtures: DOPC/Sphingomyelin/Cholesterol and DOPC/DPPC/Cholesterol, as cholesterol content is varied. Mixtures containing intermediate amounts of cholesterol exhibited two phases, and for DOPC/DPPC/Cholesterol with 12% cholesterol, three d-spacings were observed, indicating the possible coexistence of three different phases: liquid disordered (ld) phase, liquid ordered (Lo) phase, and gel phase. AFM images of supported lipid bilayers on polymer cushioned mica substrates contained clearly visible raft-like micro-domains in the similar cholesterol amount range.

This work is supported by the MARTECH and the Institute of Molecular Biophysics, both at Florida State University.

1219-Pos Relationship Between Membrane Properties And Biological Function

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Our research is related to studies elucidating the molecular properties of membranes include the stability *in vitro* and *in vivo*; the influence of cholesterol in membranes and the role of proteins in sequestering design-targeted carrier systems leading to time circulating efficacy.

The diacetylenic phosphatidylcholine (DC8,9PC) can form highly conjugated polymers within the hydrocarbon region of the bilayer. The polymerization efficiency was found to be dependent on the number of UV polymerization cycles, and the DC8,9PC and DMPC molar ratio.

Besides, DC8,9PC presents a very sharp, cooperative, thermal transition around 43.5°C. However, even samples that went through 20 cycles of UV irradiation, presented a DSC band, showing that all the DC8,9PC molecules were not polymerized. Both DSC and ESR spectra indicate the presence of domains rich in DMPC and non-polymerized DC8,9PC. When cholesterol is added ternary polymerized or non polymerized mixtures (DXPC/DC8,9PC/Chol), with DXPC corresponding to DMPC, DPPC or DSPC. Bilayer characteristics were assayed using probe MC540. Vesicle size was determined by dynamic light scattering (DLS) and citotoxicity was performed on L-929 cell line. From DSC analysis was concluded that cholesterol favors lipid miscibility independently of the saturated lipid present in the formulation. MC540 data agree for non polymerized formulations with DSC data. For polymerized formulations cholesterol addition is related to a slight tendency of increasing cell toxicity at 5 mM lipid concentration, without a clear relationship with saturated lipid carbon chain length. In the case of non polymerized samples, DSPC:chol and DMPC:chol showed the higher toxicity, this was not observed with DPPC:chol, though. When DC8,9PC was added, toxicity was significantly reduced. Cholesterol addition seems to have an influence in reducing size except for DMPC:DC8,9PC where this effect is not as clear as for other formulations.

1220-Pos Dissecting Membrane Structure And Function Using Cells From Mice Fed High Fat Diets

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Model membrane studies focus on binary or ternary lipid molecule systems in order to understand their role in organizing the structure of the plasma membrane. However, these studies cannot accurately model the diverse and complex structure of native cell membranes. In order to move toward more physiologically relevant models, we have employed a whole animal approach to understanding the effect of lipids on membrane organization. C57/BL6 mice were fed either a normal diet or high fat diets enriched in saturated (SFA) or monounsaturated fatty acids (MUFA) for 12–14 weeks. B220+ B cells were then isolated from the spleens of these mice and changes in plasma membrane structure were assessed using fluorescence anisotropy. Membranes of B cells from MUFA-fed animals showed decreased order in the acyl chain region and increased order near the headgroups, suggesting high curvature stress in vivo. Since changes in membrane microviscosity have been shown in cell culture to modify protein surface expression, we tested for changes in MHC class I and II surface expression using flow cytometry. B cells from the MUFA-fed animals had lowered levels of MHC class II and increased levels of class I, suggestive of changes in membrane vertical orientation or conformation. Changes in membrane structure were also accompanied by accumulation of fatty acids in the

cells. Quantitative analysis of images using confocal microscopy showed an increase in uptake of C1C12BODIPY and Nile Red, irrespective of the type of fat fed to the animals. Our findings suggest that dietary manipulation of animals with fatty acids offer a new approach to dissecting the role of specific lipids on membrane organization, which can be linked to physiological function.

1221-Pos Lyophilization and Storage of *E. coli* Cells from the Probiotic Formula ASAP not Affecting Bacterial Growth

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The *Escherichia coli* strain from the probiotic formula "ASAP" [1] is recommended to alleviate the symptoms of human gut abnormalities during different diseases, including FMF or IBD such as Crohn's disease and ulcerative colitis. Some differences in membrane properties of this strain have been shown might determine probiotic action [2]. To use this formula, cultivation of cells after lyophilization and storage are of practical significance.

The aim of this study was to compare bacterial cell walls' biophysical properties such as hydrophobicity and adhesion, and growth characteristics in the culture for *E. coli* strain from "ASAP", when grown or lyophilized cells were being re-cultivated.

Bacteria were grown in LB medium, pH 7.5, under anaerobic conditions [3]. Two types of cells,

(i) washed with 0.9 % NaCl before lyophilization,
(ii) lyophilized in distilled water or in 0.9 % NaCl and stored for ~1 year at 4 °C, were used.

Duration of lag phase, specific growth rate, and decrease in pH during increase in optical density (OD) at 600 nm to reach a maximal yield (bacterial mass) were determined in time intervals. These were shown to have similar dynamics closed to each other. No significant differences were revealed in bacterial cell walls' biophysical properties by the using of x-ray diffraction method under small and big angles.

The results indicate a possibility to use lyophilized and stored cells to re-cultivate *E. coli* strain from "ASAP" to get probiotic preparations.

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1222-Pos Study On Concerned Medical Parameter And Ultrastructure Of Human Erythrocyte With Atomic Force Microscopy

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The sizes and shapes of erythrocyte are important indicators of well being for humans and Atomic force microscopy(AFM) is a powerful method of detecting biological biophysical features without significant cellular damage and can resolve dynamic operations of cellular systems[1]. In this study, High resolution AFM results have provided us with the detailed quantification parameters including diameter, thickness, surface area and volume of different erythrocyte through the concerned software in the statistic level and reach the mean cell volume parameter which differs from the conclusion from other current hematological methods for immobilization procedure in AFM operation may lead to significant cellular shrinkage, but we can get much more information on surface ultrastructure in nanoscale and can resolve the shrinkage problem through do the AFM experiment in the proper physiology buffer when the erythrocyte was Immobilized appreciably[2]. Reliable cell diameter about 8±1.5μm and mean cell volume values about 80±15fl were obtained by quantifying 150 erythrocyte from 12 AFM images(12 different samples on different mica) in 55μm×55μm on Shimadzu-WET-SPM-9500J3. Experiments were carried under TM-AFM with 55μm and 55μm scanner and Si_3N_4 probes at 340–430 kHz. And the high resolution AFM results also showed that there are lots of holes in 30±5nm appeared in outer surface of the cell treated by methanol, and lots of protrude structures about 80±20nm appeared in paraformaldehyde, and some wave-liked topographies can be observed in the biconcave cells fixed by glutaraldehyde in the air at 19°C, which showed that the environmental influence to the structure and function of erythrocyte is apparent in nanoscale and the damage of methanol may provided us some testimony for the injury of methanol to erythrocyte.

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1223-Pos Viscoelastic Properties Of Interfacial Lung Surfactant Films Under The Influence Of Cholesterol

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Lung surfactant (LS) is a complex mixture of lipids and proteins originating from the type II cells that line the alveolar epithelial walls. LS reduces surface tension in the alveolar spaces, which minimizes the work of breathing and prevents alveolar collapse. A lack of functional surfactant due to premature birth leads to neonatal Respiratory Distress Syndrome (nRDS) which is routinely treated with animal derived replacement surfactant. One of the essential features of good LS is to reduce the surface tension as well as increases the surface viscosity at the alveolus air-water interface to near zero. Recently, there is no simple theory relating chemical or physical properties of a monolayer with its ability to lower surface tension or surface viscosity in the dynamic process of breathing. Monitoring the viscoelastic properties of interfacial films allows hypothesizing how surface viscosity depends on lipid and protein composition, packing properties and other variables at different states of the breathing process.

In this abstract we focus on the influence of cholesterol in a LS monolayer, as the role of cholesterol in LS is still unclear. In order to study the effect of cholesterol on the surface viscosity and surface tension, we use a modified Langmuir trough as a viscometer. Our viscometer consists of a Langmuir trough equipped with Helmholz coils which generate a controlled magnetic force to move a magnetic needle floating on the monolayer. If for example the force is constant, the surface shear viscosity can be extracted from the terminal velocity of the needle. The viscometer is a powerful tool to examine the influence of cholesterol in a LS monolayer, as the surface viscosity and surface tension can be measured simultaneously.

1224-Pos Biophysical and compositional characterization of Native Pulmonary Surfactant Membranes isolated from broncho-alveolar lavagge of wild type and SP-D knock out mice

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The particular composition of the lung surfactant (aprox. 40% saturated lipids and significant amounts of PG, mono-unsaturated PC, PI, PE and cholesterol) suggests that native surfactant bilayer-based structures could exhibit lateral segregation phenomena at physiological temperatures. This aspect has been showed before (1), where it was pointed out that liquid ordered/liquid disordered phase coexistence in this membrane was relevant for the surfactant function. The present work pretends to emphasize the idea of performing an integral study of the pulmonary surfactant membranes taking advantage of different biochemical and biophysical techniques, such as mass spectrometry on lipids, confocal single and two-photon microscopy, AFM and GUV's technology. This experimental approach is enabling us to explore the possible occurrence of phase coexistence phenomenon in bilayers and monolayers composed of native pulmonary surfactant extracted from mammalian wild type and SP-D knocked out mice lungs at physiological conditions and correlates it with the particular pulmonary surfactant membranes composition and the functional activity at air-liquid interfaces.

References

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1225-Pos Differences In Structure And Surface Behaviour Of Clinical Preparations From Porcine Pulmonary Surfactant

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Pulmonary surfactant, a lipid-protein complex secreted by the alveolar epithelium, is essential for the mechanical stabilization of breathing. The pulmonary effort during the respiratory cycle and the risk of alveolar collapse are both minimized by the adsorption of lung surfactant at the air-liquid interface of lungs, reducing its surface tension to values near 0 mN/m. Premature infants lacking an effective surfactant often suffer of Respiratory Distress Syndrome, which can be prevented or ameliorated by supplementation with some exogenous surfactant preparations. Surfacen® and Curosurf® are two of these currently in use therapeutic surfactants, both obtained from porcine lungs. The structure and thermotropic properties of membranes and interfacial films made of whole native pulmonary surfactant (NPS), Curosurf® or Surfacen® have been characterized by different techniques including epifluorescence microscopy, differential scanning calorimetry and dynamic light scattering. Epifluorescence analysis of films formed by adsorption of NPS or Surfacen® suspensions showed compression-driven segregation of condensed lipid domains, while no segregation was observed in compressed layers adsorbed from Curosurf®, a preparation obtained from lavage of minced porcine lungs. Surfacen®, Curosurf®, and NPS all showed a broad endothermic phase transition with a main calorimetric peak with maximal heat capacity at around 28.5, 26.9 and 30°C respectively. These structural and

thermotropic differences seem to correlate with important differences in the behaviour of the studied preparations once subjected to interfacial compression-expansion cycling, specially with respect to the stability of repeatedly compressed films.

Local Calcium Signaling

1226-Pos Probing Nanodomain Ca^{2+} of Ca^{2+} Channels using a Genetically Encoded Ca^{2+} Sensor (TN-XL) Fused to N-type Channels

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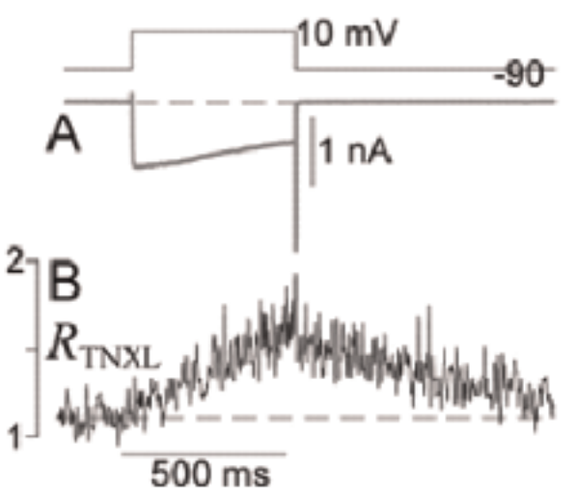
Numerous processes sense Ca^{2+} within nanometers of the cytoplasmic mouth of Ca^{2+} channels. Unfortunately, direct resolution of this 'nanodomain' Ca^{2+} has been lacking, except for a recent study wherein chemical fluorescent Ca^{2+} indicators were selectively reacted with $Ca_V1.2$ channels (Tour *et al*, *Nature Chem Biol* ***3***:423). A limitation, however, was the exceedingly low open probability P_O of these channels. Here, we undertake a different approach, fusing a genetically encoded Ca^{2+} indicator (GECI) to N-type ($Ca_V2.2$) channels. Channels are joined to TN-XL, a CFP/YFP-FRET-based GECI built around the Ca^{2+} sensing protein troponin C (Mank *et al*, *Biophys J*, ***90***:1790); and $Ca_V2.2$ TN-XL fusions maintain a high P_O ~0.6. Using TIRF imaging to enrich for GECI signals from the surface membrane, we resolve nanodomain Ca^{2+} signals, as isolated with 10 mM internal EGTA. The figure displays the whole-cell Ca^{2+} current of $Ca_V2.2$ TN-XL (A), along with the corresponding GECI Ca^{2+} readout (B, R_{TNXL}), averaged over several cells. We modeled the kinetically slowed TN-XL response (Tay *et al*, *Biophys J*, **in press**), and this analysis accords with underlying Ca^{2+} transients reaching ~25–50 μM, as predicted by prior theory.



1227-Pos Familial Hemiplegic Migraine FHM2 Mutations Disrupt Local and Global Calcium Signaling

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FHM2 is an autosomal dominant, classical migraine subtype associated with missense mutations in the a2 isoform of the Na, K-ATPase. This isoform of the Na, K-ATPase plays a role in Ca++ homeostasis, and spatiotemporal properties of Ca++ release regulate processes as diverse as differentiation, synaptic plasticity and apoptosis.

We hypothesize that perturbations in Ca++ homeostasis may be a proximal signaling defect in FHM2. We thus investigated whether FHM2 mutants disrupt Ca++ signaling in stable transformant human neuronal SH-SY5Y cells expressing either wild-type a2 or the T345A or R689Q FHM2 mutations of a2. Ca++ signals evoked by 100 μM carbachol were reduced in both mutants, and Ca++ oscillations were suppressed. Local IP3 Ca++ signals were evoked using UV-flash photolysis of caged IP3 in cells loaded with EGTA so as to 'balkanize' Ca++ waves into discrete localized Ca++ puffs. Ca++ puffs evoked in FHM2 mutant transformants occurred with a similar frequency, yet lower amplitudes than in wild-type transformant cells. Imaging by total internal reflection microscopy revealed that the majority of puffs sites are located adjacent to the plasma membrane, and these membrane-associated puffs also showed significantly reduced amplitudes in cells expressing either mutant. These FHM2 mutant effects could all be observed even without inhibiting the normal endogenous human sodium pumps, simulating the heterozygous disease state. Given that the T345A and R689Q mutations affect Na, K-ATPase enzyme kinetics and pump function in different ways and yet lead to similar disruptions in Ca++ signaling we hypothesize that alterations in Ca++ signaling may be the primary shared pathogenic mechanism common to FHM2 mutations, and may explain the similarity of that disease to FHM1, caused by dominant P/Q calcium channel mutations.

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1228-Pos Astrocyte Intracellular Calcium Dynamics Measured With Total Internal Reflection Fluorescence Microscopy

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Intracellular Ca^{2+} levels in astrocytes are set by Ca^{2+} entry through channels and transporters on the plasma membrane, in addition to