designed W-band loop-gap resonator, the sample is positioned in a gas-permeable Teflon tubing inside the resonator, which allows measurements of extremely small volume (~30 nL) of sample. We used this new design to measure properties of lens lipid membranes derived from total lipids extracted from both lenses (single donor) of a 2-year-old porcine cortex and nucleus. Detailed profiles of membrane fluidity and oxygen transport parameter were obtained from saturation recovery EPR. Analysis of conventional spectra using the microscopic-order macroscopic-disorder (MOMD) model provided rotational diffusion coefficients (R(perpendicular) and R(parallel)) and order parameters. Three different types of motion of lipid spin labels n-PC, T-PC, and CSL (ASL) with, respectively, nitroxide z-axis, x-axis, and y-axis parallel to the bilayer normal, are discussed. Results demonstrate that EPR at W-band has the potential to be a powerful tool for studying samples of small volume, ~30 nL, obtained from eye lenses of a single human donor.

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Phospholipid-Cholesterol Bilayers, Cholesterol Bilayer Domains, and Cholesterol Crystals were Detected in Lipid Dispersion Prepared from Lipids Extracted from Lens Nucleus of Old Human Donors

Laxman Mainali[1], Marija Raguz[1,2], William J. O'Brien[1], Witold K. Subczynski[1].
[1]Medical College of Wisconsin, Milwaukee, WI, USA, [2]University of Split, Split, Croatia.

Human lens lipid membranes prepared using a rapid solvent exchange method from the total lipids extracted from the clear lens cortex and nucleus of 61- to 70-year-old donors were investigated. The measured cholesterol-to-phospholipid (Chol/PL) molar ratio in these preparations was extremely high, showing values of 1.8 and 4.4 for cortex and nucleus, respectively. We expect that at this elevated Chol content, the entire membrane will become saturated with Chol (as in the case of the cortical membrane) and the excess of Chol will form Chol crystals, presumably outside the membrane (as in the case of the nuclear membrane). Properties and organization of the lipid bilayer were investigated using electron paramagnetic resonance spin-labeling methods. Formation of Chol crystals was confirmed using the differential scanning calorimetry. We showed that in the lipid dispersion prepared from nuclear lipids Chol exists in three distinguished environments: (1) Chol dispersed in PL bilayer, (2) Chol in non-crystalline membrane domains (cholesterol bilayer domains, CBDs), and (3) Chol in crystals. In cortical membranes, because of the lower Chol content, Chol crystals were not detected. Amounts of Chol in CBDs were almost the same in cortical and nuclear membranes which indicates that Chol content in both membranes is close or exceeds the Chol solubility thresholds in these membranes. Profiles of cortical and nuclear membrane properties (alkyl-chain order, fluidity, oxygen transport parameter, and hydrophobicity) were very similar to each other and to those reported for cortical and nuclear lens lipid membranes of 41- to 60-year-old donors reported earlier. This confirms our earlier statement that saturation with Chol determines properties of the PL bilayer with the minor effect of the PL composition.

3558-Pos Board B286

Be Careful When Choosing Your Dye Label: Commercial, Water-Soluble Fluorophores Often Interact with Lipid Bilayers

Robert J. Rawle, Laura D. Hughes, Steven G. Boxer.
Stanford University, Stanford, CA, USA.

Water-soluble small molecule fluorophores are widely used to label proteins, antibodies, lipids, etc. in biological systems. However, in a number of cases these fluorophores can interact strongly with lipid bilayers, influencing the interaction of the labeled target with the bilayer and/or producing misleading fluorescent signals. There is no quantitative, systematic measure of the extent of interaction between dye molecules and lipid bilayers. Here, we quantify the interaction of 32 commercially available water-soluble fluorophores with model lipid bilayers to aid in the selection of dye labels for fluorescence experiments. We also demonstrate that while calculations of a dye's hydrophobicity may be helpful in selecting a dye, those calculations are not robust in predicting the extent of dye-membrane interactions.

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Hydration and Temperature-Induced Phospholipid Phase Transitions and their Influence on Desiccation Tolerance of the Nematode Caenorhabditis Elegans

Sawsan E. Abusharkh[1], Cihan Erkut[2], Teymuras Kurzchalia[2], Karim Fahmy[1].
[1]Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Div. Biophysics, Dresden, Germany, [2]Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany.

The nematode Caenorhabditis elegans can bypass part of its normal larval development by forming a "dauer" state with arrested metabolism. The desiccation-tolerance of this state depends on the synthesis of trehalose during a preconditioning phase, where the worm is first exposed to mildly reduced RH, rendering the dauer state attractive for studying the physiology and genetics of Anhydrobiosis. Here, we correlate physical properties of phospholipids (PLs) with the desiccation tolerance of the larvae from which PLs were extracted. The response of PL structure to transient (seconds) changes in water potential and the role of trehalose in water-mediated structural transitions were addressed. Time-resolved Rapid scan FTIR spectroscopy was used in ATR geometry to record hydration-induced difference spectra. We show by chemical analysis that a reduction in choline content in the PL headgroup composition arises during preconditioning. This leads to a stronger coupling of headgroup hydration to disorder in PL acyl chains based on the time-resolved observation of PO2-, C=O and acyl CH2 stretching modes. Trehalose enhances this effect and leads to more uniform kinetics of hydration transients and lipid transitions. In combination with spectroscopic determination of altered lipid main phase transition temperatures (Tm) in PLs upon preconditioning, the data show that chemical tuning of the kinetics and the extent of coupling of headgroup hydration to acyl chain packing changes is a key process in desiccation tolerance as it may release mechanical stress from membranes during temporal changes of water potential. Additional DSC, ITC and Langmuir-Blodget data show that trehalose interacts more favourably with PLs from preconditioned larvae to support desiccation tolerance. This work highlights that chemical tuning of lyotropic phase transitions plays a fundamental role in the trehalose-dependent desiccation tolerance of C. elegans larvae.

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Physical Aspects of the Cut-Off Effect of N-Alcohols in Pure Lipid Membranes

Francisco J. Sierra-Valdez, J.C. Ruiz-Suárez.
Biophysics of Membranes, CINVESTAV, Apodaca, N.L., Mexico.

Nowadays, the molecular nature of general anesthetic target sites remains unknown; some theories profess that the action occurs in proteins, others in lipids. In particular, for nearly a century it has been known that alcohols can act as general anesthetics. However, as the chain length of an alcohol increases, so does its potency as an anesthetic, only up to a certain chain length beyond which the anesthetic activity disappears (the so-called "cut-off effect"). In the attempt to explain such phenomenon, and based on the proposed anesthetic theories, different explanations have emerged without conclusive arguments, nevertheless, the lack of sufficient evidences supporting the cut-off effect of n-alcohols in general anesthesia make this work worth to be pursue. In the present work, using calorimetry and atomic force microscopy (AFM), we show a systematic study of the interaction of n-alcohols (from methanol, C1, to eicosanol, C20) with lipid membranes, in order to collaborate in the comprehension of a physical mechanism of the cut-off phenomenon. Our results suggest that the lowering of the melting transition temperature (Tm) of lipid membranes due to short-chain alcohols, is highly related to their ability to disturb lipid membranes (as has been shown for a wide variety of anesthetics), whilst, the increase of Tm induced by long-chain ones (from C12), is caused by a stiffening of the lipid membrane. We also correlate such effects with some physical properties of n-alcohols and the lipid composition. These results concur with other findings to underwrite the idea that anesthesia does not need a specific binding site in a protein and allow us to speculate that anesthesia only depends on the ability of certain atom or molecule to solubilized in lipids increasing the disorder of the membrane.

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Interaction of Novobiocin with Salmonella Sp Outer Membrane

Thatyane M. Nobre[1], Michael Martynowycz[2], Tonya Kuhl[3], David Gidalevitz[2], Hiroshi Nikaido[1].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]Illinois Institute of Technology, Chicago, IL, USA, [3]University of California, Davis, Davis, CA, USA.

Gram-negative bacteria possess a double membrane system, with the external leaflet of the outer membrane composed of lipopolysaccharides (LPS) that act as the first barrier to drugs. The importance of studying the permeability of LPS barrier comes from its correlation with strain susceptibility. In this study, we extracted LPS from two isogenic Salmonella sp strains, that contains alterations in the lipid A portion regulated by the phoPQ-system. Interaction between phoP- (LPS with no modification) or phoPc (palmitoylation of the 3-hydroxyl group in the 3-OH-myristyl residue, addition of 4-aminoarabinose to the 4' phosphate group and addition of a 2-hydroxy group to the myristate residue at the position 3') LPS and novobiocin was evaluated by using Langmuir monolayers. Novobiocin was chosen due to its size (612 Da), large enough to permeate cells throughout the LPS, and not through the porin channels. MIC of novobiocin for phoP- was 14 μg mL-1 and for phoPc, 40 μg mL-1. p-A isotherms indicated that phoP- occupies a limiting area of 150Å2,

while for phoPc this value is 175Å2. However, addition of Mg^{2+} promotes condensation of 20A2 for phoP-, and 70A2 for phoPc. Epifluorescence microscopy reveals phoP- has fluid-like morphology .In contrast, phoPc displays circular-shaped domains that increase in size and number with the compression. The addition of novobiocin to the subphase promoted slight changes in phoP- morphology. However, for phoPc monolayer, changes in morphology were observed for higher concentrations of novobiocin at which domains started to aggregate generating structures like a "pearl necklace". Structural organization was evaluated by GIXD, and data corroborates the one obtained by epifluorescence, in which novobiocin interaction promotes liquid-crystalline phase formation. Electron density profile probed that novobiocin interacts with phoP- hydrophobic portion, but much higher concentration is needed to permeate phoPc.

3562-Pos Board B290

Size, Morphology, and miRNA Abundance of Cell-Secreted Microvesicles

Michael E. Paulaitis[1], Kitty Agarwal[2].
[1]Chemical and Biomolecular Engineering, Ohio State University, Columbus, OH, USA, [2]Chemistry, Ohio State University, Columbus, OH, USA.

Cell-secreted microvesicles consist of two populations, exosomes and shedding vesicles, that are released from all cell types in response to specific stimuli, but by entirely different mechanisms. Exosomes and shedding vesicles both contain miRNAs, although at different compositions. A feature that is thought to distinguish exosomes from shedding vesicles is their rich repertoire of miRNA. However, individual contributions from these two populations of microvesicles in transmitting differentiation-regulating signals to target cells have not been established. Asymmetric flow field fractionation-multi angle light scattering is used to fractionate the two microvesicle populations into exosomes and larger microvesicles, characterize their size distributions, and estimate absolute particle numbers in each population. The different fractions are further characterized based on their morphologies using cryo-transmission electron microscopy. In addition, qRT-PCR is used to quantify the copy numbers for selected miRNAs in the two fractions. Microvesicles secreted from different cancer cell lines in response to serum deprivation contain different populations of exosomes and larger microvesicles with the exosomes typically an order of magnitude greater in particle numbers than the larger microvesicles. The particle numbers in both populations depend on the response time. Using miR-21 as a reporter for miRNAs, we find miR-21 in both microvesicle populations, but at significantly different concentrations. Moreover, the number of secreted microvesicles is significantly greater than the total copy numbers of miRNA for all cell lines. We conclude that significant numbers of exosomes and larger microvesicles are released from cancer cell lines in response to serum deprivation, but only small numbers of these secreted microvesicles contain miRNA, suggesting the mechanism for microvesicle-mediated miRNA transfer to target cells is stochastic in nature.

Membrane Dynamics II

3563-Pos Board B291

Compositional Interface Dynamics Within Symmetric and Asymmetric Planar Lipid Bilayer Membranes

Tao Han[1], Mikko P. Haataja[2,3].
[1]Princeton University, Princeton, NJ, USA, [2]Department of Mechanical and Aerospace Engineering, Princeton Institute for the Science and Technology of Materials (PRISM), Program in Applied and Computational Mathematics (PACM), Princeton University, Princeton, NJ, USA, [3]School of Mathematics, Institute for Advanced Study, Princeton, NJ, USA.

Compositional domains within multicomponent lipid bilayer membranes are believed to facilitate many important cellular processes. In this presentation, we first outline the derivation of the general equations that describe the dynamics of compositional domains within planar membranes with asymmetry in leaflet properties and in the presence of a thermodynamic coupling between the leaflets. These equations are then employed to develop analytical solutions to the dynamics of the recurrence of registration for circular domains in the case of weak coupling. Finally, numerical solutions to the governing equations are employed to provide a deeper understanding of the domain registration process.

3564-Pos Board B292

Dynamic Implicit Solvent Coarse Grained Models of Lipid Bilayer Membranes : Fluctuating Hydrodynamics Thermostats

Paul J. Atzberger.
UC Santa Barbara, Santa Barbara, CA, USA.

Many Implicit-Solvent Coarse-Grained (IS-CG) models have been recently introduced for investigating the equilibrium properties of lipid bilayer membranes. We introduce extended IS-CG models for dynamic studies by developing fluctuating hydrodynamic thermostats. We present results for the dynamic properties of self-assembled bilayer sheets and vesicles.

3565-Pos Board B293

Dissecting the Roles of Membrane Curvature, Lipid Raft Formation and Protein-Lipid Interactions in the Clustering of Ras

Philip W. Fowler, Mark S.P. Sansom.
University of Oxford, Oxford, United Kingdom.

Membrane-associated cell signalling proteins, such as Ras, have been shown to form nanoclusters, potentially enhancing signalling efficiency and fidelity [1]. It remains unclear what process is driving this behaviour, in particular whether the separation of different lipid species cause the clustering of Ras, or whether Ras affects e.g. the curvature of the bilayer which in turn leads to aggregation. Computer simulation, validated using appropriate in vitro data, can help tease apart the interplay between the physical properties of the membrane, the individual lipids, and the perturbing effect of the proteins. Here we use a coarse-grained description of lipid bilayers and proteins that is able to model many key properties whilst also accurately reproducing the thermodynamic partitioning free energy for amino acids [2]. When combined with high performance computing, we can now begin to bridge the gap between fluorescence microscopy and molecular level structural descriptions. By simulating large patches (> 100 nm) of membranes containing different ratios of unsaturated lipids, sphingolipids and cholesterol studded with NRas, we are able to study the effect of lipidated NRas on both the curvature of the membrane and the disposition of the lipid species.

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Molecular Dynamics Simulations of Lipid-Linked Oligosaccharides in Lipid Bilayers

Nathan R. Kern[1], Emilia L. Wu[1], Sunhwan Jo[2], Kenno Vanommeslaeghe[3], Wonpil Im[1].
[1]Department of Molecular Biosciences and Center for Bioinformatics, University of Kansas, Lawrence, KS, USA, [2]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [3]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD, USA.

We have used molecular modeling and simulation to study the structure and dynamics of two lipid-linked oligosaccharides (LLO: eukaryotic Glc3-Man9-GlcNAc2-PP-dolichol and bacterial Glc1-GalNAc5-Bac1-PP-undecaprenol) in membrane bilayers of different composition. The LLOs used in this study consist of an isoprenoid moiety and an oligosaccharide linked by pyrophosphates. Each component has been studied previously as independent components, i.e., oligosaccharide in solution and isoprenoid in bilayer. However, the overall structure and dynamics of the complete LLO molecule in bilayer remain elusive. From our molecular dynamics simulations, different lipid types do not perturb the structure and dynamics of the isoprenoid moiety. The oligosaccharide conformation and dynamics appears to be similar to those measured by NMR in solution, however, preferential interaction between the oligosaccharide and bilayer interface is observed. Such preferential interaction may influence the binding to OST. Finally, the potential binding mode of the oligosaccharide moiety to bacterial OST is examined by molecular docking.

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Potential of Mean Force Calculations for Nile Red in Lipid Bilayers

Gurpreet Singh, D. Peter Tieleman.
University of Calgary, Calgary, AB, Canada.

The dye 9-diethylamino-5H-benzo[α]phenoxazine-5-one, commonly known as Nile Red, is a fluroscent molecule whose position of excitation and emission maxima are dependent on the polarity of the solvent. The dye is mainly used as a probe for the determination of the lipid microenvironment.

In this study, we present Potential of Mean Force (PMF) profiles of the Nile Red in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer, and in a bilayer containing 1:8 mixture of POPC with 1-palmitoyl-2-(9′-oxo-nonanoyl)-sn-glycero-3-phosphocholine (PoxnoPC). PoxnoPC is a stable lipid oxidation product. We use the distance and the orientation of the dye with respect to the bilayer as reaction coordinates, and compute 2D PMF profiles. These profiles provide crucial information on both the localization and the orientation of the dye in the bilayer.