2078-Pos Board B215
Eicosapentaenoic Acid-Containing Membrane Domain Involved in Cell Division of a Cold-Adapted Bacterium
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Long-chain omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in organisms from bacteria to humans as the acyl group of phospholipids in the membrane. Many biophysical studies have been conducted on model membranes and revealed that PUFAs significantly alter the basic properties of lipid bilayers, such as the fluidity, acyl chain order, phase behavior, elastic compressibility, and permeability. However, despite accumulating information on the properties of the PUFA-containing bilayers, information on the physiological role of PUFAs and their molecular mode of action in living cells is very limited. In this study, we demonstrated a novel physiological function of EPA-containing phospholipids in a cold-adapted bacterium, Shewanella livingstonensis Ac10. We previously found that lack of EPA found at the sn-2 position of glycerophospholipids causes a defect in cell division of this strain. To study the localization of EPA-containing phospholipids, we synthesized phospholipid probes labeled with a fluorescent group . A fluorescent probe in which EPA was bound to the glycerol backbone via an ester bond was found to be unsuitable for imaging because EPA was released from the probe by in vivo hydrolysis. To overcome this problem, we synthesized hydrolysis-resistant ether-type phospholipid probes. Using these probes, we found that the fluorescence localized between two nucleoids at the cell center during cell division when the cells were grown in the presence of the eicosapentaenyl group-containing probe, whereas this localization was not observed with the oleyl group-containing control probe. Thus, phospholipids containing an eicosapentaenyl group are specifically enriched at the cell division site. Formation of a membrane microdomain enriched in EPA-containing phospholipids at the nucleoid occlusion site probably facilitates cell division.

2079-Pos Board B216
Strong H-Bonds Form Bilayers: Ochromonas Danica is an Extreme Example
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Anionic lipid bilayers provide the basic structure for nearly all living membranes. Anion-anion binding in head groups? I suggest a potent H-bond, a quantized H-bond (QHb). Biomembranes use glycerol-phospholipids with vicinal anionic phosphates. Maleate, 2-cis-succinate, is archetypical for a QHb. Its 2 carboxyls trap a proton between pH 2.1 to pH 6.2, contributing quantized bond energy to the molecule. Contrasting that with 2-trans-succinate (No H-bond) it contains an extra ~15kcal. The carboxyls are forced by the structure to share resonance with each other via the, "perhaps vibrating," proton. Albeit in a narrow pH range, oleic acid and other unsaturated fatty acids do likewise. Here I report the structure of another anionic natural membrane, the chlorosulfolipid bilayer of Ochromonas danica. Its headgroups are QHb sulfates forced together by the hydrophobic chains of the bilayer. O. danica is an acidophilic (pH 4.3), fresh water alga in acid bogs. Its plasma membrane has two sets of single chain polar lipids: 2, 2, 11, 13, 15, 16, hexachloro-docosane-1,14-disulfate (>80%) and 2, 2, 12, 14, 16, 17-hexachloro-docosane-1, 15-disulfate, (<20%). Its C1-sulfates and C14/C15-sulfates are each arranged in sheets of sulfates within each monolayer. The latter sulfate sheet is made of sulfates separated by QHbs enclosed in a hydronium ion cage in the low dielectric. The chlorogroups bind hydronium ions so strongly that the CL-H3O+ bond has 0.9 the strength of a covalent bond. They are arranged along the hydrocarbon chains to lead hydroniums toward the poly-sulfate cage. They enter the bilayer by chloro pair at C2, and are then passed down to the water cages. Both primary and secondary sulfate sheets are held together by QHbs, the strength of which prevent osmotic bursting. With phospholipid bilayers such H-bonds would be moving on a nanosecond timescale.

2080-Pos Board B217
Cholesterol Flip-Flop and Lack of Swelling in Stratum Corneum Lipid Bilayers
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Atomistic simulations were performed on hydrated model lipid multilayers representative of the lipid matrix in the stratum corneum (the outermost layer of skin). We find that cholesterol transfers easily between adjacent leaflets belonging to the same bilayer via fast orientational diffusion (tumbling) in the interleaflet disordered region, while at the same time there is a large free energy cost against swelling. This fast flip-flop may play an important role in accommodating the variety of curvatures that would be required in the three dimensional arrangement of the lipid multilayers in skin, and for enabling mechanical or hydration induced strains without large curvature elastic costs.

2081-Pos Board B218
Hydration and Supramolecular Organization Studies of Lamellar Bodies in A549 Lung Cells using Laurdan Fluorescence
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The pulmonary surfactant is organized at the intracellular level in structures known as lamellar bodies (LBs). These subcellular acidic organelles have a variable size (aprox. 0.5-3 um) and based on the electron microscopy data it is proposed that they possess a concentrically membrane lamellar structure. Although the process of LB secretion by physiological inductors like ATP is well characterized, there is a lack of information related to their physical properties.
Using the well known sensitivity of LAURDAN to membrane lateral organization we study LBs organization and dynamic properties of in vivo. We use an immortalized cell line from lung carcinoma, A549, which synthesize pulmonary surfactant and stores it as LBs. The fluorescence signal from LAURDAN was analyzed using the classical generalized polarization function and a newly method based on the Fourier transformation of the emission spectrum (called spectral phasor, Fereidouni-2012). The basic improvement of the spectral phasor is related to the Fourier transformation properties, which allow us the opportunity to decompose complex emission as a linear combination of single decay.
Our results indicate that LBs have a highly packedmembrane structure with low extent of hydration in their core. This particular state is shifting to higher levels of fluidity with increased hydration levels at their periphery. The measured values of GP function indicates that the membrane in the core is in a gel like state (or liquid order state), though when the spectral data is analyzed a more complex system is identified. These results show higher lateral packing compared with DPPC vesicles but with a more heterogenic emission pattern. The interpretation opens the possibility to discuss the supramolecular organization and the role of water in these organelles.
Fereidouni et al, Optics Express-2012

2082-Pos Board B219
Modulation of Phosphoinositide Monolayer Compressibilities by Physiological Levels of Ca^{2+}
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Phosphoinositides (PIs) play a crucial role in many cellular processes that occur at the plasma membrane such as exocytosis and endocytosis. These processes not only locally enrich the membrane with PIs and are often accompanied by intracellular calcium release, but they also involve mechanical membrane deformations. Thus, the question arises how mechanical properties such as compressibility of highly negatively charged lipids such as phosphatidylinositol bisphosphate (PIP_2) are modulated by physiological levels (0-1000nM) of Ca^{2+} ions. Using pure monolayers and a Langmuir film balance, we investigated the effect of bivalent ions on the compressibility of Phosphatidylinositol (PI), Phosphatidylinositol 4,5-bisphosphate (PIP_2) , 1,2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DOPG)and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). In addition, we also present a theoretical framework that describes the relationship between electrical surface potentials and compressibilities which shows good agreement with our experimental findings.

2083-Pos Board B220
Proton Permeation through Extremophile-Inspired Lipid Membranes
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The cell membranes of thermoacidophilic archaea contain lipids with unique structural motifs such as ether linkages, branched chains, and

membrane-spanning bipolar macrocycles that may allow the organisms to maintain the large pH gradient they require to survive. We investigated the relationship between the chemical structure of a number of lipids and the proton permeability of the membranes they form by using an optimized proton permeation assay performed on liposomes containing a fluorescent indicator dye. This work focuses on the effects of tethering on proton permeability and examines lipids with membrane-spanning chains of varied length and chemical structure (e.g. number and identity of rings). We discuss the results in the context of similar chemical groups and structures found in the cell membranes of extremophiles.

2084-Pos Board B221

Vibrational Spectroscopic Studies Probing Cardiolipin Containing Liposomes with and without Cytochrome C Bound to its Anionic Surface

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Cardiolipin is an important lipid on the inner mitochondrial membrane that interacts with cytochrome c, a protein in the electron transport chain that has been recently implicated to have a role in apoptosis. To properly characterize this interaction, the protonation state of cardiolipin needs to be identified. The literature has offered two opposing views with support for both the fully protonated and the semi-protonated state at physiological pH. Cardiolipin containing liposomes have long been used as model mitochondrial membranes. We measured the FTIR spectra of 1,1′2,2′-tetraoleyl cardiolipin (TOCL), 1,2-dioleyl-sn-glycero-3-phosphocholine (DOPC), and the more physiologically relevant 20% TOCL / 80% DOPC liposomes between the pH values of 2 and 11 in the region of 1000-1300 cm^{-1}. The spectra of DOPC liposomes were found to have no noticeable pH dependence. On the contrary, several bands of the spectra of TOCL containing liposomes increase or decrease in intensity at pH values below 4. These bands were assigned to normal modes with substantial contributions from PO_4^- and P=O stretching modes, respectively, and they are diagnostic of the protonation state of the lipid. DFT based normal mode calculations revealed that the investigated spectral region is a superposition of bands assignable to collective CH deformation and PO_4 stretching modes. This study suggests that the phosphate groups of the cardiolipin molecule are fully deprotonated at physiological pH. Recently, we performed resonance Raman studies to explore conformations and spin states of ferri- and ferrocytochrome c on 20% TOCL / 80% DOPC liposomes. We found that different binding sites give rise to different ligation states of the protein.

2085-Pos Board B222

Droplet Interface Bilayer as Cell Membrane Mimics: Water Permeability Studies

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The process of water permeation across lipid membranes has significant implications for cellular physiology and homeostasis, and its study may lead to a greater understanding of the relationship between the structure of lipid bilayer and the role that lipid structure plays in water permeation. We have created a biomimetic artificial membrane, through contact of water droplets in an oil solution containing lipids. Using optical microscopy, we have measured water transport between droplets, as water moves from one droplet to another due to concentration difference. This was assessed as a function of lipid content, structure, and additives, such as cholesterol, which is an essential component of cell membrane. Our results show that cholesterol can increase the activation energy of water permeability several-fold, depending upon the structure of the lipid that makes up the bilayer, thus shedding light on how this singular sterol is vital to control of water movement. We demonstrate that the droplet interface bilayer can be employed as a convenient model membrane to rapidly explore subtle structural effects on bilayer water permeability.

2086-Pos Board B223

Comparison of Reactive Oxygen Species Production Activity and Binding Ability of Porphyrins in Cell Membrane Models

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Photo dynamic therapy (PDT) is a widespread medical treatment based on the light-triggered generation of reactive oxygen species (ROS) by porphyrin derivatives. ROS may cause oxidative damage to membranes as well as to DNA and, in consequence, ultimately kill cells. Hence, the binding ability, the location within liposomes as simple cellular membrane models, and the ROS production ability of porphyrins are of outstanding interest. Earlier we determined the location of mesoporphyrin IX dimethyl ester (MPE) and its non-esterified form, mesoporphyrin IX dihydrochloride (MPCl) in small unilamellar vesicles (SUV) with fluorescence line narrowing spectroscopy (FLN). Here we investigated the production of ROS by the photosensitizers in the aqueous medium of the vesicles and in the lipid bilayer environment. The monocomponent vesicles were formed of various saturated phosphatidylcholines. The amount of generated oxygen radicals in the aqueous media was measured on the basis of the produced tri-iodide (I3-) from potassium iodide (KI) in the presence of molybdenum (MoO4) catalyst, which was followed by absorption spectrophotometry. The ROS in the lipophilic membranes and in near-membrane regions was measured with a dihydrorhodamine derivative by fluorescence spectroscopy. We observed in general that the binding ability of MPE is considerably higher than that of MPCl. In aqueous media (without liposomes) MPCl was highly effective in ROS formation whereas in case of MPE no similar effect was observed. Liposome-incorporated MPCl produced ROS in much higher amounts than the MPE in the aqueous medium of the liposomes. In near-membrane regions MPE produced ROS in the same amount as MPCl.

Membrane Receptors and Signal Transduction III

2087-Pos Board B224

Investigating the Effect of Sodium and Voltage on δ-Opioid Receptors

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G-protein-coupled receptors (GPCRs) are the largest superfamily of membrane proteins within the human genome. They participate in numerous physiological functions, including neuronal excitability and pain signalling. Owing to their functional and structural characteristics, they are excellent drug targets. In spite of their diversity, it is thought that GPCRs share a conserved pathway of signal transduction via conformational changes in their transmembrane (TM) domain. The full range of movements leading to activation, and their interaction with external factors, are however still incompletely understood. Many GPCRs are for instance modulated by sodium. The recent high-resolution crystal structure of the δ-opioid receptor (δOR) provides detailed insight into the sodium binding site in the core of the TM domain [1]. In this work, we looked at the effect of sodium ions and transmembrane voltage on the flexibility and conformational changes of δORs. We applied a dual approach combining patch clamp electrophysiology and molecular dynamics simulations, in particular CompEl [2], to characterize the role of sodium in δOR. We studied the modulation of recombinant G-protein activated inwardly rectifying potassium (GIRK) channels by δORs in HEK cells, and simultaneously investigated the structure of δOR in double-bilayer, atomistic simulation systems under physiological and supra-physiological transmembrane electric fields. Our results implicate sodium as a key player in determining the global conformational ensemble of the δOR.

[1] G. Fenalti et al., Nature 506, 191-196 (2014).
[2] C. Kutzner et al., Biophys. J. 101, 809-817 (2011).

2088-Pos Board B225

Structure-Guided Discovery of Positive Allosteric Modulators of the Mu-Opioid Receptor

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The mu-opioid receptor (MOPr) continues to receive considerable attention in drug discovery efforts owing to its implication in pain management. Regretfully, activation of this receptor is also associated with significant adverse effects, including tolerance and abuse liability. In search for potent analgesics that are free from side effects, attention has recently shifted to allosteric modulators, that is, molecules that bind to (allosteric) sites on the receptor that are different from the orthosteric site recognized by endogenous agonists. The two recently reported positive allosteric modulators (PAMs) of the MOPr, i.e., BMS-986121 and BMS-986122, constitute the first example of such ligands. To facilitate their chemical optimization and/or discover additional PAMs of the MOPr, we searched for chemically similar compounds in the eMolecules database, and identified 1,336 molecules with a Tanimoto