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