Fluorescence is a popular family of techniques used to study membranes, however recent systematic studies show that fluorescent probe behaviour can be altered by membrane composition, probe concentration, and the presence of other probes. Using deuterium nuclear magnetic resonance spectroscopy (^{2}H NMR), we found that trace amounts of the carbocyanine probe DiIC12 are enough to alter phase coexistence behaviour of 30:30:35 DPPC-D62:DOPC:cholesterol membranes, while other probes like Laurdan, Naphthopyrene, and another carbocyanine probe DiOC18, did not affect the membrane appreciably. Laurdan is particularly well suited to the study of phase separation in lipid membranes. It partitions equally well into ordered and disordered lipid phases, and displays a phase-dependent emission spectral shift. Laurdan general polarization (GP) parameter, which characterizes said emission spectral shift, has been used to characterize membrane fluidity. We examine the relationship between Laurdan GP and ^{2}H NMR order parameters.

2572-Pos Board B264

Chemical Stress and the Cell Envelope: The Phospholipid Fraction

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Evidence from physical studies of lipids in the last decade suggests more clearly than ever before, that the phospholipid fraction of cell membranes is not an inert participant in the cell, but a sensitive, dynamic system. The simplest living examples of this are prokaryotic micro-organisms such as *E. coli*. In this project, we explore the effect of growing E. coli in the presence of chemical stress (*e.g.n*-butanol) using updated lipidomics techniques (TLC, MS and NMR) and several biophysical techniques (broad-line 31P NMR and differential scanning calorimetry). *n*-Butanol is an important product of industrial micro-organismal growth, with economically widespread uses, such as an alternative to petrol.

The combination of different experimental approaches in a chemical-biology-type strategy is designed to deliver a more fundamental understanding concerning the physical role of lipids in prokaryotes with state-of-the art accuracy. This in turn allows us to generate understanding about the effect of chemical stress agents, such as *n*-butanol, on the cell envelope.

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Profiling the Dielectric Constant at the Membrane-Peptide Interface using Ionizable EPR Probes

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Polarity, electric potentials, and hydration are the major physico-chemical characteristics of lipid membranes that govern membrane-protein and protein-protein interactions as well as small molecules transport. Insertion of transmembrane proteins perturbs membrane structure altering local dielectric environment and hydration at the membrane-protein interface. The significance of distorting local membrane structure at the lipid-protein interface for modulating protein-protein interactions should not be overlooked. In this work we report on employing pH-sensitive ionizable EPR labels to profile a heterogeneous dielectric environment along the α-helix of a WALP peptide integrated in a lipid bilayer. Labels were attached to two cysteine residues positioned equidistant from the center of the peptide so that the primary sequence of each peptide is palindromic, thus insuring symmetric location of the labels with respect to the bilayer center. The change in protonation state of the nitroxide was directly observed by EPR. Q-band double electron-electron resonance (DEER) experiments were carried out to determine the distance between spin labels when imbedded in lipid bilayers to provide information about the label location. Thus, for the first time measurements of local electrostatics at peptide-bilayer interface were based on direct distance measurements rather than on assumptions on the probe location. Two pH sensitive spin labels, methanethiosulfonic acid S-(1-oxyl-2,2,3,5,5-pentamethyl-imidazolidin-4-ylmethyl) ester (IMTSL) and S-4-(4-(dimethylamino)-2-ethyl-5,5-dimethyl-1-oxyl-2,5-dihydro-1H-imidazol-2-yl) benzylmethanethiosulfonate (IKMTSL), with intrinsic pKa's differing by approximately 2 pH units were used to expand the pH range of the titration experiments. This provided the opportunity to vary the lipid composition in order to investigate effect of the surface charge on dielectric profile at peptide-membrane interface. Water penetration at the peptide-membrane interface was assessed by hyperfine sublevel correlation spectroscopy (HYSCORE) experiment in which the hyperfine coupling between the nitroxide and hydrogen/deuterium atom of water is measured. Supported by NSF-0843632 to TIS.

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Screening the Dynamics of Membrane Constituents in Intact Microalgal Cells by Solid-State NMR

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Microalgae are unicellular organisms protected by a lipid-rich plasma membrane. In most species, this bilayer is covered by a cellulose, carbonate or silica wall. Microalgae are at the basis of the aquatic food chain; therefore the action of contaminants from human activities can impact all the trophic food web. To exert their biological effect, these molecules can target or traverse the microalgal membranes to access internal activity sites, with important consequences on cell survival. Solid-state nuclear magnetic resonance (SS-NMR) is a non- intrusive method that can provide information at the molecular level on the membrane constituents. Because of the complex composition of natural membranes, SS-NMR generally relies on the use of mimetics made of phospholipids. The objective of our work was thus to provide a tool to probe all membrane components by ^{13}C SS-NMR using isotopically-labeled intact microalgae. To do so, we have established a strategy to sort specific constituents according to their dynamics. Using experiments based on through-space (cross polarization) or through-bond (RINEPT) magnetization transfer, rigid and dynamic molecules, respectively, can be identified and studied. For this purpose, we have developed protocols to ^{13}C-enrich microalgae up to an average labeling of 90%. Our ^{13}C SS-NMR study of saltwater (*I. galbana*, *C. gracilis*) and freshwater (*C. vulgaris*, *C. reinhardtii*) microalgae revealed important differences in the dynamics of their cell wall constituents. Notably, *C. vulgaris* used in biofuel production presents the most dynamic membrane due to its high lipid content. Also, the integrity of species without cell walls, such as *I. galbana*, seems to be ensured by a more rigid organization. Our dynamic filter strategy was also tested to verify the effect of nanocrystalline cellulose and could reveal membrane stiffening at high nanoparticle concentrations.

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Docosahexaenoic Acid Affects Gel Phase by Increasing Tilt Angle

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The physical properties of docosahexasenoic acid (DHA) have dramatic effects on lipid bilayer phase behavior which could be a link to beneficial health. Its highly unsaturated structure makes fatty acid highly flexible and with a low transition temperature (Tm) of -80°C. In this investigation, we explore a binary system composed of the saturated lipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-dipalmitoyl-2-docohaxeanoyl-sn-glycero-3-phosphoethanolamine (DHA-PE, 22:6) using small angle x-ray scattering (SAXS) and atomic force microscopy (AFM). Solution SAXS is a powerful tool for structural characterization and was used to identify the different phases (Lβ' and Lα) co-existing within the membrane. In a complementary study, AFM was used to investigate the morphology of coexisting domains. In a surprising result, we observed that the DHA-PE lipid used in this study induced phase separation at concentrations as low as 0.25mol% into a DHA-PE rich liquid crystalline (Lα) phase and a DHA-PE poor gel (Lβ') phase. Also unexpectedly the thickness of the gel phase is thinner than the fluid phase as shown in the d-spacing and electron density profile. We anticipated that DHA-PE would remove or decrease the angle of tilted Lβ' phase that is composed of high concentration of DPPC, but instead it does the opposite and increase the tilt angle. However, DHA-PE still maintain its thermal property and does lower the Tm of the Lβ' phase.

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Lipopolysaccharide Induced Dynamic Lipid Organizations: Lipid Tubules, Membrane Perforations and Multi-Lamellar Stacking

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Supported lipid bilayer assemblies (sLBAs) are generally thought of as relatively stable, predictable model membranes, relevant to a biological system.