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494-Pos Board B249
Influence of Cis and Trans Unsaturated Lipids on an Interdigitated Membrane
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We have examined the effects of adding *cis*- and *trans*-unsaturated lipid to a fully interdigitated membrane using differential scanning calorimetry (DSC) and x-ray diffraction. For the interdigitated lipid, we used a monofluorinated analog of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). The single fluorine atom on the end of the *sn*-2 chain allows 1-palmitoyl-2-(16-fluoropalmitoyl)*sn*-glycero-3-phosphocholine (F-DPPC) to spontaneously form the interdigitated gel phase (L_{β}I) below the main transition temperature (T_m). Both the *cis* 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and the equivalent *trans* lipid 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC) are strongly disfavored to form the L_{β}I phase. There is a large degree of phase segregation between interdigitated and non-interdigitated lipid in DOPC/F-DPPC and DEPC/F-DPPC. The DSC thermograms reveal low miscibility and that both unsaturated lipids broaden and lower the main transition corresponding to the F-DPPC-rich component. Our WAXS data demonstrate that the unsaturated lipids progressively disrupt the intermolecular packing at higher concentrations. Furthermore, the SAXS data show that as the ratio of unsaturated lipid increases, the amount of interdigitated lipid decreases. The interdigitated gel phase formed by F-DPPC is resilient in the sense that the interdigitated phase disappears only at very high fractions of the unsaturated lipid. However, at the same concentration of unsaturated lipid, a greater percentage of the membrane remains interdigitated with DEPC than with DOPC. Therefore, the *cis* isomer is more disruptive and inhibits interdigitation more effectively than the *trans* isomer. This behavior supports the general conclusion that lipids with *trans* fatty acids have properties that are intermediate between saturated and *cis*-unsaturated lipid.

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The Permeability Coefficient of Bilayer Lipid Membrane for Cationic Porphyrins
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The interaction of porphyrins with membranes is an important part in understanding the mechanism of action of porphyrins on the biological object. Porphyrins exhibit biological effect as a result of direct interaction with biological membranes, or interaction with intracellular structures, as a result of its passing through the membrane. That's why it is very important to study the interaction of different porphyrins with membranes and to check if this interaction had any effect on the physicochemical properties of membranes. Because of the complicated organization and functioning of cellular membranes, it is appropriate to conduct experiments on model object, such is bilayer lipid membrane (BLM). The effect of meso-tetra-[4-N-(2′-oxyethyl) pyridyl] and Zn-meso-tetra-[4-N-(2′- oxyethyl) pyridyl] porphyrins on the permeability of BLM is studied using optical (UV/Vis absorption) method. The special cell adapted to UV/VIS spectrophotometer is made for the investigation of porphyrin penetration through BLM. The passage of porphyrins through the membrane is investigated by absorption in the Soret band as a function of time. As a result the permeability coefficient of BLM is calculated for these porphyrins by the method of least-squares [1].
Obtained results allow to understand the mechanisms of porphyrins passage through the cell membranes. This approach is aimed at understanding and optimizing the chemical structure and pharmacological properties of porphyrins usable in medicine. These investigations are also important in terms of interaction of porphyrins with liposomes [2], as liposomes, the structural basis of which are bilayer membranes, are widely used as transportation to deliver drugs (for example, based on porphyrins) to the target-cell.
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Comparing Phase Transition Temperatures of Giant Plasmid Membrane Vesicles with Different Preparation Methods
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Giant plasma membrane vesicles (GPMVs) are traditionally made by incubating cells with a buffer containing a reducing agent and a low concentration of formaldehyde. When GPMVs are prepared in this way from RBL-2H3 cells, the vesicles appear uniform around physiological temperatures, but phase separate into coexisting liquid-ordered and liquid-disordered phases at lower temperatures. When the reducing agent dithiothreitol (DTT) is used, the GPMVs typically phase separate in the range 15°C - 20°C, where transition temperature is defined as the temperature where half of the vesicles produced by a cell population contain coexisting liquid phases. Significantly lower transition temperatures (typically ~0C) are found when DTT is replaced with either glutathione or N-ethylmaleimide (NEM), as has been observed previously for the case of NEM (Levental et al. 2010). GPMVs were also prepared using a method that does not utilize either a reducing agent or formaldehyde, but instead uses a hypertonic chloride salt solution that results in the secretion of vesicles similar in size to those of the reducing agent methods (Del Piccolo et al. 2012). GPMVs made in this way have very low transition temperatures, typically <0C. In addition to lower transition temperatures, GPMVs prepared using NEM, glutathione, or through osmotic stress all contain a slightly increased surface fraction of liquid-ordered phase at low temperatures. These results are consistent with the previous conclusion that DTT induces biochemical changes in inner leaflet proteins that result in elevated transition temperatures and a reduced protein partitioning with liquid-ordered phase lipids (Levental et al. 2010).

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Raft Boundary Structure is Responsible for Monolayer Domains Coupling and Line Activity of Non-Bilayer Components
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In present work the structure of raft/surrounding membrane boundary is calculated basing on hydrophobic mismatch model. Raft boundary energy is minimal when boundaries of monolayer domains forming a bilayer raft are shifted by a finite distance about 4 nm. The algorithm is developed to estimate the system energy as a function of relative shift of monolayer domains centers. It is shown that for decoupling of 30 nm radius raft it is necessary to overcome the energy barrier of about 20 kBT; for reassembly of the bilayer raft it is necessary to overcome the energy barrier of about 15 kBT height. From analysis of bending stress profile across the boundary it follows that non-bilayer components possessing large spontaneous curvature tend to accumulate in narrow regions near the raft boundary. The accumulation of even small amounts of the components leads to significant change of domain line tension. This allows to explain the line activity of such components, observed experimentally.

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Probing Cholesterol-Lipid Interactions and Chemical Activity of Cholesterol in Bilayers via Cyclodextrin Depletion
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Mammalian cells regulate the concentration of cholesterol in their plasma and endoplasmic reticulum membranes (1). That regulation may be triggered by changes in the chemical activity of cholesterol in those membranes. Determining the activity of cholesterol, even in a model system, is a difficult task. In 2000, Radhakrishnan and McConnell investigated lipid monolayers at an air-water interface and presented evidence that lipids and cholesterol form complexes with a particular stoichiometry (2). By using cyclodextrin to remove cholesterol from the monolayer, they found that the chemical activity of cholesterol changed dramatically at the stoichiometry of the complex. Inspired by their work and the work of others (3), we use cyclodextrin to remove cholesterol from supported lipid bilayers that we image by fluorescence microscopy. We measure the rate at which the area of three types of bilayers decreases. The bilayers are composed of a binary mixture of cholesterol and one of three different phospholipids for which the area condensation of lipid and cholesterol in bilayers is known (4). Combining these data, we determine the rate of

cholesterol depletion, and from these rates we calculate the chemical activity of cholesterol as a function of each membrane's mole fraction of sterol. We compare results from the three bilayer types to elucidate how lipid tail structure impacts cholesterol-lipid interactions. Our ultimate goal is to compare our measured cholesterol chemical activities with recent theories based on cholesterol-lipid complexes and the umbrella model.

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Solid State ^{2}H NMR Studies of the Disordering of Raft-Like Domains by n-3 PUFA

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The health benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA) contained in fish oils continue to be investigated intensively in pre-clinical and clinical studies. The major bioactive components are docosahexaenoic acid (DHA, 22:6) with 22 carbons and 6 double bonds and eicosapentaenoic acid (EPA, 20:5) with 20 carbons and 5 double bonds. An emerging hypothesis is that n-3 PUFA are incorporated into membrane phospholipids and modify the structure and organization of lipid rafts, thus affecting cell signaling. We used solid-state ^{2}H NMR spectroscopy to compare molecular organization in mixtures of 1-palmitoyl-2-docosahexaenoylphosphatidylcholine (PDPC) and 1-palmitoyl-2-eicosapentaenoylphosphatidylcholine (PEPC) with the raft-stabilizing molecules sphingomyelin (SM) and cholesterol. Spectra for PDPC-d_{31} and PEPC-d_{31}, analogs of PDPC and PEPC with a perdeuterated palmitoyl *sn*-1 chain, revealed that DHA and EPA incorporate into raft-like domains enriched in SM and cholesterol. Greater incorporation was seen for DHA than EPA. We used PSM-d_{31}, an analog of SM with a perdeuterated *N*-palmitoyl chain, as a probe to directly investigate molecular order within raft-like domains. Our initial experiments looked at mixtures of (POPC) with SM and cholesterol as a control. In POPC/SM (1:1 mol) mixtures, the ^{2}H NMR spectra revealed segregation into POPC-rich (less ordered) and SM-rich (more ordered) domains that are nano-scale in size and contain < 180 lipids. When cholesterol (1:1:1 mol) was added, both domains became more ordered and greater mixing of POPC and SM was observed. These results will be discussed together with the results from experiments on PSM-d_{31} in mixtures with DHA- and EPA-containing phospholipids and cholesterol.

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Computational Studies of Blebbing and Vesiculation via Weak Adhesion of the Cytoskeleton in an Erythrocyte Model

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Utilizing an implicit-solvent molecular dynamics model [1], we investigated blebbing and vesiculation of the membrane-cytoskeleton complex. Blebs are extracellular balloon-like protrusion, void of cytoskeleton, which appear during cellular processes such as apoptosis, cytokinesis and cell motility. Our computational model for the lipid bilayer-cytoskeleton complex corresponds to a spherical self-assembled lipid vesicles, approximately ninety to three hundred nanometers in size, with an underlying cytoskeleton network, akin to that of erythrocytes. Two main parameters are varied in this study: (1) the ratio of the preferred membrane to cytoskeleton areas, and (2) the adhesion strength of the cytoskeleton network underpinning the membrane. Our previous work [2] established the phase behavior of blebbing with nearly permanent anchoring of the cytoskeleton-membrane complex. Further investigation has shown that blebbing can be induced by local ablation of the cytoskeleton, its peeling from the lipid bilayer and its uniform contraction. The addition of a uniformly weak and reversible adhesion between the cytoskeleton and the lipid bilayer to the model, lead to a depression of the phase behavior, and, furthermore, a vesiculation pathway due to localized decoupling between the bilayer and the cytoskeleton meshwork, which (1) modifies the ratio of preferred membrane to cytoskeleton areas, (2) induces reattachment of the cytoskeleton to the lipid bilayer, which shrinks the neck of the bleb, and (3) leads to an eventual vesiculation of the blebs. The present study may explain the vesiculation in red blood cells during their aging.

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501-Pos Board B256

Cell Cycle Phase Determines Critical Temperature in Plasma Membrane Vesicles

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Giant plasma membrane vesicles (GPMVs) isolated from RBL-2H3 cells appear uniform at physiological temperatures, contain coexisting liquid-ordered and liquid-disordered phases at low temperatures, and experience micron-sized critical fluctuations close to their critical temperature. We observe a broad distribution of critical temperatures in GPMVs isolated from a dish of cells even though individual vesicles have a well-defined critical temperature. Interestingly, we find that densely plated cells yield GPMVs with lower average critical temperatures than GPMVs from less densely plated cells. Since it is known that cellular doubling times are reduced in cells plated at high density due to contact inhibition, we hypothesized that critical temperatures are linked to the cell cycle. To test this hypothesis, we isolated GPMVs from cells at various stages in their cell cycle. We plated cells in low serum media to produce a population of cells with arrested growth, and found low critical temperatures in GPMVs isolated from these cells (~10°C). Critical temperatures recovered to typical values (~20°C) after incubating the growth-arrested cells in serum rich media for 24h. Populations of cells are synchronized at S, G2, M, and G1 stages using a double Thymidine block that arrests cells at the border between G1 and S phases. Critical temperatures are elevated in GPMVs from populations of cells in cell cycle phases that immediately precede cell division (G2 and M) compared to the other stages (G1 and S). These results suggest that the magnitude of plasma membrane heterogeneity may be dependent on the cell cycle. We are currently investigating how position in the cell cycle influences the organization of plasma membrane proteins using quantitative super-resolution microscopy with multiple colors. We are also investigating cell cycle position's impact on outcomes of functional processes known to depend on lipid heterogeneity.

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MD Simulations on Alpha-Tocopherol in PUFA Containing Lipid

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Polyunsaturated fatty acids (PUFA) are an influential constituent in cell membranes, but are extremely vulnerable to oxidation. The presumptive role for α-tocopherol (α-toc), the molecular form of vitamin E retained by the human body, is to protect PUFA-containing lipids from oxidation. To investigate whether α-toc preferentially interacts with PUFA in support of this function, we performed MD simulations on lipid bilayers composed of 1-stearoyl-2-docosahexaenoylphosphatidylcholine (SDPC, 18:0-22-6PC) and 1-stearoyl-2-oleoylphosphatidylcholine (SOPC, 18:0-18:1PC) in the presence of α-toc. SDPC with docosahexaenoic acid (DHA) for the sn-2 chain is polyunsaturated, while SOPC with oleic acid (OA) for the sn-2 chain serves as a monounsaturated control. The simulations were run at 310 K under constant pressure for 200 ns on a system comprised of 80 phospholipid molecules, 20 α-toc molecules and 2165 water molecules. In qualitative agreement with our results from solid state ^{2}H NMR and neutron scattering experiments, the simulations show that α-toc increases order inside the bilayer and that the chromanol headgroup sits near the surface in both SDPC and SOPC. Analyses of the density distribution of the lipid chains relative to α-toc are underway to determine differences in how α-toc associates with each chain. A major prediction from our simulations is that α-toc undergoes flip-flop across the bilayer, and that the rate is an order of magnitude greater in SDPC than SOPC. This is a remarkable finding that reveals a possible mechanism by which the chromanol group would not only wait at the membrane surface but would also patrol the membrane interior to meet lipid radicals and terminate the chain reaction by which lipid peroxidation proceeds.