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.