The human body is replete with fluids. Consequently, cells have adapted to respond and thrive in varying fluid flow conditions. For instance, endothelial cells align with flow direction and release vasodilators in response to increased blood pressure. However, the molecular mechanisms that cells use to detect flow remain unclear. Here, we investigate the physical forces required for one possible mechanism: lateral transport of lipid-anchored proteins by fluid flow. Unlike transmembrane proteins, lipid-anchored proteins can be moved long distances across cell surfaces by flow. Our lab has previously developed a method to experimentally measure the flow-mediated drift velocity of lipid-anchored proteins in supported bilayers. Here, we use this method to measure the force required to move protein constructs with different sizes, bound to identical lipid anchors embedded in membranes with the same lipid compositions. We generated a series of protein constructs with increasing size: monomeric streptavidin (mSA2), mSA2 attached to green fluorescent protein, and mSA2 attached to maltose binding protein. To calculate the expected hydrodynamic force on these membrane-bound proteins, accurate cross-sectional areas are required. In order to obtain these, we modeled a biotinylated lipid (bio-DPPE) and positioned it in the biotin binding site of mSA2. Next, either green fluorescent protein or maltose binding protein was attached to mSA2 to make the final constructs. Using CHARMM-GUI, we generated all-atom simulations of each bio-DPPE-mSA2 complex embedded in a POPC membrane. Using these simulations we measured the cross-sectional area and the height above the membrane. These were used to calculate an effective hydrodynamic area for each construct, which we compared to measured hydrodynamic force from our experiments.

500-Pos

Induced order domains are enriched in cholesterol

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The importance of the plasma membrane asymmetry remains without a clear understanding. Many studies pointed to coexistence of liquid phases in model membranes, and in living cells. In addition, different studies claim that domains in the cytoplasmic leaflet could elucidate events in cells, for instance virus assembly. Model bilayers of the cytoplasmic leaflet with symmetric composition show a single fluid environment. Could the cytoplasmic leaflet form a homogeneous environment? Here, we use the method of hemifusion to engineer *in vitro* asymmetric bilayers, where one phase-separated leaflet opposes a fluid leaflet. We observed that an ordered phase of an asymmetric bilayer can cause formation of *induced order domains* in the apposed fluid leaflet. The ordered phase in a separated leaflet can be either the gel or the Lo phase. Order is induced in a leaflet composed of dioleoylphosphatidylcholine (DOPC), opposed to a gel phase; or DOPC/cholesterol = 0.8/0.2 opposed to an Lo phase. In cholesterol-containing mixtures, we measured the lipid packing/order of ordered and disordered domains in asymmetric bilayers using the spectral image of the order sensor probe C-Laurdan. Our results suggest a redistribution of cholesterol, which accumulates in the *induced order domains.* We propose a previously unknown domain composed of intrinsically fluid low Tm lipids, that becomes enriched in cholesterol. The midplane interfacial tension should be lower when order is induced in these fluid lipids, because the induced order reduces the mismatch of order and density at the midplane between mismatched phases opposed to each other. Cholesterol can flow into the induced order region because of the lower cross-sectional area of induced ordered phospholipids.

501-Pos

Sterol-lipids enable large-scale, liquid-liquid phase separation in membranes of only two components

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A wide diversity of membranes, from those with hundreds of lipids (as in vacuoles of living yeast cells) to as few as three (as in artificial vesicles) phase separate into micron-scale liquid domains. This limit of three components is perplexing from a theoretical standpoint: only two components should be necessary. It is equally perplexing from an experimental standpoint: only two lipid types are required to form large-scale liquid domains in lipid monolayers. This incongruity inspired us to search for single, joined ''sterol-lipid'' molecules that replace both a sterol and a phospholipid in membranes undergoing liquid-liquid phase separation. By using sterol-lipids with long, saturated chains, we sought to mimic known preferential interactions between cholesterol and lipids with high melting temperatures. We find that membranes with only two components (one of which is a sterol-lipid) do indeed phase-separate into micron-scale liquid domains. This result mitigates experimental challenges in determining tie-lines and in maintaining constant chemical potentials of lipids as lipid ratios are changed. For one of the binary membranes, we construct a miscibility phase diagram to show how the membrane's phase separation depends on temperature and the ratio of the lipids.

502-Pos

Microfluidic measurements of diffusion and mobility of lipid anchored proteins in liquid-ordered vs. liquid-disordered supported membranes

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Lipid bilayer membranes containing cholesterol can phase separate into two coexisting liquid phases which contain different concentrations of lipids. Area per lipid, acyl chain disorder, and the diffusion constants of fluorescent lipid probes are higher in liquid-disordered phases than in liquid-ordered phases. Our group previously developed a method using microfluidic experiments on supported bilayers to characterize flow transport and diffusion of lipid-anchored proteins. Here, we probe the sensitivity of this technique by using it to determine whether the mobility of identical proteins attached to lipid anchors depends on the degree of saturation of their acyl chains. We created discrete patches of supported lipid bilayer in either a single liquid disordered phase (primarily DiphyPC) or a single liquid-ordered phase (DPPC and cholesterol). We included a small fraction (0.1%) of either saturated biotinylated lipids (bio-DPPE) or unsaturated ones (bio-DOPE) in each composition. After allowing streptavidin to bind to the biotinylated anchor lipids in the upper leaflet, we applied fluid flow and observed the resulting motion of the lipid-anchored streptavidin. This method allowed us to separately measure the diffusion constant and flow mobility for proteins attached to each lipid anchor. We found that in disordered membranes, membrane drag on saturated and unsaturated anchor lipids was the same. However, saturated anchors moved more slowly than unsaturated ones in the ordered membranes. In other words, ordered-phase membranes apply a higher drag force to individual saturated lipids than to unsaturated lipids. Our results support previous observations that heterogeneity in lipid packing and order arise in liquid-ordered membranes.

503-Pos

DPPC and cholesterol form crystalline structure responsible for pulmonary surfactant resistance to collapse

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Pulmonary surfactant is the mixture of lipids and proteins that lowers surface tension in the lungs. The material forms a thin film on the aqueous layer that lines the alveolar air-sacks. When compressed by the shrinking alveolar surface area during exhalation, the surfactant film achieves very low surface tensions. The structure of the film that sustains these low tensions remains obscure. Vesicles of pulmonary surfactant adsorb as collective packets, delivering their complete set of constituents that initially form monolayers at the interface. In this study, we modeled the alveolar films with Langmuir monolayers of DPPC mixed with variable amounts of cholesterol. When compressed at physiological temperatures, cholesterol above a mol fraction of 0.20 caused these films to collapse promptly from the surface. Collapse prevents films from reaching the low surface tensions achieved in the alveolus. The studies here at the physiological temperature determined the structural changes by which cholesterol causes faster collapse of DPPC monolayers. Grazing incidence X-ray diffraction (GIXD) showed that cholesterol incorporates into films of DPPC to form a condensed phase that preserves the same stoichiometry of cholesterol:DPPC over a broad range of cholesterol. Grazing incidence off-specular X-ray scattering (GIXOS) also determined the vertical electron density profile of the film. At surface tensions just above the onset of collapse, the films remain unchanged, with a single monolayer maintaining a constant vertical profile over the range of cholesterol up to a mol fraction of 0.40.

504-Pos

Cardiolipin acyl chain remodeling is required for inner membrane structure in saturated lipid environments

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Cardiolipin (CL) is a unique phospholipid exclusively localized and synthesized in the inner mitochondrial membrane (IMM). The acyl chain composition of CL is tightly regulated, and dysregulation of CL remodeling leads to