proteins. Because tumor cells exhibit altered membrane fluidity, we suggest this might influence pHLIP tumor targeting. We used a cell insertion assay to determine the pKa in live cells, observing that the properties in liposomes held in the more complex plasma membrane. Our results show that the formation of a TM helix is modulated by both the conformational propensities of the peptide and the physical properties of the bilayer. These results suggest a physical role for helix-membrane interactions in optimizing the function of more complex TM proteins.

2751-Plat
Structural Adaptation of Proteins to Different Biological Membranes
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Transmembrane polarity profiles and distributions of different protein groups that interact directly with lipids were calculated for 3D structuresof 187 α-helical and 73 β-barrel proteins from eight types of biological membranes. The polarity profiles were represented by H-bonding donor and acceptor capacities (α and β) and polarizability parameter (π*) calculated for protein surface. The proteins from the set have different hydrophobic thicknesses, with average values of 23.1, 27.3 and 32.5 Å for outer bacterial, inner mitochondrial and eukaryotic plasma membranes, respectively, and 29.5 to 30.6Å for endoplasmic reticulum, thylakoid and different bacterial membranes. The proteins have three distinct polarity regions: lipid head group area with peaks for positively charged groups and crystallized water (15 to 25Å from the bilayer center); interfacial ''mid-polar'' region with peaks for Tyr and Trp (8 to 15Å); and aliphatic hydrocarbon core (±8Å). Polarity of the core region was nearly identical for different membranes (α~0.01, β~0.04, π*=0.09-0.14). Main changes in polarity occur at the level of lipid carbonyl groups (~15±5Å), but the changes are more gradual than in DOPC bilayer. The locations and amplitudes of peaks for Trp, Tyr, and Phe are frequently asymmetric and dependent on the type of membrane. The distributions of crystallized water have two peaks at ±20Å. Head groups of crystallized lipids are shitted closer to the hydrocarbon boundary, especially in mitochondrial membrane and on the inner side of eukaryotic plasma membrane. All proteins have a peak of positive net charge at ~20 Å on the inner side, consistent with the ''positive inside'' rule, except single-chain β-barrels that follow ''negative inside'' rule. All curves are shifted inwards in β-barrels, consistent with their smaller thickness. Computational modeling of membrane proteins can be improved using hydrophobic thicknesses and asymmetric polarity profiles for different membranes.

2752-Plat
Predictions for Cholesterol Interaction Sites on the A2A Adenosine Receptor
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Molecular dynamics simulations of the A2A adenosine receptor totaling 1.4 μsec show clear evidence for specific sites mediating interactions between adenosine-bound A2A and cholesterol. [Lee and Lyman JACS doi: 10.1021/ja307532d] The strongest evidence is for three binding sites. Two are in the extracellular leaflet, with one site interacting with helices VII and I, and the other with helices II and III. One site is located in the intracellular leaflet, interacting with helices III and IV. One of our three predicted binding sites is confirmed by a recently-published high resolution structure of A2A cocrystallized with an antagonist. Our results demonstrate the feasibility of identifying GPCR-lipid interactions with relatively modest computational resources, offering a way to target experimental follow-on studies at specific regions of the protein.

2753-Plat
Sorting of Lipidated Peptides in Fluid Bilayers: A Molecular-Level Investigation
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Nearest-neighbor recognition (NNR) measurements have been made for two lipidated forms of GlyCys, interacting with analogues of cholesterol and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) in the liquid-ordered (Lo) and liquid-disordered (Ld) phases. Interaction free energies that have been determined from these measurements have been used in Monte Carlo simulations to quantify the distribution of the peptides between liquid-ordered and liquid-disordered regions. These simulations have shown that significant differences in the lipid chains have a very weak influence on the partitioning of the peptide between these two phases. They have also revealed an insensitivity of the peptide partition coefficient, Kp, to the size of the Lo and Ld domains that are present. In a broader context, these findings strongly suggest that the sorting of peripheral proteins in cellular membranes via differential lipidation may be more subtle than previously thought.

2754-Plat
Cation-PI Interactions as Specific Anchors for B. Thurigiensis Phosphoinositol-Specific Phospholipase-C Binding to Phosphatidylcholine Bilayer
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Phosphatidylinositol specific phospholipase Cs (PI-PLCs) from extra-cellular bacterial pathogens are associated with bacterial virulence. Membrane binding and enzymatic activity of many of these PI-PLCs are specifically enhanced by the presence of phosphatidylcholine (PC), an abundant phospholipid in the outer plasma membrane of eukaryotic cells targeted by these bacteria. The frequency of aromatic amino acids, especially tyrosines, at the interface between PI-PLC and the membrane is strikingly high. Interestingly X-ray structures of choline-containing substrates bound to their receptors have revealed tyrosine-mediated pi-cation interactions with choline moieties (Cho). In order to investigate the presence of pi-cation interactions between PI-PLC tyrosines and Cho in lipid headgroups, we performed a 500 nanoseconds-long molecular dynamics (MD) simulation of PI-PLC anchored the surface of a lipid bilayer containing 256 dimyristoylphosphatidylcholine (DMPC) lipids. The analysis of the trajectory reveals six tyrosines that are involved in pi-cation interactions with bilayer lipids. Two, Tyr88 and Tyr246, engage in long-lasting Tyr-Cho interactions that are present more than 80% of the time, while four others, Tyr251, Tyr204, Tyr86 and Tyr118, interact with Cho less frequently (<40%), but regularly during the simulation. In our simulations, all significant pi-cation interactions with cholines are thus mediated by tyrosines while tryptophans interact with the lipid tails. Together with new experimental data on wild-type and tyrosine mutants of PI-PLC our results indicate an important role for tyrosine-mediated pi-cation interactions in the specific binding of PI-PLC to PC-containing membranes. Further we suggest that tyrosine-rich interfacial binding sites in amphitropic proteins might indicate a role for pi-cation interactions.

2755-Plat
The Dengue Virus Capsid Protein Inhibitor Peptide Pep14-23 becomes Alpha-Helical upon Binding to Negative Lipids
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Dengue virus (DENV) causes a mosquito-borne disease affecting millions of people, which is spreading to temperate regions, including North America and Europe. Currently, there is no effective treatment for DENV infection. The interaction in the host liver of the viral capsid (C) protein with host intracellular lipid droplets (LDs) is crucial for virus formation [1], being previously studied in our labs [2, 3]. DENV C-LDs interaction involves a conserved segment of DENV C intrinsically disordered N-terminus [3], which led to the design of pep14-23, a novel peptide inhibitor of this critical interaction [3].
Here, we used bioinformatics tools, combined with circular dichroism (CD) and zeta-potential analysis, to determine the structural parameters and the tendency of DENV C and pep14-23 to interact and bind lipid vesicles. Bioinformatics suggests that the Flavivirus capsid protein N-terminus region, roughly corresponding to pep14-23, has a high α-helical tendency and is likely to interact with lipid systems. CD shows a conversion of pep14-23 to α-helix conformation in the presence of negative phospholipids. Zeta-potential light scattering spectroscopy supports the CD data, showing that the peptide binds strongly to negative lipid vesicles.
pep14-23 inhibition mechanism may therefore involve its binding to negative LDs phospholipids and the conversion to an α-helical peptide. This finding contributes to the design of pep14-23 based treatments of DENV and similar Flavivirus infections.
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2756-Plat
Insertion of Hsp70 into Membranes is Mediated by Negative-Charged Phospholipids and Modulated by the Fluidity of the Bilayer
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