the explicit bilayer ensembles are in very good agreement with experiment. (Crossing angles from the implicit simulations are incorrect.) The predicted helix packing residues differ from experiment. It is possible the 100 ns simulation times are insufficient for a sampling of helix rotation and thereby a satisfactory determination of the residues involved in helix-helix packing.

2535-Pos Board B305
Highly Pegylated Sterically Stabilized Micelles in Aqueous Media: Structure, Dynamics, and Storage of Therapeutic Agents
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Molecular assemblies of highly PEGylated monomers are important in many biomedical applications. For example, sterically stabilized micelles and liposomes of self-assembled DSPE-PEG2000 monomers and other phospholipids can serve as biocompatible and relatively nontoxic drug delivery nanocarriers. We perform a detailed study of the micelles formed from DSPE-PEG2000 in pure water and isotonic HEPES buffered saline solution [1]. The observed micelle sizes (5 - 15 nm) strongly depend on the solvents and the lipid concentrations used. The critical micelle concentration of DSPE-PEG2000 is ~ 10 times higher than in water and in buffer and the viscosity of the dispersion dramatically increases with the lipid concentration. To explain the experimentally observed behavior, we perform atomistic molecular dynamics simulations of the solvated micelles. Our modeling reveals that the observed assemblies have very different aggregation numbers of N ~ 90 in saline solution and N < 8 in water, due to very different screening of the charged phosphate groups in the DSPE-PEG2000 monomers. We find that in saline solution the micelle cores can inflate and their PEG coronas highly fluctuate, thus allowing storage and delivery of molecules with different chemistry. We also model the stabilization of drug molecules and small therapeutic peptides in different regions of the micelle. [1] Vukovic, L.; Drake, S. D.; Khatib, F. A.; Madriaga, A.; Brandenburg, K. S.; Kral, P.; Onyuksel, H. J. Am. Chem. Soc. 2011, 133, 13481-13488.

2536-Pos Board B306
Effect of Lipid Unsaturation on Membrane Protein Structure and Function from Multi-Microsecond Molecular Dynamics Simulations
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Lipid membrane composition is an important factor in controlling the structure and activity of many membrane proteins. This regulation can take place through the alteration of membrane physico-chemical properties or through direct protein-lipid interactions. Atomistic molecular dynamics simulations on the microsecond timescale can help uncover the general and specific mechanisms of protein function modulation. Omega-3 lipids play key roles in controlling ion channel activity in the brain and heart, with deficiencies associated with a number of a health issues, including cardiac and Alzheimer’s disease, cognitive function and vision disorders. We have explored the effects of lipid tail unsaturation by carrying out ~10 microsecond simulations of well-characterized membrane proteins rhodopsin and ion channel KcsA, incorporated into lipid bilayers containing the sn-2 chain with 0 (palmitic), 1 (oleic) and 6 (docosahexaenoic, DHA) double bonds. We observed a marked preference for DHA to solvate the trans-membrane helices of the protein and have identified protein residues preferentially interacting with the unsaturated chains. We will report calculations that reveal the effects of lipid unsaturation on the protein structure and fluctuations, with implications for protein activity. Finally we will discuss ongoing simulations of the KcsA channel in both its closed and open (active) states, to directly uncover the role of lipid unsaturation in function.

2537-Pos Board B307
Water Between Lipids: Domains For Peptides Insertion?
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Capacitance values of monolayers at different areas denote that in a state at which the area per lipid is above a critical value water path are formed beyond the hydration shell of the lipids. This area increase is 12% of the area for lipids in the expanded state and is comparable to that at which the insertion of proteins and peptides takes place in a variety of lipid composition. Therefore, it is concluded that water pathways formed by exploiting the entrance of few water molecules into the lipid bilayer is a general phenomenon. Interestingly some aminoacids having biological activity are able to induce those water paths by interacting with specific groups of the lipids, such as the amine groups in ethanolamines. The kinetics of formation; the thermodynamic and structural properties of those water pockets in the restricted microenvironments framed by lipid groups and its relevance in the selective modulation of the protein-membrane interaction is discussed considering the amount and the state of water induced by the different kinds of groups at the interface region that may act as donor or acceptors in H-bonds, for instance, PO, CO and NH. The analysis is made considering surface pressure and capacitance changes in monolayers at different areas and compared with structural data obtained by means of standard FTIR and bidimensional infrared spectroscopy. We will use this information for a further insight on the insertion of positively charged peptides into lipid membranes as described by molecular dynamics.

Membrane Fusion

2538-Pos Board B308
SNARE-Mediated Fusion Pore Dynamics from Quantitative TIRF Microscopy
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SNARE proteins are involved in most intracellular fusion events. SNAREs drive complete fusion but are also thought to generate dynamic flickering pores and reversible fusion. Live cell amperometry and simultaneous capacitance recordings indicated that the characteristic pre-spike foot may correspond to a flickering pore which later opens completely (Alvarez de Toledo et al., Nature, 1993). Here we study fusion and pore statistics between SNARE-reconstituted vesicles and supported bilayers (SBLs) using quantitative total internal reflection fluorescence microscopy (TIRFM) with single-lipid resolution. Based on the intensities of the fluorescent lipid-labeled vesicles and the course of the time-dependent intensity increase upon fusion as lipids diffuse through the pore into the SBL, we determined vesicle sizes, the delay time to create the initial pore, and the rate of intermembrane lipid mixing through the pore. These measurements required us to develop a quantitative image analysis algorithm which accounts for TIRFM effects including the spatial decay of incident light, polarization effects, fluorescence dequenching and bleaching. In cholesterol-free vesicles, lipid transfer from the SBL was slower than would be expected were the fusion pore fully open, suggesting that the pore flickers between open and closed states. To quantify these effects we developed a mathematical model of the stochastic fusion pore and the passage of lipids from vesicle to SBL through the pore. Combining the model and experimental measurements, we infer the fraction of time for which the pore is open. Without cholesterol, the pore favors the closed state. We find cholesterol has profound effects: it decreases the delay time between docking and fusion and, once formed, the pore remains fully open. Our results suggest that cholesterol favors the open pore state. Additionally, we report measurements of vesicle curvature dependence of fusion and pore flickering.

2539-Pos Board B309
Cooperativity of SNARE Complexes in Membrane Fusion: Mechanisms of SNARE Cluster-Mediated Docking and Fusion
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SNARE proteins are the core of the cellular fusion machinery. Vesicle v-SNAREs engage target membrane t-SNAREs and form SNARE complexes thought to pull the membrane surfaces together and drive fusion. Evidence suggests that many SNARE complexes participate in single fusion events and in SNARE-reconstituted vesicle-supported bilayer fusion assays 5-11 complexes were required for fusion [Karatekin et al., PNAS, 2010]. However a mechanistic understanding of this requirement is lacking. We have developed a mathematical model of SNARE cluster-driven docking and fusion which explicitly accounts for interactions between the participating SNARE complexes and the intermembrane forces that must be overcome to trigger fusion. The model is analyzed by a combination of analytical methods and computer simulations. We find that the energetically-favored cluster configuration is a ring of completely assembled SNARE complexes which dock the vesicle to the target.