bilayer as two abutted monolayers, each with a neutral surface. The constraint imposed by mathematically placing two monolayers in apposition causes minimal energy to be larger than that predicated by (incorrectly) assuming that the elastic properties of a bilayer can be quantitatively captured through a single surface. Independent of pore size, the deformation of tilt did not appreciably affect elastic energies; in other words, membrane splay dominates elastic energies. For small radii, shapes of minimal energy were close to the shape of a catenoid. For large pores, however, deviations of minimal energy shapes from catenoids were large, resulting from the necessity that the membranes be parallel and the separation between them fixed at distances far from the rim of the pore. Energies for minimal shapes were 15-60kT less than the energy of the toroidal shape for pore radii in the range of 2-16 nm and for initially parallel membranes that were separated by 2-4 nm. For the smallest pore possible (i.e., an initial pore), a toroidal geometry overestimated the minimal energy by 30 kT. For pores with radius larger than length, membrane separation near the rim of the pore exceeds the distance between the parallel membranes. These shapes of minimal elastic energy can now be used to calculate fusion pore dynamics.

480-Pos Board B249
Free Energy Landscapes of Vesicle Fusion by Umbrella Sampling MD Simulations
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Despite intensive investigation, the energy landscapes governing membrane fusion in vitro and in vivo remain uncertain. A plethora of factors including small molecules, ions, fusion proteins, and osmotic pressure gradients are known to influence fusion rates, but these perturbations only hint at the underlying molecular mechanisms.
The barriers and metastable structures that characterize fusion free energy landscapes are inherently difficult to resolve atomistically due to the fluid, disordered nature of membranes. These pathways are also difficult to access with molecular resolution simulations, namely molecular dynamics (MD), due to the time scales associated with spontaneous fusion and the lack order parameters capable of driving fusion progress through high energy intermediates.
To address this challenge, we have developed a novel umbrella sampling method paired with an order parameter capable of driving and controlling fusion progress. Our initial results for 20 nm POPC vesicles give a barrier of 43 kBT along a pathway beginning as a metastable stalk, proceeding over a barrier with a hemifused structure and then ending as an opened fusion pore. Though marginally metastable, the hemifusion diaphragm does not expand, likely due to the small vesicle size and the lack of a lipid reservoir, but instead either reverts back to a stalk or proceeds forward to form a fusion pore.

481-Pos Board B250
Local Stresses in Fusing Membranes from Molecular Simulation
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Membrane fusion involves transient and non-uniform stresses on the participating membranes. It is believed that these stresses help drive evolution of lipidic fusion intermediates and determine fusion pathways and outcomes. It has also been shown both via experiments and molecular dynamics simulations that lipid composition can dramatically affect fusion kinetics and efficiency. We have developed a means to measure locally resolved pressure in molecular dynamics simulations and implemented it in the GROMACS software. We use this to measure pressure stresses on highly-curved fusion intermediates. The non-uniform, fluctuating, and spatially curved nature of these intermediates makes measurements challenging; we utilize techniques from computational geometry to assist convergence in our measurements. We interpret our findings in the context of prior fusion theories of lipidic stalk formation, hemifusion interstitial energy, and pore formation. We also examine local membrane pressure changes near fusion peptides.

482-Pos Board B251
A Molecular Mechanism of Lipid Membrane Fusion
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Membrane fusion is an essential molecular event involved in many cellular processes, such as exocytosis, endocytosis, intracellular vesicle trafficking, fertilization, and viral infection to target cells. In spite of extensive studies of membrane fusion, however, the basic molecular mechanisms in biological systems are not well understood. Probably, it is due to the complex nature of biological membranes and the variety of possible molecular pathways for membrane fusion. We have studied the membrane fusion process, particularly ion-induced membrane fusion. Biological membrane fusion seems to occur with either ion-induced or non-ion-induced membrane process, particularly the later case is for virus membrane fusion system. Dr. Chernomordik and his co-workers have studied on non-ion-induced lipid membrane fusion and developed the so-called ''Stalk-intermediate model'' before total membrane fusion. That fusion model has been well received by many membrane fusion investigators, particularly in the virus fusion field. Stalk formation between two lipid membranes may occur due to undulation of lipid molecules or local binding of the lipid bilayers, which results in the formation of a local region of outer monolayer fusion. The stalk hypothesis can be described by macroscopic models treating bilayers and monolayers as homogeneous elastic surfaces. We have also studied non-ion-induced bilayer membrane fusion. Our membrane fusion theory is based on the interaction energies between the two membranes due to alternation of the membrane surface properties, e.g., hydrophilicity and hydrohobicity of interacting membranes, and then lipid membrane close approach and due to membrane curvature. Although the membrane interaction processes are different between the two models, these membrane fusion properties are the same as those of our ion-induced lipid membrane fusion.

Protein-Lipid Interactions I

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Influence of pH and Side-Chain Negative Charge on the Behavior of Designed Transmembrane Peptides in Lipid Bilayers
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GWALP23 (acetyl-GGALW^{5}LALALALALALALW^{19}LAGA-amide) is a favorable model peptide for investigations of single-residue effects on protein-lipid interactions and the properties of membrane-spanning helices (J. Biol. Chem. 285, 31723). GWALP23 has favorable properties in bilayer membranes because the peptide exhibits only limited dynamic averaging of NMR observables such as the ^{2}H quadrupolar splitting or the ^{15}N-^{1}H dipolar coupling (Biophys. J. 101, 2939). To investigate the potential influence of negatively charged side chains upon system properties, we have substituted a single Leu residue with Glu at different positions and incorporated specific ^{2}H-Ala labels in the core of the single-Trp peptide Y^{5}GWALP23 (see Biochemistry 51, 2044). Solid state ^{2}H NMR experiments were used to examine the peptide orientation and dynamics as functions of the lipid bilayer thickness and pH in hydrated lipid bilayer membranes. We observed well defined ^{2}H quadrupolar splittings for Y^{5}GWALP23-E16 in the pH range from 4.0 to 8.2, suggesting that the peptide helix is well oriented in DOPC lipid bilayers. The glutamic acid residue, though protonated, seemed to confer multi-state behavior at pH 2.5, and the resulting populations exhibited slow exchange on the NMR time scale. The deprotonation of E16 at pH 8.2 did not have any effect on the peptide orientation, perhaps suggesting that the close proximity of E16 to W19 (on the next helical turn) could provide stability to the peptide helix. We are also studying the peptide-lipid behavior when Glu is substituted in position 12 and/or 14, individually or together.

484-Pos Board B253
Functional Consequences of Incomplete Hydrophobic Matching at TM1 of the LeuT Transporter
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The Leucine Transporter (LeuT) is the prototype for structure-function studies of mammalian Neurotransmitter: Sodium Symporters such as DAT, SERT and NET, the transporters for dopamine, serotonin, and norepinephrine, respectively. Its functional sensitivity to the environment, i.e., membranes or detergents in various compositions, has engaged much recent research. As the role of the environment in the function and organization of transmembrane proteins has been shown to involve hydrophobic mismatch, we investigated the membrane deformation and extent of hydrophobic matching for LeuT with the recently described hybrid Continuum-Molecular Dynamics (CTMD) method that combines elastic continuum formulations with an atomistic description of the lipid-protein interface from molecular dynamics simulations. The analysis was performed for functionally relevant conformations of LeuT embedded in two different model membranes: a POPC lipid bilayer and a model bacterial bilayer composed of a 3:1 mixture of POPE and POPG lipids. In both bilayers we found significant membrane thinning and water penetration near the membrane-facing Lys288 of TM7, a positively charged residue embedded

deep inside the bilayer. This generates a polar environment near Lys288, but leads to unfavorable hydrophobic-polar interactions at neighboring membrane-facing hydrophobic residues in the cytoplasmic-end segments of TM1 (TM1a) and TM7. Analysis of the K288A mutant MD simulations showed that both membrane deformation and water penetration were eliminated, together with the unfavorable hydrophobic-polar interaction at TM1a and TM7. These results connect the hydrophobic mismatch due to the non-conserved Lys288 residue to a key structural element mediating transport, the TM1a segment that has been shown to move outward during substrate transport. In so doing, the results also provide mechanistic insights into how the K288A mutation, a background mutation used in some recent experimental studies, leads to significantly improved transport properties in LeuT.

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Comparison of Interfacial Tyrosine, Tryptophan and Phenylalanine Residues as Determinants of Orientation and Dynamics of Transmembrane Peptides

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Aromatic amino acids often flank the transmembrane alpha helices of integral membrane proteins. By favoring locations within the membrane-water interface of the lipid bilayer, the aromatic residues Trp, Tyr, and Phe may serve as anchors to help stabilize a transmembrane orientation. In this work, we compare the influence of interfacial Trp, Tyr or Phe residues upon the properties of helical transmembrane peptides. For such comparisons, it is critical to start with no more than one interfacial aromatic residue near each end of a transmembrane helix, for example that of GWALP23 (acetyl-GGALW^5(LA)$_6$LW^{19}LAGA-[ethanol]amide; see J. Biol. Chem. 285, 31723). To this end, we have employed ^{2}H-labeled alanines and solid-state NMR spectroscopy to investigate the consequences of replacing W5 in GWALP23 with Tyr or Phe residues at the same or proximate locations. We find that GWALP23 peptides having Y5, F5, or W5 exhibit essentially the same average tilt in bilayer membranes of DLPC, DMPC, and DOPC. When double Tyr anchors are present, in Y^{4,5}GWALP23, the NMR observables are more subject to dynamic averaging and at the same time less responsive to the bilayer thickness than when a single Tyr or Phe residue occupies position 4 or 5. Interestingly, Y^{4}GWALP23 and Y^{5}GWALP23 show similar low levels of dynamic averaging and have a difference of about 30°-40° in the preferred helix azimuthal rotation angle in each lipid. Decreased dynamics are observed when ring hydrogen bonding is removed as in the case of F^{4,5}GWALP23. We conclude that, in the absence of other functional groups, interfacial aromatic residues determine the preferred orientations and dynamics of membrane-spanning peptides.

486-Pos Board B255

Cyanylated Cysteine used to Observe the Interaction between the CM15 Antimicrobial Peptide and Neutral Lipid Membranes

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Antimicrobial peptides, the first immune response, are known to provide protection against pathogens; there are three proposed pore formation models by which antimicrobial peptides are believed to disrupt membranes. Previous work used the artificial amino acid cyanylated cysteine to confirm that the synthetic hybrid antimicrobial peptide CM15 porates anionic lipid vesicles by the barrel stave mechanism, which requires a single dominant orientation of the peptide on the lipid surface. Recent molecular dynamics results suggest that CM15 might adopt a number of different structures when binding to neutral lipid bilayers, including those made primarily of phosphatidylcholine (PC) lipids. Infrared spectrometry of CM15 variants provides evidence that the lipid bound conformation of CM15 to neutral bilayers varies significantly from that in contact with anionic bilayers. Additionally, circular dichroism experiments give further insight into the different structures of CM15 in contact with neutral lipid membranes.

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Supported Lipid Bilayers at the Air-Water Interface: A Comprehensive Mechanistic Study of the Cyclic Peptoid Ml 2-6 on Model Bacteria Membrane Systems

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In this investigation, three complimentary experimental techniques including atomic force microscopy (AFM), X-ray reflectivity (XR), and epiflourescence microscopy (EFM) were employed to determine the mechanism of action of the antimicrobial cyclic peptoid ML2-6 on model mammalian and bacteria membrane systems. Mammalian and bacterial membranes were mimicked with Langmuir monolayers and supported bilayers both at solid support and at the air-water interface. We introduce a novel approach in which octadecyltrimethoxysilane (OTMS) supported lipid bilayers are used to mimic mammalian and bacterial membranes, which can be probed with X-ray scattering at the air-water interface. ML2-6 was found to be active on all bacteria membrane models, which was deduced by morphological changes observed from AFM and EFM images following the introduction of the peptoid into the system. In addition, XR revealed changes in film thickness and electron-density profile after the addition of ML2-6, consistent with peptoid insertion into phosphatidyl-glycerol (PG) lipid headgroup in both monolayer and bilayer mimics. Conversely, ML2-6 was found to be inactive on all mammalian membrane models investigated, which was concluded form the lack of morphological changes observed from AFM and EFM images when the peptoid was introduced into the system. Furthermore, XR revealed little change in DPPC/Cholesterol film thickness and electron-density profile after the addition of ML2-6. This suggests that ML2-6 may exhibit potential as an antibacterial agent with low cytoxicity to mammalian host cells.

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Phytochemicals Promiscuously Alter Membrane Protein Function and Bilayer Properties

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Biologically active plant phenols (phytochemicals) are a cornerstone of traditional medicine. Phytochemicals have attracted increasing attention from Western medicine, and thousands of studies on their activity are published each year. Phytochemicals exert a broad range of pharmacological effects including being antioxidant, anti-inflammatory, anticarcinogenic and antimicrobial, yet their mechanism(s) of action are usually ill defined. Some better-studied phytochemicals modulate the function of a multitude of unrelated proteins, with few identified binding sites. Different phenolic compounds often affect the same proteins, many of which are membrane-associated. Additionally, in spite of large variations in chemical structure, plant phenols often have synergistic effects. In this context, it may be relevant that phytochemicals generally are hydrophobic/amphipathic and tend to adsorb to lipid bilayers. Phytochemicals therefore could exert some of their actions indirectly by perturbing membrane properties. To test the hypothesis that plant phenols exert their action on protein function by altering lipid bilayer properties, we chose five heavily studied phytochemicals: capsaicin (chili peppers), curcumin (turmeric), EGCG ((-)-epigallocatechin gallate, green tea), genistein (soybeans), and resveratrol (grapes). We measured their propensity to alter bilayer properties using gramicidin A channels, as probes for changes in bilayer material properties, and explored the phytochemicals' effect on various membrane proteins: potassium channels, membrane-anchored metalloproteases, mechanosensitive channels of large conductance and voltage-gated sodium channels. All the tested compounds alter bilayer properties at concentrations consistent with their reported biological activity; they also altered the function of the membrane proteins tested, albeit at varying concentrations. We show that phytochemicals can alter protein function through a bilayer-mediated manner; therefore, any studies on phytochemicals should keep their promiscuity in mind and before claiming specific interactions evaluate the possibility of an indirect membrane mediated mechanism.

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Lipid Membrane Binding of Computationally-Designed DNA Aptamers Specific for Phosphatidylserine

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Recently we applied an entropy based seed-and-grow strategy to design a set of short DNA aptamers (Chem. Biol. Drug Des. 78:1-13). Each member consisting upto 12 nucleotides binds specifically with phospholipid phosphatidylserine (PS). The PS binding aptamers can be used e.g., to diagnose the PS