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Association of Transmembrane Helices in Viral Fusion Peptides Suggests a Protein-Centric Mechanism of Membrane Fusion

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A broad range of biological functions, from neurotransmitter release to infection by enveloped viruses, is achieved by fusogenic proteins, which increase the intrinsically slow rate of membrane fusion by using their energetically downhill conformational changes. Among viral fusogenic proteins, many are identical trimers that feature a fusion mechanism reminiscent of the SNARE proteins responsible for vesicle fusion. In this mechanism, the N-terminal hydrophobic fusion peptide domains (FP) of the three protein chains insert into the host cell's membrane, and then zipper with the viral-membrane attached TM domains of the same protein, driving the two membranes together in the process. So far, only high resolution structures of the soluble portions exist, and the minimal number of trimeric proteins is also undetermined. By analytical ultra-centrifugation and polarized infrared spectroscopy, we proved that the FP of the parainfluenza virus (PIV) fusogenic protein forms hexameric helical bundles that lie transverse to lipid bilayers. We modeled the FP hexamer's structure and refined it by molecular dynamics simulations, observing an association mode mediated by water and a glutamine amino acid, frequently occurring in diverse fusion peptide sequences. This structure suggests that as few as two trimeric proteins may form a hexameric bundle of their FP domains, and induce curvature on the cellular membrane through a mechanism analogous to mechanosensitive channels. The FP hexamer is at the geometric center of such fusion mechanism at all times. By simulations at atomic detail of the fusion process, we identified the amino acids that control the associated hydration and dehydration events, and suggest new strategies to inhibit viral infections.

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High-Resolution Secondary and Tertiary Structure of the Membrane-Associated HIV Fusion Peptide by Itself and in Large gp41 Ectodomain Constructs: Correlation Between Beta Sheet Registry, Membrane Insertion and Perturbation, and Fusion Catalysis

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The initial step of HIV infection is fusion between the viral and target cell membranes. Fusion is mediated by the HIV gp41 protein and its N-terminal ''fusion peptide'' (FP) which binds to target cell membranes. The FP by itself catalyzes membrane fusion and the secondary and tertiary structure of membrane-associated FP was probed at high-resolution using solid-state NMR spectroscopy. For membranes with biologically-relevant cholesterol content, the FP forms antiparallel β sheet structure with a wide distribution of antiparallel registries. The population of each registry was quantified and a good correlation was observed between registries that were populated and those which had negative free energies of membrane insertion. A very different registry distribution was detected for the non-functional V2E mutant which binds to membranes but is not membrane-inserted. These results support a general structure-function model which correlates β sheet FP registry, FP membrane insertion and membrane perturbation, and fusion catalysis. This model is currently being tested for large membrane-associated ectodomain constructs of gp41 that contain the FP.

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Individual Vesicle-Vesicle and Vesicle-Planar Bilayer Fusion Events Mediated by DNA

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We have previously reported that DNA-lipid conjugates, when inserted into lipid vesicles, can mediate interactions between vesicles such as docking and fusion*. These DNA-mediated interactions are a model for the biological machinery (SNARE proteins) employed in synaptic vesicle fusion. We can also use DNA-lipids to build model membrane platforms, such as tethered vesicles and tethered free-standing membranes, which are employed in these studies to observe individual fusion events using fluorescence microscopy. Two systems will be described: vesicle to vesicle fusion between mobile, tethered vesicles, and vesicle to planar bilayer fusion of small vesicles to a DNA-tethered free-standing bilayer. Fusion of individual vesicles is observed in real time using lipid and content mixing assays, as well as FRET upon hybridization of labeled DNA-lipids. These studies provide insight into the relative timescales and extents of docking, lipid mixing, and content exchange. In addition, using different sequences of fluorescently labeled DNA to mediate fusion gives insight into the importance of the rates and number density of hybrid formation at the fusion pore relative to the rate of fusion pore opening. This fusion machinery can also be used to deliver membrane proteins from small vesicles into GUVs or DNA-tethered planar bilayers, allowing membrane proteins to be studied in a free-standing bilayer.

*Chan et al, PNAS 2007 and 2009; Chan et al, Biointerphases 2008.

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DNA-Machinery for Delivering Membrane Proteins into Free Standing Lipid Bilayers

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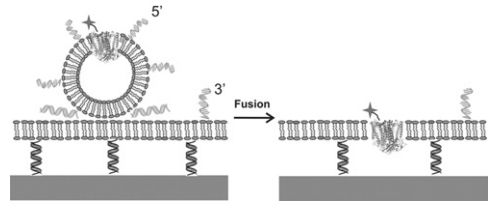
Free standing lipid bilayers tethered to a glass surface by short DNA duplexes provide an excellent environment for studying transmembrane proteins free from any surface interactions. However, membrane protein dynamics in such model membranes are by far less studied due to the difficulty of protein incorporation into GUVs and tethered membranes while maintaining their function. Inspired by previous work on DNA-mediated membrane fusion study of our group*, we applied a DNA-machinery to achieve fusion of proteoliposomes containing photosyntethic reaction center to either GUVs or DNA-tethered lipid membrane patches formed by GUV rupture onto DNA coated glass surfaces**. The diffusion behavior of delivered proteins is measured and compared with those in supported bilayers. Also, the protein activity and orientation before and after fusion is analyzed. This will offer a feasible method to incorporate intact membrane proteins to already formed model membranes. In addition, the behavior of proteins during the fusion event will provide insight into the mechanism of DNA-mediated lipid membrane fusion.



* Chan *et al., PNAS*, **106**, 4, 979-984 (2009)

** Chung *et al., Journal of Structural Biology*, **168,** 190-199 (2009)

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Interaction Forces Between Model Myelin Membranes

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Myelin dysfunctions vary from deterioration of signal transduction to demyelinating diseases such as multiple sclerosis (MS). MS is characterized by a change in the lipid composition of the myelin membrane which leads to the appearance of lesions reflecting loss of inter-membrane adhesion. We want to understand the molecular synergistic interaction between lipids responsible for a healthy myelin membrane structure.

We have performed Surface Force Apparatus measurements on supported myelin membranes, in order to study the effect of lipid composition and calcium on the interaction forces. Interaction forces between cytoplasmic healthy model membranes were found to be repulsive and electrostatic in nature at large separation distances. At smaller separation distances, steric forces due to the compression of the lipid headgroups and the hydration layer take over. Addition of calcium affected the effective surface potential of the membrane and its thickness. At high compressive loads and long contact time, adhesion forces appear but remain quite low (<2mN/m). Van der Waals and hydrophobic interaction forces are the main components of this adhesive force. Lipid composition of the membrane was changed to model the cytoplasmic membrane composition found in patients having MS. The interaction forces between the membranes in calcium depleted medium were found to be quite similar to healthy model. Addition of calcium in the medium resulted in a large increase of the adhesion force which magnitude appears to be time and load dependent. This enhancement of the adhesion energy is accompanied by a thinning of the membrane during contact time indicating that hydrophobic interaction is responsible for the increase in the adhesion force.

These results show how the molecular forces between model myelin membranes depend on the lipid composition which will allow us to develop a molecular model of the early stages of demyelination.

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The Accelerated Late Adsorption of Pulmonary Surfactant

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The rate at which surfactants lower surface tension (γ) during adsorption to an air/water interface normally slows progressively as γ falls. With vesicles of pulmonary surfactant, the slope of the γ-time isotherm initially decreases, but it then becomes steeper just before reaching the equilibrium γ. To determine the mechanisms that produce the accelerated drop in γ, we tested whether