3496-Pos
Fusion Activity of HIV Gp41 Fusion Domain Is Related to its Secondary Structure
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The HIV gp41 fusion domain plays a critical role in membrane fusion during the viral entry process. A thorough understanding of the relationship between the structure and activity of the fusion domain in different environments helps to formulate mechanistic models on how it might function in mediating membrane fusion. The secondary structure of the fusion domain in small unilamellar vesicles composed of different lipid compositions was investigated by circular dichroism spectroscopy. In membranes containing less than 30% cholesterol, the fusion domain forms an alpha helix, and in membranes containing more than 30% cholesterol, the fusion domain forms a beta structure. EPR spectroscopy of spin-labeled fusion domains is consistent with two different conformations in membranes with and without cholesterol and further determines the orientation and depth of the fusion domain in membranes. The fusion and membrane penetration activity of this domain in different membranes was measured by fluorescent lipid mixing and contents leakage assays. Interestingly, the fusion domain fuses membranes in both its helical and beta-structured forms. A high percentage of cholesterol, which promotes beta structure, promotes fusion, but a high concentration of acidic lipids, which in the absence of cholesterol leads to membrane insertion as an alpha helix, also promotes fusion. The results indicate that the HIV gp41 fusion domain is plastic in different membrane environments and that both the alpha helical and beta structured forms may contribute to fusion. Supported by NIH grant AI030557

3497-Pos
Voltage Across the Target Cell Membrane is a Strong Regulator of Fusion of Virus Containing Class II or Class III Proteins
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Simultaneous electrophysiological voltage clamp and confocal microscopy were used to investigate the dependence of fusion on the voltage across the membrane of the target cell for class II and class III fusion proteins. Cells expressing the fusion protein of Semliki Forest Virus or Venezuelan Equine Encephalitis Virus, both class II, or Vesicular Stomatitis Virus, class III, fused to target cells that were voltage-clamped to a trans-negative potential. Fusion did not occur for positive potentials. This pattern also held for fusion of pseudotyped virions to cells. These virions contained one of the three types of fusion proteins and a fluorescent lipid in the envelopes, and GFP within their core, allowing both hemifusion and fusion to be monitored after acidification of the external medium. Fusion of the pseudovirions exhibited the same voltage-dependence as found for cell-cell fusion. Hemifusion occurred for either voltage polarity at physiological temperature, but the kinetics of hemifusion depended on polarity and lipid dye transfer from virus to cell was aborted for positive, but not for negative potentials. The latter strongly indicates that hemifused membranes separate back into two distinct membranes for positive potentials, accounting for the block of fusion for this polarity. Creating hemifusion at low temperature allowed the hemifusion stage to be captured and studied. Fluorescent lipid dye clearly left the virus and content mixing was subsequently observed for trans-negative potentials, showing that hemifusion is a functional intermediate of fusion in this system. Voltage may be an important driving force for infection by all viruses that utilize class II and III proteins. Supported by NIH GM 27367.

3498-Pos
Conformational Sampling of Influenza Fusion Peptide in Membrane Bilayers as a Function of Termini and Protonation States
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Influenza virus fusion peptide (IFP) consists of the last 20 amino acid residues of the HA2 subdomain of influenza virus hemagglutinin. IFP is critical in mediating fusion of viral and host cell membranes during viral entry. The interaction of monomeric influenza fusion peptide with membranes is studied with replica exchange molecular dynamics simulations using a new implicit membrane model to reach sub-millisecond time scales. The conformational sampling of the fusion peptide was studied as a function of different N- and C-termini, including an experimental construct with an additional C-terminal tag, as well as a function of protonation of acidic residues. It is found that the influenza fusion peptide mostly adopts helical structures with a pronounced kink at residues 11-13 with both N-terminal and C-terminal helices oriented mostly parallel to the membrane surface. A charged C-terminus and the presence of a charged C-terminal tag significantly alters the conformational sampling of the fusion peptide while altered protonation states at pH=5 appear to have only a minor effect.

3499-Pos
Membrane Fusion Enhanced!
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Membrane fusion is essential for the life of eukaryotic cells. Fusion proteins play an essential role in membrane fusion by overcoming a variety of energetic barriers when mediating fusion.
Here, we focus on the energetic barriers in the fusion pathway and the tactics which fusion proteins use to overcome those barriers. More specifically, we study the effects of fusion mediators on the formation of the stalk, hemi-fused state and fusion pore by means of molecular dynamic simulations. Our results elucidate the physical principles which fusion proteins use to enhance membrane fusion.

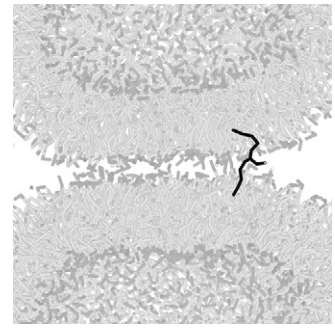

Figure 1. A single lipid initiates the stalk in protein free membrane fusion. What would a protein do?

3500-Pos
Inaccuracy of the Helfrich Equation for the Curvature Energy of Fusion Intermediates
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We must know the curvature energy of fusion intermediates in order to understand how fusion-mediating proteins overcome the energy barrier to fusion. As calculated with present models, stalk energies are too high to explain observed fusion rates, by $> 30\ k_BT$ for pure lipid stalks (Siegel, Biophys. J. 95:5200 [2008]). One possible explanation is that the curvature energy of stalks has been calculated using the Helfrich expression (Z. Naturforschung C 28:693 [1973]), which is based on a continuum model. That equation is quadratic (second order) in curvature, and was derived for low-curvature surfaces. Here I test whether the Helfrich equation is accurate for high-curvature surfaces like the monolayers of ''stalk'' fusion intermediates. I apply a fourth-order model which is successful in describing Q_{II} phase stability (Biophys. J. 91:608 [2006]). There is only enough data to do this for one lipid; DOPE-Me. For a surface like a ''non-smooth'' monolayer stalk (e.g., Kozlovsky et al., Biophys. J. 87:2508 [2004]), the fourth-order model reduces the total curvature energy by ca. 15 k_BT. In contrast, for a surface resembling a ''smooth'' (continuous) monolayer stalk (e.g., Markin & Albanesi, Biophys. J. 82:693 [2002]), results from the fourth-order model show that the continuum theory breaks down: both models are inaccurate by at least several tens of k_BT (the fourth-order result is lowest). The breakdown is due to the high density of Gaussian curvature. The fourth-order results for actual non-smooth stalks need to be validated with the minimization procedure applied by Kozlovsky et al., which accounts for the additional effect of lipid tilt. The fourth-order model is valid for catenoidal fusion pores with small radii. it shows that in DOPE-Me, nascent pores spontaneously expand from water channel radii of 1 nm to > 10 nm.

3501-Pos
Fusogenic Activity of Cationic Lipids Correlates with Lipid Shape Distribution
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Cationic lipids are efficient and popular agents to transport anionic molecules (DNA, RNA, anionic proteins) into cells. Addition of the co-lipid DOPE into cationic lipid formulations is considered as promoting cell delivery of DNA by enhancing fusion processes with cell membranes. Here we demonstrate, by combining FRET and confocal microscopy, that some cationic lipids do not require a co-lipid to fuse efficiently with cells. These cationic lipids are able to self-organize into bilayers that are stable enough to form liposomes, while presenting some destabilizing properties reminiscent of the conically-shaped fusogenic co-lipid, DOPE. We therefore analyze the resident lipid structures in cationic bilayers by molecular dynamics simulations, clustering the individual lipid structures into populations of similarly shaped molecules, as opposed to the classical approach of using the static packing parameter to define the lipid shapes. Comparison of fusogenic properties with these lipid populations suggests that the ratio of cylindrical vs. conical lipid populations correlates with the ability to fuse with cell membranes.