

3502-Pos**Membrane Tension Drives Expansion of Hemifusion Diaphragms Nucleated by Influenza Hemagglutinin**

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Infection by enveloped viruses such as HIV and influenza requires fusion of viral and host membranes. The influenza virus fuses with the membrane of the host endosome whose low pH activates the viral protein hemagglutinin (HA). HA may initiate fusion by pulling membranes together and destabilizing bilayer structure. Considerable evidence suggests two-stage fusion: first hemifusion where only the proximal leaflets fuse, followed by full fusion [Floyd et al, *Proc Natl Acad Sci USA*, 2008]. Much debate surrounds the structure and dynamics of hemifusion intermediates. The hemifusion diaphragm (HD) may be produced when fused proximal leaflets are pushed aside and compressed, allowing the separate distal monolayers to meet forming a sealed diaphragm. In classic experiments by Melikyan et al [Melikyan et al, *J Cell Biol*, 1995] strikingly direct and quantitative observations of HDs were achieved using HA-expressing fibroblasts: they reported ~20-micron HDs with suspended bilayers and inferred micron-sized HDs with red blood cells. Here we show membrane tension is the driving force for HD growth and determines final equilibrium size. Applying principles of membrane physics we mathematically modeled HD equilibrium and growth kinetics as observed in these experiments. The principal force resisting growth is proximal leaflet compression which generates interleaflet tension, with lesser contributions from membrane-cytoskeleton and membrane-membrane adhesion forces. HD growth is dynamically resisted by interleaflet friction. Using independently measured physical parameters, our model results for equilibrium HD size and growth rates agree closely with measurements in Melikyan et al. Applying our theory to *in vivo* viral fusion we propose that virus-endosome HDs equilibrate on millisecond timescales, much faster than the ~10-s timescales for fusion pore formation seen *in vitro*. We discuss mechanisms whereby viruses may harness membrane tension driving HD growth and RNA release.

3503-Pos**Multi-Scale Modeling of the "Contact-Facilitated" Delivery Mechanism of Perfluorocarbon-Based Nanoemulsions**

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Perfluorocarbon-based nanoemulsions with stabilizing surface monolayers of emulsifying phospholipids are promising platforms to carry diagnostic and therapeutic agents for cancer. However, to achieve their full therapeutic potential will require investigating the microscopic mechanism of nanoemulsion interactions with biological membranes and the forces that govern cargo transfer. From such investigations and the resulting mechanistic understanding it will be possible to exploit cargo and nanoemulsion characteristics to use them more effectively in imaging and therapeutic applications. Experimental observations suggest a distinctive "contact-facilitated" nanoemulsion delivery mechanism in which cargo diffuses to the targeted cell membrane through a lipid complex formed between a nanoemulsion and the target bilayer. This complex is hypothesized to be structurally comparable to the hemifusion stalk formed during membrane fusion. We are investigating this contact-facilitated delivery mechanism at a molecular level by employing multi-scale molecular dynamics simulations. Force field parameters for the nanoemulsion perfluorocarbon molecule were developed at multiple resolutions to give good agreement to experimental data at all scales of simulation. The structural and dynamical details of the nanoemulsions were characterized at an atomic level. However, in order to access larger time and length scales, the interactions between a nanoemulsion and a target bilayer were simulated using a coarse-grained model to directly examine lipid complex formation hypothesized to precede contact-facilitated delivery. In particular, various phospholipid compositions of the surface monolayer were tested for the lipid complex formation.

3504-Pos**Fusion Between Intraluminal Vesicles of Late Endosomes as a Possible Mechanism of Endosomal Escape by Cell-Penetrating Peptides**

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Escape from endosomes is a major limiting step in the delivery of various bioactive molecules such as proteins and nucleic acids by cationic cell-penetrating peptides including HIV Tat-derived peptide (TAT). In this work we explore the mechanism of TAT escape from endosomes using protein-free liposomes. We found that TAT induces vesicle content leakage and membrane fusion of liposomes mimicking late endosomal lipid composition. Extent of both leakage and fusion increases with the increase in the content of bis(monoacylglycerol)phosphate (BMP), which is a characteristic lipid of late endosomal membrane. TAT-induced membrane fusion and leakage of BMP-containing liposomes was promoted by acidic pH. Replacement of BMP by its structural isomer phosphatidylglycerol (PG) significantly inhibits TAT-induced membrane rearrangements. While there was no significant difference between BMP and PG in the binding affinity of TAT, effects of BMP and PG on the L_α to H_{II} phase transition of egg PE suggested that BMP is more fusogenic than PG. Modifications of liposome composition that inhibited TAT-induced lipid mixing (incorporation of either PEG-lipid or LPC) also inhibited TAT-induced leakage. We demonstrate that fluorescein labeled TAT efficiently translocates across lipid bilayer of liposomes that mimic intraluminal vesicles of late endosomes and are highly enriched in BMP. Based on these results, we propose that TAT induces leaky fusion between BMP-containing bilayers of late endosomal membranes first to deliver TAT into the intraluminal vesicles and then, upon vesicle fusion with the limiting membranes, to release the peptide into cytosol.

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3505-Pos**Fission of Membrane Nanotubes Caused by Osmotic Stress**Alexey Evseev¹, Pavel Bashkurov^{1,2}.

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Membrane fission is a crucial stage in production of all kinds of intracellular vesicle carriers. To detach the newly formed vesicle from mother membrane the lipid neck connecting them should be cut. To avoid the escape of contents of the vesicle the cleavage of the neck is going by constitution of hemifission structure coupled with generation of locally highly bent membrane surfaces. The special protein machinery are designed in cells to produce the curvatures necessary for realization of unleaky membrane fission. To study the behavior of lipid bilayer subjected to extremely high curvatures we designed an experimental system of lipid nanotubes (NT) exposed to osmotic pressure. NT were pulled from bilayer lipid membranes. Osmotic pressure was caused by the difference of salt concentration inside and outside of NT. The equilibrium form of NT subjected to the fixed osmotic pressure depends on the length and the radius of NT and the water permeability properties of its membrane. The squeezing of NT begins only after it reaches the certain length. We show that above the critical length of NT increase of osmolarity of outside solution led to narrowing of NT. We found that high osmotic pressure could squeeze NT to a critical radius of lumen where instantaneous fission of NT took place. Fission of NT was leakage free what was the evidence of formation of hemifission structure. Estimations of the critical radius of lumen revealed that it was less than 2 nm. We varied the amount of cholesterol in NT membrane to increase rigidity and equilibrium radius of NT but the value of the critical radius of fission remained the same. Thus we conclude that membrane rearrangements leading to non-leaky membrane fission can be initiated by a critical squeezing of the membrane tubule.

3506-Pos**Decreasing Temperature Below T_i or Increasing Cholesterol Enhance Vesicle-Bilayer Membrane Fusion**

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Lipid composition plays an important role in fusion of vesicles to membranes, an essential process for exocytosis. Lipid head group, tail structure, and sterol content all impact the complex phase behavior of membranes. To determine the effect of lipids on fusion, we utilized the nystatin/ergosterol (nys/erg) fusion assay and stimulated fusion with a salt (osmotic) gradient. With this assay, vesicles containing nys and erg fuse with a planar membrane producing characteristic spike increases in membrane conductance.

Using PE/PC (7:3) membranes, we varied cholesterol from 0-40 mol% and observed significant increases in fusion rates. In one series of experiments, membranes were formed with 0 mol% cholesterol, repainted with 20 mol%, then repainted with 0 mol%. The 20 mol% cholesterol membrane showed a marked increase in fusion rates over both pre- and post- controls. Likewise, increased fusion rates were observed in DPPC/cholesterol (9:1) membranes upon lowering temperature below the phase transition (T_i). These data are consistent with a liquid disordered lipid phase suppressing vesicle fusion, and shows how membrane fusion can be affected by lipid behavior.

3507-Pos**Membrane Fusion Assay Based on Pore-Spanning Lipid Bilayers**

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Fusion of biological membranes is a central requirement for many cellular processes. It involves at least two distinct steps, binding or apposition of