3502-Pos

Membrane Tension Drives Expansion of Hemifusion Diaphragms Nucleated by Influenza Hemagglutinin

Jason M. Warner, **Ben O'Shaughnessy**.
Columbia University, New York, NY, USA.

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 hemaggluttinin (HA). HA may initiate fusion by pulling membranes together and destabilizing bilayer stucture. 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 equlibrium 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

Sun-Joo Lee, Brett N. Olsen, Paul H. Schlesinger, Nathan A. Baker.
Washington University in St. Louis, St. Louis, MO, USA.

Perfluorocarbon-based nanoemulsions with stabilizing surface monolayers of emulsifying phospholipids are promising platforms to carry diagnostic and therapeutic agents for cancer. However, to acheive 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

Sung-Tae Yang, Leonid Chernomordik, Kamran Melikov.
NICHD, Bethesda, MD, USA.

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(monoacylglycero)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.

3505-Pos

Fission of Membrane Nanotubes Caused by Osmotic Stress

Alexey Evseev[1], Pavel Bashkirov[1,2].
[1]Frumkin Institute of Physical Chemistry and Electrohemistry of RAS, Moscow, Russian Federation, [2]National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, USA.

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 localy 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 extremly high curvatures we designed an exeperimental 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 equillibrium 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 equillibrium 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_t or Increasing Cholesterol Enhance Vesicle-Bilayer Membrane Fusion

Kevin J. Tuttle, David E. Lee, Reed A. Doxey, Dixon J. Woodbury.
Brigham Young University, Provo, UT, USA.

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/ergesterol (nys/erg) fusion assay and stimulated fusion with a salt (osmotic) gradient. With this assay, vesicles containing nys and erg fuse with a planer 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_t). 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

Ines Höfer, **Claudia Steinem**.
Georg August Universität, Göttingen, Germany.

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

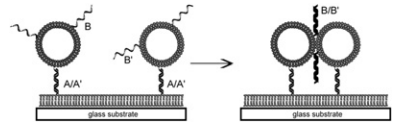
two membranes and their subsequent merger followed by the mixing of aqueous compartments encapsulated by these membranes. Experiments based on artificial membrane systems have significantly contributed to our current knowledge on membrane fusion processes. However, there are still a number of drawbacks associated with these assays. Thus, we aim to establish a new vesicle-planar membrane fusion assay to be able to gain insight into protein-mediated fusion processes starting from docking, via hemifusion to full fusion. To achieve this goal, membranes suspending the pores of a highly ordered porous material were established, which have the advantage that they are very robust, and mechanically stable. Moreover, both membrane sides can be addressed individually allowing the application of a transmembrane potential, fusion modulating compounds or an electrochemical gradient.
Our results show that the fusion of unilamellar vesicles with these pore suspending membranes can be readily followed by time-lapsed fluorescence microscopy. Pore-spanning membranes are achieved by painting a lipid dissolved in n-decane on a functionalized porous substrate. The membrane is doped with the fluorescence dye Oregon Green DHPE, which allows following the membrane formation process by means of fluorescence microscopy. Single fusion events are observed upon the addition of large unilamellar vesicles doped with Texas Red DHPE in a time resolved manner. Lipid mixing during the fusion process is followed by the occurring Förster resonance energy transfer (FRET), from which the diffusion constant of the lipids in the plane of the bilayer can be obtained. Simultaneously, the release of a water soluble dye entrapped in the vesicle lumen is observed.

3508-Pos
Direct Observation of Intermediate States in Membrane Fusion by Photonic Force Microscopy
Andrea Keidel, Tobias F. Bartsch, Ernst-Ludwig Florin.
University of Texas at Austin, Austin, TX, USA.
Protein-free lipid bilayer fusion is an important model system for studying fundamental properties of biological membrane fusion. Fusion intermediates, as observed in viral or synaptic fusion, have been shown to be similar to those found with phospholipid membranes (Chernomordik and Kozlov, (2008), Nat. Struct. Mol. Biol., 15(7):675-683) To determine what role proteins play in the fusion process, one must understand the intermediate steps of protein-free fusion first. However, the characterization of all fusion intermediates in a single fusion event is difficult because some intermediates are expected to occur only on fast time scale.
Fusion of a vesicle to a target membrane can be modeled by bringing an optically trapped and lipid bilayer-coated silica bead onto a membrane-coated glass coverslide. We use a Photonic Force Microscope to measure the position of the trapped bead in three-dimensions with microsecond temporal and nanometer spatial resolution (Bartsch et al., (2009), ChemPhysChem, 10(9-10):1541-1547). These position traces contain a wealth of information about the fusion event.
In our assay, fusion can be induced with thermal energy alone; no additional force is needed. Fusion intermediates (transient fusion, stalk formation, hemifusion and full fusion) are clearly distinguishable. They are stable over periods of time and transitions between them are shorter than the lifetime of the intermediates by orders of magnitude. Each intermediate shows characteristic features in the bead's thermal fluctuation amplitude and position distribution. For instance, the confinement of the particle by the membrane in a hemifused stage shows typical features expected for a two-dimensional fluid.
Our assay is general, as it allows one to study the influence of lipid composition, protein content, or buffer conditions on the intermediates of membrane fusion. The assay can be combined with other established methods for monitoring membrane fusion.

3509-Pos
Lipid Bilayer Rigidity Affects the Fusion Kinetics of Individually Observed Influenza Particles
Jason J. Otterstrom[1], Daniel L. Floyd[1], John J. Skehel[2],
Stephen C. Harrison[1], Antoine M. van Oijen[1].
[1]Harvard Medical School, Boston, MA, USA, [2]MRC National Institute of Medical Research, Mill Hill, London, United Kingdom.
Infection by an enveloped virus begins with fusion of the lipid bilayer covering a virus particle to that of a target cellular membrane. This process passes through a hemifusion intermediate (mixing between the outer membrane leaflets of the virus and cell) and results in the formation of a fusion pore (inner leaflet mixing), which permits passage of viral contents into the cellular cytoplasm. Our lab has developed an in vitro, two-color fluorescence assay that monitors the hemifusion and pore formation kinetics of single virus particles fusing with a planar, fluid target bilayer. The rigidity of this bilayer, as measured by its bending modulus, can be controlled by adjusting the length and saturation of the acyl chains comprising the membrane [1]. Using a flexible C18:3 membrane and a rigid C22:1 membrane, we find that the average time to hemifusion is increased when using the rigid membrane relative to the flexible membrane.
[1] - Rawicz, W., Olbrich, K.C., McIntosh, T., Needham, D., Evans, E. Biophys J. v. 79 pp. 328-39

3510-Pos
Site-Specific DNA-Controlled Fusion of Single Lipid Vesicles to Supported Lipid Bilayers
Lisa V. Simonsson[1], Peter Jönsson[1], Gudrun Stengel[1,2], Fredrik Höök[1].
[1]Chalmers University of Technology, Gothenburg, Sweden, [2]University of Colorado, Department of Chemistry & Biochemistry, Boulder, CO, USA.
Membrane fusion is widely studied, both to gain insights in natural processes like exocytosis and viral fusion, as well as a tool to deliver membrane constituents to preformed lipid bilayers, for example to study ion channels. We recently demonstrated efficient fusion of suspended phospholipid vesicles induced by cholesterol-modified oligonucleotides, which hybridize in a zipper-like fashion, mimicking the natural protein-based fusion machinery in a reductionist way.[1, 2] In this work we make use of a unique feature of DNA, namely the power of sequence-specific hybridization, enabling site-specific fusion of lipid vesicles (~100 nm) to DNA-modified supported lipid bilayers (SLBs). A simplistic DNA-array was formed in a microfluidic device with four channel arms. A mixture of vesicles modified with different DNA strands, complementary to the different DNA strands of the array was added. Using differently dye-labeled lipids (2% w/w) in the vesicles, sorting was visualized using total internal reflection fluorescence microscopy (TIR-FM) and different sets of filter cubes. Addition of Ca^{2+} (10 mM), induced fusion of vesicles modified at a DNA-to-vesicle ratio of 10:1, whereas for all other DNA coverages evaluated (1, 5, 25 and 50 DNA duplexes per vesicle), no significant fusion was observed. By studying the diffusive behavior of the tethered vesicles prior to Ca^{2+} addition, we gain some more insights in the nature of the tethers and hence, the prerequisites of DNA-controlled fusion of lipid vesicles to SLBs. We anticipate that with site-specific DNA-controlled fusion realized, the concept of DNA-controlled sorting of membrane-protein containing vesicles on DNA-arrays could soon be extended to retroactive delivery of membrane proteins to preformed SLB arrays.
[1] Stengel, G. et al. (2007). *J. Am. Chem. Soc.* **129**(31): 9584-5.
[2] Stengel, G. et al.(2008) *J. Phys. Chem. B* **112**(28): 8264-74.

3511-Pos
DNA-Mediated Fusion between Individual Tethered Vesicles
Robert J. Rawle, Bettina van Lengerich, Steven G. Boxer.
Stanford University, Stanford, CA, USA.
We have previously shown that DNA-lipid conjugates mediate fusion between vesicles displaying complementary binding partners in a geometry that mimics that of SNARE-mediated vesicle fusion*. Using a new tethering strategy that allows simultaneous deposition of cognate vesicle partners, we can create well-mixed populations of tethered vesicles that are laterally mobile. DNA-mediated interactions between vesicles, such as docking and fusion, can be triggered by changing the salt concentration immediately prior to observation on a fluorescence microscope. We demonstrate the ability to initiate and observe individual docking events between tethered vesicles and also demonstrate the effect of DNA sequence and geometry on the docking reaction. Preliminary results of the observation of DNA-mediated fusion between individual tethered vesicles at the single event level are discussed.



*Biointerphases, 3, FA17 (2008); PNAS, 106, 979 (2009)

3512-Pos
Covalent Tethering of Lipid Vesicles to a Supported Lipid Bilayer by a DNA-Templated Click Reaction
Bettina van Lengerich, Robert J. Rawle, Steven G. Boxer.
Stanford University, Stanford, CA, USA.
Previously, our lab reported a technique for studying DNA-mediated docking reactions between individual tethered vesicles using fluorescence microscopy.* To prevent these interactions from occurring in the bulk solution prior to tethering and subsequent observation, vesicle partners were spatially separated in a microfluidic device during the tethering process, and subsequently allowed to encounter each other by random diffusion. However, this diffusion is very