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 vi-
ral 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 hemifu-
sion 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 dy-
amics of hemifusion intermediates. The hemifusion diaphragm (HD) may be
produced when fused proximal leaflets are pushed aside and compressed, al-
lowing 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 bi-
layers and inferred micron-sized HDs with red blood cells. Here we show mem-
brane tension is the driving force for HD growth and determines final equilib-
rium size. Applying principles of membrane physics we mathematically
modeled HD equilibrium and growth kinetics as observed in these experiments.
Principal force resisting growth is proximal leaflet compression which gen-
erates interleaflet tension, with lesser contributions from membrane-cytoskele-
ton and membrane-membrane adhesion forces. HD growth is dynamically re-
sisted 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 ~10s-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 po-
tential 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 understand-
ing it will be possible to exploit cargo and nanoemulsion characteristics to
use them more effectively in imaging and therapeutic applications. Exper-
imental 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 bila-
ayer. This complex is hypothesized to be structurally comparable to the bio-
membrane phospholipid nanoemulsion system. Our model is validated with
bioembrane experiments, employing multi-scale molecular dynamics simula-
tions. Force field parameters for the model of perfluorocarbon nanoemulsions
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 charac-
terized 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
of the vesicle the cleavage of the neck is going by constitution of hemifusion
structure coupled with generation of locally highly bent membrane surfaces.
We propose a mechanism in which cargo transfers from the nanoemulsion to
the target membrane as the nanoemulsion undergoes structural changes.

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 bio-
active 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 lipo-
somes mimicking late endosomal lipid composition. Extent of both leakage and
fusion increases with the increase in the content of bis(monoacylglycerol)phos-
phate (BMP), which is a characteristic lipid of late endosomal membrane. TAT-
induced membrane fusion and leakage of BMP-containing liposomes was pro-
moted by acidic pH. Replacement of BMP by its structural isomer phosphati-
dyglycerol (PG) significantly inhibits TAT-induced membrane rearrange-
ments. While there was no significant difference between BMP and PG in the
binding affinity of TAT, effects of BMP and PG on the Lα to HII phase tran-
sition of egg PE suggested that BMP is more fusogenic than PG. Modifications of
liposome composition that inhibited TAT-induced lipid mixing (incorpora-
tion of either PEG-lipid or LPC) also inhibited TAT-induced leakage. We dem-
onstrated that TAT efficiently translocates across lipid bila-

3505-Pos
Fission of Membrane Nanotubes Caused by Osmotic Stress
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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 hemifusion
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 exper-
imental 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. Fis-
sion 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 fis-
sion 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 Tt on Intraluminal Vesicles of Late Endosomes
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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 as-
say and stimulated fusion with a salt (osmotic) gradient. With this assay, ves-
icles containing nys and erg fuse with a planar membrane producing character-
istic 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, increases in fusion rates were observed in DPPC/cholesterol (9:1) membranes upon lowering
temperature below the phase transition (Tt). These data are consistent with a liquid disordered lipid phase suppressing vesicle
fusion, and shows how membrane fusion can be affected by lipid behavior.
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 pores 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 

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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

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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.


3510-Pos Site-Specific DNA-Controlled Fusion of Single Lipid Vesicles to Supported Lipid Bilayers

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Membrane fusion is widely studied, both to gain insights in natural processes like exocytosis and viral fusion, as well as 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 dyelabeled 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 Ca2+ (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 Ca2+ 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.


3511-Pos DNA-Mediated Fusion between Individual Tethered Vesicles

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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

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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