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
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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.
[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
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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
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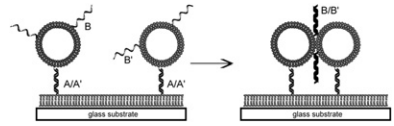
We have previously shown that DNA-lipid conjugates mediate fusion between vesicles displaying complementary binding partners in a geometry that mimics that of SNARE-mediated vesicle fusion*. Using a new tethering strategy that allows simultaneous deposition of cognate vesicle partners, we can create 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

slow compared to the docking reaction, so actual observation of single docking events in real time was extremely rare. Here, we present a new method of tethering vesicles that is orthogonal to the DNA-mediated docking and fusion reactions. Vesicles are covalently attached to the supported lipid bilayer by a DNA-templated click reaction, allowing simultaneous deposition of cognate vesicle populations displaying complementary DNA. This results in well-mixed populations of tethered vesicles, which diffuse randomly in two dimensions. Upon raising the salt concentration, tethered vesicles can be triggered to interact with each other via DNA hybridization. An additional parameter probed with this strategy is the location of the templating DNA-lipid, which can be anchored in the vesicle or in the supporting bilayer. The mobility, as measured by single particle tracking, of vesicles anchored by two lipids is approximately 1.6-fold slower than that of vesicles anchored only with a single lipid.

* Chan, Y-H M *et al*. 2007. *Proc Natl Acad Sci USA* 104:18913.

3513-Pos

Role of Curvature in PEG-Mediated Fusion Between Highly Curved and Un-Curved Membranes

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During neurotransmitter release, curved synaptic vesicles fuse with un-curved pre-synaptic plasma membrane, leading to the merger of two lipid bilayers and the release of neurotransmitters. Our previous efforts to model this system have employed two populations of highly curved vesicles, while others have used two populations of vesicles having ill-defined but lower curvature. Here we examine poly ethylene glycol (PEG)-triggered fusion of highly curved (SUV) with relatively un-curved (LUV) vesicles, composed of a mixture of DOPC/DOPE/sphingomeylin/DOPS/cholesterol (32/25/15/8/20), which closely models the lipid composition of synaptic vesicles. Lipid mixing (LM), contents mixing (CM) and leakage (L) time courses were fitted globally to 3- or 4-state sequential models (Weinreb, Biophys. J., 2007), from which we obtained estimates of rate constants for conversion between states as well as probabilities of LM, CM and L for each state. As expected, un-curved LUV-LUV fusion was barely detectable, while highly curved SUV-SUV fusion was reasonably efficient, saturating at ~50% LM and ~40% CM. Remarkably, SUV-LUV fusion was decidedly more efficient, saturating at nearly 100% LM and CM. Analysis of the fusion kinetics at different temperatures (17°-42°C) revealed complex activation thermodynamics. The rate of the first fusion intermediate formation is decidedly faster in the highly curved (stressed) SUV-SUV system than in the mixed system, with increasing temperature shifting the probability of CM toward earlier steps; whereas the probability of CM in SUV-LUV fusion shifts towards fusion pore. This suggests that mismatched curvature promotes more efficient and productive fusion events.

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3514-Pos

VSV Trans-Membrane Domain Promotes Content Mixing to occur Early in the Fusion Process

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Polyethylene glycol (PEG)-mediated fusion of 25 nm vesicles was studied at different temperatures in the presence of the trans-membrane domain (TMD) of the G protein (fusion protein) of vesicular stomatitis virus (VSV). Vesicles were composed of dioleyol-phosphatidylcholine (DOPC), dioleyol-phosphatidylethanolamine (DOPE), bovine brain sphingomyelin (SM), and cholesterol (CH) in a molar ratio of 35:30:15:20. Kinetic parameters of the fusion process were determined by fitting lipid mixing (LM), content mixing (CM), and content leakage (L) time courses globally to a three state sequential model that allowed for leakage from the final fusion pore state. This yielded the rate constants for conversion between different states as well as the probabilities of the occurrence of LM and CM in each state. The TMD enhanced the initial rate of lipid mixing and content mixing in agreement with our previous report (Biochemistry, 2002, 14925). This resulted partly from enhanced extent of CM, although the extent of LM remained unaltered. In addition, the rate of intermediate formation was increased slightly, although, the principal effect of TMD was to increase the probability of CM in the initial intermediate at the expense of the final step of the process. This suggests that the presence of TMD enhanced flickering pore formation, leading to an increase in CM early in the process. Supported by NIGMS grant 32707 to BRL.

3515-Pos

Effect of HIV Gp41 Fusion Peptide and its Cross-Linked Oligomers in Membrane Fusion

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Virus-cell membrane fusion is an essential and important step for the infectious entry of a virus into a host cell. During HIV infection gp41 fusion protein plays an essential role in membrane fusion. Recently, it has been reported that a trimeric form of gp41 fusion peptide is optimally catalytically efficient for lipid mixing between lipid vesicles. We have characterized the effect of different oligomers (monomer, dimer, trimer and tetramer) of gp41 fusion peptide on the entire process of 23 nm vesicle fusion. Time courses of lipid mixing, content mixing, and content leakage were determined for control vesicles as well as for vesicles in presence of different oligomers. We were able to describe data for all systems using a one-intermediate leaky fusion model, and obtained thereby rate constants of the three steps in this model. An increase in the extent of lipid mixing was observed in presence of the different chemically cross-linked oligomers, as well as a very small amount of content mixing (~2%). All species induced substantial content leakage, with the trimer and tetramer being most membrane disruptive. The rate of lipid mixing and content mixing increased in the order of monomer $<$dimer $<$trimer $\approx$ tetramer. The oligomers affected fusion induced by 5% poly(ethylene) glycol (PEG) in a completely opposite fashion (i.e., they inhibited both). Quasi-elastic light scattering (QELS) and static 900 light scattering data in presence of different oligomers suggested formation of larger aggregates in the presence of all oligomers, and the size and polydispersity of the aggregates followed the same trend seen for lipid mixing. Supported by USPHS grant GM32707 to BRL.

3516-Pos

Fusogenic Activity of PLA_2-IIA and SMase in PEG-Mediated Membrane Fusion

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Phospholipase A_2-IIA (PLA_2-IIA) and sphingomyelinase (SMase) have been shown to induce fusion of membranes both *in vivo* and *in vitro*. The fusogenic potential of these two enzymes has been observed, for example, during neurotransmitter release. It is also believed that the products of hydrolysis of these two enzymes induce a reduction in the free energy cost of the fusion event. Assessment of the biophysical mechanisms by which these enzymes propel membrane fusion may provide relevant information for a variety of biological scenarios including neurotransmitter release. Poly(ethylene glycol)-(PEG-) mediated fusion of small unilamellar vesicles (SUVs) was studied in the presence of PLA_2-IIA and SMase at varying temperatures between 17C and 40C. Lipid composition was dioleoylphosphatidylcholine (DOPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), porcine brain sphingomyelin (SM), and cholesterol (CH) in a ratio of 35:30:15:20. Content mixing (CM), lipid mixing (LM) and leakage (L) time courses were obtained and globally fitted to a 3 or 4-state sequential vesicle fusion model introduced by Weinberg and Lentz (Weinberg and Lentz, Biophys. J., 2007, 92; 4012). Based on the global fits, rate constants for the transitions between states, and LM, CM and L occurrence probabilities are obtained. We observe that both LM and CM are enhanced by enzyme activity in SUVs. In addition, the exogenous addition of hydrolysis products also promotes LM and CM in the absence of the enzymes. A careful study of the temperature dependent plots is used to explore alterations in the energy barriers between intermediate states. These findings may shed light into the biological mechanisms wherein specific vesicle lipid structure and enzyme catalysis become interrelated to induce membrane fusion.

3517-Pos

Membrane Fusion is Spatially Controlled by Modification of Phosphoinositides

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Nuclear envelope assembly is an essential event in cell cycle but its mechanism and regulation remain mostly unknown. Using a cell-free system derived from sea urchin gametes we report that nuclear envelope formation involves the fusion of membrane vesicles highly enriched in phosphoinositides via the production of a fusogenic lipid, the diacylglycerol. By performing time course fluorescence lifetime imagery, we measured the kinetic of this process and demonstrate that nuclear envelope assembly is polarised. It is initiated at the poles of the nucleus where the nuclear envelope remnants are located. This study provides a mechanism for temporal control of NE assembly and offers an explanation for how such a process of membrane fusion can be spatially regulated.