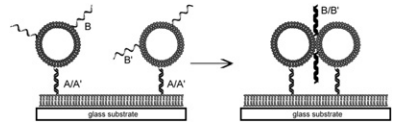
two membranes and their subsequent merger followed by the mixing of aqueous compartments encapsulated by these membranes. Experiments based on artificial membrane systems have significantly contributed to our current knowledge on membrane fusion processes. However, there are still a number of drawbacks 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