Communication

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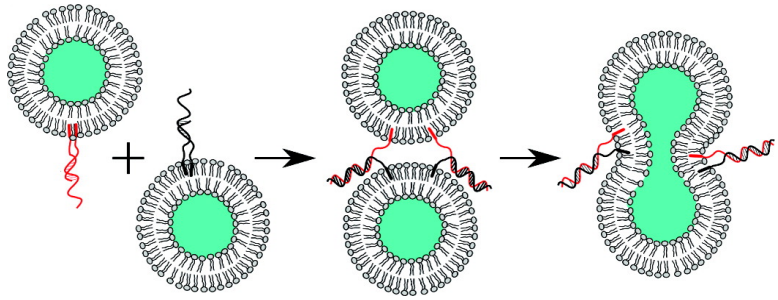

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DNA-Induced Programmable Fusion of Phospholipid Vesicles

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Biological membrane fusion is strictly regulated by members of the SNARE family (soluble N-ethylmaleimide-sensitive factor attachment protein receptors); molecular recognition between proteins located at the transport vesicle and the target membrane leads to the formation of a SNARE protein complex that catalyzes the lipid rearrangements necessary to merge adjacent lipid bilayers.[1,2] Lipid vesicles also play a key role in the design and construction of lipid-based platforms for the screening of membrane proteins such as lipid vesicle arrays or supported lipid bilayers.[3,4] Although the ability to multiplex is at the heart of any array technology, the physical properties of lipids make it particularly difficult to incorporate this feature into membrane protein arrays. In this report, we present a method to selectively fuse lipid vesicles by the hybridization of membrane-anchored DNA strands. This concept will provide a tool to manipulate the composition and content of artificial and biological lipid compartments and a model for membrane fusion in cells. Recently, other groups have designed recognition motifs that induce membrane fusion,[5,6] but this is, to our knowledge, the first method that takes advantage of the encoding potential of DNA in this context.

The fusion of lipid bilayers is a two-step process. First, the bilayers are brought into close proximity, overcoming the repulsive hydration forces between the lipid headgroups. Second, the boundary between the hydrophilic and the hydrophobic part of the bilayer is destabilized, which results in the opening of a rapidly expanding fusion pore. According to the stalk hypothesis,[7] these events proceed through the stalk transition state, a structure in which the proximal outer lipid leaflets have merged but not the inner ones.[1,2] SNARE proteins typically consist of a hydrophobic C-terminal transmembrane domain and a water soluble N-terminal domain, the SNARE motif. Upon recognition, the initially unstructured SNARE motifs assemble into a four helix bundle that can only be dismantled by specialized proteins in a process coupled to energy consumption.[1,2] The formation of the helix bundle is thought to start at the N-terminus and to proceed toward the transmembrane domains in a zipperlike fashion, which imposes pulling stress on the membrane.

Inspired by this geometry, we used a special design of cholesterol (CH) modified DNA strands to mimic this recognition process. The hydrophobic CH moieties spontaneously incorporate into the bilayer and serve to anchor the DNA.[8,9] While single CH tagged DNA binds to egg-PC bilayers with a K_D of ~17 nM, the coupling of double CH anchored DNA is significantly stronger.[9] In our approach, the orientation of the DNA strands with respect to the CH anchor was designed such that hybridization occurs in a zipperlike fashion and forces vesicles modified with complementary DNA into close contact (Figure 1).

The lipid rearrangements taking place as a consequence of the forced bilayer contact were investigated using fluorescence resonant energy transfer (FRET) between donor (D) and acceptor (A) dyes. We tested model membranes composed of different lipid classes: (a) DOPC/DOPE, (b) DOPC/CH, (c) DOPC/DOPE/CH, and (d) DOPC/DOPE/SM/CH (DOPC = 1, 2-dioleyl-*sn*-glycero-3-phosphocholine, DOPE = 1,2-dioleyl-*sn*-glycero-3-phospho-ethanolamine, SM = bovine brain sphingomyelin). Unilamellar lipid vesicles with a nominal diameter of 100 nm were prepared by extrusion and modified either with ds-1/4 or with ds-2/3 DNA (Figure 1). The vesicle population carrying ds-2/3 was labeled with Bodipy-500/510 (D) and Bodipy-530/550 (A) (dye lipid acronyms in the Supporting Information), while the vesicle population with ds-1/4 was unlabeled. Mixing of both vesicle species resulted in an increase in donor emission due to the decrease in FRET efficiency caused by merging of labeled and unlabeled lipid bilayers (Figure 2a). The presence of complementary DNA strands rendered vesicles of all four lipid compositions fusogenic, whereas the presence of non-complementary DNA did not (Figure 3a). However, lipid mixing

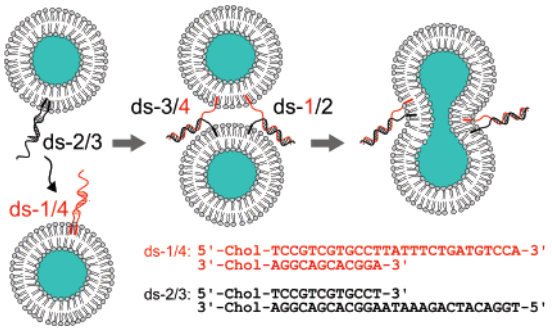

Figure 1. DNA-induced vesicle fusion. Initially, vesicles are modified with the double CH terminated DNA strands ds-1/4 and ds-2/3 (left side). As ds-1/4 and ds-2/3 encounter each other, they hybridize in a zipperlike fashion, thereby forming blunt-ended duplexes with 27 base pairs (ds-1/2) and 12 base pairs (ds-3/4) (middle). In this geometry, the bilayers are thought to contact each other, which eventually enables opening of the fusion pore (right side).

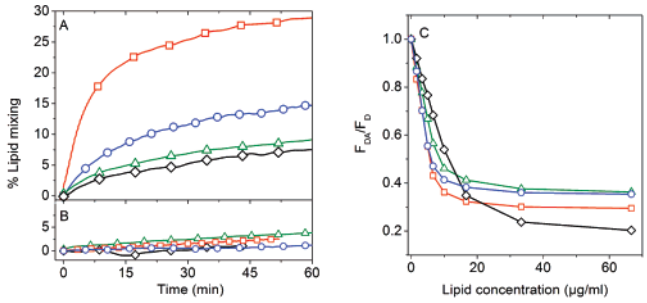

Figure 2. FRET experiments measuring lipid mixing and DNA binding for vesicles composed of DOPC/DOPE/CH (50/25/25) (red square), DOPC/DOPE/SM/CH (35/30/15/20) (blue circle), DOPC/CH (75/25) (green triangle), and DOPC/DOPE (75/25) (black diamond). (A) Lipid mixing between vesicles modified with complementary DNA strands. 100 strands of ds-2/3 were incorporated into the Bodipy-vesicles and 50 strands ds-1/4 were anchored in the unlabeled vesicles. (B) Negative controls using DNA-free vesicles. (C) Incorporation of CH-anchored DNA into vesicles measured by FRET. Donor-labeled ds-2/3 was titrated with increasing amounts of acceptor-labeled vesicles. F_D refers to the donor intensity in the absence, F_{DA} to the donor intensity in the presence, of acceptor-labeled vesicles.

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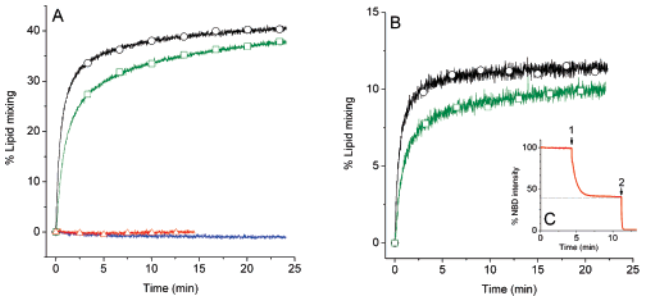

Figure 3. Outer and inner leaflet mixing for DOPC/DOPE/CH vesicle. (A) Total lipid mixing. Unlabeled vesicles were mixed with NBD/Rh-labeled vesicles under conditions identical to those described in Figure 2 (black squares). Lipid mixing was slowed down at a lower DNA coverage of 25 DNA strands (green squares). If noncomplementary DNA was used instead (100 DNA strands), no lipid mixing was observed (blue line), as was the case when employing only 1 DNA strand per vesicle (red line). (B) Inner leaflet mixing. NBD/Rh-labeled vesicles were treated with sodium dithionite and purified by size exclusion chromatography. Subsequently, reactions were performed in analogue to panel A. (C) Display of the drop in NBD intensity upon addition of sodium dithionite [**1**] and complete elimination of fluorescence after denaturing the vesicles with 0.08% TritonX-100 [**2**].

proceeded the fastest and most efficient with vesicles composed of DOPC/DOPE/CH and DOPC/DOPE/SM/CH.

The fusion rate depended critically on the number of DNA strands incorporated into the membrane. To determine whether the affinity for the incorporation of CH DNA, and thus the number of DNA strands engaged in the process, depends on the lipid composition, we performed FRET titrations to monitor the DNA uptake. Increasing amounts of rhodamine (Rh, A)-labeled vesicles were added to a fixed concentration of fluorescein (D)-labeled ds-2/3 DNA, and the corresponding decrease in D emission was recorded (Figure 2c). The curves reveal that the initial DNA uptake is faster for the lipid compositions containing CH. However, at the DNA-to-lipid ratio employed in the lipid mixing assay (the molar ratio was 1:1310 corresponding to ~100 DNA strands per 100 nm sized vesicles), all four curves have leveled off and, hence, all available DNA strands are bound to the vesicles. Consequently, the observed difference in lipid mixing originates from differences in the physical properties of the model membranes rather than from differences in the number of fusion sites present.

However, true bilayer fusion is defined as the merger of both the inner and the outer lipid leaflet of a membrane. To test for mixing of the inner leaflet, we repeated the experiments for the most potent lipid composition, DOPC/DOPE/CH, using a FRET pair, NBD−PS (D) and Rh-DHPE (A), whose fluorescence can be selectively eliminated from the outer leaflet by treatment with sodium dithionite.[10] Because sodium dithionite cannot penetrate the vesicle membrane, only the NBD molecules located in the outer leaflet are rendered nonfluorescent, leading to a 60% drop in NBD intensity (**1**, Figure 3c). This is expected because the number of lipids in the outer leaflet is slightly higher than in the inner one for a 100 nm vesicle, predicting a change of 56%.[11] Efficient lipid mixing was observed for both untreated and for dithionite-treated vesicles using the same DNA concentrations like in Figure 2a (Figure 3, black squares) or only 25 DNA strands per vesicle (Figure 3, green squares). Lipid mixing was observed for DNA concentrations as low as 5 DNA strands (not shown). The relative increase in NBD intensity for total lipid mixing was ~75% (in 25 min), which is large compared to the ~33% (in 2 h) reported by Gong et al. using the same FRET scheme[5] but a 10-fold higher concentration of recognition motifs (vancomycin/magainin). However, in the inner leaflet mixing assay (Figure 3b) the efficiency is reduced by two-thirds compared to the total lipid mixing (Figure 3a). This observation allows us to conclude that at least one-third of the observed total lipid mixing represents complete vesicle fusion.

In summary, we introduce a new concept for programmable lipid vesicle fusion that relies on the zipperlike hybridization of membrane-anchored DNA strands. DNA-induced fusion was demonstrated to be sensitive to the presence of cone shaped lipids such as DOPE and CH. This suggests that DNA-mediated fusion traverses through the stalk intermediate whose geometry is known to favor the incorporation of those lipids.[12] In addition, CH has the tendency to form inverted hexagonal phases, which destabilizes lamellar bilayers.[13] Ternary lipid mixtures containing CH, like the most potent mixtures DOPC/DOPE/CH (and SM), frequently display complex phase behavior that is characterized by the coexistence of liquid disordered and ordered phases. These so-called raft structures have been observed in biological and model membranes,[12,14−16] and their involvement in membrane fusion is currently under investigation.

In the presented model system, force is generated through DNA duplex formation and exerted on the membrane. Preliminary results indicate a dependence of fusogenity on the strength of the membrane anchor and on the DNA length (will be addressed in forthcoming publications). Because these components can both be conveniently modulated, the system is expected to provide new insights into the role of proximity and force transmission in membrane fusion.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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