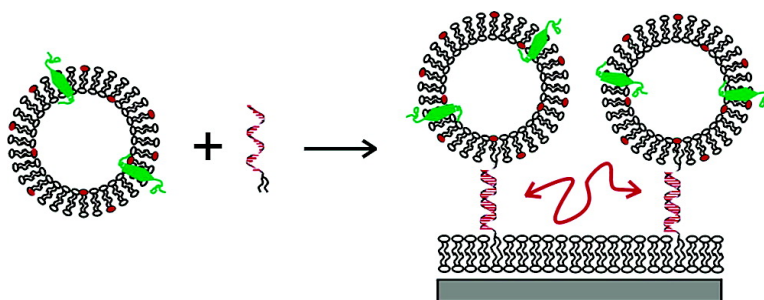Communication

General Method for Modification of Liposomes for Encoded Assembly on Supported Bilayers

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General Method for Modification of Liposomes for Encoded Assembly on Supported Bilayers

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We recently reported a novel system shown schematically in Figure 1A in which intact lipid vesicles were assembled on a fluid-supported bilayer using oligonucleotide tethers.[1] Functionalized oligonucleotides were covalently attached to the surface of pre-formed lipid vesicles by incorporating a small fraction of lipids with reactive headgroups during vesicle assembly. Vesicles displaying oligonucleotides were then tethered to a fluid-supported bilayer displaying oligonucleotides of complementary sequence. These tethered vesicles retain their integrity and diffuse parallel to the plane of the supporting bilayer. Encoded arrays of tethered vesicles were created by displaying orthogonal sequences of oligonucleotides on a patterned bilayer surface.[2,3] A major drawback of this method is the requirement for the inclusion of a reactive lipid during the vesicle assembly process. This may be incompatible with vesicles containing proteins (proteoliposomes), the ultimate target of the tethering strategy, due to side reactions with the protein or special features of the proteoliposome assembly. Furthermore, it is desirable to control as much as possible the number of oligonucleotides displayed on the surface and to avoid side reactions such as hydrolysis of the reactive headgroup that leaves unwanted and uncontrolled levels of impurities on the vesicle surface. We now report the synthesis of an amphiphilic oligonucleotide species (Figure 1B) which is soluble in buffer but inserts cleanly into preformed vesicles and proteoliposomes of varying composition under mild conditions for sequence-specific tethering onto a fluid-supported bilayer (Figure 1C). This method should be more generally useful not only for synthetic vesicles and proteoliposomes but also for native vesicles and cells.

To achieve this goal, we utilized a simple method for functionalization and subsequent modification of oligonucleotides on the 5′-end prior to cleavage from the DNA synthesis column.[4] The terminal dimethoxytrityl (DMT) group was removed and reacted with an iodination reagent, $(PhO)_3PCH_3I$, to render the 5′-end electrophilic. Treatment with a lipid-thiolate[5] followed by deprotection, cleavage, and reverse-phase HPLC purification yielded the desired product (Figure 1B) (see Supporting Information for details). A complementary set of 24-mer oligonucleotides (sequences A and A′) were synthesized and modified in this way (($C_{18})_2$-A, ($C_{18})_2$-A′).[7] Egg yolk phosphatidylcholine (PC) vesicles containing Texas Red 1,2-dihexadecanoyl-phosphatidylethanolamine (Texas Red DHPE) (1 mol %) for visualization and unlabeled PC vesicles containing 2 mol % 1,2-dipalmitoyl phosphatidylserine (DPPS) were prepared by extrusion through 100 nm polycarbonate membranes at approximate lipid concentrations of 10 mg/mL. ($C_{18})_2$-A and ($C_{18})_2$-A′ were dissolved in a 50/50 (v/v) mixture of buffer and acetonitrile to 10 μM and added to these preformed Texas Red DHPE and unlabeled PC/DPPS vesicles, respectively, and incubated at 4 °C for 4 h.[8] The ($C_{18})_2$-DNA solution added never exceeded 10% of the total volume.

A glass-supported phospholipid bilayer was formed by fusion of the unlabeled PC/DPPS small unilamellar vesicles to which
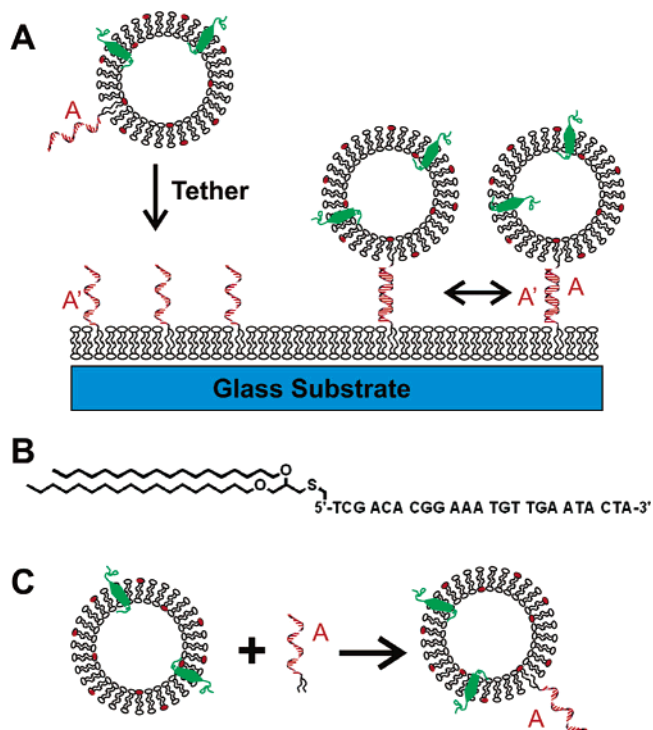

Figure 1. (A) Vesicles displaying DNA on the surface are tethered to a supported bilayer displaying the complementary sequence (A′) and allowed to hybridize (schematic transmembrane protein shown in green). Vesicles tethered to the surface diffuse laterally. Drawings are not to scale. (B) Structure of the lipid−DNA conjugate, ($C_{18})_2$-A. (C) Lipid−DNA reagent with sequence A inserts into preformed vesicles or proteoliposomes.

($C_{18})_2$-A′ had been added with a cleaned glass coverslip and washed extensively with buffer. Subsequent incubation at room temperature for 30 min with Texas Red DHPE vesicles to which ($C_{18})_2$-A had been added and washing resulted in mobile tethered vesicles, visualized by epi-fluorescence video microscopy. A series of control experiments demonstrated that nonspecific binding is reduced when negatively charged DPPS is included in the supporting bilayer. Vesicle purification after incubation with ($C_{18})_2$-A by gel filtration chromatography did not improve or change tethering behavior, suggesting that very little ($C_{18})_2$-A remains free in solution, that is, it is entirely incorporated into the preformed vesicles. Patterning the substrate prior to bilayer formation by microcontact printing of gridlines with fibronectin[10] resulted in micrometer-scale arrays of mobile tethered vesicles. As in earlier work, vesicles were observed to collide with each other and with barriers reversibly, without observable loss of content or mixing of lipids.[9]

A second set (sequences B and B′) of complementary modified lipid−oligonucleotide conjugates (($C_{18})_2$-B, ($C_{18})_2$-B′) with an orthogonal sequence to the first set were prepared[11] to demonstrate encoding capability. An array of unlabeled supported bilayer on a
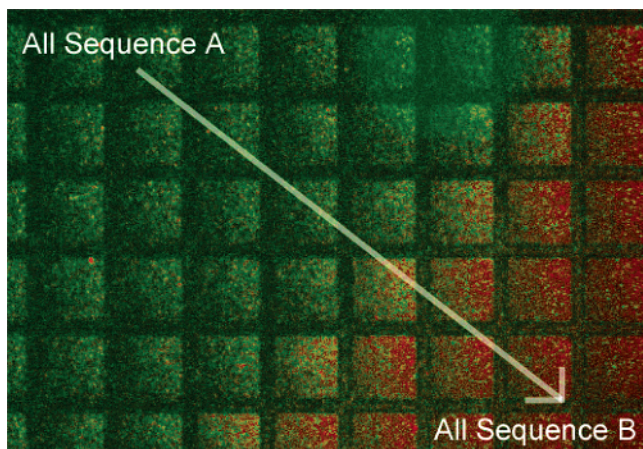
Figure 2. Array of unlabeled supported bilayer on a pattern of 50-μm grids displaying different amounts of $(C_{18})_2$-A and $(C_{18})_2$-B from top left to bottom right was prepared. A mixture of $(C_{18})_2$-A' displaying Texas Red DHPE vesicles (100 nm) and $(C_{18})_2$-B' displaying Oregon Green DHPE vesicles (100 nm) is sorted according to sequence, demonstrating the encoding capability of the oligonucleotide tether.

fibronectin-patterned surface displaying different amounts of $(C_{18})_2$-A and $(C_{18})_2$-B from top left to bottom right was prepared[12] and exposed to a mixture of Texas Red DHPE-labeled vesicles to which $(C_{18})_2$-A' had been added and Oregon Green DHPE-labeled vesicles to which $(C_{18})_2$-B' had been added. Sorting on the surface according to sequence was observed as expected (Figure 2). The success of the sorting experiment without the need for purification is also a demonstration of the essentially irreversible and complete insertion of $(C_{18})_2$-DNA into vesicles on the time scale of these experiments.[13] Further experiments showed unequivocally that stable insertion of $(C_{18})_2$-DNA occurs.[9]

The DNA–lipid conjugate described here can be considered a reagent for tethering preformed vesicles or proteoliposomes of arbitrary composition, content, and origin (e.g., native vesicles or cells) to supported bilayer surfaces in a manner that encodes the identity of the vesicle or proteoliposome in the DNA sequence. We envision this architecture to be particularly useful for studying membranes and integral membrane proteins and their interactions with each other and the components in solution under conditions that approach a native environment. Proteoliposomes based on several different transmembrane proteins have been tethered using this strategy and will be reported separately. Likewise, the two-dimensional mobility of the tethered vesicles as a function of the amount of $(C_{18})_2$-DNA, at the air–water interface, and the coupled motion of tethered vesicles and components in the supported bilayer in response to electric fields will be described in detail in subsequent papers.

Supporting Information Available: Experimental details of the synthesis of $(C_{18})_2$-A and MALDI-TOF data of the product; experimental evidence to further support the insertion of lipid–DNA into vesicles. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Yoshina-Ishii, C.; Boxer, S. G. *J. Am. Chem. Soc.* **2003**, *13*, 3696–3697.

(2) Groves, J. T.; Boxer, S. G. *Acc. Chem. Res.* **2002**, *35*, 149–157.

(3) Surface immobilizations of intact vesicles and proteoliposomes have been reported. However, to our knowledge, lateral mobility had not been previously demonstrated. Examples include: (a) Jung, L. S.; Shumaker-Parry, J. S.; Campbell, C. T.; Yee, S. S.; Gelb, M. H. *J. Am. Chem. Soc.* **2000**, *122*, 4177–4184. (b) Boukobza, E.; Sonnenfeld, A.; Haran, G. *J. Phys. Chem. B* **2001**, *105*, 12165–12170. (c) Stanish, I.; Santos, J. P.; Singh, A. *J. Am. Chem. Soc.* **2001**, *123*, 1008–1009. (d) Svedhem, S.; Pfeiffer, I.; Larsson, C.; Wingren, C.; Borrebaeck, C.; Höök, F. *ChemBioChem* **2003**, *4*, 339–343. (e) Stamou, D.; Duschl, C.; Delamarche, E.; Vogel, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 5580–5583. (f) Patolsky, F.; Lichtenstein, A.; Willner, I. *Angew. Chem., Int. Ed.* **2000**, *39*, 940–944. (g) Okumus, B.; Wilson, T. J.; Lilley, D. M. J.; Ha, T. *Biophys. J.* **2004**, *87*, 2798–2806.

(4) (a) Miller, G. P.; Kool, E. T. *Org. Lett.* **2002**, *4*, 3599–3601. (b) Miller, G. P.; Kool, E. T. *J. Org. Chem.* **2004**, *60* (7), 2404–2410.

(5) Vederone, G.; Craynest, N. V.; Boussif, O.; Santaella, C.; Bischoff, R.; Kolbe, H. V. J.; Vierling, P. *J. Med. Chem.* **2000**, *43*, 1367–1379.

(6) Synthesis of other lypophilic DNA reagents have been reported for antisense applications but self-assembly of membrane structures have not been demonstrated with them. Examples include: (a) Shea, R. G.; Marsters, J. C.; Bischofberger, N. *Nucleic Acids Res.* **1990**, *18*, 3777–3783. (b) Vinogradov, S. V.; Doan, R. L.; Hélène, C. *Tetrahedron Letters* **1995**, *36*, 2493–2496. (c) Guzaev, A.; Lönnberg, H. *Tetrahedron* **1999**, *55*, 9101–9116. (d) Krieg, A. M.; Tonkinson, J.; Matson, S.; Zhao, Q.; Saxon, M.; Zhang, L.-M.; Bhanja, U.; Yakubov, L.; Stein, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1048–1052.

(7) $(C_{18})_2$-A sequence: 5'-lipid-TCC TGT GTG AAA TTG TTA TCC GCA-3'. $(C_{18})_2$-A' is the complementary sequence, also with a 5'-lipid modification.

(8) The density of tethered vesicles on the surface can be controlled by either changing the amount of DNA on the surface or by changing incubation conditions which result in incomplete hybridization (once tethered, vesicles on a patterned surface can be concentrated by applying an electric field parallel to the plane of the bilayer). The amount of DNA displayed on the surface is easily changed with this new method by adding varying amounts of the amphiphilic DNA to vesicles (typically 0.1–100 per vesicle) or by mixing vesicles with no DNA during supported bilayer formation.

(9) See Supporting Information for further discussion.

(10) Kung. L. A.; Kam, L.; Hovis, J. S.; Boxer, S. G. *Langmuir* **1999**, *16*, 6773–6776.

(11) $(C_{18})_2$-B sequence: 5'-lipid-TAG TAT TCA ACA TTT CCG TGT CGA-3'. $(C_{18})_2$-B' is the complementary sequence, also with a 5'-lipid modification.

(12) Kam, L.; Boxer, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 12901–12902.

(13) Insertion and exchange of the $(C_{18})_2$-DNA construct described here into preformed vesicles is a delicate balance between its solubility in buffer and the bilayer environment of a vesicle and supported bilayers. We have found that other molecules, for example a commercially available cholesterol–DNA conjugate (Trilink Biotechnologies, San Diego, CA) displaying the same oligonucleotide sequence, do not partition as effectively into vesicles; to the extent by which these associate with bilayers, this association is reversible so that they cannot be used to form arrays. (Two cholesterol anchors have been shown to be a more stable anchor. See Pfeiffer, I.; Höök, F. *J. Am Chem. Soc.* **2004**, *126*, 10224–10225.) Variations in the coupling and the $(C_{18})_2$- composition may enhance or degrade the utility of this reagent. We note that DNA–lipid conjugates have been described as tools for gene therapy (Soutschek, J. et al. *Nature* **2004**, *432*, 173–178), and the $(C_{18})_2$-DNA associated with vesicles may prove useful in such applications.

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