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# Arrays of Mobile Tethered Vesicles on Supported Lipid Bilayers

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We report a novel system for creating two-dimensional arrays of mobile vesicles spatially encoded on the surface of a fluid-supported lipid bilayer by oligonucleotide tethers.[1] This architecture should have a wide range of applications in membrane biophysics, biotechnology, and colloid and interface science.

The concept of oligonucleotide-tethered arrays of mobile vesicles is schematically represented in Figure 1A. Fluid-supported lipid bilayers are first prepared by vesicle fusion to a glass substrate using vesicles that display short oligonucleotides (sequence A and/or B) on their surface.[2] Subsequently, fresh vesicles displaying the antisense oligonucleotide (denoted A′ and B′, respectively) are flowed over the planar supported bilayer leading to sequence-specific tethering of these intact vesicles to the supported bilayer.[3] The tethered vesicles can be individually visualized by fluorescence microscopy, either by labeling a small fraction of the lipids comprising the vesicle or by entrapping a water soluble fluorescent dye as illustrated in Figure 1A and described below.[4]

Control experiments demonstrating the specificity of binding show that intact vesicles do not become associated with the supported bilayer unless an oligonucleotide with the correct complementary sequence is present. Furthermore, vesicle tethering is not observed when the supported bilayer displaying an oligonucleotide sequence is first exposed to the free antisense oligonucleotide, which blocks further binding. Because the tethering mechanism contains information encoded in the oligonucleotide sequence, the location of vesicle tethering, including whatever lipids, proteins, and/or contents are associated with the particular oligonucleotide sequence displayed by the vesicle, can be controlled with precision. This is demonstrated by creating patterned bilayer surfaces[5] displaying different oligonucleotide sequences in different corralled regions, as illustrated in Figure 1A, using methods described earlier for simple lipids.[6] Figure 1B shows an example where two different 24-mer sequences are displayed on the bilayer in a spatially defined array of a patterned bilayer surface from left to right. Upon adding a mixture of vesicles, each bearing one or the other antisense oligonucleotide headgroup, one with a green content label and the other with a red lipid label, the mixture is sorted by the patterned surface, reflecting the positions of their compliment on the surface. This sorting process demonstrates the sequence-specific binding of different vesicles to the surface.

Individual tethered vesicles can be distinguished as granularity in Figure 1B. Vesicles diffuse parallel to the plane of the fluid-supported bilayer because they are tethered to a lipid, and this is best visualized by video microscopy.[7] The trajectories of individual diffusing vesicles can be measured by single particle tracking[8,9] over long periods of time because many dyes are present per vesicle.[4] A few example trajectories are shown in Figure 2A, the mean square displacement as a function of time in Figure 2B, and a histogram of diffusion constants in Figure 2C. The average value for the typical experiment shown is 0.9 $\mu$m$^2$/s, comparable to those observed for lipids in a supported bilayer.[10−12] The diffusion properties of tethered vesicles as a function of tethering length,

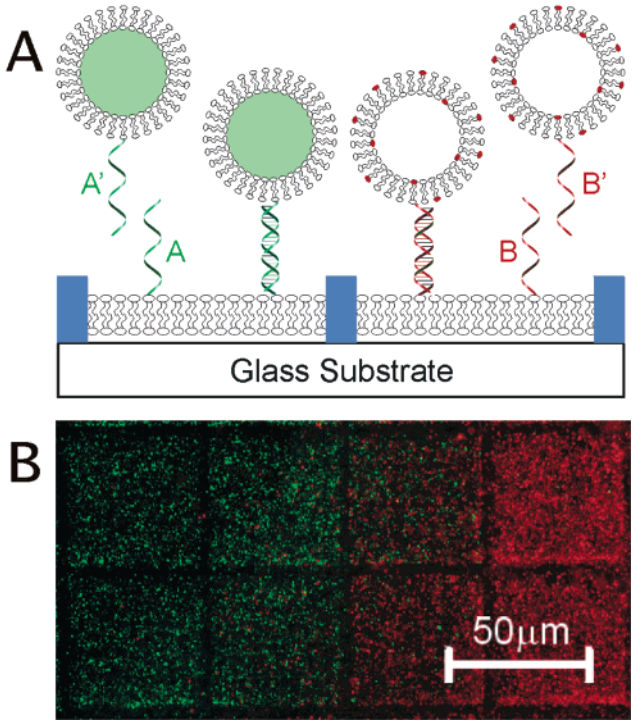


***Figure 1.*** (A) Schematic diagram of the tethering of vesicles displaying different oligonucleotides (denoted A′, green content labeled, and B′, red lipid labeled) on their lipid headgroups to complementary sequences (denoted A and B, respectively) displayed on a fluid-supported lipid bilayer formed on a patterned surface.[5] The information encoded in the oligonucleotide sequence results in specific attachment of the vesicles to the supported lipid bilayer while maintaining the ability to diffuse in two dimensions. Note that the drawing is not to scale: vesicles ranging in diameter from 30 to 200 nm have been tethered, while a typical 24-mer is about 8 nm in length and the bilayer is approximately 5 nm thick. (B) Self-sorting of vesicles displaying different oligonucleotides and corresponding different compositions (as in A′ and B′ in Figure 1A) onto a patterned supported bilayer surface displaying different amounts of the complementary oligonucleotide from all A on the left to all B on the right. Gridlines of fibronectin, 50 $\mu$m apart, were stamped on the surface of a cleaned glass substrate by microcontact printing[15] and a supported bilayer was formed displaying two different 24-mer oligonucleotides (as in A and B, Figure 1A) in a spatially defined array using flow techniques.[15] Two types of vesicles displaying the complementary oligonucleotides, green content labeled[4] 100 nm diameter vesicles with oligonucleotide A′ = 5′-GAG TAT TCA ACA TTT CCG TGT-3′ and red lipid labeled[4] 50 nm diameter vesicles with oligonucleotide B′ = 5′-TCC TGT GTG AAA TTG TTA TCC GCT-3′ on its surface, were premixed and then incubated with the patterned bilayer. After rinsing away excess unattached vesicles, the vesicles tethered vesicles are arrayed, reflecting the oligonucleotide composition displayed on the patterned bilayer array.

vesicle size, vesicle composition, density, and confinement on the surface will be reported separately. Tethered vesicles are observed to collide reversibly on the surface without loss of contents or mixing of lipids, and they move rapidly in response to an electric field.[13] If the surface is patterned by mechanically scratching the


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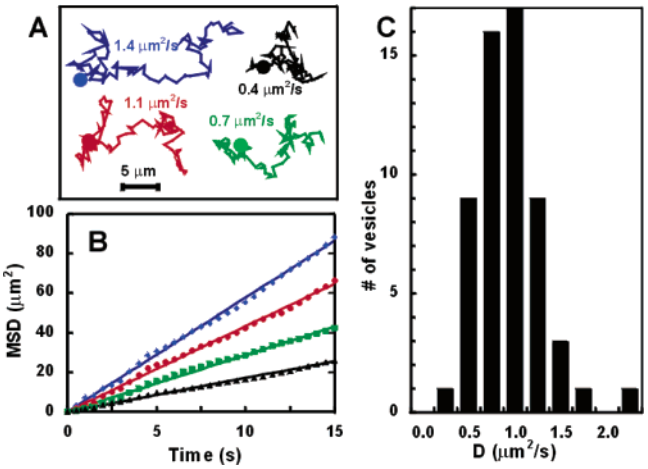


***Figure 2.*** Visualization of individual tethered vesicles and their mobility. (A) Individual egg phosphatidylcholine vesicles containing 1% Texas Red DHPE[2] and displaying the 24-mer sequence 5′-AGC GGA TAA CAA TTT CAC ACA GGA-3′ were tethered to the complementary sequence displayed on a supported lipid bilayer. The 2D diffusive motion of some vesicles is shown and is best visualized by video microscopy.[7] (B) Examples of mean-square displacement (MSD) versus time plots of four vesicles analyzed using unweighted internal averaging over all time pairs[17] and their least-squares fits up to the 15th time interval (lines through the data, $T = 25$ °C). The diffusion coefficient is given by $D = \mathrm{MSD}/4t$ for a random walk in two dimensions.[17] Each vesicle was tracked for 100 time steps at 500 ms intervals. (C) Histogram of diffusion coefficients of 57 vesicles for a typical experiment using 100 nm vesicles labeled with 1% Texas Red DHPE attached to a supported bilayer. The mean value of $D = 0.9$ $\mu$m$^2$/s.

supported bilayer,[5,14] which leaves a gap on the surface, or with microcontact printed protein barriers,[15] the tethered vesicles do not stick at the edges.

Since it is straightforward to incorporate integral membrane proteins into vesicles (but not into supported bilayers), this strategy provides a direct route for preparing arrays of integral membrane proteins or more complex membrane-associated assemblies. Furthermore, because the vesicles are mobile and can be individually visualized, this system is well suited for studying interactions between membrane-associated proteins on different vesicles, for example, during the process of protein-mediated vesicle fusion; this will be reported separately.

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**Supporting Information Available:** Additional images and video microscopy of tethered vesicles. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

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(2) An example preparation of oligonucleotide-modified vesicles. A mixture of lipids containing egg phosphatidylcholine and 0.5 mol % of reactive lipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[3-(2-pyridyldithio)propionate] (sodium salt) (N-PDP-PE, Avanti Polar Lipids), and fluorescently labeled lipid, Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TR DHPE, Molecular Probes) in chloroform, is dried to a film, reconstituted in buffer (100 mM borate, 50 mM citrate, 100 mM NaCl, 2 mM EDTA, pH 8.0) to 25 mM and extruded through a 50 nm polycarbonate membrane (Avanti) to form vesicles. An oligonucleotide modified with a disulfide group on the 5′-end (IDT DNA technologies) is first reduced to expose a free sulfhydril functionality with 10 molar excess tris(2-carboxyethyl)phosphine (TCEP), then added to the vesicle solution to a final DNA concentration of 50 $\mu$M and lipid concentration of 13 mM. The DNA attaches to the outside surface by a disulfide exchange reaction on average 1−2 per vesicle, estimated using an assay based on the fluorescence of a labeled antisense oligonucleotide. Vesicles are isolated on a Sepharse CL-4B gel filtration column with the same buffer as eluant. Supported bilayers displaying oligonucleotides are formed on a cleaned glass coverslip by vesicle fusion as described earlier for simple lipids (Salafsky, J.; Groves, J. T.; Boxer, S. G. *Biochemistry* **1996**, *35*, 14773−81), and excess vesicles are rinsed away with copious amounts of buffer. Vesicles, at approximately 70 $\mu$M in lipids, displaying the complementary oligonucleotide, are incubated with this bilayer at room temperature for 30 min followed by further rinsing with buffer to remove free, unattached vesicles. Similar procedures were used to prepare vesicles varying in size from 30 to 200 nm and oligonucleotide lengths of 16 to 24 bases.

(3) Intact, tethered vesicles have been previously reported in the literature. These systems do not to our knowledge show lateral mobility, and because a single linkage type was used, multiple types of vesicles encoded by the linkage could not be displayed. Systems include the following: Biotin/Avidin−monolayer on Gold: (a) Jung, L. S.; Shumaker-Parry, J. S.; Campbell, C. T.; Yee, S. S.; Gelb, M. H. *J. Am. Chem. Soc.* **2000**, *122*, 4177−84. Biotin/Avidin−supported bilayer: (b) Boukobza, E.; Sonnenfeld, A.; Haran, G. *J. Phys. Chem. B* **2001**, *105*, 12165−70. Chemisorption of vesicles onto gold films: (c) Stanish, I.; Santos, J. P.; Singh, A. *J. Am. Chem. Soc.* **2001**, *123*, 1008−9.

(4) A typical lipid labeled vesicle preparation includes 1 mol % Texas Red DHPE, so a single 100 nm diameter vesicle contains approximately 1000 fluorophores. A 100 nm vesicle entrapping 10 mM Oregon Green 488 carboxylic acid contains approximately 2300 dye moledules.

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(6) Kung, L. A.; Kam, L.; Hovis, J. S.; Boxer, S. G. *Langmuir* **1999**, *16*, 6773−6.

(7) See Supporting Information for a video of diffusing vesicles, Movie 1.

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(12) The lipid compositions of the supported bilayer and the vesicles are independent. Fluorescently labeled lipids in the underlying supported bilayer are observed by FRAP to diffuse in the presence of tethered vesicles as in their absence.

(13) Single and double stranded oligonucleotide lipid headgroups in the supported bilayer subjected to a lateral electric field move as expected towards the positive electrode. By contrast, tethered vesicles move rapidly in the direction of the field (see Supporting Information for a movie demonstrating this, Movie 2). This is opposite to the direction expected based on their net negative charge, suggesting that they respond to electroosmotic flow as has been observed for some tethered proteins (Groves, J. T.; Wülfing, C.; and Boxer, S. G. *Biophys. J.* **1996**, *71*, 2716−2723). Note that the double helical oligonucleotide tether extends less than 10 nm from the supported bilayer surface, whereas a tethered vesicle extends out further by its diameter. Because electrophoretic drift is very rapid, it is possible to concentrate tethered vesicles along corral boundaries (see Supporting Information). Even when highly concentrated, tethered vesicles are observed to retain their contents and no lipid leaflet mixing is observed. When the field is turned off, the vesicles relax back to randomness by lateral diffusion or they can be moved in the opposite direction by reversing the bias of the field.

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