Controlling Two-Dimensional Tethered Vesicle Motion Using an Electric Field: Interplay of Electrophoresis and Electro-Osmosis

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We recently introduced methods to tether phospholipid vesicles or proteoliposomes onto a fluid-supported lipid bilayer using DNA hybridization (Yoshina-Ishii, C.; Miller, G. P.; Kraft, M. L.; Kool, E. T.; Boxer, S. G. *J. Am. Chem. Soc.* **2005**, *127*, 1356−1357). These intact tethered vesicles diffuse in two dimensions parallel to the supporting membrane surface. In this article, we report the dynamic response of individual tethered vesicles to an electric field applied parallel to the bilayer surface. Vesicles respond to the field by moving in the direction of electro-osmotic flow, and this can be used to reversibly concentrate tethered vesicles against a barrier. By adding increasing amounts of negatively charged phosphatidylserine to the supporting bilayer to increase electro-osmosis, the electrophoretic mobility of the tethered vesicles can be increased. The electro-osmotic contribution can be modeled well by a sphere connected to a cylindrical anchor in a viscous membrane with charged headgroups. The electrophoretic force on the negatively charged tethered vesicles opposes the electro-osmotic force. By increasing the amount of negative charge on the tethered vesicle, drift in the direction of electro-osmotic flow can be slowed; at high negative charge on the tethered vesicle, motion can be forced in the direction of electrophoresis. The balance between these forces can be visualized on a patterned supporting bilayer containing negatively charged lipids that reorganize in an externally applied electric field to create a gradient of charge within a corralled region. The charge gradient at the surface creates a gradient of electro-osmotic flow, and vesicles carrying similar amounts of negative charge can be focused to a region perpendicular to the applied field where electrophoresis is balanced by electro-osmosis, away from the corral boundary. Electric fields are effective tools to direct tethered vesicles and concentrate them and to measure the tethered vesicle's electrostatic properties.

1. Introduction

Intact lipid vesicles can be tethered to 2D supported lipid bilayer membranes using oligonucleotide hybridization at the membrane surface as shown in Figure 1.[1] Lipids in the supported membrane are fluid, and tethering whole vesicles to oligonucleotides anchored in such a membrane with lipidlike hydrophobic anchors gives 2D mobility to the vesicles.[1a,c] Many tools have been developed to pattern and manipulate the composition of supported lipid bilayers,[2] and these can also be transferred to the supporting membrane for the tethered vesicles. This system is expected to be particularly useful for studying membrane−membrane interactions, such as those mediated by integral membrane proteins, because the interacting vesicles are confined to an experimentally convenient 2D format, and individual vesicle behavior is easily observed in real time by techniques such as epifluorescence microscopy. Small electric fields (10−20 V/cm) applied parallel to the support can bias the drift of the vesicles.[1a] In this article, we show that this is due to two mechanisms that may compete or work in concert: electrophoresis of charged vesicles and electro-osmosis caused by the charged supporting surface. This competition leads to novel behavior that is interesting both for understanding the dynamics of complex fluid interfaces and as a model for the behavior of membrane-anchored components.

2. Background and Theory

Electrophoresis[3] is the movement of a charged particle relative to a stationary phase by an applied electric field. In our case, this is the movement of a negatively charged lipid in a supported membrane, a membrane-anchored oligonucleotide, or a DNA-tethered net negatively charged vesicle toward the positive electrode when a field is applied parallel to the substrate. A freely suspended charged particle (for example, a charged vesicle) in an electrolyte solution subjected to an electric field moves though the stationary solution by electrophoresis as a result of three main forces. The force in the direction predicted from the charge of the particle is the product of the electric field and the total charge on the vesicle within the plane of shear. This force is opposed by Stokes friction, which scales linearly with the velocity of the particle in the low Reynolds number regime applicable to our system. The applied field also exerts a force on the counterions in the diffuse double layer of the vesicle, in the opposite direction to the force on the charged vesicle, slowing down its motion.

A charged colloidal particle in an electrolyte solution is surrounded by a diffuse electrical double layer with a characteristic thickness or Debye length, κ^{-1}, defined for a 1:1 electrolyte by

$$\kappa = \sqrt{\left(\frac{2e^2CN}{\epsilon_r\epsilon_0 kT}\right)} \tag{1}$$

where e is the unit of charge, C is the concentration of cations, N is Avogadro's number, ϵ_r and ϵ_0 are the dielectric constant and the permittivity of free space, respectively, k is the Boltzmann constant, and T is the absolute temperature. Typical Debye lengths are 1 nm for 100 mM, 3 nm for 10 mM, and 10 nm for 1 mM NaCl solutions. If the double-layer thickness is small compared to the radius of the particle, a (i.e., $\kappa a \gg 1$), then the electrophoretic

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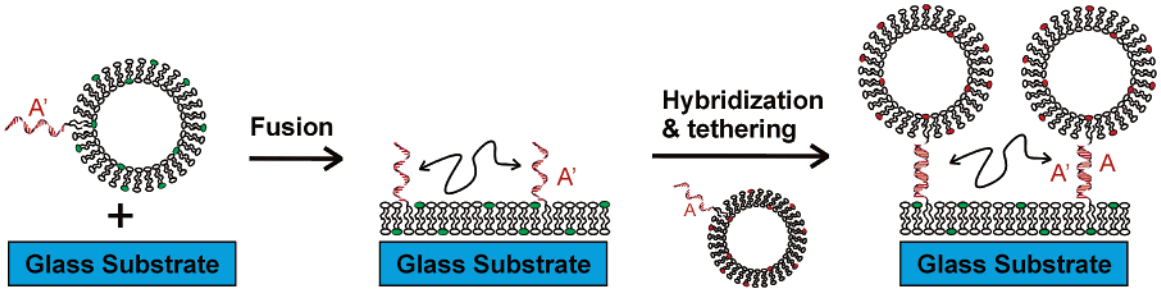

Figure 1. Schematic of the tethered vesicle assembly process. Vesicles displaying oligonucleotides with sequence A′ are exposed to a cleaned glass substrate to form a supported bilayer displaying mobile (indicated with the double arrow) oligonucleotides on the surface. Subsequent incubation with fresh vesicles displaying oligonucleotides with the complementary sequence A results in the assembly of mobile tethered vesicles by hybridization and tethering.

mobility, u_e, of freely suspended charged particles can be described by the Helmholtz−Smoluchowski equation

$$u_e = \frac{v_e}{E} = \frac{\epsilon_r \epsilon_0 \zeta}{\eta_w} \tag{2}$$

where v_e is the electrophoretic velocity, E is the applied field, ζ is the zeta potential, which is the electrostatic potential at the hydrodynamic plane of shear, and η_w is the viscosity of the aqueous medium. Theoretical calculation of the zeta potential of vesicles is discussed in detail in the Appendix.

Electro-osmosis is the movement of bulk liquid relative to a stationary charged surface in an electric field. A region of excess charge occurs adjacent to charged surfaces, where a diffuse cloud of oppositely charged counterions screens the surface charge. An externally applied electric field exerts a force on this diffuse, charged layer and their associated water molecules, which gives rise to a fluid flow relative to the surface. In our experiments, the supporting bilayer surfaces are negatively charged, and an electro-osmotic flow of bulk fluid is induced toward the negative electrode because of the excess of positive ions in the diffuse double layer.

The electro-osmotic velocity, v_{EO}, given the surface zeta potential, ζ_{EO}, and the viscosity of the aqueous medium, η_w, is given by

$$\frac{v_{EO}}{E} = \frac{-\epsilon_r \epsilon_0 \zeta_{EO}}{\eta_w} \tag{3}$$

The flow induced by electro-osmosis is independent of the distance from the charged surface outside the region of excess charge (characterized by the Debye length) and results in a plug flow. Freely suspended particles outside of the diffuse double layer are expected to move with this bulk flow. The tethered vesicles in our system protrude well beyond the electrical double layer; however, because the vesicles are tethered to a supported membrane, the drift of vesicles is retarded by a viscous drag contribution from the hydrophobic core of the supporting membrane.

To predict the electrophoretic behavior of tethered vesicles, we model our system as a charged sphere anchored to a viscous membrane by an uncharged cylinder (Figure 2). The Stokes drag on the sphere is

$$F_1 = 6\pi\eta_w a_v (v_{EO} - v_v) \tag{4}$$

where v_{EO} is given by eq 3 and a_v and v_v are the radius and drift velocity, respectively, of the tethered vesicle.

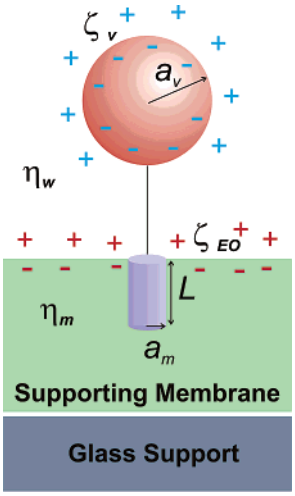

Figure 2. Model of a tethered vesicle for the theoretical prediction of its electrophoretic behavior.

The drag on the cylinder of length L inside the membrane is[4]

$$F_2 = \frac{-4\pi\eta_m L v_v}{\ln\left(\frac{L}{2a_m}\right) + 0.84} \tag{5}$$

where a_m is the radius of the cylinder in the supporting lipid membrane and η_m is the microviscosity of the membrane lipid region. The electrophoretic retardation force on the charged sphere[18] is

$$F_3 = 6\pi a_v \epsilon_r \epsilon_0 \zeta_v E \tag{6}$$

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where ζ_v is the zeta potential of the tethered vesicle surface. At steady state, the sum of all forces is zero

$$F_1 + F_2 + F_3 = 0 \tag{7}$$

which gives an expression for the electrophoretic mobility of a tethered vesicle

$$u_v = \frac{v_v}{E} = \frac{3\epsilon_r\epsilon_0 a_v(\zeta_v - \zeta_{EO})}{3a_v\eta_w + \dfrac{2\eta_m L}{\ln\left(\dfrac{L}{2a_m}\right) + 0.84}} \tag{8}$$

In the tethered vesicle system, the charges in the fluid-supporting bilayer as well as the charged components in the tethered vesicles can rearrange in response to an externally applied field. The consequences of these rearrangements will be discussed further below.

3. Materials and Methods

3.1. Vesicle Preparation. A mixture of lipids containing 5 mg of zwitterionic (neutral at pH 7.2) egg phosphatidylcholine (egg PC, Avanti Polar Lipids) and 90 μg (1 mol %) of fluorescently labeled lipid, Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TR-DHPE, Molecular Probes, single negative charge), in chloroform was dried to a film and reconstituted in buffer (10 mM phosphate, 100 mM NaCl, pH 7.2) to 10 mg/mL. The lipid mixture was extruded through a 100 nm polycarbonate membrane (Avanti) to form small unilamellar vesicles. Lipophilic oligonucleotide, $(C_{18})_2$-A (Figure 1)[5] where sequence A is found in ref 5, was dissolved in 1:1 acetonitrile/water to a concentration of 10 μM. The $(C_{18})_2$-A solution (0.6 μL, on average 0.1 DNA/vesicle) was added to the vesicles prepared above at room temperature while mixing and incubated at 4 °C for 3 h. Similarly, vesicles used to form the supporting bilayer were made using 5 mg of egg PC, 115 μg of 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC, 2 mol %, neutral at pH 7.2), and 101 μg of 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-L-serine] (DPPS, 2 mol %, single negative charge). $(C_{18})_2$-A′ solution (6 μL, 10 μM, where sequence A′ is complementary to A) was added to these vesicles to yield vesicles with an average number of one A′ DNA per vesicle. For experiments in Figures 4 and 10, a reactive lipid, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[4-(*p*-maleimidophenyl)butyramide] (sodium salt) (MPB-PE), was used to react 5′-functionalized DNA on the surfaces of vesicles for tethering, as described in ref 1. Similar procedures were used to prepare vesicles varying in size from 30 to 200 nm and different amounts of $(C_{18})_2$-DNA.

Supported bilayers displaying oligonucleotides are formed on a cleaned glass coverslip with fibronectin gridlines, patterned by microcontact printing,[6] by vesicle fusion as described earlier for simple lipids[7] by adding 35 μL of vesicles displaying $(C_{18})_2$-A′ and labeled with NBD-PC diluted to 2 mg/mL to a CoverWell perfusion chamber gasket (9 mm diameter, 0.5 mm thickness, Molecular Probes) and rinsing with buffer after a 15 min incubation period. Vesicles (4 μL) displaying $(C_{18})_2$-A and labeled with TR-DHPE are injected into the gasket and mixed. After a 45 min incubation period at room temperature, the gasket is thoroughly washed with buffer, and the tethered vesicles are observed with a Nikon TE300 inverted epifluorescence microscope and a 100× oil immersion objective.

To perform electrophoresis, the assembled tethered vesicle system was assembled into a sandwich with another clean coverslip and mounted in an electrophoresis cell as described earlier.[9,10]

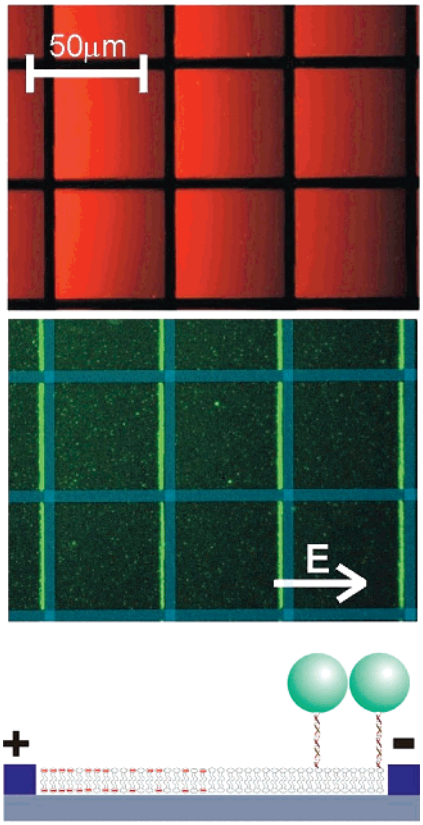

Figure 3. (Top): Steady-state image of a 1% Texas Red DHPE (negatively charged fluorescent lipid) subjected to an externally applied field of 10 V/cm. The red fluorescence is observed to form a gradient toward the positive electrode. (Middle): Egg PC vesicles containing Oregon Green content dye (10 mM) were tethered to the same supporting bilayer and visualized through a green filter. The vesicles accumulate in a narrow region along the barriers toward the negative electrode. The fibronectin barriers have been highlighted in blue in this image. (Bottom): Cartoon representation of the behavior of components in one corralled region. Note that the electrodes are far from the patterned surface to the left and right.

4. Results and Discussion

4.1. Basic Phenomenology. We have previously demonstrated that charged lipids in a supported bilayer, visualized with a fluorescent label, migrate toward the electrode of opposite charge by electrophoresis.[8] When the substrate is patterned and fluid membranes are confined into corrals, charged lipids accumulate along one edge of the corral, forming a gradient whose shape depends sensitively on the properties of the charged components and their interactions with other components as well as diffusion.[8] An example is shown in the top panel of Figure 3 where 1% Texas Red DHPE, bearing a single negative charge, in an egg PC supporting bilayer patterned with fibronectin grid lines was subjected to a field of 13 V/cm: the red fluorescence forms a gradient toward the positive electrode, that is, toward the barriers on the left, as expected. When egg PC vesicles labeled with a green fluorescent content dye (10 mM) are tethered to the same supporting bilayer and visualized through a green filter, the vesicles are observed to move rapidly in the direction of the field and accumulate in a narrow region along the barriers on the right (bottom panel, Figure 3). The sharpness of a gradient depends in large measure on the competition between random 2D diffusion and the directional force exerted by the field;[8] in the case of the tethered vesicles, the sharpness reflects their relatively slower diffusion and large force, whereas in the case of the Texas DHPE lipid, diffusion competes much more effectively with the force so the gradient is much more shallow.

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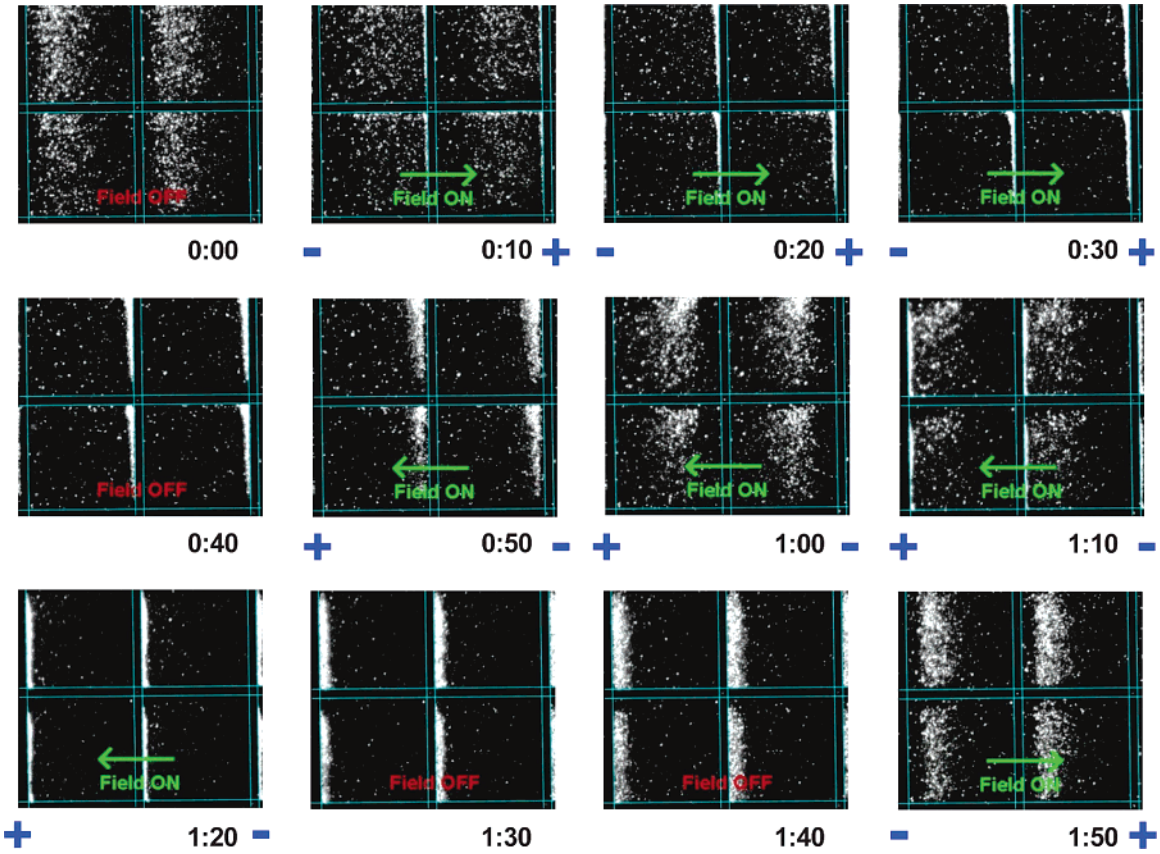

Figure 4. Upon application of an electric field, tethered vesicles accumulate against a barrier reversibly. The panels show still frames from a video acquisition (movie in Supporting Information) of egg PC tethered vesicles labeled with 1% Texas Red DHPE on a patterned supporting bilayer (egg PC bilayer with 1% MPB-PE for DNA tethering, fibronectin gridlines, 50 μm). Even when the tethered vesicles are compressed together, they are not observed to stick to barriers or fuse with each other. When the field is turned off, the vesicles diffuse by random motion.

The accumulation of vesicles against a barrier is fully reversible by switching the polarity of the electrodes. Even when the tethered vesicles are compressed together by an electric field against a barrier, they are not observed to stick to barriers or fuse with each other, as shown in Figure 4 and the movie in Supporting Information. When the field is turned off, the vesicles diffuse in two dimensions. The electric field is also a gentle method for removing vesicles that are nonspecifically adsorbed, for example, onto some defects. Such immobile vesicles are often observed to detach from the surface into solution and out of the microscope focus when a field is applied. This can be seen in Figure 4 by comparing the frame at $t = 0{:}40$ min, following one application of the field to concentrate the tethered vesicles against the right barriers, with that at 1:30 min when the field has been reversed. Fewer bound vesicles remain (seen as white dots in the regions away from the barrier where the tethered vesicles have been concentrated).

The direction of vesicle motion can be explained by electro-osmosis, where the excess cations in the electrical double layer adjacent to the negatively charged supporting bilayer cause the bulk flow of liquid toward the negative electrode. The flow velocity is zero at the surface and develops to a value given by eq 3 beyond the region of excess charge. Therefore, in cases where the mobile charged component is well within the double layer, such as for fluorescently labeled negatively charged lipid Texas Red DHPE, electro-osmosis has no effect, so this component moves electrophoretically (i.e., as expected from its charge toward the positive electrode). The Debye screening length in our experiments is typically 1−2 nm, and tethered vesicles that are 30−200 nm in diameter in addition to the oligonucleotide tether, which is approximately 8 nm long, protrude well beyond this region. Tethered vesicles move in response to hydrodynamic flow (not shown), and as a result on a patterned surface, vesicles concentrate against a barrier as they move with the flow. Some membrane proteins on the surface have been observed to move with electro-osmosis.[10,18] In contrast to a typical protein, we have great flexibility in modifying the charge on the tethered vesicle, and this allows a much more detailed quantitative analysis as discussed in the following text.

4.2. Analysis of Tethered Vesicle Drift Velocity on Unpatterned Bilayers. The drift velocity of vesicles can be determined by an analysis of their mean square displacement (MSD) over time obtained from individual particle trajectories. The inset to Figure 5A shows example tracks of three vesicle trajectories moving in an electric field. Figure 5A is a plot of their MSD versus time. The mean square displacement is fitted to the equation

$$\mathrm{MSD} = 4Dt + (v_v t)^2 \qquad (9)$$

to obtain both the diffusion coefficient, D, and vesicle drift velocity, v_v. In the case of random diffusion with no external field, the MSD is a linear function of time, as demonstrated by extensive single-particle tracking measurements on this system.[21] Figure 5B shows a histogram of drift velocities for a set of vesicles drifting under the influence of an externally applied field of 13 V/cm. The spread in Figure 5B is a convolution of intrinsic

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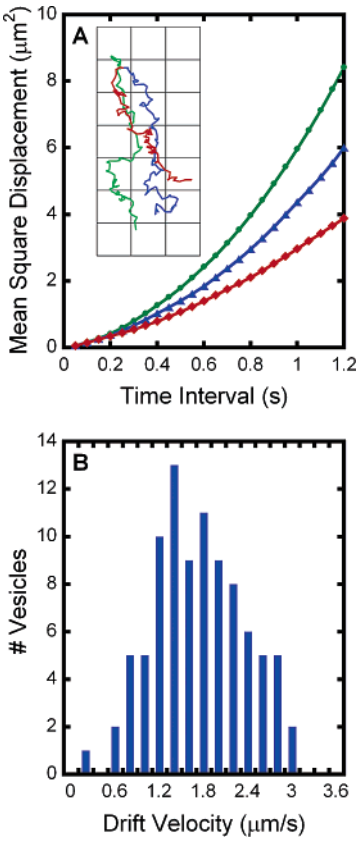

Figure 5. Quantitative analysis of the drift velocity of tethered vesicles on an unpatterned surface subjected to an electric field. (A) MSD vs time interval showing a quadratic dependence corresponding to the trajectories shown in the inset of A—three example trajectories of tethered vesicles moving under the influence of an externally applied field of 13 V/cm (down). The tethered egg PC vesicles contain 1% Texas Red DHPE for visualization and are tethered to an egg PC bilayer containing 2% DPPS. (B) Histogram of drift velocities of 91 vesicles, with an average drift velocity of 1.5 μm/s.

statistical spread associated with the finite number of measurements[19] as well as experimental variations in size, charge, and experimental errors.

If electro-osmosis is the mechanism by which the vesicles are moving, then it is expected that the drift velocity of vesicles should increase linearly with the field, and increasing the charge on the *supporting* membrane should increase the observed drift velocity of the tethered vesicles. Parts A and B of Figure 6 show the dependence of the tethered vesicle drift velocity on the applied field for 100 nm egg PC vesicles labeled with 1% Texas Red DHPE tethered on *un*patterned 5% DPPS + egg PC bilayer and 2% DPPS + egg PC bilayer, respectively. The data show that the drift velocity increases linearly with the field; the electrophoretic mobilities (the slopes of these plots) are 0.20 (μm/s)/(V/cm) for 5% DPPS in the supporting bilayer and 0.12 (μm/s)/(V/cm) for the 2% DPPS bilayer. This is consistent with our hypothesis that electro-osmosis is driving vesicle drift. It is interesting that when vesicles are tethered to an egg PC bilayer, which should have no net charge, they are still observed to move by electro-osmosis. There are several possible sources of this electro-osmosis: negatively charged impurities in an egg PC mixture, excess charge at the surface associated with unhybridized negatively charged oligonucleotides, and counterions associated with the negatively charged glass surface.

Because the vesicles that we tether carry net negative charges from added negatively charged lipids (e.g., DPPS), negatively charged fluorescent labels (e.g. Texas Red DHPE), and/or

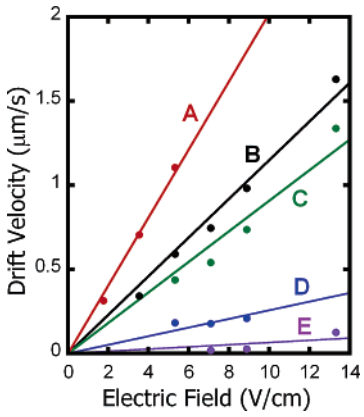

Figure 6. Average drift velocity of tethered vesicles at different applied electric fields as a function of the charge on the supporting bilayer (A vs B) and the charge on the vesicle (B compared with C, D, and E). The slope of the linear fit gives the electrophoretic mobility of tethered vesicles for a given composition. (A) Supporting bilayer: egg PC + 5% DPPS. Tethered vesicle: egg PC + 1% Texas Red DHPE. (B) Supporting bilayer: egg PC + 2% DPPS. Tethered vesicle: egg PC + 1% Texas Red DHPE. (C) Supporting bilayer: egg PC + 2% DPPS. Tethered vesicle: egg PC + 3% DPPS + 1% Texas Red DHPE. (D) Supporting bilayer: egg PC + 2% DPPS. Tethered vesicle: egg PC + 5% DPPS + 1% Texas Red DHPE. (E) Supporting bilayer: egg PC + 2% DPPS. Tethered vesicle: egg PC + 10% DPPS + 1% Texas Red DHPE.

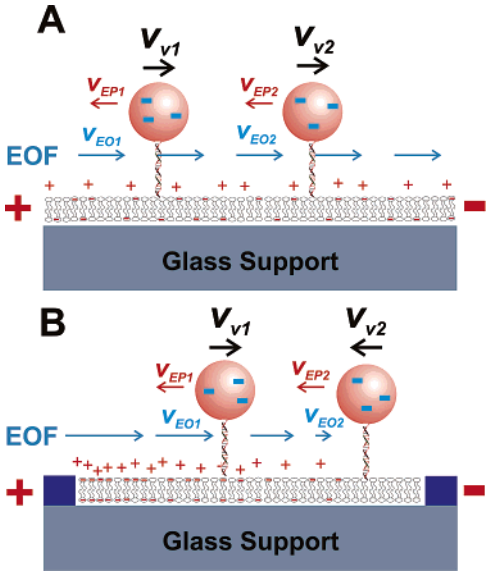

Figure 7. (A) Schematic illustration of the counteracting electrophoretic and electro-osmotic forces that tethered vesicles experience when an electric field is applied to the system. (B) Schematic illustration of tethered vesicles experiencing uneven electro-osmotic flow on a patterned supported bilayer with negatively charged lipids. Upon application of an electric field, charged components within the supporting lipid bilayer reorganize, creating uneven electro-osmotic flow over the surface. Thus, vesicles in different positions with respect to the barriers (vesicles 1 and 2) experience different amounts of electro-osmotic flow (v_{EO1} and v_{EO2}). The result is that the vesicles move toward each other (v_{v1} and v_{v2}). At steady state, the interplay of these counteracting forces results in the focusing of tethered vesicles into a line perpendicular to the direction of the field.

oligonucleotides associated with the vesicle, we expect electrophoretic forces on the tethered vesicles to counter the forces from electro-osmotic flow caused by the negatively charged surface, as shown schematically in Figure 7A. We studied this systematically by holding the charge on the *un*patterned supporting bilayer constant (2% DPPS in egg PC) and varying the charge

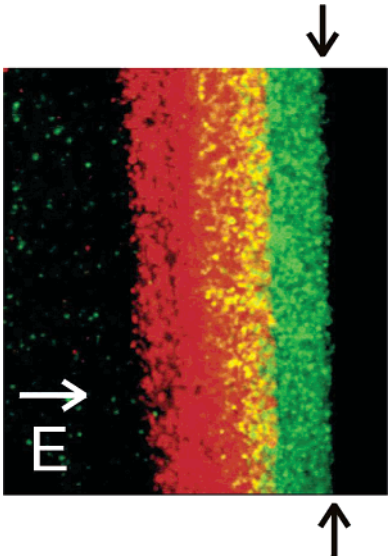

Figure 8. Separation of tethered vesicles based on charge. Red vesicles (egg PC + 1% Texas Red DHPE + 4% DPPS, 50 nm) and green vesicles (egg PC + 2% Oregon Green DHPE) were tethered to an unlabeled supporting bilayer (egg PC + 2% DPPS). Scratch barriers were introduced (position marked by black arrows above and below the image). After first moving all of the mobile vesicles in this corral toward a scratch barrier on the left (outside the field of view of this image) by an electric field applied toward the left, the field was reversed. At steady state, stripes of red and green were observed because of their different electrophoretic mobilities.

on the 100 nm egg PC vesicles labeled with 1% Texas Red DHPE by adding negatively charged DPPS in amounts from 0 to 10%. As the negative charge on the vesicle is increased, the average drift velocity is reduced as shown in Figure 6B−E. At 10% DPPS, the vesicles are almost stationary in the field strength range studied. Increasing the charge on the vesicles further requires a higher ionic strength buffer for efficient and stable tethering, presumably because of electrostatic repulsion with the supporting membrane, and the behavior of this system in an electric field was not studied.

Figure 6 shows that drift velocities of tethered vesicles can vary significantly on the same surface if their charges are different. This suggests that a separation on the surface may be possible on the basis of the charges carried by the vesicles. In the experiment in Figure 8, two types of vesicles with different compositions and colors were tethered simultaneously onto a surface with an egg PC/2% DPPS supporting bilayer: 50 nm vesicles with 4% DPPS and 1% Texas Red DHPE and 50 nm vesicles labeled with 2% Oregon Green DHPE. Scratch barriers were introduced after the system was assembled.[8] A uniform mixture of red and green vesicles was tethered, and then an electric field was applied toward the left of Figure 8 to sweep all vesicles to a barrier (outside of the field of view of Figure 8) so that most vesicles were within a few micrometers of each other. The electric field was then reversed, and stripes of red and green were observed at the right barrier, with the green vesicles traveling to the barrier first because they are less negatively charged than the red vesicles, which have a larger contribution from electrophoresis countering electro-osmosis.

Figure 9 is a plot of the observed electrophoretic mobilities of 100 nm tethered egg PC vesicles containing 1% Texas Red DHPE for visualization and varying amounts of phosphatidylserine (0, 3, 5, and 10 mol %) against their calculated zeta potentials, assuming that the negative charges associated with Texas Red DHPE behave like those on phosphatidylserine. The vesicles are tethered to an unlabeled egg PC supporting bilayer

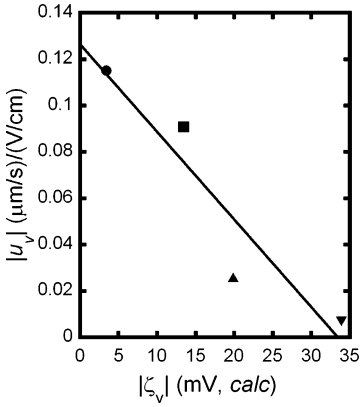

Figure 9. Observed electrophoretic mobilities of 100 nm tethered egg PC vesicles containing 1% Texas Red DHPE for visualization and varying amounts of negatively charged DPPS, plotted against their calculated zeta potentials (Appendix). Circle, square, triangle, and inverted triangle points indicate 0, 3, 5, and 10 mol % DPPS, respectively.

containing 2% phosphatidylserine.[13] In the case where the tethered vesicle is uncharged, $\zeta_v = 0$ and the intercept of the plot in Figure 9, from eq 8, should be

$$|u_v| = \frac{3\epsilon_r\epsilon_0\zeta_{EO}}{3a_v\eta_w + \dfrac{2\eta_m L}{\ln\left(\dfrac{L}{2a_m}\right) + 0.84}} \qquad (10)$$

The value calculated for the electrophoretic mobility of the uncharged vesicle is 0.14 (μm/s)/(V/cm), close to the value of the intercept predicted by a linear fit of the data extrapolating to zero charge on the vesicle. It is interesting that the size of the membrane-bound cylinder used in this model is the size of a single lipid, consistent with a single anchor for the tethered vesicles. Details of the method for these calculations and values of the parameters used can be found in the Appendix.

This analysis leads us to believe that the electro-osmotic component of vesicle drift is well described by theory, even with this simplified model of the anchor in the membrane. It is predicted by the modified Helmholtz−Smoluchowski equation (eq 8) that the electrophoretic velocity of a particle and the electro-osmotic velocity of the fluid of a surface made from the same material would cancel if the radius of the particle is large compared to the Debye screening length and is independent of particle size or surface morphology. In our system, when vesicles are tethered to a surface with the same composition composed of egg PC and visualized by an internally incorporated dye in the vesicle, it is observed to move in the direction of electro-osmotic flow instead of remaining stationary, as the theory would predict. A balance of these forces is observed only when the vesicle charge is much greater than the surface charge. In Figure 6E (vesicle composition 10% PS, 1% TR-DHPE), where the electrophoretic mobility is low, the surface bilayer is mostly composed of neutral egg PC with an added 2% negatively charged phosphatidylserine. In other words, the electrophoretic component in the observed mobility in response to an electric field is apparently muted. Note that this effect is not due to the charge structure of the phospholipid bilayer. McLaughlin et al. studied the movement of large vesicles by electrophoresis and electro-osmosis and showed that their behavior could be explained by the difference in zeta potentials, as expected.[13]

There are several possible sources of the lower-than-expected contribution from electrophoresis in our experiment. One factor

may be the "relaxation" effect of the ionic atmosphere described by Wiersema et al.[14] The ionic atmosphere of a charged particle undergoing uniform motion lags behind the center of the particle and produces an electrical force that retards the motion of the particle. This relaxation effect has not been considered in the derivation of the Helmholtz−Smoluchowski equation. Another factor contributing to the slowing of vesicles may be the mobility of the charged lipids within a vesicle. Individual lipids in the vesicle can rearrange and give rise to a vesicle with a nonuniform distribution of charge, which if large enough will also have a slowing effect on the vesicles. Finally, our vesicles are tethered to a highly charged DNA that may disturb the structure of the double layer enough to reduce the electrophoretic mobility.[15]

4.3. Reorganization Dynamics on Patterned Bilayers. It is clear from these experiments that if the negative charge in the supporting membrane can be sufficiently depleted and electro-osmosis suppressed then negatively charged vesicles should move by electrophoresis. A unique characteristic of our system is that the charged components in the supporting membrane are also mobile and reorganize upon application of an electric field. On a *patterned* surface, at steady state, each membrane corral containing a mixture of neutral and negatively charged lipids reorganizes to create a composition gradient from almost completely charge depleted toward the negative electrode to highly charged toward the positive electrode, as seen for the Texas Red DPHE in the top panel of Figure 3. We have shown previously that any unhybridized single-stranded anchored DNA also moves by electrophoresis toward the positive electrode. Thus, the application of an electric field to a patterned supported bilayer generates a charge-modulated surface that should lead to uneven electro-osmotic flow: fast flow in regions of high membrane charge and slow or no flow on the opposite side of the membrane corral where charge is depleted. This concept is illustrated schematically in Figure 7B. A related effect has been presented by Ajdari, who showed that a surface with periodic charge modulation results in convective rolls.[16]

When a field is applied to randomly distributed tethered vesicles on a patterned supporting membrane containing negatively charged mobile lipids, an even electro-osmotic flow sweeps vesicles against each barrier in the direction of the field (Figures 3 and 4) because the reorganization of lipids in the supporting membrane is slow relative to the development of electro-osmotic flow in response to the external field. If the amount of charge in the supporting membrane is sufficiently low and the electric field sufficiently large such that a region of the supporting membrane becomes so depleted of charge as to cause a significant reduction in electro-osmotic flow in that region, then negatively charged tethered vesicles should reverse direction and begin to move by electrophoresis until the electrophoretic force is balanced by electro-osmosis. At steady state, the interplay of these counteracting forces results in a focusing of tethered vesicles into a narrow region perpendicular to the applied field in a region away from either barrier, as illustrated schematically in Figure 7B. The vesicle on the left (vesicle 1) experiences a larger electro-osmotic force because of its position with respect to the barrier in a region of supporting membrane containing charged lipids. This results in the net motion of the vesicle to the right. The vesicle on the right (vesicle 2) is in a region of depleted supporting membrane charge, and the electrophoretic force is larger than electro-osmosis, resulting in a drift to the left. Both vesicles become stationary at steady state when they have reached a position where electro-osmosis and electrophoresis are balanced. The focused line is broadened by packing, inhomogeneity in the charge per vesicle, and diffusive motion.

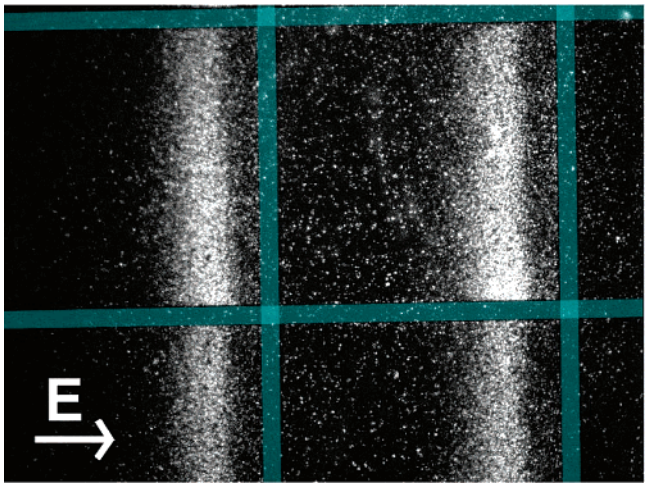

Figure 10. Steady-state image after applying 10 V/cm to 100 nm Texas Red-labeled vesicles containing 0.5% MPB-PE tethered to an egg PC supporting bilayer on a 100 μm fibronectin gridline patterned surface. The fibronectin barriers have been highlighted in blue.

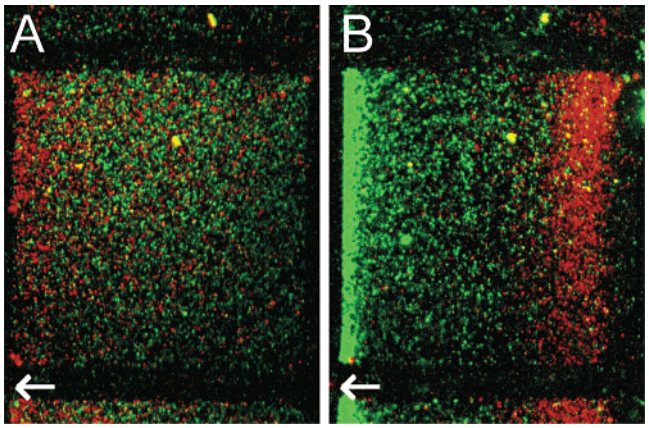

Figure 11. Two different vesicles are tethered to a fibronectin patterned (100 μm gridlines) egg PC supporting bilayer: red (50 nm, egg PC + 1% Texas Red DHPE + 4% DPPS) and green (50 nm, 1% Oregon Green DHPE). An electric field of 20 V/cm was applied in the direction indicated by the white arrow in each panel. Initially, both red and green vesicles move toward the left by electro-osmosis (A), but as negative charges in the supporting bilayer reorganize, the red vesicles slowly begin to move by electrophoresis toward the right. Green vesicles carry less charge than red vesicles, show no tendency to move by electrophoresis, and remain concentrated against the left barrier by electro-osmosis. At steady state (B), the green and red vesicles are well separated, and the red vesicles are focused into a line near the right barrier.

Figure 10 shows the experimental realization of this effect. When the field is first turned on, the vesicles are quickly swept toward the negative electrode (cf. Figure 3), and then after a few minutes, a line of vesicles moves away from the barrier and focuses there at steady state. If two populations of tethered vesicles carrying different charges are subjected to a field, then the vesicles experience different amounts of electrophoretic force on them (Figure 11). In the experiment in Figure 11, the red vesicles focus, whereas the green vesicles bearing less charge are swept to the barrier by electro-osmosis. This experiment achieves separation of tethered vesicles based on charge, and because the balance of electrophoresis and electro-osmosis results in vesicles accumulating at different positions relative to a barrier, the position of the line is an indicator of the electrophoretic component and thus the charge of the tethered vesicles. Therefore, a change in the charge of tethered vesicles, for example, by the reaction of proteins in tethered vesicles, may be detectable as a change in

the position of the focused line. This property may be useful as a sensor of vesicle charge and also as a detector for the change in vesicle charge.

Although we did not perform experiments using positively charged lipids in either the supporting membrane or the tethered vesicle, we predict that if the net charges on the vesicle and the supporting bilayer are opposite then electro-osmosis and electrophoresis will work together and no focusing effects will be observed.

Because the method for the assembly of the tethered vesicle system allows independent control of the compositions of the supporting bilayer and tethered vesicle, the response of vesicles to an electric field can be finely tuned. This increased ability to control the motion of tethered vesicles with electric fields expands the versatility of DNA-tethered vesicles for studying membrane−membrane interactions. For example, the ability to direct vesicles to a new region of the membrane, exposing them to a new supporting membrane environment, concentrating vesicles to enhance vesicle−vesicle interaction, and using the focusing effect as a detector of the change in vesicle properties may all be useful. We also note that the underlying phenomenology described here applies to any membrane anchored or associated object subjected to electric fields, whether in artificial systems as here or in fields that may be generated by charge-transport mechanisms in cells. Likewise, corralling effects are likely to be common so that the reorganization of membrane-associated components based on the mechanisms described here and anticipated in earlier work[18,19] may be an important contribution to cell surface organization.

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Supporting Information Available: Video microscopy of vesicles undergoing drift under an applied external electric field. This material is available free of charge via the Internet at http://pubs.acs.org.

Appendix

Calculation of the Zeta Potential and Predicted Electro-Osmotic Velocity of Phosphatidylserine-Containing Phosphatidylcholine Membranes. We use the Stern equation to determine the surface potential of phosphatidylcholine-containing membranes[20] in an electrolyte with sodium ions. This model assumes that the surface is uniformly charged and the ions in the diffuse double layer are point charges and distributed according to the Boltzmann distribution. The solvent is assumed to influence the double layer only through its dielectric constant, which is assumed to have the same value throughout the diffuse part.

We begin first by determining the electrostatic potential on the surface of the membrane, ψ_0. For a given bulk aqueous concentration of monovalent electrolyte, C, the concentration of monovalent cations at the membrane solution interface, C_0, is given by the Boltzmann equation

$$C_0 = Ce^{(-e\psi_0/kT)} \tag{A1}$$

Table A1: Values of Constants and Variables Used in This Experiment and for Computing the Theoretically Predicted Electrophoretic Mobility According to Our Model

aqueous viscosity	η_w	8.937×10^{-4}	Pa·s
membrane microviscosity[21]	η_m	1.2×10^{-1}	Pa·s
concentration of monovalent cations	C	6.0×10^{-2}	mol/L
Z		1	
lipid cylinder radius	a_m	1.0	nm
cylinder length	L	1.5	nm
Debye length	κ^{-1}	1.2	nm
vesicle radius	a_v	50	nm

where e is the electronic charge, k is the Boltzmann constant, and T is the absolute temperature. Sodium ions are observed to interact with phosphatidylserine. To account for the cations adsorbed onto the surface of the membrane, the Langmuir adsorption isotherm is used to calculate the effective charge density on the surface, σ, from the charge density of the membrane due to the negatively charged phosphatidylserine, σ^{max},

$$\sigma = \frac{\sigma^{max}}{1 + KC_0} \tag{A2}$$

where K is the intrinsic association constant. For sodium ions adsorbing onto phosphatidylserine membranes, the value of K is 0.6 M^{-1}.[20]

The Gouy equation

$$\sinh\left(\frac{e\psi_0}{2kT}\right) = \frac{\sigma}{\sqrt{8CN\epsilon_r\epsilon_0 kT}} \tag{A3}$$

is then used in combination with equations (x and y) to evaluate ψ_0 numerically. N is Avogadro's number, ϵ_r is the dimensionless dielectric constant, and ϵ_0 is the permittivity of free space.

The Gouy−Chapman model of the diffuse double layer describes the electrostatic potential near a charged surface as a function of distance, $\psi(x)$,

$$\psi(x) = \frac{2kT}{e}\ln\left[\frac{1 + \alpha e^{(-\kappa x)}}{1 - \alpha e^{(-\kappa x)}}\right] \tag{A4}$$

where

$$\alpha = \frac{e^{([(e\psi_0)/(2kT)] - 1)}}{e^{([(e\psi_0)/(2kT)]+1)}} \tag{A5}$$

and κ, the inverse Debye length, is given by

$$\kappa = \sqrt{\left(\frac{2e^2CNz^2}{\epsilon_r\epsilon_0 kT}\right)} \tag{A6}$$

The zeta potential, ζ, the potential at the hydrodynamic plane of shear, is approximately 2 Å from the surface[19] and is calculated from eq A4 and using values in Table A1.

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