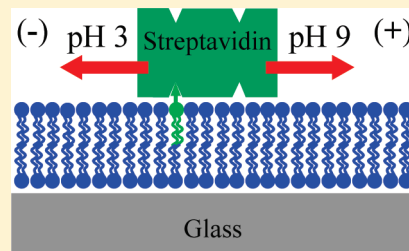


Supported Bilayer Electrophoresis under Controlled Buffer Conditions

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ABSTRACT: A pH controlled flow cell device was constructed to allow electrophoretic movement of charged lipids and membrane associated proteins in supported phospholipid bilayers. The device isolated electrolysis products near the electrodes from the electrophoresis process within the bilayer. This allowed the pH over the bilayer region to remain within ± 0.2 pH units or better over many hours at salt concentrations up to 10 mM. Using this setup, it was found that the electrophoretic mobility of a dye conjugated lipid (Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TR-DHPE)) was essentially constant between pH 3.3 and 9.3. In contrast, streptavidin, which was bound to biotinylated lipids, shifted from migrating cathodically at acidic pH values to migrating anodically under basic conditions. This shift was due to the modulation of the net charge on the protein, which changed the electrophoretic forces experienced by the macromolecule. The addition of a polyethylene glycol (PEG) cushion beneath the bilayer or the increase in the ionic strength of the buffer solution resulted in a decrease of the electroosmotic force experienced by the streptavidin with little effect on the Texas Red-DHPE. As such, it was possible in part to control the electrophoretic and electroosmotic contributions to streptavidin independently of one another.



The use of supported lipid bilayers (SLBs) as model cell membranes is well-established.¹ There are multiple reports in the literature of attempts to use SLB-based techniques to investigate lipids, transmembrane proteins, and membrane-associated proteins. There have been a number of significant successes in these efforts. In particular, the use of cushioned bilayers often results in a substantial fraction of the proteins in the membrane showing mobility by fluorescence recovery after photobleaching (FRAP) measurements.^{2–7} Other techniques, such as surface acoustic wave generation and quartz crystal microbalance with dissipation (QCM-D), have been used to pattern and monitor lipids, lipid-bound proteins, and membrane proteins.^{8–10} Additionally, electrophoresis has shown promise as a method for separating charged membrane components in SLBs.^{11–14} The motivation for these efforts stems in part from a desire to find new methods to separate membrane proteins in a lipid bilayer environment, rather than under harsher and more denaturing conditions.^{15,16}

Early SLB electrophoresis moved or separated charged fluorescently labeled lipids.^{17,18} In later reports, SLB-based separations were developed to the point that lipid molecules exhibiting only minor differences such as the *ortho* and *para* isomers of Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (TR-DHPE) could be separated.¹⁹ Membrane-associated proteins have also been analyzed using SLB electrophoresis with some success. Groves et al.¹¹ first demonstrated that proteins covalently attached to lipids through a glycan-phosphatidyl inositol linkage could be moved electrophoretically on an SLB. More recently, a number of groups have reported the electrophoretic motion of proteins.^{12–14,20} Of particular note, Han et al.¹⁴ were able to control the electrophoretic motion of streptavidin bound to biotinylated lipids by varying the composition and thus the amount of charge in the bilayer.

When a potential is applied along an SLB, there are at least two possible mechanisms by which membrane-bound species may be transported. First, it is by the electrophoretic force. The magnitude of the electrophoretic force is a function of the charge on the molecule and the potential gradient. Thus, a difference in charge can be used to separate molecules. The second is the electroosmotic force. This is a byproduct of having a fixed charge, either on the glass substrate or on the bilayer itself. The fixed charge attracts counterions. When a potential is applied, the fixed charge remains stationary (or moves relatively slowly if in a bilayer), while the counterions migrate electrophoretically. This mass directional movement of ions induces an electroosmotic flow, which can in turn exert a force on molecules that protrude above the plane of the bilayer (e.g., membrane proteins). Glass surfaces are negatively charged under most conditions, and thus, the electroosmotic flow normally pushes material toward the cathode.²¹

In supported bilayer electrophoretic experiments, a potential is applied between two electrodes. This hydrolyzes water and generates protons and oxygen gas at the anode and hydroxide and hydrogen gas at the cathode. The SLB should, therefore, be isolated from these electrolysis products. In fact, the bubbles generated at an electrode can directly delaminate the SLB. Moreover, the pH swings induced by the electrogenerated ions can alter the electrophoretic mobility of pH-sensitive bilayer species. Proteins are particularly susceptible to pH changes due to the large number and variety of amino acids that can be protonated or deprotonated near physiological pH.

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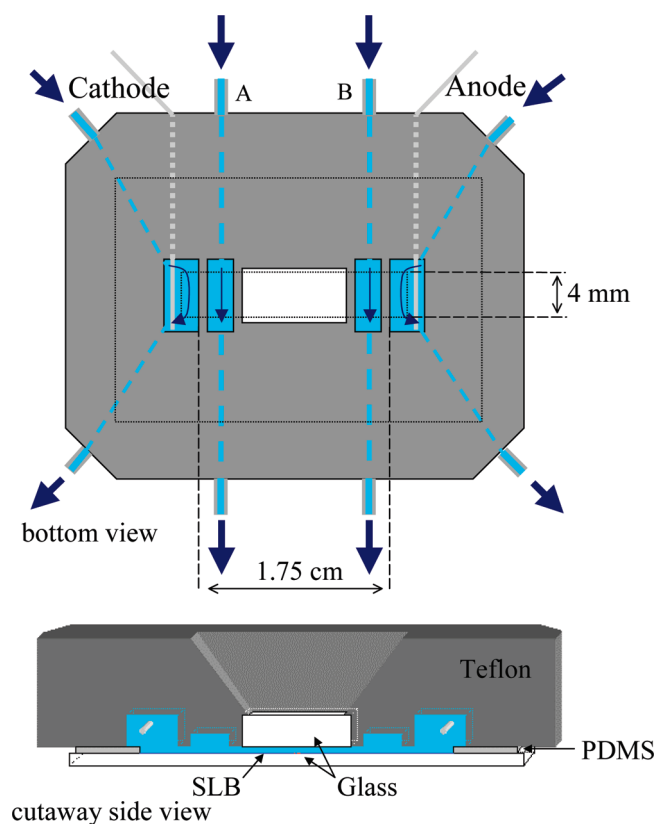


Figure 1. Schematic diagram of the flow cell used for separations in the SLB. Buffer flows in tubes and holes (blue) in the Teflon into and out of 4 channels (Cathode, A, B, Anode) in the bottom face of the device. These channels clear electrolysis products generated by the electrodes (light gray lines) before they can reach the active area of the device holding the SLB components to be electrophoresed. The top schematic shows a view of the flow cell from below, while the bottom schematic shows a cutaway view.

Traditionally, two strategies have been employed to minimize the effects of electrolysis. First, the experiment is performed with a high resistance between the two electrodes. This is done by constricting the aqueous volume above the bilayer and working at low ionic strength. This reduces the current that passes between the electrodes and, thus, the quantity of electrolysis products. It also reduces Joule heating, which can damage the SLB. A constricted solution volume additionally ensures that the majority of the potential drop occurs across the area of interest. Second, buffer reservoirs are placed around the electrodes in order to mitigate pH changes that result from the electrogenerated ions. The buffer is normally present only at low ionic strength (often 1 mM or less), but the relatively large volumes used provide some time-limited buffering capacity. This prevents large pH changes over the SLB until the buffering capacity of the reservoirs has been exhausted.

Combining thin volumes above the bilayers with larger reservoirs adjacent to it should allow the pH of the supported bilayer to remain relatively unchanged for several minutes. This is sufficient to separate lipids, which generally have relatively high electrophoretic mobilities in SLBs. Membrane-associated proteins, on the other hand, can have lower mobilities. Thus, longer runs can be necessary to separate proteins. In order for the electrophoretic mobility of a protein to remain constant during

an experiment, the pH must remain unchanged. Otherwise, the net charge on the protein will continuously be altered over the course of the experiment. Additionally, some proteins require relatively high ionic strength solutions to remain in their native state. These parameters are, unfortunately, incompatible with traditional SLB electrophoresis setups.

To enable longer electrophoretic runs at constant pH, we have developed a new flow cell (Figure 1). This device puts double channels on each side of a supported bilayer. The electrodes are embedded in the outer channels, and buffer is flowed through both the inner and outer channels. This constant buffer flow ensures that the pH in the aqueous solution above the membrane remains within ± 0.2 pH units of the initial pH (pH 3.3 to 9.3) at ionic strengths at or below 10 mM. As such, the apparatus enables electrophoresis experiments to be run for long time periods under controlled pH conditions. An additional benefit in these experiments is that higher ionic strengths can be employed than in previous setups. This is the case because the flowing solution constantly extracts heat generated by the electrodes. This setup was employed to monitor the electrophoretic mobility of Texas Red-DHPE and streptavidin linked to the bilayer via biotin-phosphoethanolamine (biotin-PE). It was found that, by altering the pH, the direction of travel of the streptavidin could be reversed. Moreover, the electroosmotic contribution could be separately controlled by tuning the distance between the bilayer and the underlying support.

EXPERIMENTAL SECTION

Glass Cleaning. Glass coverslips (Corning, NY, 24 \times 40 mm No. 1.5) were cleaned by boiling in 7 \times solution (MP Biomedicals, Solon, OH) diluted 1:5 with distilled water. They were then rinsed thoroughly with purified water (Nanopure Ultrapure Water system, Barnstead) and blown dry with nitrogen. The coverslips were annealed at 530 $^{\circ}$ C for 5 h and stored for up to 2 weeks before use.²²

Vesicle Preparation. Vesicles were prepared via the freeze-thaw/extrusion method.^{23,24} Lipids were mixed at the desired ratios in chloroform solutions. The chloroform was evaporated under a stream of nitrogen, and the lipids were placed under vacuum for 4 h to remove any remaining solvent. The lipid mixture was rehydrated with tris(hydroxymethyl)aminomethane buffer (10 mM Tris, 100 mM NaCl, pH 7.4, Tris/NaCl buffer) and subjected to ten freeze/thaw cycles in liquid nitrogen and warm water. The solution was extruded ten times through a track-etched polycarbonate membrane with 100 nm pores (Whatman), diluted to 1 mg/mL, and stored at 4 $^{\circ}$ C until use. The mean vesicle size from each batch was found to range between 80 and 120 nm by dynamic light scattering using a 90Plus Particle Size Analyzer (Brookhaven Instrument Corp., Holtsville, NY).

SLB Formation. Supported lipid bilayers were formed via the vesicle fusion method as described previously.^{25–27} A narrow line of analyte material could be added to the membrane by employing the scratch and backfill method (Figure 2).¹⁹ To do this, an initial bilayer (shown in blue) was formed at the liquid/solid interface using a 1 mg/mL solution of 100 nm diameter vesicles within the confines of a polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI) well on a clean glass coverslip. The vesicles, which were made of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC, Avanti Polar Lipids, Alabaster, AL), were incubated over the surface for at least 10 min

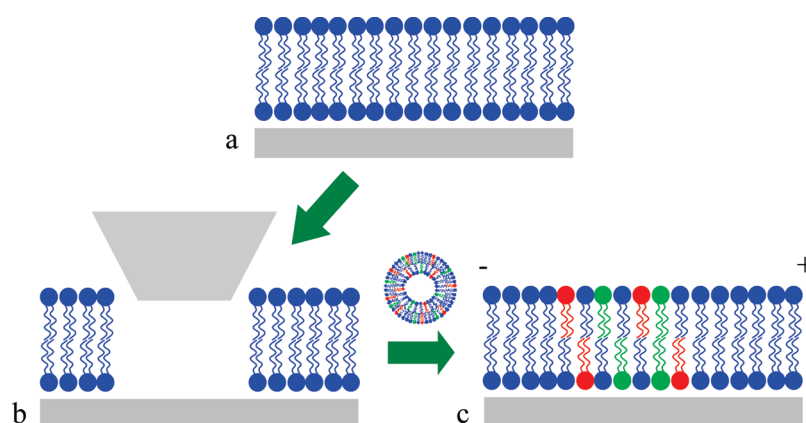


Figure 2. Scratch and backfill method of producing a band of lipids in a matrix for separation. (a) Vesicles are fused to a glass coverslip to form an SLB. (b) A Teflon-coated metal spatula is used to scratch the slide, removing a band of matrix material. (c) Vesicles containing a lipid mixture to be separated are then fused into the vacant area, and a potential is applied along the bilayer.

Table 1. Performance of the Flow Cell under Different Buffer and pH Conditions^a

buffer	initial pH	buffer capacity (mM/pH)	pH of outlet buffer			
			cathode	A	B	anode
1 mM citrate	3.0	2.9	3.0	3.0	3.0	3.0
1 mM citrate	5.2	0.62	5.3	5.2	5.2	5.1
1 mM citrate	7.7	0.11	11.0	7.7	7.7	6.7
1 mM Tris	9.2	0.15	9.2	9.2	9.2	9.2
1 mM Tris and 10 mM NaCl	7.4	0.34	9.0	7.5	7.3	3.3
1 mM Tris and 100 mM NaCl	7.7	0.49	11.6	10.0	7.7	2.2

^a Sodium was the cation in the citrate buffers.

before being washed away with fresh Tris buffer. These vesicles were doped with 0.5 mol percent C16 mPEG 5000 Ceramide (polyethylene glycol (PEG), Avanti Polar Lipids, Alabaster, AL) in runs requiring a polymer cushion. Next, a solution containing the analyte vesicles was introduced above the surface, and the surface was scratched with a Teflon-coated metal spatula as shown in Figure 2b. This removed a line of lipids and allowed the vesicles containing the analyte lipids to embed into the matrix SLB. Incubation of these vesicles was allowed to proceed for 8 min. After this, the slide was again rinsed with purified water followed by Tris/NaCl buffer. This produced a narrow band of labeled lipids as illustrated in Figure 2c. This analyte band consisted of POPC doped with 1.0 mol % 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(cap biotinyl) (b-DOPE, Avanti Polar Lipids, Alabaster, AL) and 0.1 mol % TR-DHPE (Invitrogen, Carlsbad, CA). Streptavidin could be bound to the surface by incubating a Tris/NaCl solution containing 0.01 mg/mL labeled streptavidin over the surface for 10 to 20 min followed by rinsing away excess protein with Tris/NaCl buffer.

Flow Cell. The electrophoresis flow cell setup (Figure 1) was manufactured in-house. It consisted of a Teflon body in which a series of channels, tubes, and a viewing window were machined. The SLB was held on a separate glass coverslip in a long, narrow well (20 mm by 4 mm) carved into a polydimethylsiloxane (PDMS) slab (30 mm × 20 mm × 0.1 mm). The coverslip/PDMS system was held tightly against the Teflon body by clamps. The two outer channels (labeled “Cathode” and “Anode” in Figure 1) were 1 mm deep and also held the platinum electrodes (0.25 mm diameter, shown in gray). The inner two

channels (labeled “A” and “B” in Figure 1) were only 100 μm deep, which forced buffer to flow close to the coverslip supporting the bilayers. The inner channels ensured that any electrolysis products that escaped from the outer channels would be swept away before reaching the central region where the SLB was housed. Inlet and outlet buffer was carried by gravity in Teflon tubing to and from the device. A height difference of ~75 cm between the buffer reservoir and the device was employed to adjust the flow rate to ~0.6 mL per minute in each tube. It was found that the flow rate increased in a linear fashion with height. A 1 mm thick glass observation window was placed over the central region of the flow cell so that the bilayer could be visualized by fluorescence microscopy. This allowed the electrophoretic separation to be observed in real time using either an upright or an inverted microscope.

The PDMS well used in these experiments was fabricated by allowing the PDMS to polymerize between two glass slides separated by the thickness of No. 1 coverslips (100–150 μm thick). A section of the PDMS sheet was cut out and removed to make the well. Thus, during an experimental run, the SLB was held in a channel that was 100–150 μm high. The PDMS well was rinsed with ethanol and purified water. Additional dust and particulates were removed with adhesive tape before it was affixed to the coverslip.

Streptavidin Labeling. Streptavidin with varying numbers of dye labels was used in this work. Unlabeled streptavidin was purchased from Sigma. A portion of this protein was labeled with 0.3 Alexa Fluor 488 dyes per molecule using a protein labeling kit (Molecular Probes, Carlsbad, CA). A second portion was labeled

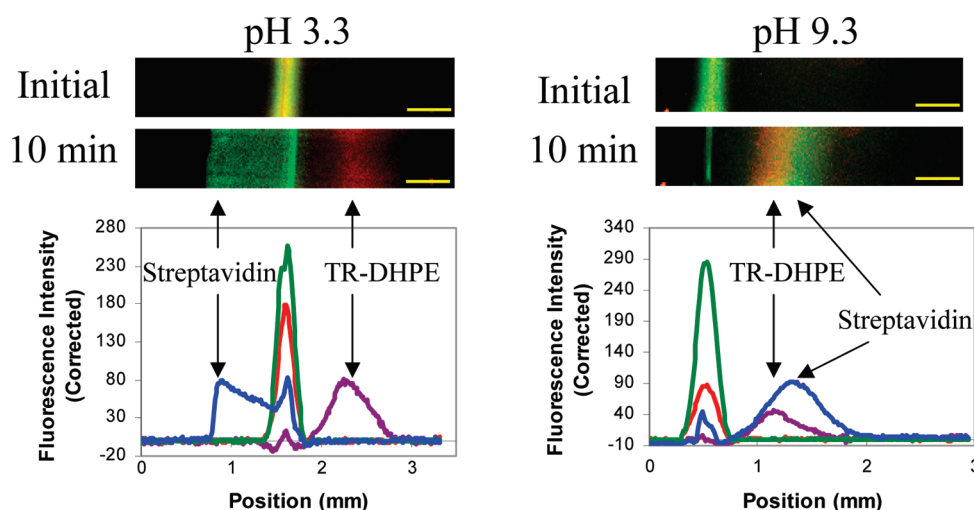


Figure 3. Typical electrophoresis results in 1 mM, pH 3.3 sodium citrate (left) and 1 mM, pH 9.3 Tris (right). False color fluorescence images (top) show the position of TR-DHPE (red) and streptavidin (green) initially (upper images) and after 10 min of applying a 170 V/cm field (lower images). Below these images are the corresponding fluorescence linescans with the initial intensities being depicted in green (streptavidin) and red (TR-DHPE) and the final intensities in blue (streptavidin) and purple (TR-DHPE).

with 4.0 Alexa Fluor 488 dyes per protein also using the protein labeling kit. In all labeling experiments, the dye labeled protein was separated from unreacted dye molecules using a size exclusion column provided as part of the labeling kit with Tris/NaCl buffer as the eluent. The number of dyes per protein molecule was determined by measuring the UV/vis absorbance of the labeled, purified streptavidin solution at 280 and 494 nm with an Agilent 8453 UV/vis spectrometer. The degree of labeling was calculated as per the instructions in the protein labeling kit, using an extinction coefficient of 3.2 mL cm/mg for streptavidin.²⁸

RESULTS AND DISCUSSION

Flow Cell pH Control. In a first set of experiments, the pH in the four-channel flow cell was tested at a variety of values. The results are listed in Table 1. The outermost channels are designated “Cathode” and “Anode” as this was where the electrodes were housed. In contrast, the inner channels are designated “A” and “B”. As can be seen, solutions containing 1 mM of a buffering agent were used at 4 different pH values. In the case of sodium citrate at pH 7.7, the salt was intentionally well outside of its buffering range to measure the pH variation in an essentially unbuffered solution. Under a final set of conditions, 10 mM and 100 mM NaCl were added, respectively, to 1 mM Tris buffer, which substantially increased the solution conductivity. In all cases, a 250 V (140 V/cm) potential was applied across the device and maintained for at least 30 min before the pH was measured in each of the four channels. A solution flow rate of 0.6 mL/min was constantly maintained in all channels. This meant that the flow velocity was actually an order of magnitude faster in the inner channels than in the outer channels. This faster flow rate in the inner channels kept the pressure higher there and forced a net solution flow from the inner channels to the outer channels. The control of electrolysis conditions by means of channel depth is similar in concept to work done in free-flow electrophoresis, which suffers from similar problems due to electrolysis products.²⁹

As can be seen from Table 1, the pH of the inner channels never deviated within experimental error from the initial pH

value of the solutions for all low ionic strength experiments. The outer channels showed more variances, especially in the cathode chamber for sodium citrate buffer at an initial pH of 7.7. As expected, substantial variance from the initial pH could also be seen in the anode and cathode chambers when 10 or 100 mM NaCl was added. Nevertheless, the two inner channels maintained pH values within 0.1 pH unit of the initial solution in the 10 mM case. Such a result ensured that the SLB chamber between these two channels would only be subjected to very small pH swings up to 10 mM ionic strength. Since a pH variation of ~2 pH units in the A channel was observed with 100 mM NaCl, salt concentrations were held to only 10 mM in all subsequent electrophoresis experiments with bilayers.

It should be noted that the flow of buffer through the device helped in two ways to counter heating that is often detrimental to electrophoretic devices. First, a significant source of heating at longer times is the exothermic reaction of water electrolysis products: protons and hydroxide ions. When these ions are removed before they can react, this heating should be completely avoided. Other electrolysis products, particularly oxygen and hydrogen gas, were also removed in this step as previously noted. Second, the continual flow of room temperature buffer through the device carried away heat generated by Joule heating (resistive heating). The flow cell tolerated relatively high potentials and currents. With 1 mM buffer, the current was around 100 μ Amps at an applied potential of 250 V (140 V/cm). When 10 mM NaCl was employed at an applied potential of 250 V, the current was ~1 mAmp. Finally, a current of ~10 mAmps was found with 100 mM NaCl in 1 mM Tris at the same applied potential.

TR-DHPE and Streptavidin Migration as a Function of pH. The mobility of Texas Red-DHPE and Alexa Fluor 488 labeled streptavidin with 4.0 dyes per protein molecule were observed at pH 3.3 and 9.3. These experiments were performed with 1 mM sodium citrate buffer and 1 mM Tris buffer, respectively. A field of 170 V/cm was applied for 10 min, and the fluorescence images both before and after electrophoresis are shown along with the corresponding line profiles. Initially, both the streptavidin (green curves) and Texas Red-DHPE (red

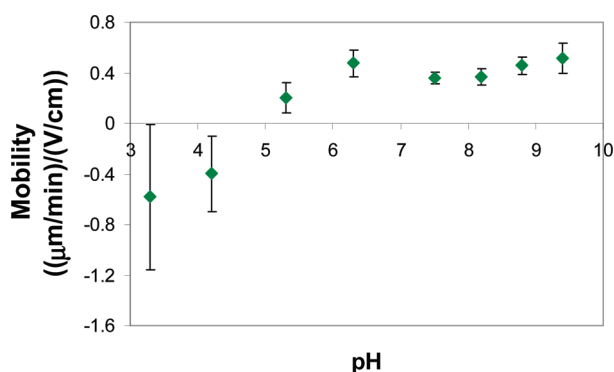


Figure 4. Mobility of streptavidin labeled with an average of 4.0 dyes/molecule as a function of pH at 170 V/cm. All runs were performed at 1 mM buffer concentration in a POPC SLB. Sodium citrate was used under acidic conditions (below pH 6), sodium phosphate at near-neutral conditions (6–7), and Tris under basic conditions (above pH 7). In this and all other figures, the error bars represent one standard deviation.

curves) were confined to approximately 400 μm wide strips. Under both sets of conditions, the Texas Red-DHPE migrated to the right toward the anode (purple curves). This occurred because the net charge on the dye-labeled lipid molecule remained negative over this pH range. Throughout the pH range tested, the mobility of the Texas Red-DHPE was 0.32 ± 0.04 ($\mu\text{m}/\text{min})/(\text{V}/\text{cm})$. One can also observe a small immobile fraction of the dye in both cases as a small peak left at the origin. This may be due to adsorption at defects caused by scratching. The immobile fraction represented no more than 2% of the total Texas Red-DHPE under all circumstances.

In contrast with Texas Red, the biotin-bound streptavidin behaved quite differently. The biomacromolecule moved to the left toward the cathode in the image at pH 3.3, while it moved to the right toward the anode at pH 9.3 (blue curves). In other words, the protein appeared to bear a net positive charge at pH 3.3 and a net negative charge at pH 9.3. The mobility of the streptavidin varied between -0.6 ($\mu\text{m}/\text{min})/(\text{V}/\text{cm})$ at pH 3.3 and 0.5 ($\mu\text{m}/\text{min})/(\text{V}/\text{cm})$ at pH 9.3. In both cases, it should be noted that a somewhat more substantial fraction of the membrane-bound biomacromolecules was immobile compared with the lipid ($\sim 20\%$).

The electrophoresis experiments shown in Figure 3 were repeated as a function of pH between 3.3 and 9.3. The mobility values of the 4 dye-labeled streptavidin are plotted as a function of pH in Figure 4. The streptavidin mobility switched from a negative value to positive value just below pH 5.0. It should be cautioned that this does not represent a true isoelectric point for the protein because the macromolecule was also subject to electroosmotic forces.¹¹ Indeed, the negatively charged substrate attracted cations (Na^+), which flowed toward the cathode. This electroosmotic flow also exerted a net force toward the cathode on the protein. The electroosmotic force on the streptavidin originated principally from the glass coverslip supporting the bilayer. An additional electroosmotic force could be generated at the glass viewing window of the device. However, silanizing the viewing window was found to have no effect on the streptavidin mobility. It should also be noted that the number of bound biotinylated lipids should affect the pH at which streptavidin shows no net migration. This should occur because each biotinylated lipid had a minus one charge. There can be either one or two bound

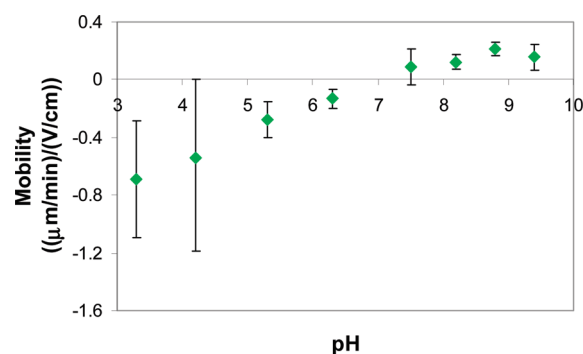


Figure 5. Mobility of streptavidin labeled with an average of 0.3 dyes/molecule as a function of pH at 170 V/cm. All runs were performed at 1 mM buffer concentration in a POPC SLB. Sodium citrate was used under acidic conditions (below pH 6), sodium phosphate at near-neutral conditions (6 to 7), and Tris under basic conditions (above pH 7).

biotin-DOPE molecules per streptavidin.^{30,31} These bound lipid molecules will somewhat offset the effect of electroosmosis.

The electrophoretic mobility experiments with labeled streptavidin were repeated with 0.3 dye molecules per protein (Figure 5). Under these circumstances, the pH of zero net streptavidin migration became far more basic ($\sim \text{pH } 7$). In fact, most of the observed protein molecules should have possessed a single label as the unlabeled molecules were not visible and very few molecules contained more than one label. The large shift in the isoelectric point was expected because the Alex Fluor 488 dyes were conjugated to free lysine residues on the protein surface via a succinimidyl ester. This is significant, because the free lysine bears a positive charge below $\text{pH } \sim 10.3$, while the dye is negatively charged. As such, four positive charges are converted to negative charges when the protein has four labels, but only one residue has its charge flipped when one dye is used. The pI of streptavidin has been reported to be between 5 and 6,^{30,31} but this value clearly depends upon the degree of labeling, as more labeling should make the value more acidic. Such a result is consistent with literature data involving capillary electrophoresis measurements of green fluorescent protein as a function of labeling degree.³²

Curiously, at pH 3 and 4, the electrophoretic behavior of streptavidin differed from its behavior under more basic conditions. Specifically, at higher pH values, the streptavidin migrated in a gradually broadening Gaussian peak as shown in Figure 3 at pH 9.3. At low pH, however, the streptavidin migrated as a front that slowed and stopped after 5 to 10 min. This led to the decidedly non-Gaussian shape seen at pH 3.3 in Figure 3. Both streptavidin with an average of 0.3 and 4.0 labels behaved identically in this respect. This implies that the effect was not only due to protein labeling. Rather, it seems likely that some structural change or partial unfolding may be occurring near pH 4 and below regardless of the labeling extent. This change may cause the streptavidin to aggregate and eventually stop electrophoretic motion completely. Aggregation of streptavidin under somewhat acidic conditions has been reported before, supporting this hypothesis.³³ Control experiments at a higher concentration of biotin in the bilayer (2% as opposed to 1%) were performed. These systems, in turn, had higher number densities of bound streptavidin and resulted in higher immobile fractions of the protein, further supporting the aggregation hypothesis. It should be noted that the electrophoretic mobility values reported

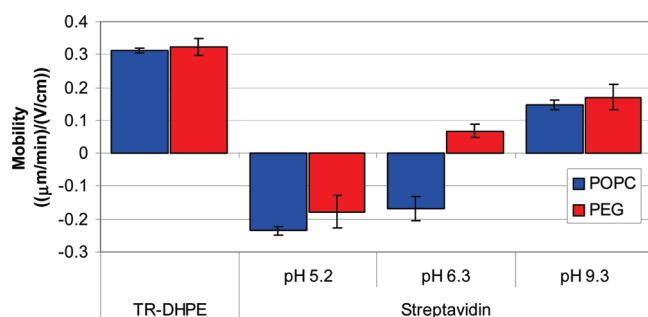


Figure 6. Mobility of TR-DHPE and streptavidin in POPC (blue) and in a POPC/PEG bilayer (red). The runs were performed in 1 mM sodium citrate (pH 5.2), sodium phosphate (pH 6.5), and Tris (pH 9.3) buffer with an applied potential of 140 V/cm.

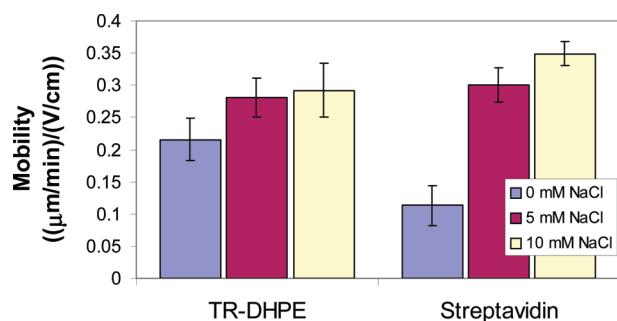


Figure 7. Mobility of TR-DHPE and streptavidin in POPC at different ionic strengths. All runs were performed in a pH 7.9, 1:1 sodium citrate/Tris buffer mixture for a total buffer strength of 0.5 mM. NaCl was added to the 0.5 mM buffer to make the 5 or 10 mM solutions. Electrophoresis was performed at 140 V/cm.

in Figures 4 and 5 were taken within the first 5 min for the runs at pH 3 and 4. At all other pH values, the mobilities remained consistent over the course of 30 and even 60 min runs.

Electrophoresis in PEG-Containing SLBs. In a next set of experiments, we wished to attenuate the electroosmotic contribution to the mobility of the fluorescently labeled membrane components. To do this, 0.5 mol % polyethyleneglycol linked phosphatidylethanolamine was added to the membrane. This concentration, near the mushroom to brush transition, lifted the bilayer up away from the underlying negatively charged silica substrate by an amount similar to the Flory radius, in this case ca. 6 nm.^{34,35} As such, it was removed from the diffuse double layer and the source of electromotive flow. Figure 6 compares the results of adding PEG to the separation SLB with simple POPC membranes. The data were taken with 0.3 dye/molecule streptavidin at 140 V/cm potentials in 1 mM buffers. As can be seen, the mobility of Texas Red-DHPE was essentially unchanged by the addition of PEG. In contrast, the results for streptavidin are more pronounced. Under conditions far from the transition between the anodic and cathodic directions of travel, adding PEG slightly shifted the streptavidin mobility in the anodic direction (pH 5.2 and 9.3). Near the anodic to cathodic transition, adding PEG to the SLB completely reversed the direction of movement from cathodic to anodic (pH 6.3). Such a result is consistent with the notion that biotin-bound streptavidin bears a slight negative charge under these conditions.^{30,31} When the polymer cushion was absent, the protein moved toward the cathode because the electroosmotic force was dominant. In the

presence of the PEG layer, this force was attenuated and the protein moved toward the anode based on its net charge.

Electrophoresis as a Function of Ionic Strength. A similar effect to that of adding PEG can be observed merely by modulating the ionic strength of the buffer. To do this, the electrophoretic mobilities of streptavidin and Texas Red-DHPE were observed with 0.5 mM citrate/0.5 mM Tris buffer containing 0 mM NaCl, 5 mM NaCl, and 10 mM NaCl. The mobility results at pH 7.9 with a field of 140 V/cm are given in Figure 7. As can be seen, the mobility of the Texas Red-labeled lipid was only slightly affected by the addition of salt. On the other hand, the streptavidin migration rate more than doubled. This occurred because increasing the ionic strength reduced the Debye length in the solution from 10 nm with the 0.5 mM buffer to 3 to 4 nm with the addition of 5 or 10 mM of NaCl.³⁶ Therefore, the charge on the glass support was more strongly screened at higher ionic strength. Also, there were probably more counterions between the bilayer and the support, which should mitigate the field normal to the bilayer. As the streptavidin was confined to the upper leaflet of the bilayer, this reduced the electroosmotic force and allowed the negatively charged streptavidin to migrate faster toward the anode.

CONCLUSIONS

A flow cell has been constructed that allows the pH above a supported lipid bilayer to be continuously controlled during electrophoresis. Using this device, the response of membrane-bound streptavidin was investigated as a function of pH, the presence or absence of a PEG cushion support, and the ionic strength. TR-DHPE served as a reference compound that was relatively insensitive to these variables. The electrophoretic migration of streptavidin was found to be highly pH dependent. At acidic pH values, the streptavidin migrated toward the cathode. At basic pH values, the protein migrated toward the anode. In both cases, electrophoretic and electroosmotic forces were present. The change in direction occurred as the charge on the streptavidin became sufficiently negative to counteract electroosmotic flow. Adding a PEG cushion to the bilayer or increasing the ionic strength also attenuated the electroosmotic force. Thus, both the electrophoretic and electroosmotic forces could be altered independently of one another. This suggests intriguing possibilities in the ability to precisely control the movement of membrane bound species in supported lipid bilayers.

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