Supporting Information for:

Protein Separation by Electrophoretic-Electroosmotic Focusing on Supported Lipid Bilayers

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Supporting Information

The volume of an IgG molecule is about 350nm^3.[1] Assuming that the protein were spherical, which is a crude approximation, the radius of an IgG would be about 4.5 nm.

The area of a lipid is about 0.66nm^2; therefore, the surface charge density contributed by a 10% POPG bilayer:

$$(0.1 \times -1.6\times10^{-19}\ \text{C})/0.66\ \text{nm}^2 = -24.3\text{mC/m}^2$$

Equation Derivation

The electrophoretic force on a membrane-bound protein is:[2]

$$\overrightarrow{F_E} = 6\pi r_m \varepsilon_r \varepsilon_o \zeta_m \vec{E} \qquad (S1)$$

r_m is the radius of the protein, ε_r is dielectric constant of aqueous phase and ε_o is relative permittivity of free space, ζ_m is the zeta potential of the protein and E is the external electric field .

The drag force (caused by electroosmotic flow) on a membrane-bound protein is:[2]

$$\overrightarrow{F_{EO}} = 6\pi\eta_w r_m \overrightarrow{v_{EO}} \qquad (S2)$$

η_w is the viscosity of the solution, r_m is the radius of the protein and v_{EO} is the electroosmotic flow rate. v_{EO} can be obtained from equation (S3):

$$\overrightarrow{v_{EO}} = -\frac{\vec{E}\varepsilon_r\varepsilon_o\zeta_{EO}}{\eta_w} \qquad (S3)$$

ζ_{EO} is the zeta potential at the surface, ε_r is relative permittivity of the liquid and ε_o is the dielectric constant of vacuum. Putting equation (S3) into equation (S2) yields equation 4:

$$\overrightarrow{F_{EO}} = -6\pi r_m \varepsilon_r \varepsilon_o \zeta_{EO} \vec{E} \qquad (S4)$$

The drag force on biotinylated lipids by the hydrophobic core of the lipid bilayer should be considered. However, all components stop moving in our EEF experiment. As such the drift velocity becomes zero at the focusing point. Therefore this drag force is set to zero at steady state.

Moreover at steady state, $\overrightarrow{F_E} + \overrightarrow{F_{EO}} = 0$ and equation (S1) is equal to equation (S4):

$$\zeta_m = \zeta_{EO} \qquad (S5)$$

Theoretically, the focusing takes place at the iso-zeta potential position, where the zeta potential of the protein is equal to the local zeta potential on the SLB.

The Debye length κ^{-1} can be written as follows:

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_r \varepsilon_o kT}{2e^2 I N_A}}$$

k is Boltzman constant, T is temperature, I is the ionic strength and N_A is Avogadro's number.

Below the focusing positions for individual components used in the main manuscript are shown:

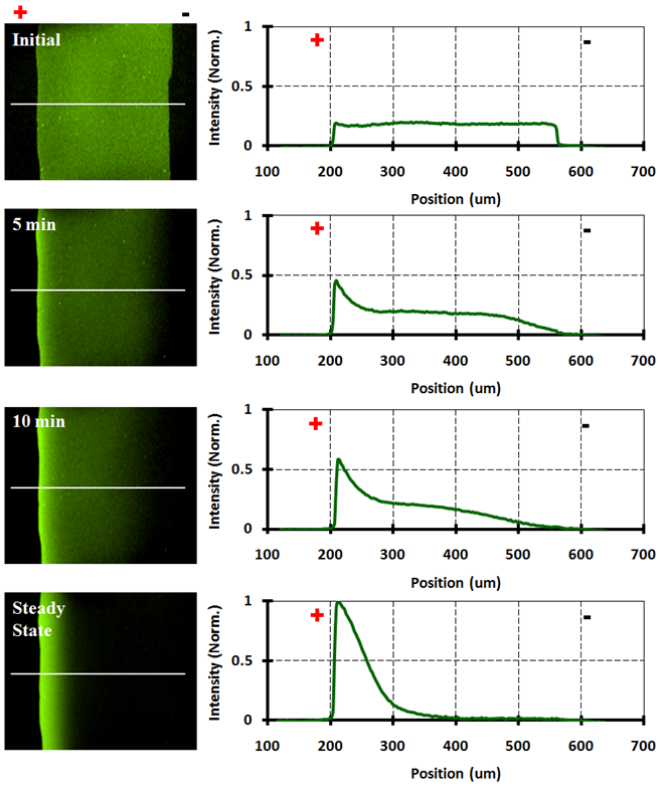

Figure S1. Migration of NBD-DPPE in a negatively charged lipid bilayer (10% POPG) with a 50 V/cm electric field at pH 7.3 controlled by 10 mM Tris buffer. The corresponding fluorescence line scan profile is on the right of each image.

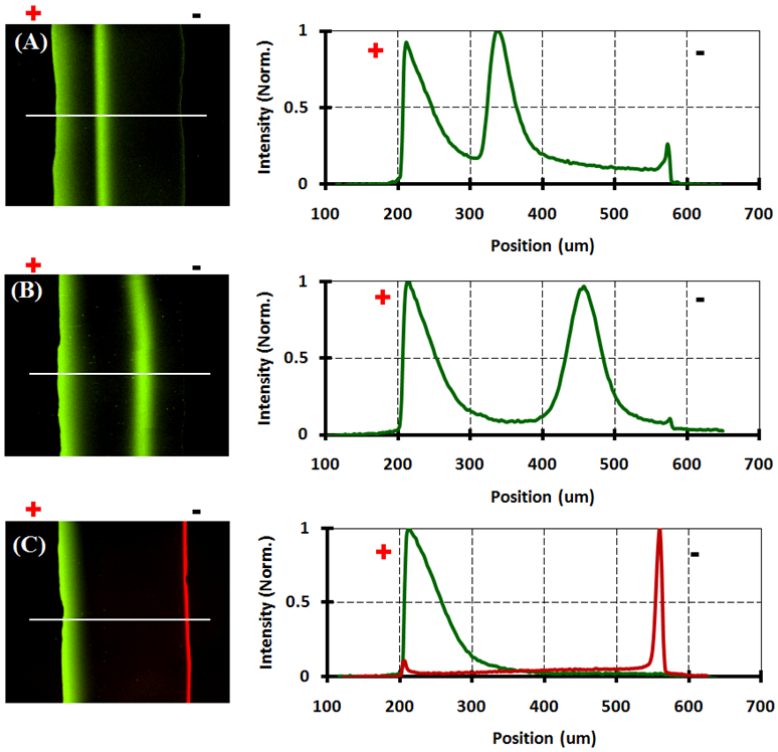

Figure S2. Separation of NBD-DPPE and different membrane-bound proteins, (A) StrA-4, (B) StrA-1 (B) and (C) IgG anti biotin from a homogeneous mixture in a negatively charged lipid bilayer (10% POPG) in a 50 V/cm electric field. The pH was 7.3 in a 10 mM Tris buffer. The corresponding line scan profile is on the right of each image.

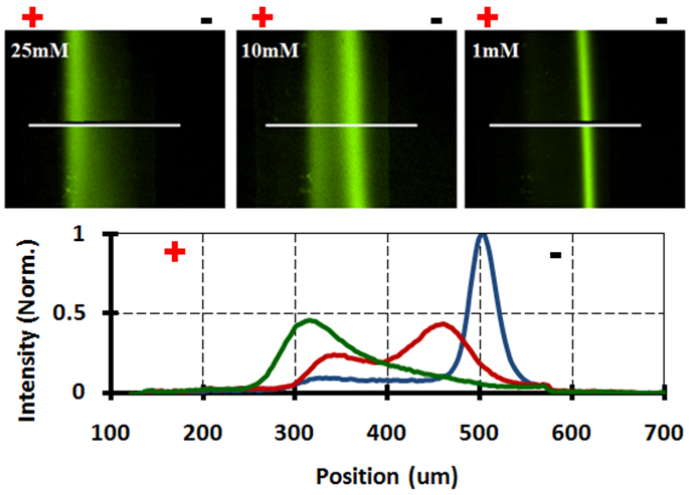

Figure S3. The migrations of StrA-4 and StrA-1 bands are shown at steady state under three buffer concentrations in a 10% POPG SLB with a 50 V/cm electric field. The pH was 7.3 and the ionic strengths were 25 mM, 10 mM and 1 mM, respectively. The change was made using different concentrations of Tris buffer. The fluorescence pictures are on the top with the given buffer concentration written in the upper left side of each image. The fluorescent line scan profiles of 25 mM (Green), 10 mM (Red) and 1 mM (Blue) buffer concentration are shown

together beneath the fluorescence images. Based on **Eq. 5**, increasing the ionic strength (decrease of Debye length) should move all peaks to more negatively charged regions on the SLB as observed.

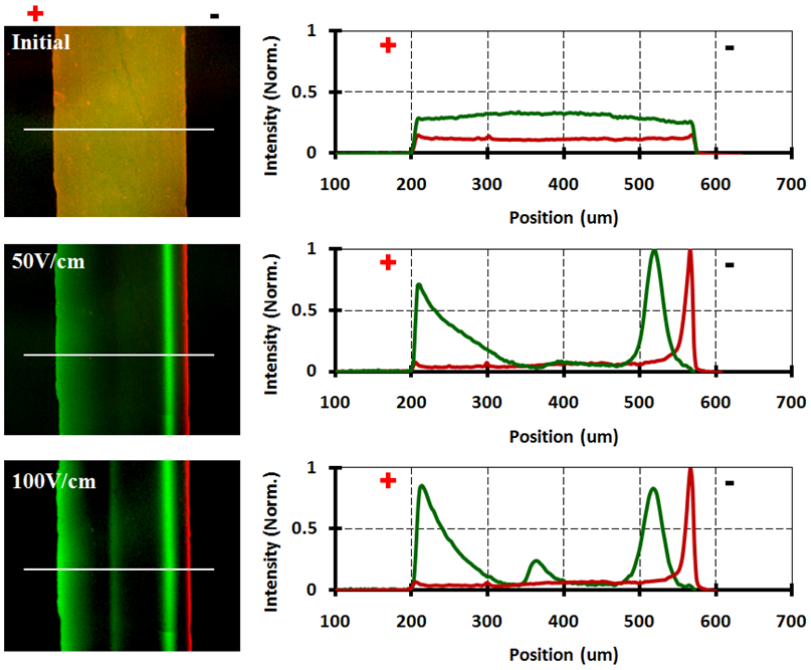

Figure S4. Separation of a protein and lipid mixture on a 20% POPG SLB. The separation conditions were: 10 mM Tris buffer at pH 7.3 with both 50 V/cm and 100 V/cm electric fields as noted in the upper right hand slide of the images. The fluorescent images are on the left and the corresponding line profiles are on the right. The bands on the bottom image from left to right are NBD-DPPE, StrA-4, StrA-1 and IgG. At 100 V/cm, the negative charge gradient was more compressed compared with 50 V/cm, so the streptavidin peaks moved to the left and the two components were very well separated.

REFERENCE:

(1) Schneider, S. W.; Lärmer, J.; Henderson, R. M.; Oberleithner, H. *Pflügers Archiv European Journal of Physiology* **1998**, *435*, 362.

(2) McLaughlin, S.; Poo, M. M. *Biophys. J.* **1981**, *34*, 85.