Supporting Information

Electrophoretic Measurements of Lipid Charges in Supported Bilayers

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Fluorescence Recovery After Photobleaching (FRAP) Analysis

Supported bilayers were bleached with a mixed gas Kr+/Ar+ laser at an intensity of 25-50 mW. The laser spot had a radius of approximately 18 µm. Images were taken immediately after photobleaching using an epifluorescence microscope under 10X magnification as a function of time in order to image the fluorescence recovery process (Fig. S1). Over time, the fluorescence in the area of the bleached spot area (indicated by red arrows) recovered due to the diffusion of unbleached fluorophore-labeled lipids into this area.

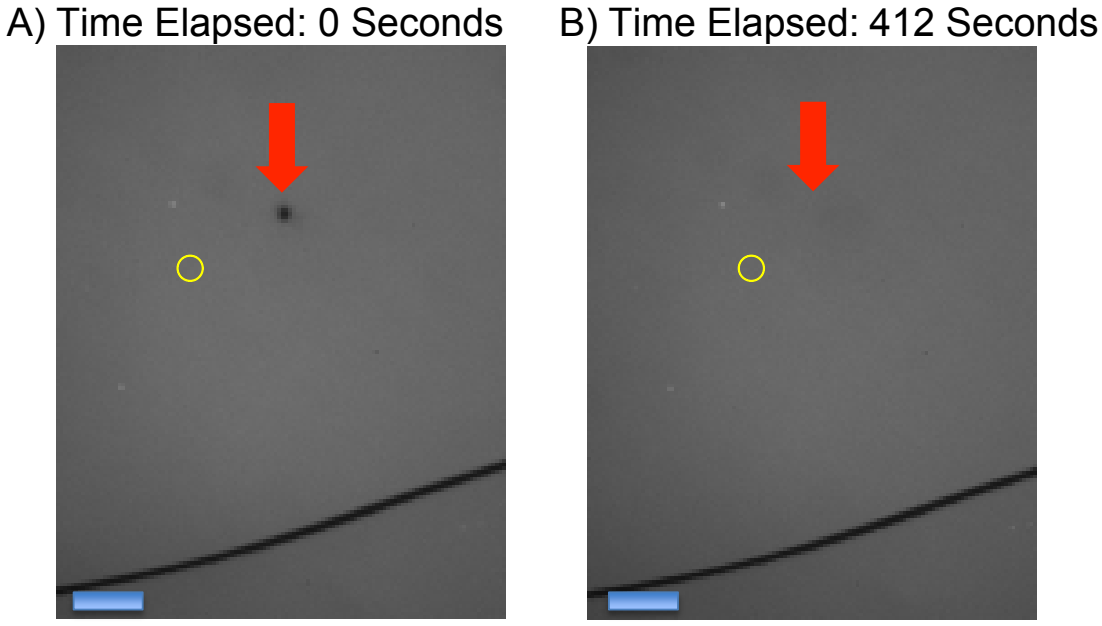

Figure S1. Two images of a 1 mol % NBD-PS/ 99% POPC supported bilayer immediately after photobleaching (A) and 412 seconds after photobleaching (B). The bleached spot is visible as a dark circle in the center of (A) as indicated by the red arrow and has faded away in B. The yellow circles encompass the control spot that was used for normalization and to correct for any photobleaching, which was negligible. The blue scale bar represents a distance of 190 µm.

The fluorescence recovery was analyzed using the method developed by Axelrod and Soumpasis.[1,2] To do this, the averaged intensity over the area of the bleached spot, B_t, was normalized at each point in time. This was done by divided B_t over the area of an unbleached spot, U_t, of the same size at each time point as well. This ratio provided the corrected fluorescence, F_t, (Eqn. S1). The control and unbleached spot had the same size area. It should be noted that photobleaching was negligible over the course of these measurements, although random fluctuations in integrated intensity were found to be on the order of ± 1%.

$$F_t = \frac{B_t}{U_t} \qquad \text{Eq. S1}$$

The normalized fluorescence intensity was converted to a FRAP ratio ($F_{FRAP(t)}$) using Eq. S2. Note, F_t is the normalized fluorescence of the spot as a function of time, t, and F_0 is the normalized fluorescence intensity of the first image taken after photobleaching.

$$F_{FRAP(t)} = \frac{F_t - F_0}{1 - F_0} \qquad \text{Eq. S2}$$

The FRAP ratio was plotted in Figure S2 as a function of time and fit to a single exponential function (Eq. S3):

$$F_{FRAP(t)} = a(1 - e^{-bt}) \qquad \text{Eq. S3}$$

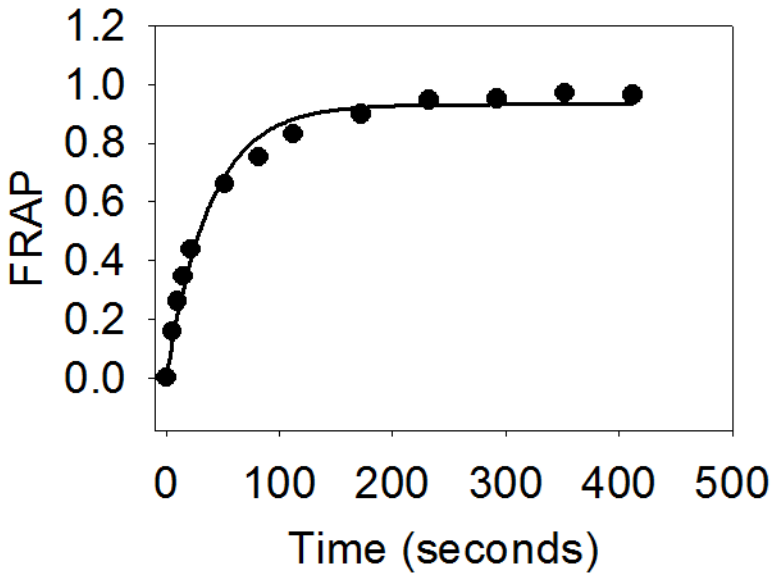

Figure S2. The FRAP ratio as a function of time for the bilayer shown in Fig. S1. The data was fit to a single exponential curve (black curved line) using Eq. S3.

It should be noted that the variable, *a*, in Eq. S3 is the percent recovery and is used to define the fraction of fluorophores that are mobile in the supported bilayer. The constant, *b*, in the exponent is employed to determine the $t_{1/2}$ value from the FRAP curve (Eq. S4):

$$t_{1/2} = \frac{\ln(2)}{b} \qquad \text{Eq. S4}$$

The $t_{1/2}$ value for the FRAP curve is then used in Eq. S5 to determine the self-diffusion coefficient *D*. The constant, ω, in Eq. 5 represents the radius of the laser spot size used to bleach the bilayer, which was 18 μm. A correction factor γ was used to correct for deviations from a Gaussian intensity profile across the laser spot. The value of γ used was 1.2.

$$D = \frac{\omega^2}{4t_{1/2}}\gamma \qquad \text{Eq. S5}$$

Measured Zeta Potentials

Table I

Debye Length	Lipid	Measured Zeta Potential (mV)
3.4 nM	NBD-PS	36
	ortho Texas Red-DHPE	17
	para Texas Red-DHPE	15
10 nM	NBD-PS	11
	ortho Texas Red-DHPE	9
	para Texas Red-DHPE	11

This table shows the zeta potentials calculated from the drift velocity measurements for each lipid. The calculation was performed from the charge on the lipids using Eq. 12 and plugging that charge value into Eq. 7. All zeta potentials are low enough so that polarization of the double layer around the lipid is small. As such, the apparent reduction in electrophoretic mobility caused by relaxation effects should be minimal.

References

(1) Axelrod, D.; Koppel, D. E.; Schlessinger, J.; Elson, E.; Webb, W. W. *Biophys J* **1976**, *16*, 1055.

(2) Soumpasis, D. M. *Biophys J* **1983**, *41*, 95.