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Supplementary Information

1. FRAP data on lipid bilayer and lipid monolayer regions on photo patterned TMS SAM surface.

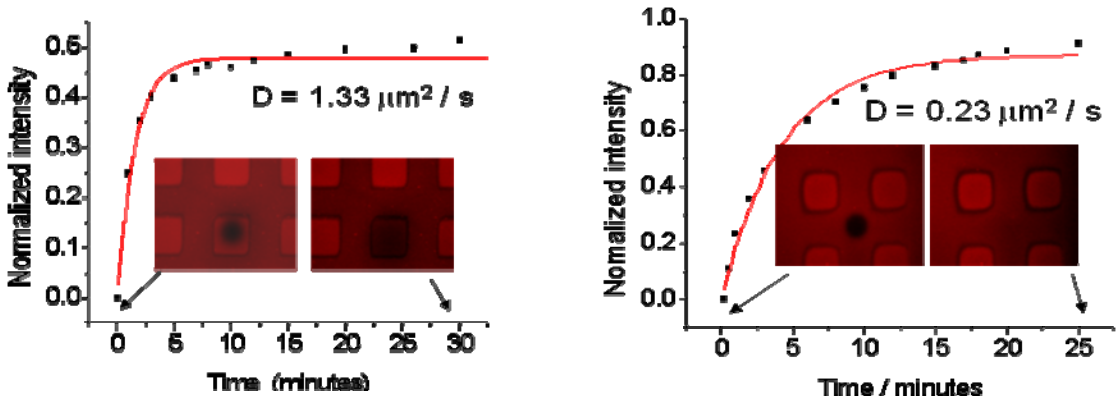

Fig. S1 FRAP data of lipid bilayer (a) and lipid monolayer (b) on photo patterned TMS SAM surface.

2. Charged lipid separation in an electric field.

Fig. S2 demonstrates the separation of oppositely charged lipids in the membrane. TR-DHPE (q = -1e) (red color) and D 291 (q = +1e) (green color) lipids were separated upon application of a 50 V/cm for 10 minutes. The TR-DHPE moved to the positive electrode side, while D 291 moved to the negative electrode side. The separation was clearly shown by the fluorescent intensity line profile of these two dye labeled lipids (the inset of Fig. S2). We also note that the lipids in the monolayer regions undergo separation in the electric field.

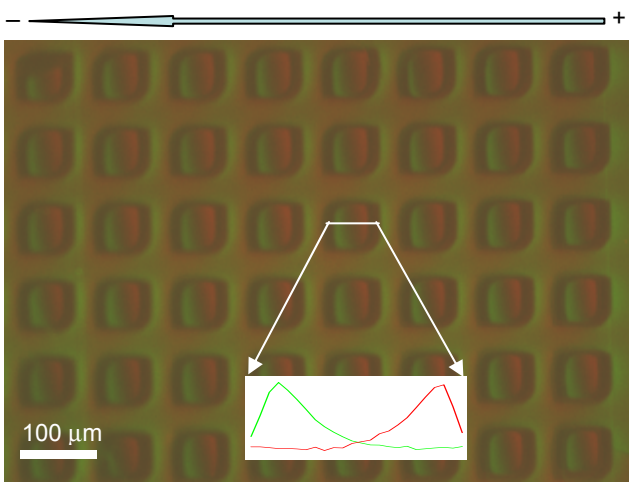

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Fig. S2 Separation of charged lipids of TR DHPE (-1) and D 291 (+1) under the application of a 50 V /cm field for 10 minutes.

Lipids with the same charge polarity can also be separated. Figure S3 shows the separation of TR-DHPE (-1) and NBD PS (-2) under an electric field. Initially both TR-DHPE and NBD PS were driven to the left side of the square by applying a 30 V/cm (left to right direction) for 90 minutes. After changing the field direction, the field strength was increased to 75 V/cm to rapidly separate the two lipids. From the line profiles, a gradual peak separation can be seen (Figure 5 S3b, c and d). The red and the green points correspond to TR DHPE and NBD PS respectively. This system was simulated (solid curves in Figure S3) using the finite element method (FEM) (see experimental section in the paper). The model was solved using the diffusion coefficients obtained from FRAP (1.33 ± 0.07 $\mu m^2/s$ for TR-DHPE and 1.50 ± 0.14 $\mu m^2/s$ for NBD PS), electric field strengths of 30 V/cm for the buildup and 75 V/cm for the separation, and initial concentrations of 1.91 x 10^{-8} mol/m^2 and 3.80 x 10^{-8} mol/m^2 for TR-DHPE and NBD PS, respectively. For the purpose of the simulation, it was assumed that the electric field strengths were as applied, and the mobilites of the charge carriers were reduced. The effective mobilites that gave the best fit to the experimental data were found to be 0.78 x 10^8 s/kg for TR-DHPE, and 1.20 x 10^8 s/kg for NBD PS. These values for the effective mobilities gave a good fit to the experimental data in terms of the peak position (Figure S4a), but the profiles did not appear to spread as fast as in the experiment. The full width half maximum (FWHM) of simulated peaks are consistently narrower than those of experimental peaks (Figure S4b), indicating that diffusion is not the sole cause for the spread in the profile and that other factors might need to be considered, eg. multicomponent charge species. From Figure 6a, the drift velocity of TR DHPE is 0.14 ± 0.01 μm/s, which is around half of 0.29 ± 0.02 μm/s for NBD PS. This is consistent with what we expect from the charge numbers of these two charged species, and also with the value obtained from the data shown in Figure 3d.

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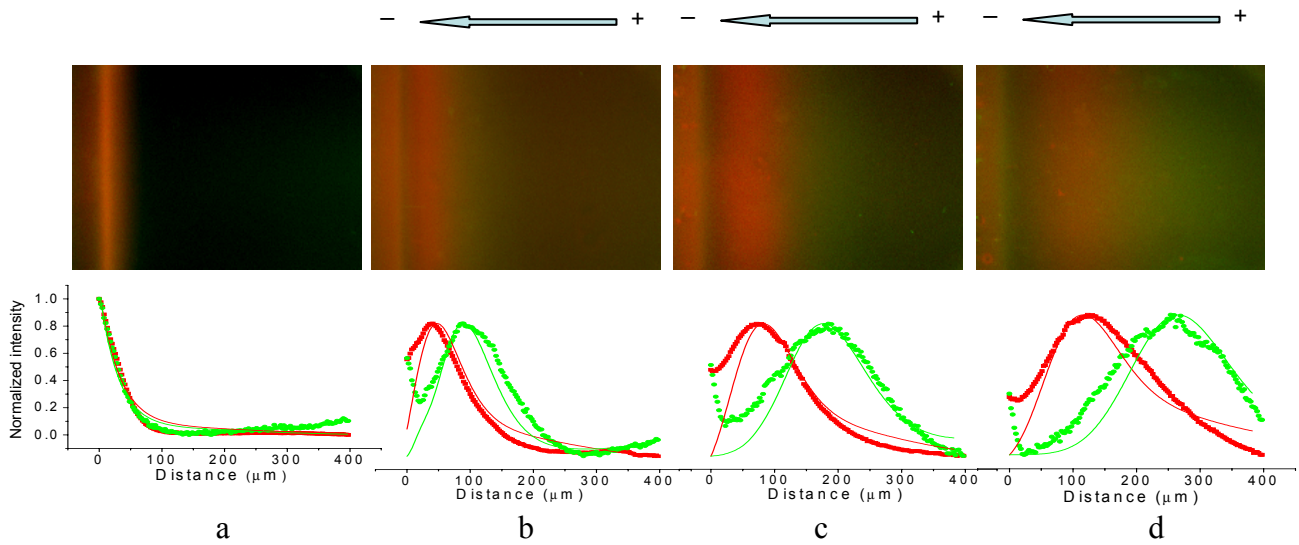

Fig. S3 Fluorescent images of lipid bilayer containing 0.67% TR-DHPE and 1.33 % NBD PS before (a) and after applying 75 V/cm for 5 (b), 10 (c), and 15 minutes (d). The red and green dotted curves under each image represent the average intensity profile of TR DHPE and NBD PS respectively, while the solid curves under each image represent the corresponding simulation results.

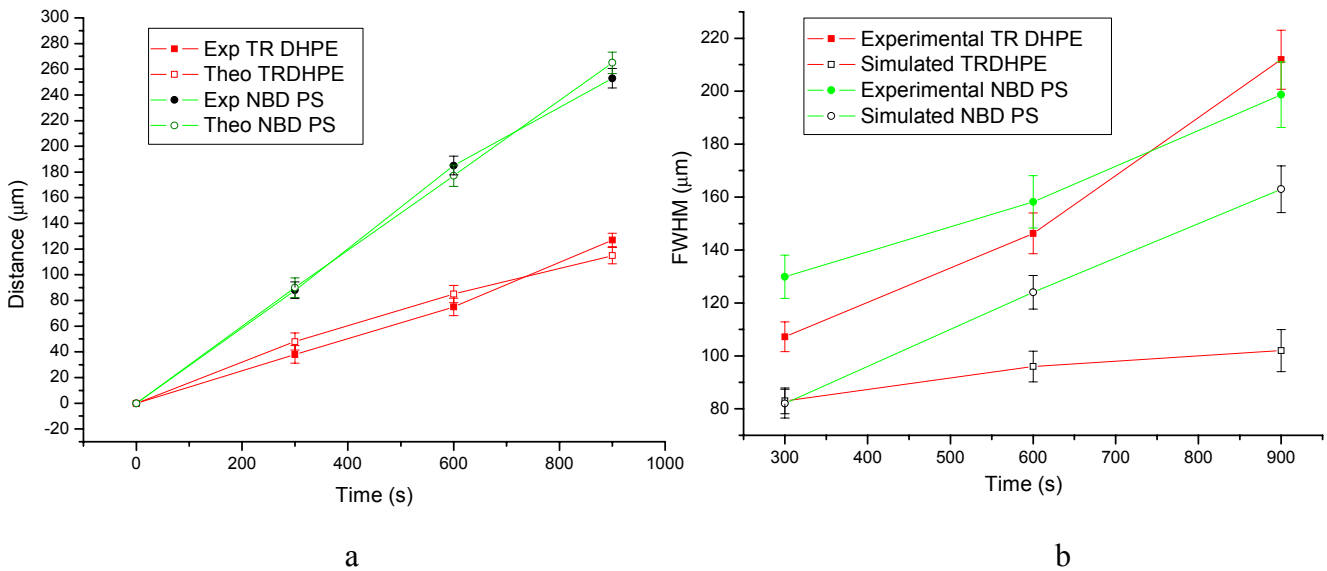

Fig. S4 Experimental and simulated peak positions a) and FWHM of experimental and simulated peaks b) were plotted as a function of time when applying 75 V/cm.

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3. The calculation for overall charge density, surface potential and zeta potential of membrane.

At the point of $\zeta_1 \approx \zeta_2$, the lipid membrane contains neutral lipids (egg PC), 0.5% negatively (-1) charged biotin lipid, and 0.25% positively charged (+1) DOTAP. The overall charge situation is -0.25%, which indicates -1e per 400 lipids. The overall charge density is given as -1e / (400 × 58.5 Å^2), ie, -6.84 × 10^{-4} Cm^{-2} (1C = 6.25×10^{18} e).

According to equation 7($\sigma_o = \varepsilon\kappa\psi_0$), $\psi_0 = \sigma_0/\varepsilon\kappa$ where ε is the permittivity of electrolyte medium [$80\varepsilon_0$ = 80 × (8.854×10^{-12}) Fm^{-1}]; κ is the reciprocal of the Debye length(0.1×10^{9}m^{-1}).

Thus

$$\psi_o = \frac{\sigma_0}{\varepsilon\kappa} = \frac{-6.84\times10^{-4}\,Cm^{-2}}{(80\times8.854\times10^{-12}\,Fm^{-1})(0.1\times10^{9}\,m^{-1})} \cong -10mV$$

According to equation 9:

$$\gamma = \frac{\exp[ze\psi_0 / 2kT]-1}{\exp[ze\psi_0 / 2kT]+1} \qquad (9)$$

γ was calculated to be -0.097.

The zeta potential of membrane, ζ_2, is equal to the potential, ψ, at the hydrodynamic plane of shear (x= 2Å from the surface) and is calculated to be -0.98 mV after bringing the γ value to equation 8 below.

$$\psi = \frac{2kT}{ze}\ln\left(\frac{1+\gamma\exp[-\kappa x]}{1-\gamma\exp[-\kappa x]}\right) \qquad (8)$$

4. The 'volume exclusion' effect of DOTAP in the lipid bilayer membrane in the electrical field.

Fig. S5 shows the 'volume exclusion' effect of positively charged DOTAP in the egg PC lipid bilayer containing 0.5% neutral fluorescenctly labelled lipid (D3793). When the positively charged DOTAP moves towards the negative electrode in the electric field, it can exclude other lipids from the negative electrode region and effectively displace them towards the positive electrode. From the curve of Fig. S5a we do not see any 'volume exclusion' effect at low concentration of DOTAP (in this case 0.5% DOTAP). However, the 'volume exclusion' effect becomes significant at higher concentration, eg. 20% DOTAP. In this case the fluorescently labelled region is observed to be excluded during charged lipid migration, see Fig. S5b.

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Therefore, the contribution of 0.5% DOTAP to the gradient in the case of our streptavidin studies can be considered negligible.

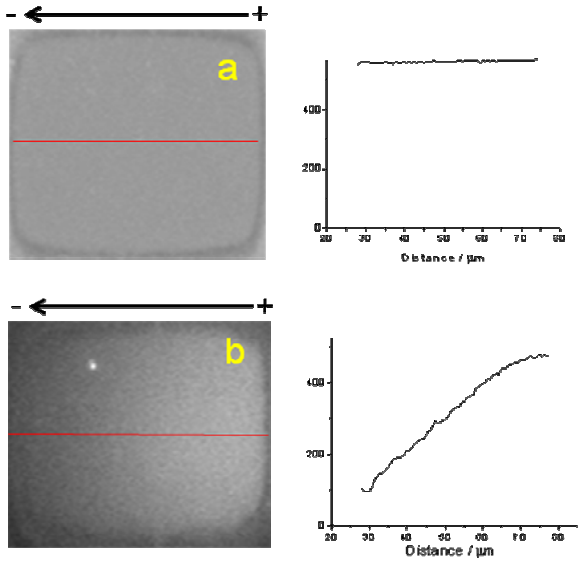

Fig. S5 the 'volume exclusion' effect of a) 0.5% DOTAP, and b) 20% DOTAP in the egg PC lipid bilayer membrane for 5 minutes after applying 5 V/cm field in 1 mM PBS buffer. The lipid bilayers contain 0.5% D 3793. The curves are the line profiles along the red line in the images.