On-Chip Electrophoresis in Supported Lipid Bilayer Membranes Achieved Using Low Potentials

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Experimental section

Materials. All starting materials and chemicals were purchased from Sigma Aldrich, Fluka, Serva, Becton Dickinson, Avanti Polar Lipids, Microchem, Invitrogen and Fujifilm, and they were used as received, unless otherwise stated. MilliQ water with a resistivity higher than 18 $M\Omega cm^{-1}$ was used in all experiments.

Chip fabrication. A bilayer lift-off recipe was used for fabricating Au electrodes on borofloat glass wafers. First, LOR 5A (MicroChem) was spin-coated, after which normal lithography was performed on top with Olin OiR 907-17 photoresist (FujiFilm) to create a bilayer resist stack. Electrode patterns were made by exposing the photoresist through a patterned photomask and developing in Olin OPD 4262 (FujiFilm). The develop step washed away the exposed photoresist, and etching through the LOR 5A layer created an undercut. Then, 5 nm Ti and 95 nm Au were deposited via e-beam evaporation (BAK 600, Balzers) The bilayer resist was then removed by sonication in acetone (20 min) and isopropanol (10 min) followed by 5 min immersion in OPD 4262, serving as a sacrificial layer to leave patterned Au electrodes on borofloat glass. To fabricate the Cr corrals (10 nm thick) in between the Au electrodes, the same procedure was performed a second time, but in this case following alignment with respect to the Au electrodes.

PDMS flow channel. A silicon flow channel master was produced by standard photolithography steps and deep reactive ion etching. The polydimethylsiloxane (PDMS) flow channels were prepared from a degassed mixture of 10:1 Sylgard 184 elastomer and curing agent (Dow Corning Corp., Midland, MI), which was casted onto the silicon master and cured at 80°C for 1 h. The flow channels were cut to size and inlets and outlets were punched using a 1 mm Ø punch (Harris Uni-core, Sigma-Aldrich).

PDMS bonding. Chips were rinsed and sonicated extensively with acetone, ethanol and MilliQ water, and dried prior to UV-ozone exposure (UV/Ozone Procleaner plus, Bioforce Nanosciences) for at least 20 min. After UV exposure, the chips were rinsed with ethanol, water and dried under a stream of nitrogen. Both cut-out PDMS flow channels and cleaned chips were treated with oxygen plasma for 30 s at 40 W (Plasma prep II, SPI supplies) after which they were bonded immediately. The chips were placed on a hot plate for 10 min at 70^{0}C to increase the binding strength. Tygon tubing (VWR, 0.25 mm inner Ø and 0.76 mm outer Ø) was inserted into the PDMS. The assembled μSLB electrophoresis chip was placed in an oven at 60^{0}C for 1 h. Leakage-free operation was shown for flow rates up to 2 mL/min.

Vesicle preparation. 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) (Avanti Polar Lipids Inc., Alabaster, AL) was stored as a 25 mg/mL stock solution in chloroform at -20^{0}C. 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine-N-biotinyl (Biotinyl-PE) (Avanti Polar Lipids Inc., Alabaster, AL) was stored as a 10 mg/mL stock in chloroform at -20^{0}C. The charged lipid-dye conjugate, Texas Red-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (TR-DHPE) (Invitrogen Ltd.,Paisley, UK) was stored as a 1 mg/mL stock solution in methanol at -20^{0}C. Aliquots of the DOPC (99.8 mol%) and TR-DHPE (0.2 mol%) or DOPC (98.8 mol%), TR-DHPE (0.2 mol%) and Biotinyl-PE (1 mol) stock solutions were mixed and dried under a flow of nitrogen in a glass vial, and subsequently placed under vacuum for at least 1 h. The resulting lipid film was re-suspended by vortexing in MilliQ water to form multilamellar vesicles (MLVs) at 1 mg/mL. The MLV solution was extruded 11 times through a 100 nm polycarbonate membrane (Avanti Polar Lipids Inc., Alabaster, AL) at room temperature. The resulting large unilaminar vesicles (LUVs) were characterized by dynamic light scattering (DLS, Nanotrac, Microtrac) (Figure S3) and stored until use at 4°C for a maximum of two weeks.

SLB formation. Supported lipid bilayer (SLB) formation was achieved by dilution of the LUV solution to 0.5 mg/mL in 1x phosphate-buffered saline (0.01 M PBS, Gibco, lacking $MgCl_2$ and $CaCl_2$). Prior to LUV incubation, the chips were flushed briefly with 1x PBS. Afterwards the chips were incubated with the vesicle suspension for a least 30 min to allow for vesicle adsorption and rupture to occur. Subsequently, the chips were washed with MilliQ water or aqueous 0.5 mM hydroxymethylferrocene ($FcCH_2OH$).

Streptavidin. Alexa fluor 488 labeled streptavidin (Invitrogen Ltd.,Paisley, UK) was dissolved in PBS (0.01 M PBS, Gibco, lacking $MgCl_2$ and $CaCl_2$), and stored at -20°C in aliquots until further use. Based on A280 and A494 absorption measurements (NanoDrop 1000, Thermo Scientific) 1.6 dyes/protein was deduced. Biotinylated SLBs were incubated with 100 μg/mL for 15 minutes after that unbound streptavidin was washed away with MilliQ.

Fluorescence Recovery After Photobleaching (FRAP). FRAP measurements were conducted using a Nikon A1 CSLM with a 20x objective. To derive the diffusion coefficient, modified Bessel functions as described by Soumpasis et al. 1983, were used. Data was corrected for acquisition bleaching and normalized.

μSLB electrophoresis. Prior to SLB formation, wires were soldered onto the gold contact pads. Electric fields were generated and currents were measured with a CH Instruments 760D potentiostat with the counter and reference connections shorted. During μSLB electrophoresis, a freshly prepared solution of 0.5 mM of $FcCH_2OH$ in MilliQ water was flushed through the chip at 100 μL/min unless otherwise stated.

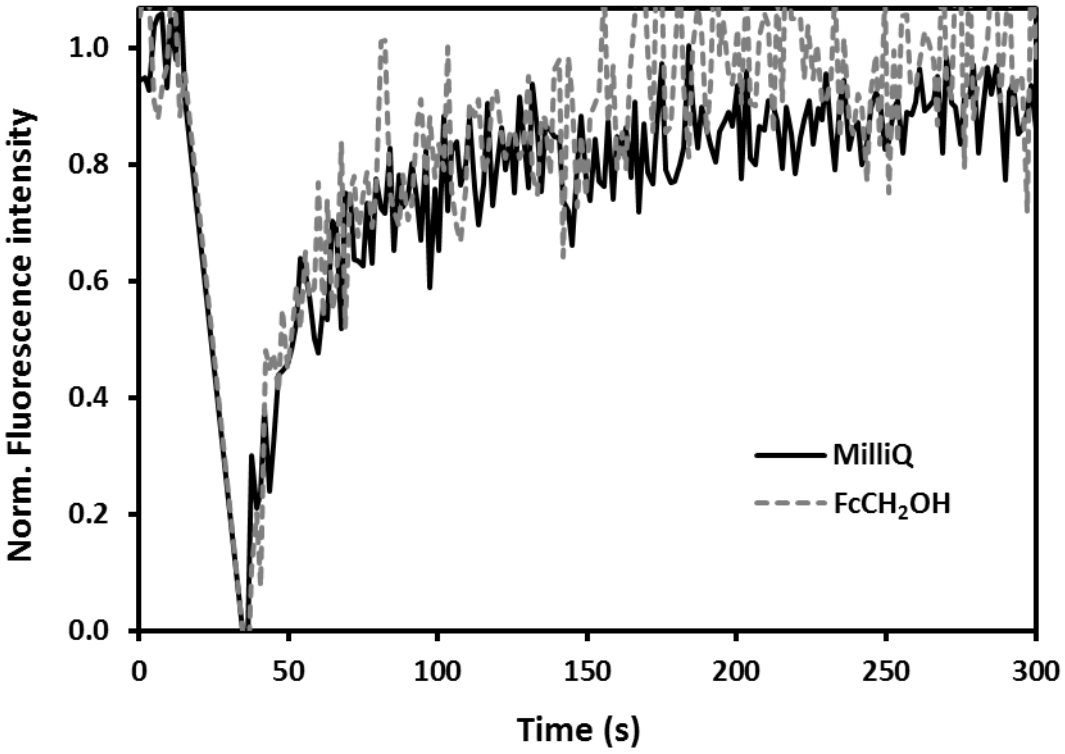
Fluorescence microscopy. An Olympus inverted IX71 epi-fluorescence research microscope with a Xenon X-cite 120PC as light source and a digital Olympus DR70 camera for image acquisition was used to acquire fluorescence micrographs during μSLB electrophoresis at 10x magnification. Green excitation ($510 \leq \lambda_{ex} \leq 550$ nm) and red emission (λ_{em}>590 nm) was filtered using the U-MWG2 Olympus filter

cube. ISO200 camera setting was used to record high quality, low noise images and care was taken to ensure image acquisition was performed in the linear response regime. Per corral the mean pixel intensity was used to correct the profile plot after which a background correction was performed. To retrieve the relative background, the steady-state fluorescence profiles at 1.2, 1.0 and 0.75 V were fitted to an exponential growth function, via the equation

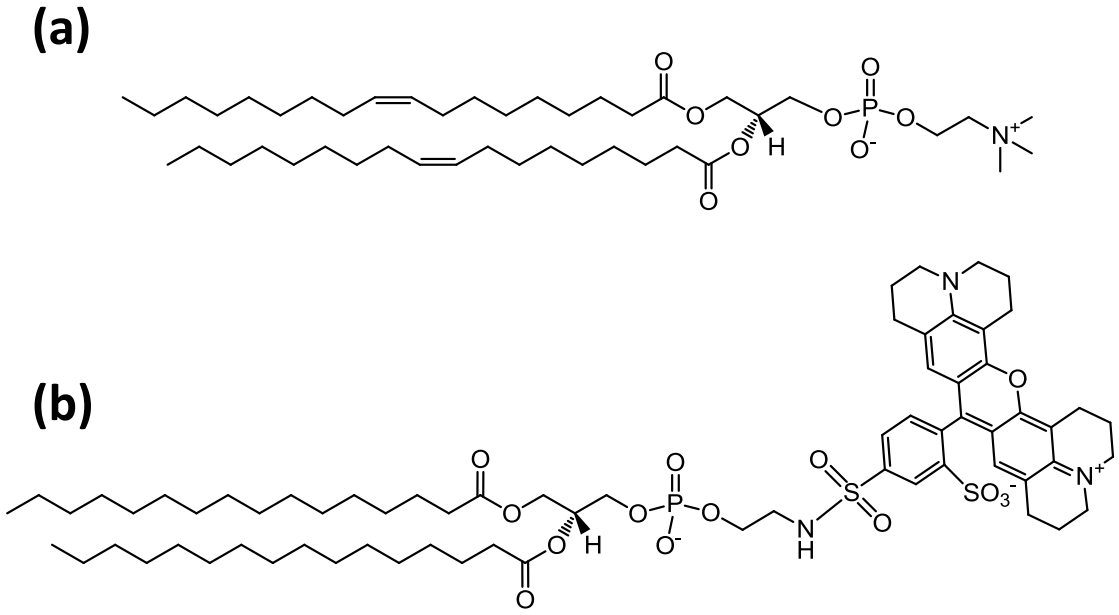
$$I(x) = I(0)(1+r)^{x} \qquad (5)$$

where I(x) is the normalized fluorescence intensity at position x, I(0) the background fluorescence intensity, r the growth rate and x the distance (μm). Data fitting was conducted on the average profile plots deduced per column of corrals perpendicular to the electrodes. Subsequently, the background fraction (BF) of the mean pixel intensity was calculated. The background fractions of aforementioned conditions were used to calculate an average BF of 62 ± 4%, that was used to calculate and correct the background in all of the obtained profile plots, per corral. To obtain the final, averaged, steady-state fluorescence profiles at one potential, at least 14 normalized and background corrected corrals were averaged.

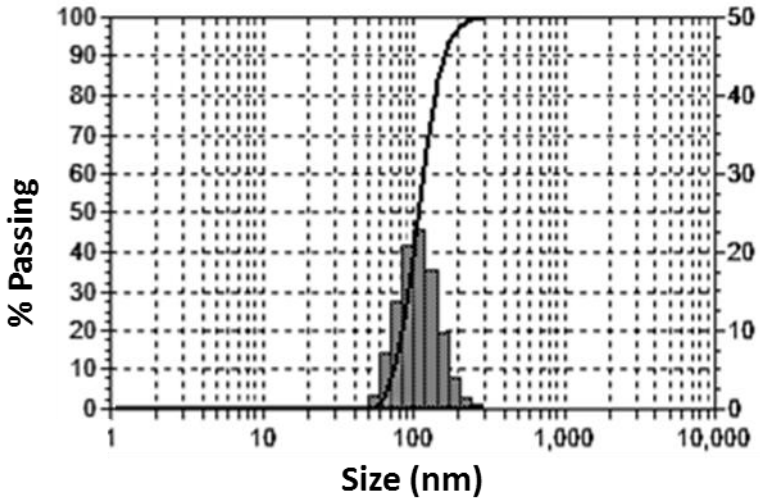
Data analysis. Image analysis was performed using ImageJ (NIH), Origin (OriginLab) and Excel (Microsoft). FRAP data was analyzed with FRAPAnalyser (University of Luxembourg).



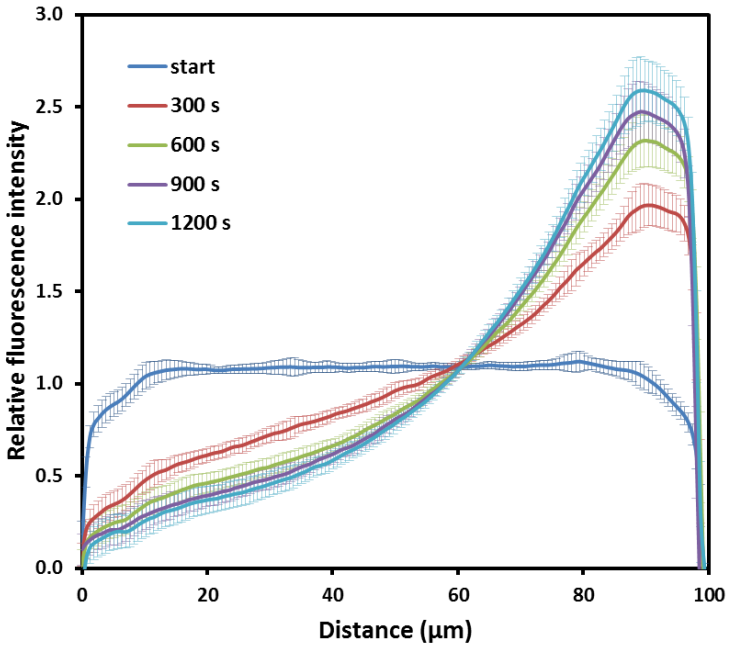
Supplementary Figure S1. FRAP recovery curves. Comparison between MilliQ water and aqueous 0.5 mM $FcCH_2OH$.



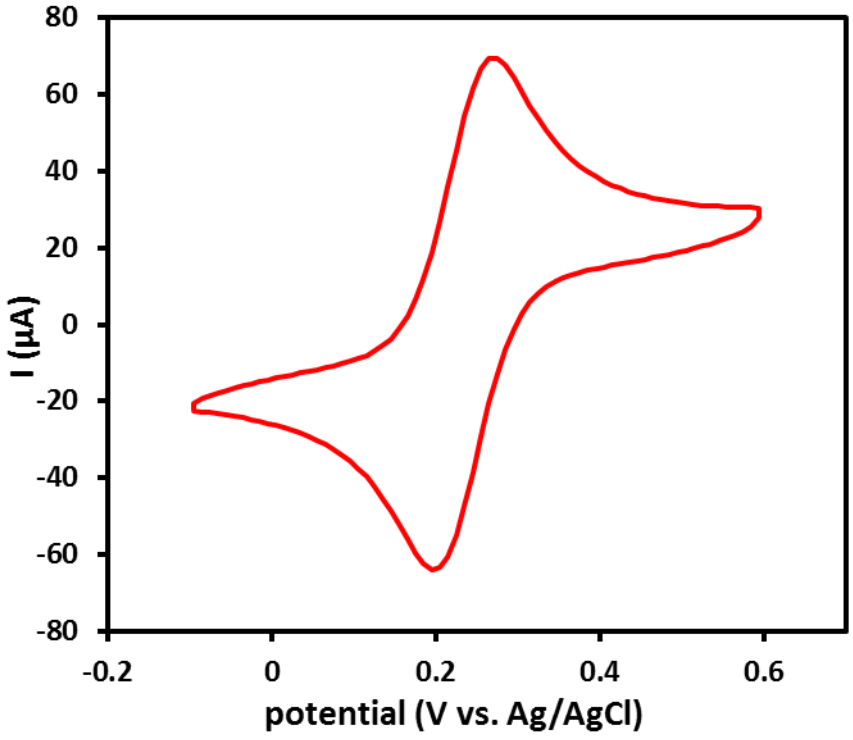
Supplementary Figure S2. Chemical structure of a) DOPC lipid, Tm -20^{0}C and b) TR-DHPE.



Supplementary Figure S3. DLS of DOPC LUVs doped with 0.2 mol% TR-DHPE at 1.0 mg/mL after 11 times of extrusion.



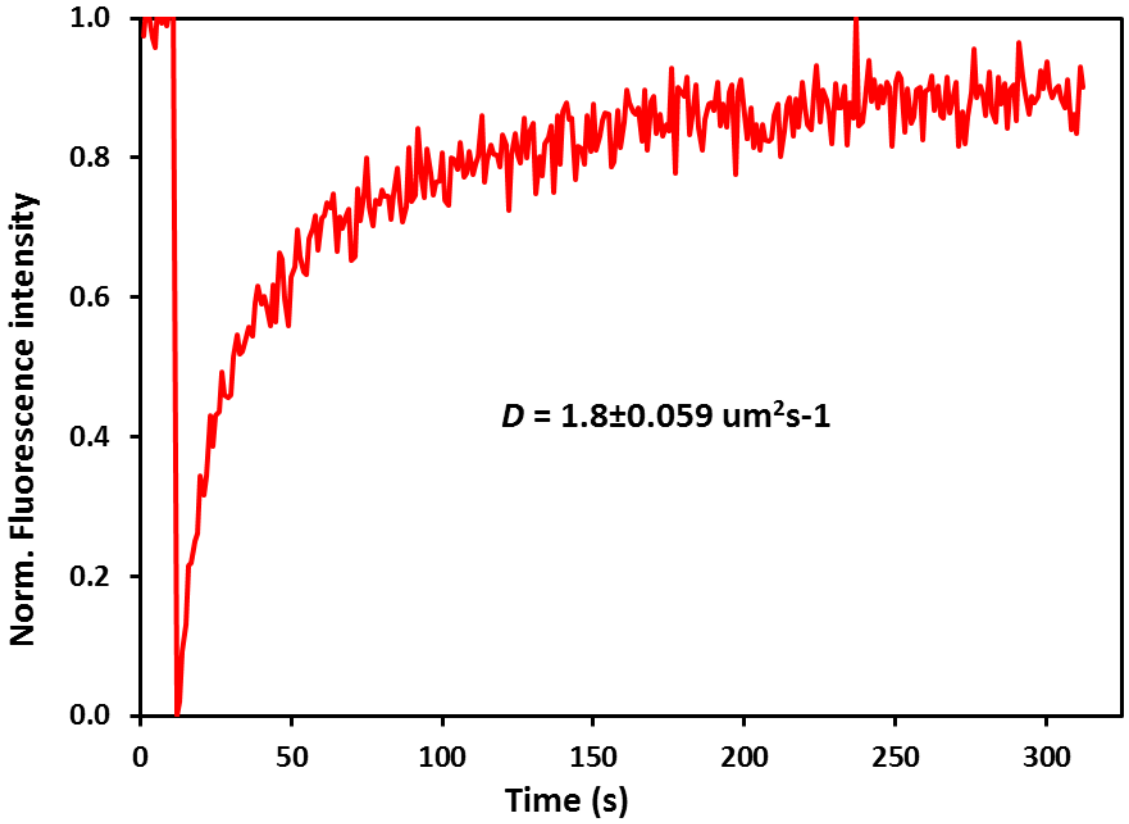
Supplementary Figure S4. Electrophoretic build-up of TR-DHPE over time using a 1 V potential difference. Data presented a mean ± SD.



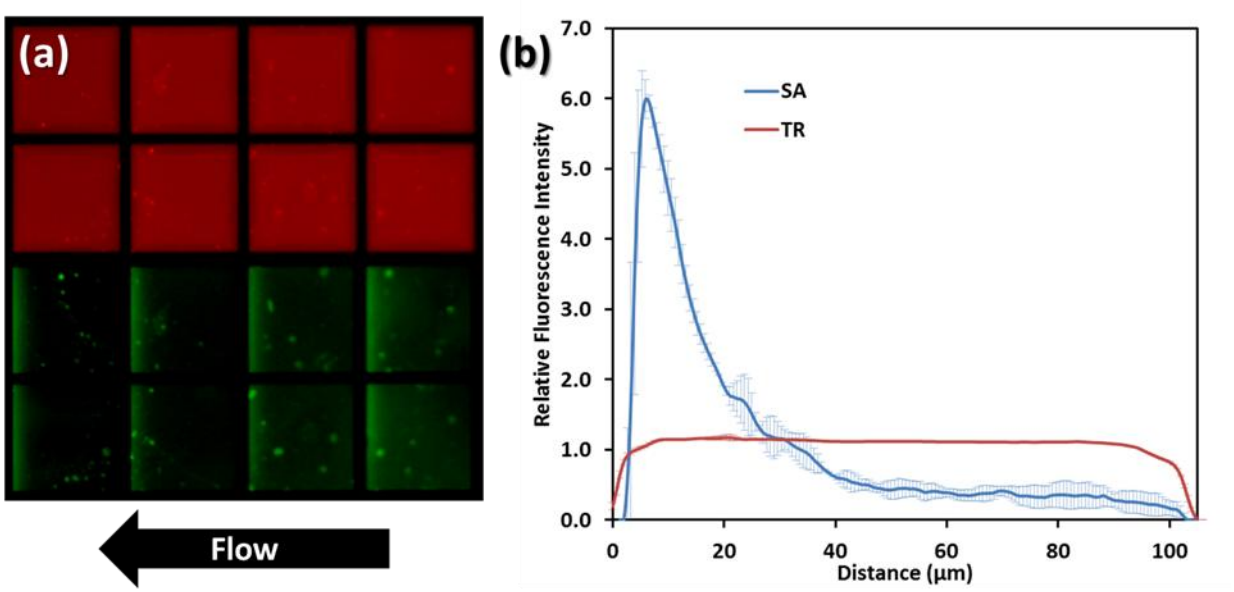
Supplementary Figure S5. Cyclic voltammogram of 1.0 mM $FcCH_2OH$ in 0.1 M K_2SO_4 vs. Ag/AgCl.

Applied potential	Calculated E-fields	Fitted E-fields
1.20	15.73	16.27
1.00	13.10	13.04
0.75	9.83	8.65
0.50	6.53	6.97
0.25	3.27	4.17

Supplementary Table S1. Comparison between calculated and fitted E-fields, r^2 = 0.97.



Supplementary Figure S6. FRAP recovery curve of a biotinylated SLB.



Supplementary Figure S7. Effect of $FcCH_2OH$ flow. Streptavidin migration due to sheer stress from bulk flow at a flow rate of 100 µL/min. (a) Fluorescent micrographs of a SLB doped with 0.2 mol% TR-DHPE (red) and 1.0 mol% Biotinyl-PE after incubation with Alexa fluor 488 labeled streptavidin (green) under flow. (b) Corresponding profile plots, data presented as mean ± SD of four center corrals.