Measuring the Potential Energy Barrier to Lipid Bilayer Electroporation

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Supplementary Material

Experimental Methods

Droplet interface bilayer setup

Glass coverslips (24 $\times$ 40 mm, thickness No.1; Menzel-Gläzer, Thermo Scientific) were subjected to an oxygen plasma for several minutes. 140 μL of 0.75% (wt/vol) ultra-low gelling agarose, homogenised at 90 °C, was spun onto the coverslip at 4000 r.p.m. for 30 s. The coverslip was then affixed to a poly(methyl methacrylate) device (Figure S1) using sticky tape. This aids in preventing the drying out of the agarose. Via the filling holes (Figure S1A), 170 μL of 2% (wt/vol) agarose containing 750 mM $CaCl_2$, 10 mM HEPES was pipetted into the device. This is the hydrating agarose. A solution of DPhPC (diphytanoyl phosphatidylcholine; Avanti Polar Lipids, Alabama, USA, and Lipoid, Ludwigshafen, Germany) in hexadecane at 8.7 mg·mL^{-1} was added to the wells, and the device allowed to incubate for 15 minutes, to allow lipid monolayer formation at the oil-water interface above the agarose. Droplets of $\sim$50 nL in volume containing 1.5 M KCl, 10 mM HEPES, 370 μM EDTA and 50 μM of the Ca^{2+}-sensitive dye Fluo-8 (AAT Bioquest, California, USA) were incubated in the same lipid-in-oil solution for 30 minutes. Bilayers formed within minutes when the droplet was allowed to sink to into the well and make contact with the monolayer on the substrate.

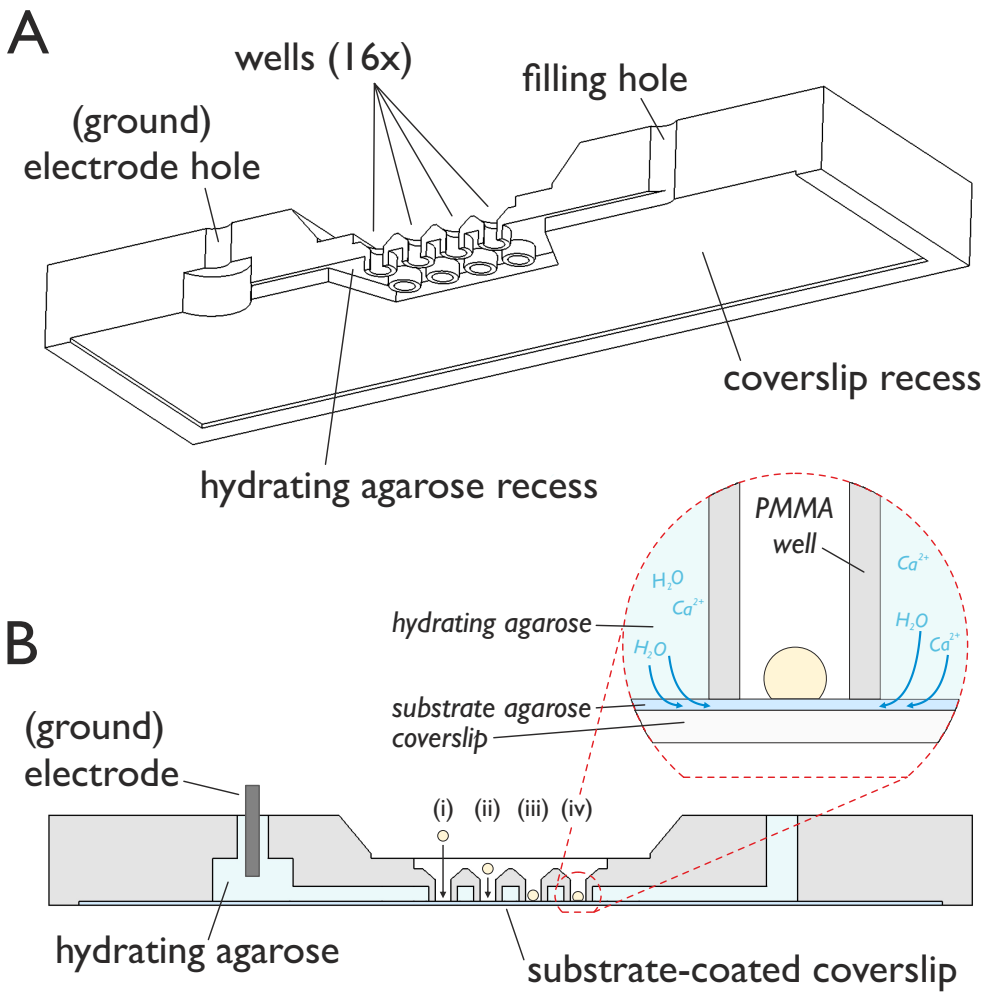

Figure S1: (A) Cut-through view of the device, angled towards the underside. (B) Cut-through side elevation, showing the device when filled; the hydrating agarose surrounds the wells but does not cover the substrate. *i*—*iv* represent the stages of bilayer formation: an aqueous droplet that has been incubated in lipid-in-oil (for monolayer formation) is placed in a well that has been incubated in the same solution (*i*) where it sinks due to gravity (*ii, iii*), forming a bilayer with the monolayer assembled on the substrate agarose (*iv*).

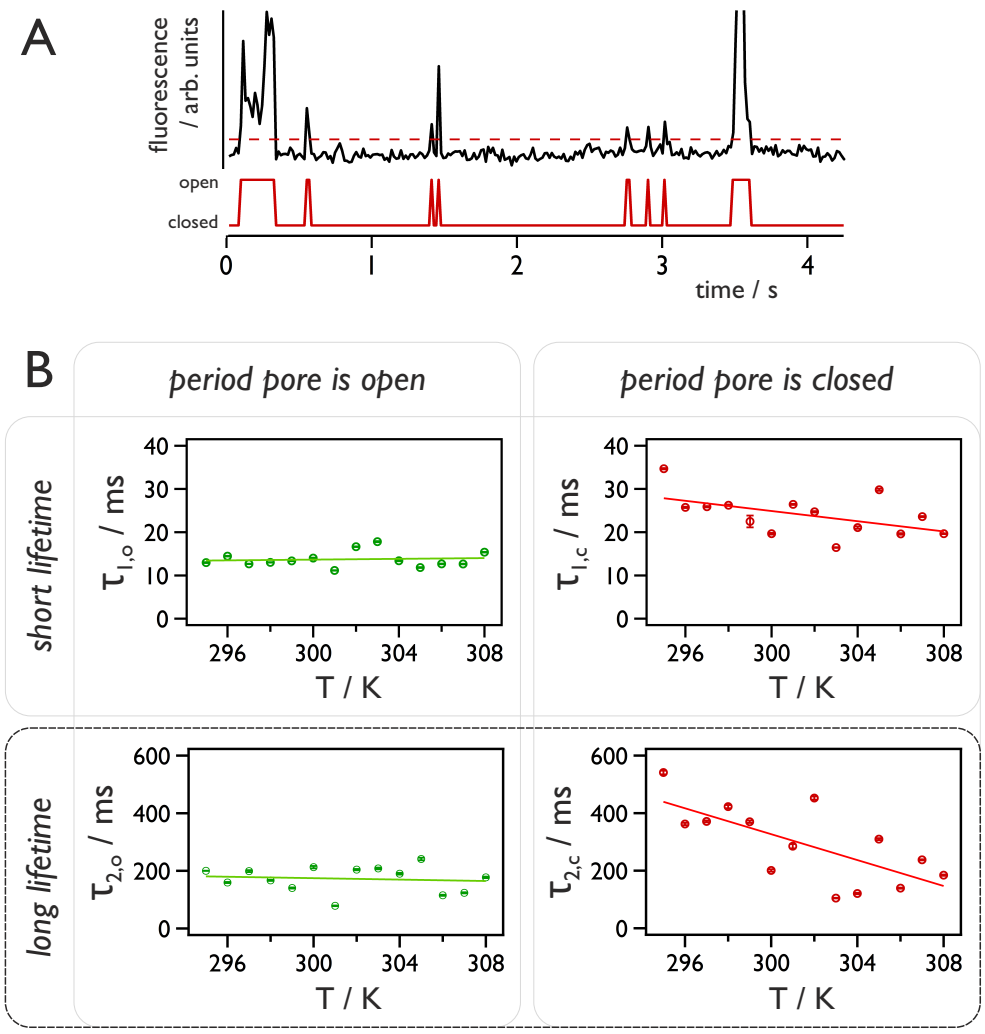

Figure S2: (A) Pore open and closed lifetimes were defined as the period above (open) or below (closed) a threshold oSCR intensity value, set at 2.5 times the standard deviation of the background. The *black* trace is that from a single pore; *red dashed* line indicates the location of the threshold; *solid red* line beneath shows the detected open and closed pore states. (B) Temperature dependence of the open and closed pore times. Double exponentials were fitted to histograms of these periods, yielding a pair of tau values characteristic of those functions, for both the open ($\tau_{1/2,\mathrm{o}}$) and closed periods ($\tau_{1/2,\mathrm{c}}$) at each temperature (see also main text). These are plotted here against the temperature at which the oSCR recording was made. The open lifetimes appear to vary only slightly with increasing temperature, whereas there is a clear temperature dependence of the closed lifetimes. Error bars are the standard deviation of the tau values derived from the histogram fits; straight lines are linear regression fits to the data points. The data presented within the black dotted area is the same as that in Figure 3 of the main text.