Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

A New Approach for the Fabrication of Microscale Lipid Bilayers at Glass Pipets: Application to Passive Permeation Visualization

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S1. Incorporation of Gramicidin into DPPC bilayer membrane

To form gramicidin incorporated bilayer membranes, a 1mg/ml solution of DPPC containing 0.5 % (w/w) gramicidin in chloroform was used. The pipet filled with 0.1M KCl solution was held in the lipid solution for approximately one minute to allow the monolayer to assemble, after which the pipet was removed, leaving the monolayer intact on the meniscus of the pipet and allowing any residual volatile solvent molecules to easily evaporate. The pipet was then positioned above an electrolyte solution, onto the surface of which a small amount of lipid solution (10 µl) was dropped, forming a monolayer at the air/water interface. The pipet was slowly lowered until the two monolayers made contact and a bilayer was formed. After bilayer formation, the current between the QRCEs in the pipet and a third QRCE in the bulk solution (i_{bulk}) was monitored as the potential is scanned linearly to determine the resistance of the bilayer from the slope of the current-voltage curves produced. Figure S1 shows the current-voltage curve recorded for a normal bilayer and that of a gramicidin incorporated bilayer membrane. The increase in ion current for gramicidin incorporated membrane is a

direct consequence of the ion flow across the gramicidin channels which is diagnostic of phospholipid assembly at the pipet tip being in a bilayer configuration.[S1, S2]

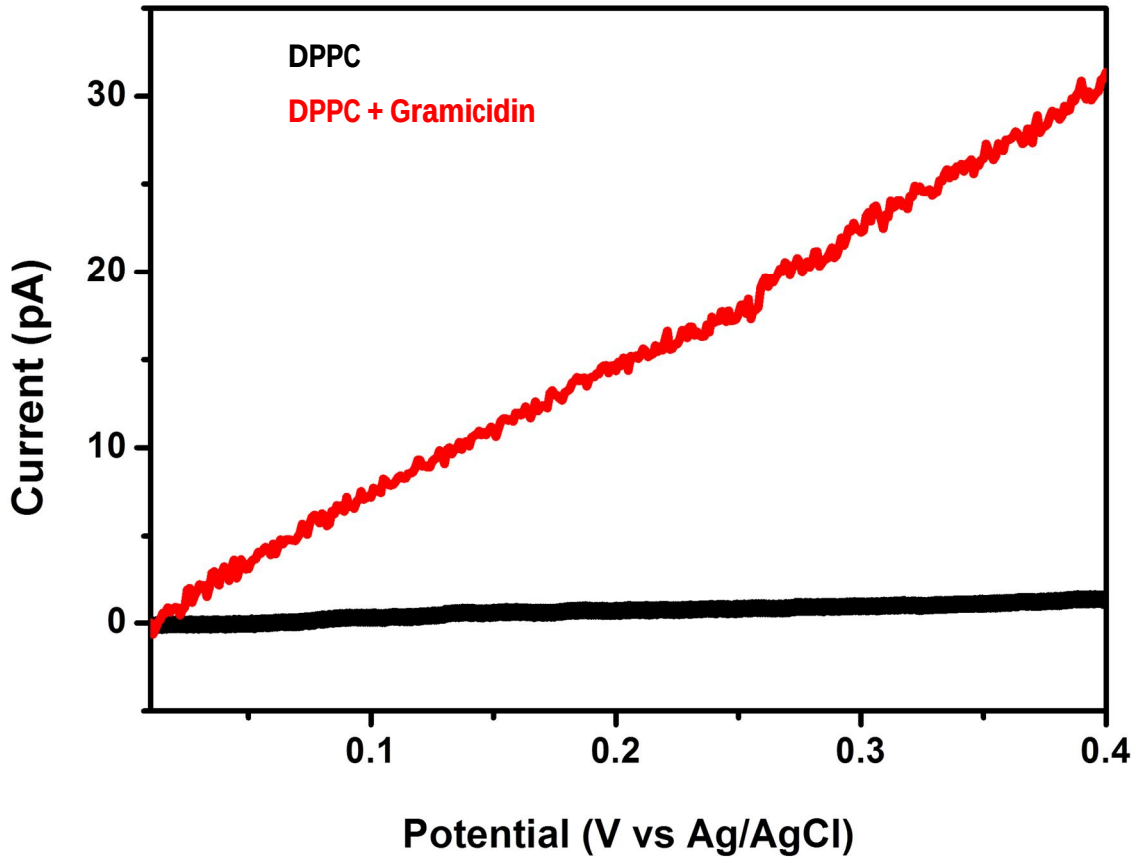

Figure S1. Current-voltage (I-V) curves for DPPC and gramicidin incorporated DPPC bilayer membranes.

S2. Pressure/Area Isotherms for DPPC

Pressure/area isotherms for DPPC monolayers on aqueous electrolyte solution were recorded using a Langmuir trough (Nima Technology, Model 611D), with surface pressures measured using a Wilhemy balance. The Langmuir trough was first thoroughly cleaned with chloroform, and a pressure/area isotherm was run to check that there was no surface contamination. After cleaning, 50 µl of 0.5 mg/ml DPPC in chloroform was deposited onto the 0.1 M KCl subphase, and the solvent allowed to evaporate before compression was

initiated. These isotherms indicated that the area per molecule in a fully assembled monolayer is ~35 $Å^2$ with a surface pressure of ~50 mN m^{-1} (Figure S2). To ensure full monolayer coverage on the surface of the bulk electrolyte solution in the CLSM cell, 10 µl of 1 mg/ml lipid solution was added, yielding an area per lipid of 34 $Å^2$.

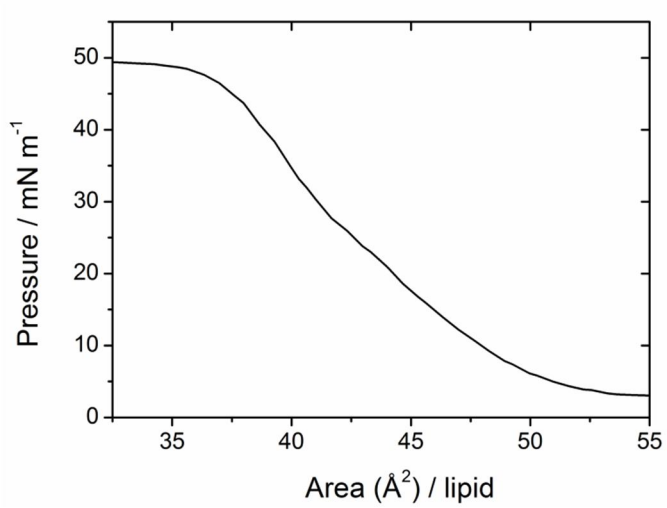

Figure S2. Pressure/area isotherm for the compression of a DPPC monolayer.

S3. Simulation Details

The diffusion coefficients and pK_a values for each of the weak acids are listed in Table S1.[S3]

Table S1: Diffusion coefficient, D_{HX}, and pK_a values for each weak acid studied

Carboxylic Acid	pK_a	D_{HX} (10^{-6} cm^2 s^{-1})
Acetic	4.76	12.71
Propanoic	4.83	9.18
Butanoic	4.83	8.17
Hexanoic	4.85	7.84

To correlate experimental data with the simulations, CLSM images were analyzed to produce fluorescence profiles normal to the end of the pipet. To calculate the average fluorescence, a cone of pixels was selected normal to the end of the pipet and the fluorescence intensity was plotted against the absolute distance from the end of the pipet. A polynomial fit was then applied to reduce the experimental noise and this fit was matched to simulated profiles to extract a permeation coefficient. A typical raw fluorescence profile for the permeation of 100 mM propanoic acid is shown in Figure S3 along with the polynomial fit which allows for easier comparison with the simulated data, while retaining the main features of the profile.

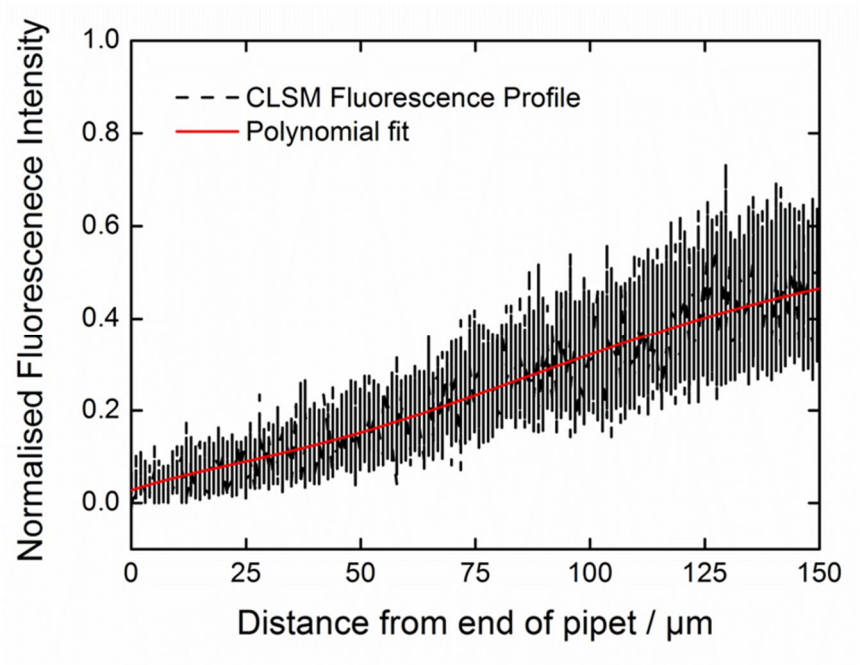

Figure S3. Fluorescence intensity-distance plot normal to the end of a pipet extending into the bulk solution, together with the corresponding polynomial fit for the permeation of propanoic acid (100 mM in the pipet).

S4. Analysis of CLSM Profiles

Figure S5 (a) shows experimental fluorescence data for the case where a bilayer at the end of a pipet separates a solution of pH 4.2 in the pipet (HCl) from bulk solution at pH 8. This

firstly confirms that without a weak acid carrier, protons are confined to the pipet, as evidenced by a fairly sharp change in pH between the interior and exterior of the pipet. However, closer inspection of the profile in the region of the bilayer (pipet end) reveals a change in fluorescence (increase over a finite distance of *ca.* 25 µm) from the end of the pipet. This is an artefact of CLSM imaging at this magnification (10× objective). Light from outside the focal plane is not perfectly rejected, such that in the region around the end of the pipet the measured fluorescence is a combination of that from inside and outside the pipet. If this profile is compared to that of acetic acid, which shows the steepest change in fluorescence between the inside and outside of the pipet (because of the lowest permeation coefficient), there is a clear difference between the two, and it is evident that this imaging artefact does not significantly affect the shape of the measured fluorescence profile over most of the distance (Figure S5 (b)). However, in order to reduce the error in fitting a simulated profile to the experimental data, the first 25 µm of each of the profiles was discarded; after this point the profile without weak acid has reached 90 % of its maximum value and so the contribution of this effect for further distances in the weak acid profiles can be reasonably assumed to be minimal.

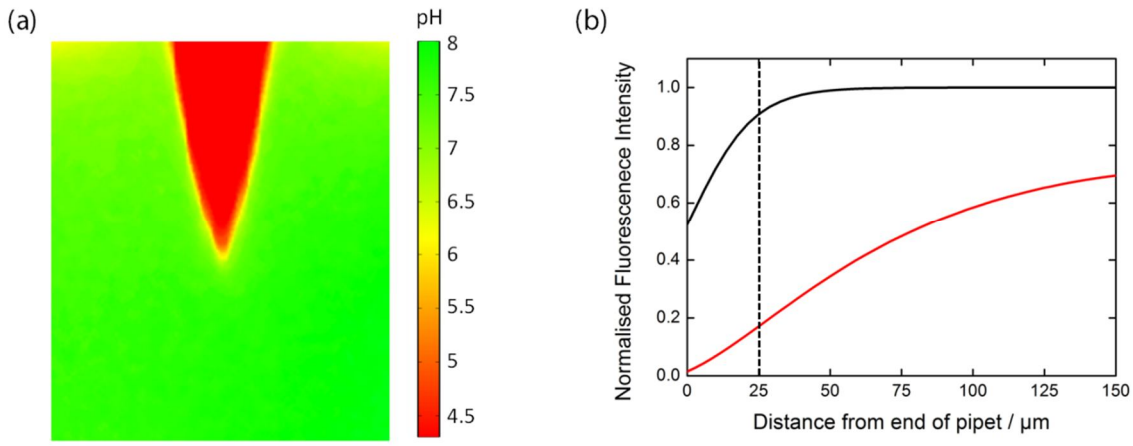

Figure S5. (a) CLSM image of a pipet containing only 0.1 M KCl at pH 4.2 after bilayer formation. (b) Fluorescence intensity profiles normal to the pipet orifice for the same pipet (black line) compared to that for 100 mM acetic acid at pH 4.2 (red line).

References

S1. O. S. Andersen, *Biophysical Journal*, 1983, **41**, 119-133.
S2. A. Hirano, M. Wakabayashi, Y. Matsuno and M. Sugawara, *Biosensors & Bioelectronics*, 2003, **18**, 973-983.
S3. J. M. A. Grime, M. A. Edwards, N. C. Rudd and P. R. Unwin, *Proc. Natl. Acad. Sci.*, 2008, **105**, 14277-14282.