Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Multiplexed Droplet Interface Bilayer Formation

Nathan E. Barlow,[a b] Guido Bolognesi,[a b] Anthony J. Flemming,[b c] Nicholas J. Brooks,[a b] Laura M. C. Barter [a b] and Oscar Ces[*a b]

[a] Department of Chemistry, Imperial College London, Exhibition Road

South Kensington, London, UK, SW7 2AZ. E-mail: o.ces@imperial.ac.uk.

[b] Institute of Chemical Biology, Imperial College London, Exhibition Road

South Kensington, London, UK, SW7 2AZ. E-mail: l.barter@imperial.ac.uk

[c] Syngenta, Jealott's Hill International Research Centre,

Bracknell, Berkshire, RG42 6EY, UK. E-mail: anthony.flemming@syngenta.com

Test Setup and Imaging

Once the chip wells are immersed in oil, the droplets are loaded with a pipette at 200 nL into each well individually. Proper pipetting techniques are required here to obtain a distribution of droplet sizes that are as monodisperse as possible, i.e. the pipette tip must be attached with uniform force, pipetting must be performed in a vertical orientation and the tips must be clean of residual sample before reuse. As the droplets are deposited into the hexadecane filled wells (set in an *offset* orientation), care must be taken to prevent air bubbles from forming which can carry the sample droplets out of the sample wells. Once all droplets are loaded the movable wells are shifted laterally into the *contact* orientation to start the assay as shown in Fig. S-1.

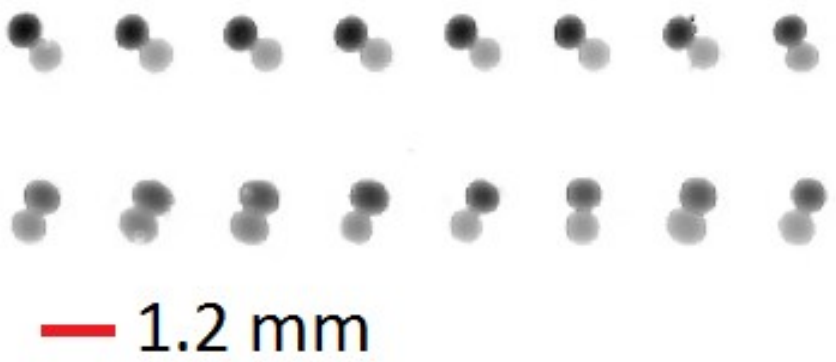

Fig. S-1: A fluorescence image taken at 15 minutes during a DIB permeability assay with aqueous droplets of DPhPC (top row) and DOPC (bottom row) membranes in hexadecane. The fluorophore resorufin is used at concentration gradients in the linear fluorescence region of 5.00 to 0.63 μM. The fluorophore concentrations are deposited in the opposing chambers. Note also that 1 pixel has a length of 50 μm in the original image.

Resorufin Fluorescence Intensity Calibration in Linear Region, dye leakage and DIB formation

The concentration of resorufin was chosen to maintain a linear relationship with fluorescence intensity, which was found to be in the low micromolar region. Four sets of 15 droplets were analysed for intensity at concentrations of 0.625, 1.25, 2.5 and 5 μM as shown in Fig. S-2.

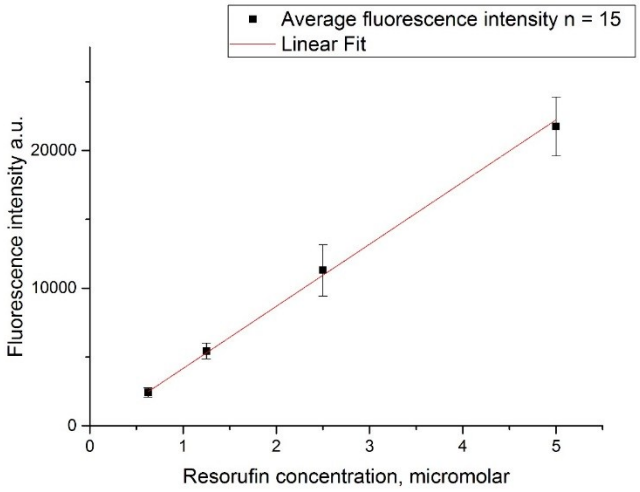

Fig. S-2: Fluorescence intensity is plotted against resorufin concentration to show a linear relationship.

Note also that dye leakage was not observed from individual droplets into the surrounding aqueous phase. After 1 and 2-hour incubation the average fluorescence intensity of six 0.2 μL droplets with resorufin did not change significantly.

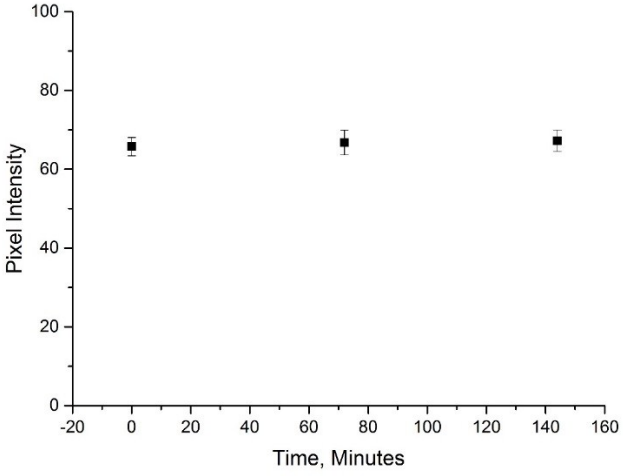

Fig. S-3: Average fluorescence intensity (greyscale pixel intensity) of an array of six droplets is plotted over time to verify minimal resorufin leakage.

Furthermore, to verify that firstly there is minimal dye leakage, calibration droplets in close proximity with two different concentrations of dye were monitored for 15 minutes. As shown in Fig. S-4, it is clear that droplets in close proximity do not translocate solute significantly.

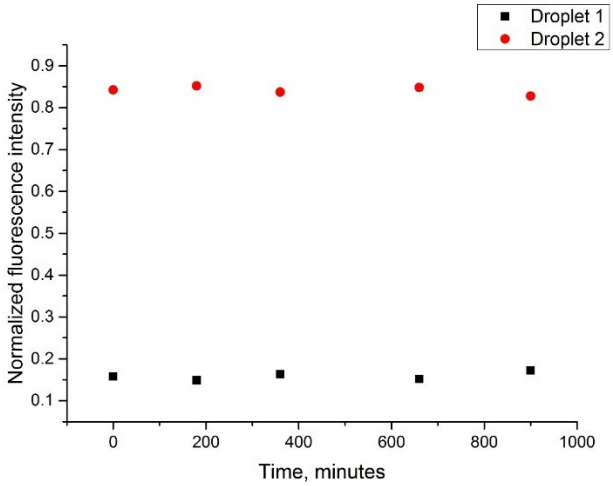

Fig. S-4: Normalized fluorescence intensity of droplets in close proximity that did not form interface bilayers is plotted over time to verify the existence of a DIB by permeation.

Movable Chamber Chip Fabrication

The linear consist of three laser cut PMMA parts: a 0.2 mm thick base, a 1 mm thick stationary well chamber, and a 1mm thick movable chamber array where the chambers are cut to 1.2 mm diameter. It is noted that the movable chambers are limited in thickness by material brittleness for it was observed that array chamber manifolds thinner than 1 mm tended to be more breakable during operation. Note also that a frame can also be fabricated to hold excess fluid in the chip if necessary. The PMMA sheeting is purchased from Weatherall Equipment and Instruments UK and the parts are designed on AutoCAD and laser cut on a 20 Watt universal VLS Laser Platform with a spot size of 200 micron. The chambers are laser cut to a diameter of 1 mm. The stationary parts are solvent bonded with acetone. The process is performed by hand under a fume hood by applying a layer of acetone between cleaned parts. After excess acetone is removed, the bonded parts are held together tightly until the solvent between the chips has evaporated. Note that a minimal volume of acetone is used to prevent discoloration of the PMMA surface. The finished chips are cleaned with ethanol and dried in an oven at 70˚C for several hours before use. A screw adjustment mechanism can be optionally added to control the lateral movement of the chamber chip Fig S-5.

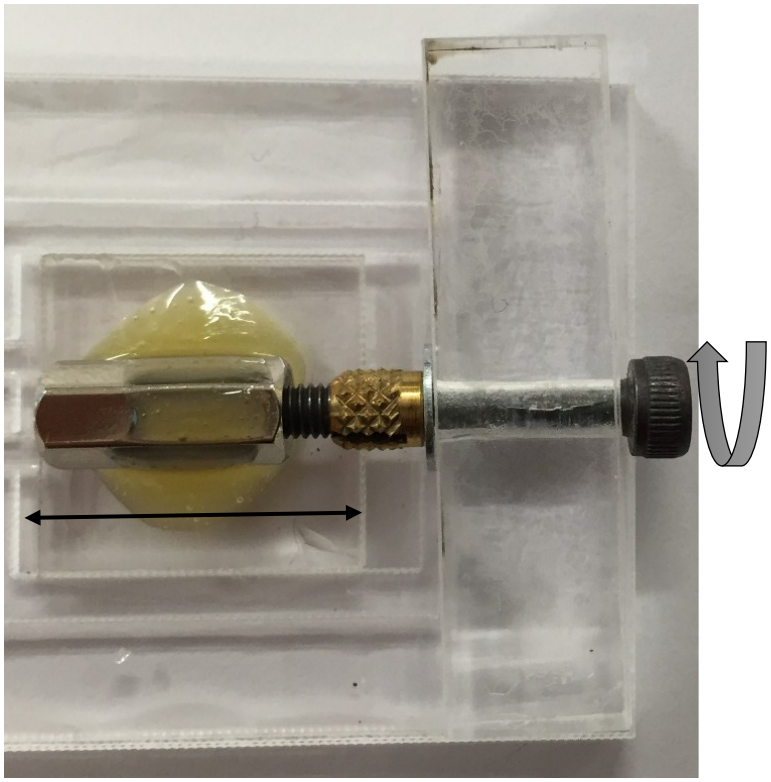

Fig. S-5: Screw adjustment mechanism with hex-nut glued to movable chip controlled by screw adjustment.

Derivation of Permeability DIB Assay Fluorescence Intensity Dynamics

Fick's law of diffusion is given by equation (S1) as a function of droplet concentration C_i, mass diffusivity D and membrane thickness δ.

$$J = \frac{D}{\delta}(C_2 - C_1) \tag{S1}$$

Permeability here is defined as $P = \frac{D}{\delta}$, and the flux equation can be modified to the first order ordinary differential equation (S2).

$$\frac{dC_1}{dt} = P\frac{A}{V}(C_2 - C_1) \tag{S2}$$

In the solute concentration range that is linear with respect to fluorescence intensity, the concentration is directly proportional to the relative intensity $C_{1,2} \propto [I]_{1,2}$. By substitution equation (S2) becomes equation (S3).

$$\frac{d[I]_1}{dt} = k([I]_2 - [I]_1) \tag{S3}$$

Here the proportionality constant k has units of inverse seconds, where the effective permeability value can be attained by the ratio of the droplet volume over the interfacial area. A non-reactive, incompressible system mass balance is imposed, which implies that the total moles from both droplets is conserved, or $[I]_2 + [I]_1 = 1$. This is valid under the presupposition that the fluorophore does not leak into the oil phase and there is minimal photobleaching of the fluorophore. Thus, the relative intensity can be calculated from the absolute values by equation (S4), which range from 0 to 1.

$$[I]_1 = \frac{[I]_1{}^{abs}}{[I]_1{}^{abs} + [I]_2{}^{abs}} \tag{S4}$$

From the mole balance, equation (S3) can be stated as a function of one variable by equation (S5), which has a standard solution (S6), where the initial intensity value $[I]_{1_0}$ is a known constant.

$$\frac{d[I]_1}{dt} = k(1 - 2[I]_1) \tag{S5}$$

$$[I]_1 = c_1 e^{-2kt} + \frac{1}{2};\ [I]_{1_0} - \frac{1}{2} = c_1 \tag{S6}$$

Statistical Analysis

The permeability measurement comes from the rate constant k from (S6) and the droplet volume V and interfacial surface area A in (S7).

$$P = \frac{kV}{A} \tag{S7}$$

The error propagation can be measured in a standard way by equation (S8).

$$\sigma_P = P\sqrt{\left(\frac{\sigma_k}{k}\right)^2 + \left(\frac{\sigma_A}{A}\right)^2 + \left(\frac{\sigma_V}{V}\right)^2} \tag{S8}$$

This comes from the Taylor series approximation and partial derivatives of (S7) by (S8a,b).

$$\sigma_P^2 = \left(\frac{\partial P}{\partial k}\sigma_k\right)^2 + \left(\frac{\partial P}{\partial V}\sigma_V\right)^2 + \left(\frac{\partial P}{\partial A}\sigma_A\right)^2 \tag{S8a}$$

$$\frac{\partial P}{\partial k} = \frac{V}{A} \qquad \frac{\partial P}{\partial V} = \frac{k}{A} \qquad \frac{\partial P}{\partial A} = -\frac{kV}{A^2} \tag{S8b}$$

The rate constant k is measured from a non-linear least squares optimization, the error of this type of analysis has been discussed by Burrell.[1] The χ^2 functional is minimized for the model equation $f(t_i,k)$ for time sample t_i by varying k in (S9).

$$\chi^2 = \sum_{i=1}^{n} \frac{1}{\sigma_i^2}\left(y_i - f(t_i,k)\right)^2 \tag{S9}$$

The matrix α_{jl} can be obtained from the formula (S10), which in this case breaks down to a single value as there is only one parameter to be solved in $f(t_i,k) = c_1 e^{-2kt} + \frac{1}{2}$.

$$\alpha = \sum_{i=1}^{n} \frac{1}{\sigma_i^2}\left[\left(\frac{\partial}{\partial k} f(t_i,k)\right)^2 - \left(y_i - f(t_i,k)\right)\frac{\partial^2}{\partial k^2} f(t_i,k)\right] \tag{S10}$$

This becomes equation (S11).

$$\alpha = \sum_{i=1}^{n} \frac{1}{\sigma_i^2}\left[\left(-2tc_1 e^{-2kt_i}\right)^2 - \left(y_i - c_1 e^{-2kt_i} - \frac{1}{2}\right)\left(4t^2 c_1 e^{-2kt_i}\right)\right] \tag{S11}$$

The standard error on the rate constant k is then given by (S12).

$$(\sigma_k)^2 = \sum_{i=1}^{n}\left(\frac{\alpha^{-1} 2tc_1 e^{-2kt_i}}{\sigma_i}\right)^2 \tag{S12}$$

The volume V and area A come from geometric measurements. The area measurement is estimated by taking the interface diameter under the assumption that the contact area is uniformly circular. This can be confirmed by looking at Fig. S-6b, which shows that there is no disruption of the interface from the PMMA surface. Additionally, as shown in Fig. S-7, it is confirmed that the interface of a DIB is circular on PMMA in hexadecane. The diameter of a DIB

membrane or droplet is measured $d = \sqrt{\Delta x^2 + \Delta y^2}$ with a pixel graduation accurate to 50 micron (Typhoon Fluorescence Imager). Taking a measurement implies making two choices, a start and end point, thus if the error on picking a pixel point is 50 micron, the error on picking the distance between two pixel points is 70 micron. Given that the area and volume are measured by as a function of interfacial diameter d_{int} and droplet diameter d_{diam} by $A = \frac{\pi}{4} d_{int}^2$ and $= \frac{\pi}{6} d_{diam}^3$, the standard errors are $\sigma_A = \frac{2A}{d_{int}} \sigma_{\Delta d_{int}}$ and $\sigma_V = \frac{3V}{d_{diam}} \sigma_{\Delta d_{diam}}$ respectively. Note that for N experimental repeats, the error propagation becomes $\sigma_{P_{overall}} = \frac{\sigma_P}{\sqrt{N}}$.To compare the permeability results of DOPC and DPhPC, a Welch's Student t-test can be performed by equation S13.[2]

$$t_{value} = \frac{\bar{P}_{DOPC} + \bar{P}_{DPhPC}}{\sqrt{\frac{\sigma_{DOPC}}{N_{DOPC}} + \frac{\sigma_{DPhPC}}{N_{DOPC}}}} \quad \text{(S13)}$$

For a value permeability value of *0.78 x 10^{-4}cm s^{-1} (±0.17 x 10^{-4}) with 4 samples and 1.26 x 10^{-4}cm s^{-1} (±0.24 x 10^{-4}) with 16 samples,* this means that the t value is 4.8. For a df of 14, this implies over 99.8% confidence (p=0.001).

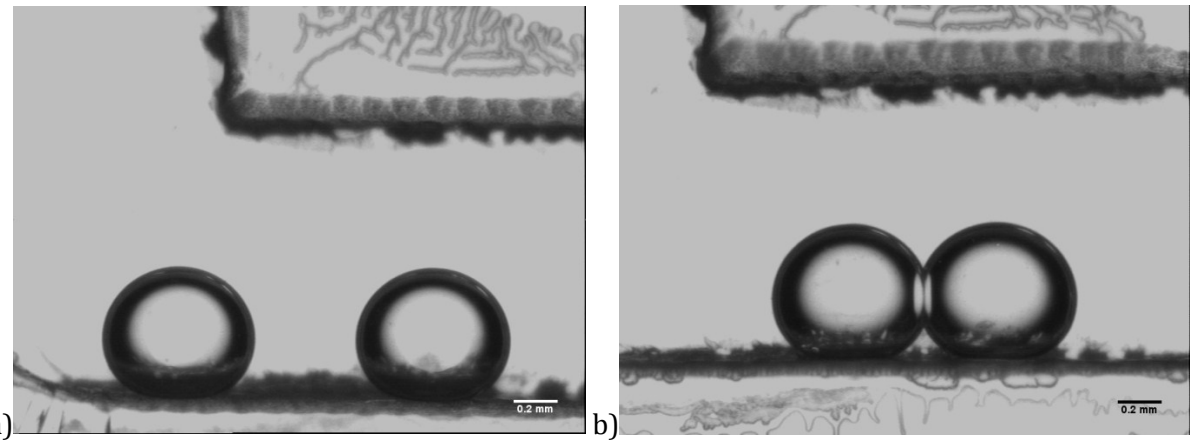

Fig. S-6: Side-view brightfield image of aqueous DPhPC lipid emulsion droplets in hexadecane (a) that are formed into a DIB pair (b).

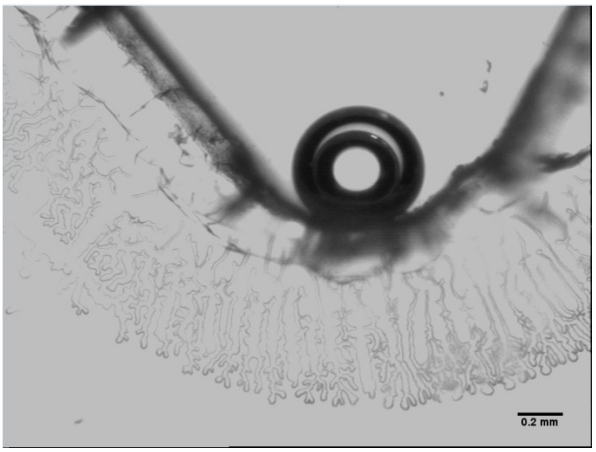

Fig. S-7: Side-view brightfield image of DIB pair showing a circular interface.

References

(1) Burrell, K. H. Error analysis for parameters determined in nonlinear least-squares fits. *American Journal of Physics* **1990**, *58*, 160-164.

(2) Welch, B. L. The generalization of 'Student's' problem when several different population variances are involved. *Biometrika* **1947**, *34*, 28-35.