Electronic Supplementary Material (ESI) for ChemComm.

Microfluidic generation of encapsulated droplet interface bilayer networks (multisomes) and their use as cell-like reactors

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Supplementary Information

S1: Lipid Preparation

Unless otherwise noted all reagents were purchased from Sigma Aldrich. In all experiments DPhPC lipid (Avanti Polar Lipids) was dissolved in mineral oil (25 mg ml $^{-1}$) by sonication at 50˚C for 60 minutes in a bath sonicator. In fluorescence microreactor experiments 10 mg ml $^{-1}$ DPhPC was also present in the internal aqueous droplets in the form of 100 nm small unilamellar vesicles, formed by extruding lipid-in-water suspension 11 times through a polycarbonate membrane. Aqueous solutions were composed of de-ionised (DI) water unless otherwise mentioned.

S2: Device Design

The design of the microfluidic chip is shown in Fig. SI 1. The portion of the device where w/o droplets were present was patterned to be hydrophobic, and where w/o/w droplets were present patterned to be hydrophilic. Meanders were present after the droplet generation junction to give droplets times to be effectively stabilised by lipid monolayers. Channel geometries for the w/o generation region were 100 µm in width and depth. These dimensions were larger in the second encapsulation region of the device, with a width and depth of 150 µm and 200 µm respectively to reduce droplet confinement. This was important to prevent droplet squeezing, which led to bilayer rupture.

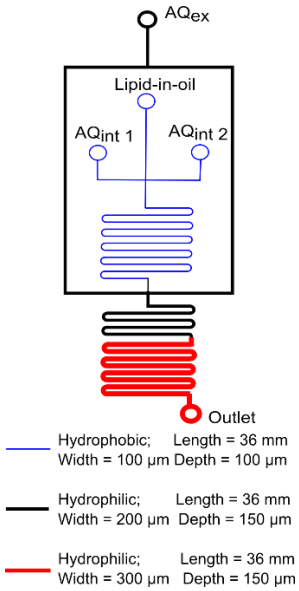

Fig. S 1 – Schematic of device channel dimensions and surface properties. Surface chemistry was appropriately patterned to be hydrophilic in regions where water was the continuous phase.

S3: Device Fabrication

A PDMS based microfluidic device was fabricated in a cleanroom using standard soft lithography techniques.[1] A silicon master was first made by photoresist deposition (Su8 2050, MicroChem, MA, USA), selective UV exposure using a photomask, baking and development. Channels with different depths were constructed using a double lithography strategy, by sequential photoresist deposition and exposure before development and silanization. Polydimethylsiloxane (PDMS, Sylgard 184 elastomer kit, Dow Corning) was poured on a microfabricated master and left to cure at 50˚ C for at least three hours. PDMS was then released form the underlying substrate, and irreversibly bonded to a second thin sheet of PDMS to seal the channels. This was achieved by exposure in a plasma oven (Harrick Plasma, NY, USA) for 30 seconds, and then pressing the surfaces together.

A prerequisite for successful droplet generation is that the continuous (i.e. external) phase must preferentially wet the channel walls. As water was the continuous phase in the second half of the device and PDMS is hydrophobic, the channels were selectively modified to yield hydrophilic surfaces in these regions. This was achieved via sequential layer-by-layer deposition of charged polymers, as reported by Bauer *et al.*[2] Immediately after plasma treatment, a positively charged polymer, polyallylamine hydrochloride (PAH, 0.1% w/v) in 0.5 M NaCl was selectively applied to the parts of the chip that were intended to be hydrophilic for two minutes. It was prevented from reaching other parts of the chip by applying an air stream (200 µL min $^{-1}$) through those regions (Figure S2). The PAH was then removed using a steam of air, followed by flowing through 0.1 M NaCl. The same process was repeated with a negatively charged polymer, polystyrenesulfonate (PSS, 0.1% w/v) in 0.5 M NaCl. The above procedure was performed three times, followed by washing through with DI water before the device was used. A commercial hydrophobic agent, Rain-X (Kraco Enterprises, CA, USA) was then applied to the hydrophobic part of channel to stop the aqueous phase wetting the PDMS channels.

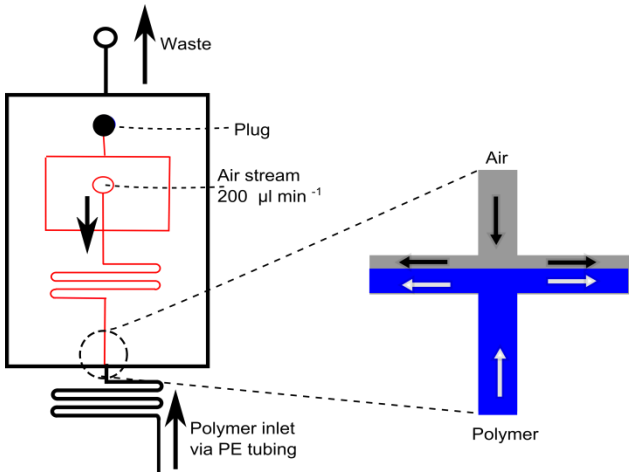

Figure S2 – Schematic of method used for selective surface treatment of the device. Only the channels coloured in black came into contact with the polymer solutions to become hydrophilic.

S4: Contact Angle Measurements for Treated PDMS

Successful surface treatment was confirmed by contact angle analysis (static sessile drop method) using a drop shape analyser (EasyDrop FM40, Kruss, Germany), and the provided software (Drop Shape Analysis DSA 100). A sheet of PDMS was made hydrophilic by the same method used for the channel treatment. The average water-air contact angle was found to change from 105˚ ± 6.1 ˚, to 43 ˚± 6.8˚ upon hydrophilic treatment (n =10, errors = one S.D).

S5: Double emulsion generation

To produce stable two-drop double emulsions, flow rates were 0.5,1, and 8 µL min^{-1} for the inner-aqueous, oil, and outer-aqueous phases respectively. Precision pumps used to drive the flow (Chemyx Fusion 100, Chymix Inc. Texas, USA), and polyethylene tubing (Harvard Apparatus, Kent, UK) was used to deliver solutions to the device. To monitor droplet in the channels in the chemical synthesis experiments, flow was stopped at the pumps and the outlet was sealed, leading to stationary multisomes.

S6 Chemical synthesis experiments

In multisome microreactor experiments both inner and outer aqueous phases contained 0.1M $NaHCO_3$, made to pH 8.3 by the addition of NaOH. Double emulsions were formed with one droplet containing 0.23 mM fluorogenic Chromeo P540 (Active Motif, La Hulpe, Belgium), and a second 16 mM ethanolamine. All reactions were performed at ambient temperatures. Fluorescence was monitored using Nikon Eclipse TE2000-E fluorescent inverted microscope, an illuminating mercury arc lamp, and a tetramethylrhodamine (TRITC) filter (long bandpass). Images were taken with a QICAM camera (QImaging) at 100 ms exposure and were analysed using ImageJ software.

S7: Fluorescence characterisation of fluorogenic pyrylium reagent (P540)

The excitation and emission profile of fluorogenic pyrylium reagent (P540) and fluorescent pyridinium product is shown in Fig. S3. This data was collected on a Cary Eclipse Fluorescence Spectrophotometer (Agilent, Santa Clara, USA).

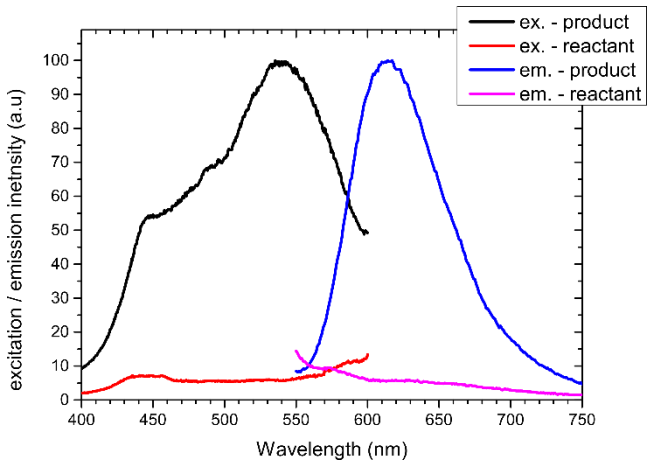

Figure S3 – Excitation and emission profile of the pyrylium reagent before and after reacting with ethanolamine to yield the fluorescent pyridinium product.

S8: Video of multisome generation

SI References

[1] Y. Xia and G. M. Whitesides, *Annual review of materials science* **1998**, *28*, 153-184.
[2] W.-A. C. Bauer, M. Fischlechner, C. Abell and W. T. S. Huck, *Lab on a Chip* **2010**, *10*, 1814-1819.