Supplementary Information

Production of Giant Unilamellar Vesicles and Encapsulation of Nematic Lyotropic Liquid Crystals

Peng Bao\textsuperscript{a}, Daniel A. Paterson\textsuperscript{a,b}, Sally A. Peyman\textsuperscript{a,c}, J. Cliff Jones\textsuperscript{a}, Jonathan A. T. Sandoe\textsuperscript{c}, Helen F. Gleeson\textsuperscript{a}, Stephen D. Evans\textsuperscript{a}, Richard J. Bushby\textsuperscript{a}\textsuperscript{*}

\textsuperscript{a} School of Physics and Astronomy, University of Leeds, Leeds, LS2 9JT, UK
\textsuperscript{b} School of Chemistry, University of Leeds, Leeds, LS2 9JT, UK
\textsuperscript{c} Leeds Institute of Medical Research, University of Leeds, Leeds, LS2 9JT, UK

*Author for Correspondence: r.j.bushby@leeds.ac.uk
Supplementary Information

Contents

1. Materials and Methods:
   1.1. Materials
   1.2. PDMS device fabrication
   1.3. Coating of the exit channel with PVA
   1.4. Solutions
   1.5. Flow system
   1.6. Microscopy observation of double emulsions and GUVs
   1.7. Fluorescence leakage assay
   1.8. Osmolarity measurements
   1.9. Osmotic Shrinkage of the Sunset Yellow containing GUVs

2. Figures:
   Figure S1. Schematic showing the method used for PVA-coating of the exit channel.
   Figure S2. Fluorescence image of a sample made with a lipid concentration of 15 mg/mL viewed shortly after collection
   Figure S3. Calcein leakage assay with alpha-hemolysin.
   Figure S4. Measured osmolarities of glycerol, SSY and DSCG in water.
   Figure S5. Image of the SSY-containing GUVs at the beginning of the osmotic shrinking process.
   Figure S6. The diameter of a double emulsion ‘droplet’ containing DSCG plotted as a function of time.

3. Videos:
   Video SV1. The formation of double emulsion droplets.
   Video SV2. Tumbling motion of a single GUV showing the presence of a small residual droplet of octanol in the bilayer
   Video SV3. The leakage of calcein from a GUV after inserting hemolysin.
   Video SV4. The effect of heating on DE droplets containing DSCG solution which were made using oleic acid as the ‘oil’ rather than octanol.
   Video SV5. Observation of the swelling of DE droplets of DSCG solution (made using octanol as the ‘oil’) as a function of time.

4. References:
1. Materials and methods

1.1. Materials

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), glucose (purity >99.5%), sucrose (purity >99.5%), HEPES (purity >99.5%), disodium cromoglycate (DSCG) (purity > 95%) and octanol (>90%) were purchased from Sigma-Aldrich. Texas Red™ 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Texas Red-DHPE) and calcein (>93%) were purchased from Fisher Scientific (UK). Sunset Yellow (SSY, >90%), was purchased from TCI and hydroxypropyl cellulose nanocrystals (HPC) from Alfa Aesar. Premium glass microscope slides used, were obtained from Fisher Scientific (Pittsburgh, PA). Sylgard 184 silicone elastomer was purchase from Farnell, UK. All aqueous solutions were prepared with deionized water, using a Milli-Q water purification system (Millipore, Bedford, MA).

1.2. PDMS device fabrication

The detail of the fabrication of the polydimethylsiloxane (PDMS) devices used can be found in our previous paper. Briefly, the software “CleWin” was used for the design. An MW2 laser direct-write laser system (Durham Magneto Optics Ltd, Durham, UK) was used to pattern the SU8 (thickness of 25 µm) creating an ‘SU8 on silicon’ master. A PDMS (PDMS monomer and curing agent used in a weight ratio of 10:1) copy of this silicon master was cross-linked at 75 °C for 1 h. Afterwards, access holes were punched into the PDMS copy, which was then given 1 min of O₂ plasma treatment and bonded onto a glass slide. Post-bonding baking in an oven at 75 °C for 30 min increased the bonding strength and resulted in a device ready for use. For the flow focus design used in our experiments, the width of nozzle is about 15 µm and the width and length of channel after the nozzle are 100 and 4000 µm, respectively. The depth of all the channels is 25 µm.

1.3. Coating of the exit channel with PVA

The inside wall of the channel after the nozzle needs to be made hydrophilic by coating with PVA. We have developed a new and convenient PVA partial coating protocol by backfilling trace amounts of PVA solution (1 µL) from the outlet and stopping the PVA solution at the nozzle by surface tension. In detail, two plastic syringes with 5 mL air inside were connected to two of the three inlets and connected to mechanical syringe pumps. 1 µL of 2% PVA (M_w 30-70 kg/mol, hydrolysis 87-90%) solution was added to the outlet of the device. As a result of capillary action, the PVA solution slowly progressed into the channel until it reached the nozzle/outlet channel interface where, because of surface tension it stops, as shown...
schematically in the Supplementary Information Figure S1. After an incubation of 8 min, the PVA solution was blown out by airflow from the two syringes using a sudden reduction of the volume of the air within them. Finally, the PDMS device was placed on a hotplate and dried at 120 °C for 30 min to enhance the adhesion of the PVA to PDMS. The resulting device was then ready for use.

1.4. Solutions
The lipid mixture (DOPC:DOPG 1:1 with 0.1 mol% Texas Red-DHPE) was dissolved in chloroform in a glass vial and dried under nitrogen flow for 1 h. The required amount of octanol was added to the dried lipid mixture to achieve the concentration of lipid needed (between 2 and 30 mg/mL). The lipid-oil mixture was treated in a sonication bath for 30 min before use. For the double emulsion formation shown in Figure 3, the internal aqueous solution was 600 mM sucrose with 0.05 mM calcein, the external aqueous solution was 600 mM sucrose and oil-lipid phase was 2 mg/mL DOPC: DOPG (1:1) with 0.1 mol% Texas Red-DHPE in octanol. For the GUV formation shown in Figure 4, the internal aqueous solution was 600 mM sucrose solution with 0.05 mM calcein, the external aqueous solution was 600 mM glucose and oil-lipid phase was 15 mg/mL DOPC: DOPG (1:1) with 0.1 mol% Texas Red-DHPE in octanol. For the GUVs shown in Figure 6a, the internal solution was 7 wt% of SSY, the external aqueous solution was 1% glycerol and the oil-lipid phase was 15 mg/mL DOPC: DOPG (1:1) with 0.1 mol% Texas Red-DHPE in octanol. For GUVs used in Figure 6b-6d, the internal solution was 15 wt% of DSCG, the external aqueous solution was 1.2 M sucrose and the oil-lipid phase was 30 mg/mL DOPC: DOPG (1:1) with 0.1 mol% Texas Red-DHPE in octanol.

1.5. Flow system
Two mechanical syringe pumps (Standard infuse/withdraw PHD ultra syringe pump, Harvard Apparatus, USA) were used for pumping the three fluidic phases. Typical flow rates for internal water phase, oil/lipid phase and external water phase were 0.1/0.1/1.5 µL/min respectively (producing a total volume of 1 mL in ca. 10 h).

1.6. Microscopy observation of double emulsions and GUVs
An inverted Nikon microscope (Nikon TiU, Nikon Instruments Europe B. V., Kingston, UK) equipped with a high-speed camera (Photron SA5, Japan) was used for monitoring and recording the formation of double emulsions in-situ. A polarized fluorescence microscope (Nikon N90, Nikon Instruments Europe B. V., Kingston, UK) was used to obtain the fluorescent and polarized microscopy images. Unless otherwise stated, all measurements were made at room temperature.

1.7. Fluorescence leakage assay
GUVs with aqueous solution (600 mM sucrose and 0.05 mM calcein) inside and aqueous solution (600 mM glucose) outside, were produced. To remove the calcein in the external solution this was exchanged with 600 mM glucose (centrifugation and replacement of the supernatant liquid five times). The GUVs were pipetted into a well for imaging with a fluorescent microscope (Nikon E600, Nikon Instruments Europe B. V., Kingston, UK). The channel-forming protein (alpha-hemolysin) was added to the external solution at a concentration of 25 μg/mL. The GUV samples were imaged every 20 s and the intensity of fluorescence signal inside the GUVs was analysed using the software ImageJ. For a control experiment, another sample without hemolysin was recorded and analysed in the same manner.

1.8. Osmolarity measurements
The osmolarity of the aqueous solutions and of the lyotropic liquid crystal solutions were measured using a Loser type 15 osmometer (Camlab, UK).

1.9. Osmotic Shrinkage of the Sunset Yellow containing GUVs
The GUVs containing sunset yellow (0.16 M) were produced in 1 vol% glycerol solution. After leaving overnight, the GUVs were collected from the bottom of Eppendorf. small portions (2 μL) of this GUV solution were added to more concentrated solutions of glycerol in water (18 μL, 4 vol% to 15 vol%) the mixture gently agitated and, after 10min, pipetted into a PDMS well contained within glass coverslips for polarizing microscope observation.
2. Figures

**Figure S1.** Schematic showing the method used for PVA-coating of the exit channel in the PDMS microfluidic chip (see experimental section). Device (a) before and (b) after filling the exit channel with PVA solution. 1 μL of a 2% PVA solution in water was added at the outlet of the device. The exit channel then spontaneously fills with the PVA solution, which (because of surface tension) stops at the nozzle.

**Figure S2.** Fluorescence image of a sample made with a lipid concentration of 15 mg/mL viewed shortly after collection showing a mixture of GUVs and double emulsion droplets which have partially de-wetted and which still have a large oil droplet attached.
Figure S3. (a) Schematic of calcein leakage induced by adding alpha-hemolysin. (b) The intensity change of the fluorescence signal of calcein inside of the GUVs in the presence of alpha-hemolysin (black), compared with reference sample without alpha-hemolysin (red). The small decrease in intensity seen in this control experiment is probably due to irreversible photo-bleaching. Compare Figure 5c, Nature Comms., 2016, 10, 1800.

Figure S4. Measured osmolarities of glycerol, SSY and DSCG in water
Figure S5. Image of the SSY-containing GUVs at the beginning of the osmotic shrinking process. Diameter $19.0 \pm 1.8 \, \mu m$.

Figure S6. The diameter of a double emulsion ‘droplet’ containing DSCG plotted as a function of time. In this case, the internal aqueous phase was 15.2% DSCG in de-ionized water; the oil phase was octanol with 2 mg/mL DOPC:DOPG (1:1 molar ratio). The external aqueous solution was 1.2 M sucrose.
Supplementary Information

3. Videos

Video SV1. The formation of double emulsion droplets.

Video SV2. Tumbling motion of a single GUV showing the presence of a small residual droplet of octanol in the bilayer

Video SV3. The leakage of calcein from a GUV after inserting hemolysin.

Video SV4. The effect of heating on DE droplets containing DSCG solution which were made using oleic acid as the ‘oil’ rather than octanol.

Video SV5. Observation of the swelling of DE droplets of DSCG solution (made using octanol as the ‘oil’) as a function of time.

4. References