## Supporting Information

# Encapsulation of compartmentalized cytoplasm mimic within lipid membrane by microfluidics†

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**Materials**

GTP nucleotide, dextran 500 (500 kDa), PEG 8 (8 kDa) and other analytical grade chemicals were from Sigma. Polar extract phospholipids from *E. coli* (EcL) and DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine), both from Avanti Polar Lipids (Alabama, USA), were stored in chloroform at -20$^{o}$C. Shortly before use EcL, doped when required with 20% DMPC, was thoroughly dried in a Speed-Vac device and the resulting film resuspended in mineral oil by two cycles of vortex and 15 min sonication in a bath. Final concentration of EcL in mineral oil was 20 g/L.

**Protein purification and labelling of FtsZ and PEG**

FtsZ was purified by the calcium precipitation method as described[1] and stored at -80$^{o}$C until used. The protein was covalently labelled with Alexa 488 carboxylic acid succinimidyl ester dye (Molecular Probes/Invitrogen) as earlier stated[2,3] and stored frozen at -80$^{o}$C. The ratio of labelling of FtsZ was 0.5-0.9 moles of fluorophore per mole of protein. For the experiments, protein solutions were equilibrated in 50 mM Tris-HCl, 300 mM KCl, 1 mM $MgCl_2$, pH 7.5. Labelling of PEG with Alexa 647 was done as described[4].

**Preparation of phases for LLPS systems**

Enriched phases were prepared as earlier described[4]. Briefly, dextran 500 and PEG 8, equilibrated by extensive dialysis in 50 mM Tris-HCl, 300 mM KCl, pH 7.5, were thoroughly mixed to yield final nominal concentrations rendering phase separation, 82 g/L dextran 500 and 53 g/L PEG 8. Separation was expedited by centrifugation at 3000 rpm for 5 min in a bench centrifuge. Final concentrations of the isolated enriched phases, determined from the refractive index increment, were around twofold their nominal concentration in the mixture[4].

**Microfluidic chip fabrication**

The devices used were constructed by conventional soft lithographic techniques from masters (chip design detailed elsewhere[5]). PDMS base Sylgard$^{TM}$ 184 and curing agent (Dow Corning GmbH, Germany) were mixed in a 10:1 (w/w) ratio, degassed, decanted onto masters and heated at 65$^{o}$C for at least 4 hours. Inlet and outlet holes were punched in the PDMS peeled from the master, and the channels were sealed by a glass slide, activating the surfaces by oxygen plasma (Diener electronic GmbH, Germany). Devices were coated with a hydrophobic layer flushing Aquapel (Pittsburgh Glass Works, LLC) in the channels and allowing it to dry overnight at 65$^{o}$C.

**Encapsulation of PEG/dextran solutions by microfluidics**

Encapsulation was conducted by mixing two streams of dispersed aqueous phases, one with dextran 500 and the other with PEG 8, in an approximately 1:1 ratio prior to the droplet formation junction. Alexa-647 labelled PEG (2 μM) was included in the PEG solution. FtsZ (25 μM) with a tracer amount labelled with Alexa 488 (1 μM FtsZ-Alexa 488) was added to the two aqueous phases. When induction of protein polymerization before encapsulation was required, one of the solutions contained FtsZ (25 μM) with FtsZ-Alexa 488 (2 μM) and the other the nucleotide GTP (4-6 mM). The buffer for the aqueous solutions was 50 mM Tris-HCl, 300 mM KCl, 1 mM $MgCl_2$, pH 7.5. The third stream supplied the *E. coli* lipid mixture at 20 g/L in mineral oil. We found no differences in the microdroplet formation step derived from the presence of DMPC, included for the subsequent generation of permeable vesicles. All experiments were conducted at room temperature. Data presented correspond to experiments delivering solutions at 120 μL/h (oil phase) and 5 and 7 μL/h

(dextran and PEG aqueous phases, respectively) by automated syringe pumps (Cetoni GmbH, Germany) yielding uniform droplets with average diameter of 16 μm (~2 pL). In some instances the final flows of the two aqueous phases were slightly different but no significant effect was observed in the encapsulation. Decreasing of the oil phase flow resulted in average diameters somewhat increased.

**Formation of Giant Unilamellar Vesicles and triggering of FtsZ polymerization**

For their subsequent conversion into GUVs, droplets were collected, once stable production was achieved, introducing the outlet tubing from the microfluidic chip into 700 μL of oil phase stabilized for at least 1 hour over 400 μL of outer solution. Collection was initially tested positioning the outlet tubing either slightly above or slightly below the oil/air interface, in both cases close to the centre of the tube section avoiding contact with the walls. In the first case the encapsulated material accumulated mostly within the oil surface and subsequent steps rendered very low amounts of GUVs. Positioning of the tubing slightly below the oil surface proved to be more suitable for successful generation of GUVs. Upon delivery of the microdroplets from the outlet tubing, they migrated throughout the whole oil volume until reaching the oil/outer solution interface, where they accumulated. Probably this migration through the oil phase helped further stabilizing the lipid monolayer before subsequent centrifugation into the aqueous solution, plus avoids impact of microdroplets against the oil/air interface and their accumulation into a less friendly environment. Composition of the outer solution was that of the solution inside the vesicles (50 mM Tris-HCl, 300 mM KCl, 1 mM $MgCl_2$, pH 7.5), supplemented with 117 mM sucrose that rendered an osmolarity ~25 mOsmol/Kg above the highest osmolarity of the encapsulated solutions. The osmolarities, measured in a Osmomat 3000 (Gonotec GmbH, Germany), of the PEG and dextran solutions were close to each other (PEG ca 740 mOsmol/Kg, dextran ca 710 mOsmol/Kg). Similar results were obtained when glucose was used instead of sucrose to adjust the osmolarity of the outer solution. Droplet collection times were varied from 10 to 30 min, being 30 min the one selected to improve the yield of the method. The solutions were then centrifuged (15 min, 1500 rpm in a bench centrifuge), the oil phase removed and the droplets washed with outer solution and centrifuged again (15 min, 2000 rpm). For triggering of FtsZ polymerization, 20 mM GTP was added in this last step. Pelleted vesicles were visualized as described below. All experiments were conducted at room temperature.

**Fluorescence microscopy and measurement of droplets diameter**

Production of the lipid droplets in the microfluidic chip was monitored using an Axiovert 135 fluorescence microscope (Zeiss). The resulting droplets and giant vesicles were visualized by confocal microscopy with a Leica TCS-SP2-AOBS or a Leica TCS-SP5-AOBS inverted confocal microscope with a HCX PL APO 63x oil immersion objective (N.A. = 1.4–1.6; Leica, Mannheim, Germany). Ar (488 nm) and He-Ne (633 nm) ion lasers were used to excite Alexa 488 and Alexa 647 dyes, respectively.

The droplets were imaged directly in the chip, keeping them in the device reservoir by simultaneously cutting the inlet tubing before stopping the flow. The vesicles were visualized by placing ~70 μL in chambers fabricated by gluing a silicone isolator (Molecular probes/Invitrogen) to coverslips as previously described[4].

ImageJ (National Institutes of Health, USA) was used to measure the distribution of diameters applying the line tool of the software through the equatorial section of the droplets/vesicles and to obtain the intensity profiles in the red and green channels. Size distributions shown correspond to 3 representative experiments for the droplets and 2 different experiments for the GUVs, independent from the ones corresponding to the droplets.

**Behaviour of FtsZ in PEG/dextran systems**

The distribution of FtsZ in the PEG 8/dextran 500 LLPS system is uneven and largely determined by its association state, as previously characterized[4]. The fraction of unassembled FtsZ (unassembled meaning monomer to short oligomers) is higher in the dextran compared to the PEG phase, as expected for a native protein and in contrast to the behaviour observed with denatured proteins that tend to accumulate in the more hydrophobic PEG phase that stabilizes their exposed hydrophobic residues[6,7]. The formation, under crowding conditions, of thick bundles of the FtsZ polymers induced by GTP favours their location at the interface between these two crowding polymers, likely because of the reduction of interfacial tension at the aqueous/aqueous interface between them, as previously described for other large structures as cells and nanoparticles[8-11].

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