Supplementary information

Synthesis of Microcapsules using Inverse Emulsion Periphery RAFT Polymerization via SPG Membrane Emulsification

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Experimental

1. Materials

All materials were reagent grade and used as received, unless otherwise specified: Di(ethylene glycol) methyl ether methacrylate (DEGMA, 95%, Sigma-Aldrich), sodium chloride (NaCl, Univar), sulforhodamine B (SRB, Sigma-Aldrich), diethyl ether (Et_2O, 99%, Univar), toluene (99.5%, Univar) and *N*,*N*-dimethylacetamide (DMAc, 99.9%, Sigma-Aldrich) were used without further purification. Deuterated NMR solvents ($CDCl_3$) were purchased from Cambridge Isotope Laboratories. Methyl methacrylate (MMA, >99%, Sigma-Aldrich) and ethylene glycol dimethacrylate (EGDMA, 98%, Sigma-Aldrich) were deinhibited by passing through a column of activated basic alumina. Deinhibited monomers were stored below 4 °C and used within 7 days. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized twice from methanol. Deionized (DI) water was produced by a Milli-Q reverse osmosis system and had a resistivity of 19.6 mΩ cm^{-1}. The RAFT agent, 4-cyanopentanoic acid dithiobenzoate (CPADB), was synthesized according to the literature.[1] Shirasu porous glass (SPG) membranes (hydrophobic) with average pore size of 0.1, 0.2, 0.8 and 3.0 μm and SPG module were purchased from SPG Technology Co. Ltd.

2. Analyses

2.1. Size exclusion chromatography

Size exclusion chromatography (SEC) was performed using a Shimadzu modular system, comprising an SIL-10AD autoinjector, an LC-10AT pump, a DGU-12A degasser, a CTO-10A column oven, and an RID-10A differential refractive index detector. A column arrangement consisting of a Polymer Laboratories 5.0 μm bead size guard column (50 × 7.8 mm), followed by four linear PL column (300 × 7.8 mm, 500, 10^3, 10^4, and 10^5 Å, 5 μm pore size) was used for the analysis. *N,N*-Dimethylacetamide (DMAc, 0.03% w/v LiBr, 0.05% w/v 2,6-dibutyl-4-

methylphenol(BHT)) was used as the mobile phase at a constant temperature of 50 °C and a constant flow rate of 1 mL min^{-1}. The SEC system was calibrated using linear polystyrene standards, ranging from 500 to 10^6 g mol^{-1} (Polymer Laboratories). Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

2.2. Nuclear Magnetic Resonance (NMR)

NMR was utilized to analyse the structure of the synthesized compounds as well as to determine the monomer conversion in the block copolymer synthesis. ^{1}H NMR spectroscopy was carried out using a Bruker Avance III 300 MHz, equipped with an autosampler system. Chemical shifts are reported in parts per million (ppm), relative to the residual solvent peak. The theoretical molecular weight ($M_{n,th}$) was calculated according to the following equation:

$$M_{n,th} = \frac{[monomer]}{[RAFT]} \times \text{conversion} \times M_{monomer} + M_{RAFT} \quad (1)$$

where M_{RAFT} denotes the molecular weight of the RAFT agent or the macroRAFT agent.

2.3. Particle size measurements

Particle/droplet size was measured using two different instruments depending on size. For smaller droplet/particles (prepared by membranes with pore size of 0.2 and 0.8 μm), DLS measurements were conducted on a Zetasizer Nano ZS (Malvern), with a 4 mV He−Ne laser operating at λ = 632 nm and noninvasive backscatter detection at 173°. Measurements were conducted in a quartz cuvette at 25°C, with 30s equilibration period prior to each set of measurements. For a given sample, a total of three measurements were conducted. In each measurement, the number of runs, attenuator, and path length used were automatically adjusted by the instrument, depending on the quality of the sample. The presented results are averages of the three measurements. For larger droplet/particle, the size was measured by a mastersizer S (Malvern), equipped with 300 mm lens.

2.4. Fluorescence spectroscopy

Encapsulation efficiency (EE) was measured using fluorescence spectroscopy (Cary Eclipse). After synthesis of microcapsules with guest molecules, an aliquot (1.4 mL) of sample was transferred to a centrifuge tube and centrifuged at 7000 rpm for 2 min to collect microcapsules. After drying, microcapsules were redispersed in water and centrifuged again at 7000 rpm for 2 min. A supernatant was collected for fluorescence spectroscopy. Fluorescence intensity from the supernatant was measured (ex 490 nm, em 526 nm for FBSA, ex 565 nm, em 586 nm for SRB). EE and loading content (LC) are calculated using the following formula:

$$EE\ (\%) = (1 - \frac{Fluorescence\ \ from\ non-encapsulated\ dyes}{Fluorescence\ from\ loaded\ dyes}) \times 100$$

$$LC\ (\%) = (\frac{mass\ of\ encapsulated\ dyes}{mass\ of\ capsules}) \times 100$$

Mass of encapsulated dyes was estimated from EE.

2.5. Optical Microscopy and graphical analysis

Optical micrographs were obtained by an optical microscope (Leica DM2500 M). The average droplet diameters for Exps 3, 4 and 6 were calculated by analysing at least seven hundreds of droplet using Image J. For Exp 2, the diameter of droplets was estimated from Fig. 2a by selecting random droplets with Image J.

2.6. Transmission Electron Microscopy (TEM)

TEM images were obtained by using a JEOL1400 TEM operating at an accelerating voltage of 100 kV. Images were recorded via the Gatan CCD imaging software. All TEM samples were prepared by dropping a 1 mg mL^{-1} emulsion on a formvar-supported copper grid. Excess solvent was drained using filter paper after 1 min.

2.7. Cryo Electron Microscopy (cryo-EM)

The vitrified specimens analysed in cryo-TEM were prepared using the following method; lacey carbon grids (Pro Sci Tech, QLD, Australia) were hydrophilized by glow discharge for 60 s (Pelco easiglow; Ted Pella Inc., CA, USA). Specimens were then prepared within a controlled environment using the Leica EM GP (Leica, NSW, Australia), relative humidity 80% at 25°C. Two and a half microliters of the sample solution was then pipetted onto the hydrophilized lacey grid, and blotted for 1.5s. The grid was then automatically plunged into liquid ethane at its freezing temperature (- 183°C) to form a vitrified layer. The grids were then transferred to a Dewar of liquid nitrogen (- 196°C) for transport and storage. The vitrified samples were examined using a FEI Tecani (FEI, OR, USA), operating at an accelerating voltage of 200 kV. We used a Gatan 626 cryo holder (Gatan, CA, USA), which maintained the sample below -172°C during both the transfer and imaging. Images were recorded digitally using an Eagle 2k CCD camera (FEI) and Digital Micrograph (Gatan). All samples were analysed using the low-dose software (FEI) to minimise beam exposure and electron beam radiation damage.

2.8. Confocal laser scanning microscopy

The microdroplets were spread on slides glass and mounted with coverslips. A LSM780 (Carl Zeiss) laser scanning confocal microscope was used to observe the samples. The system was equipped with an argon laser and a DPSS 561-10 laser connected to a Zeiss Axio Observer.Z1 inverted microscope. Zen imaging software was used for image acquisition and analysis.

3. MacroRAFT synthesis

3.1. RAFT polymerization of DEGMA using CPADB

DEGMA (10 g, 5.369 $\times 10^{-2}$ mol), CPADB (0.3 g, 1.074×10^{-3} mol) as RAFT agent, and AIBN (0.0176 g, 1.074×10^{-4} mol) as initiator were dissolved in toluene (21.5 mL) to give a [monomer]:[RAFT]:[initiator] molar ratio of 50:1:0.1. The reaction mixture was thoroughly purged with nitrogen gas for 30 min before being placed in an oil bath at 70 °C for 4h. After polymerization, the reaction was stopped by placing the mixture in an ice bath for 30 min. The polymer was isolated by precipitation in diethyl ether to yield poly(DEGMA) as a viscous red liquid. The monomer conversion was determined to be 35% via ^{1}H NMR ($M_{n,th}$ = 3536 g mol^{-1}, $M_{n,sec}$ = 4370 g mol^{-1}(with respect to PMMA standards), $Đ$ =1.1). ^{1}H NMR (300 MHz, $CDCl_3$):δ (ppm) = 4.1 (2*n*H, −COOC**H**$_2$-), 3.7-3.5 (6*n*H, −C**H**$_2$O), 3.4 (3nH, −OC**H**$_3$), 2.1−1.7 (2*n*H, CH_2 of the main chain), 1.1−0.9 (3*n*H CH_3 of the main chain), where n is the degree of polymerization.

3.2. *Synthesis of PDEGMA-b-PMMA macroRAFT agent*

Poly(DEGMA) macroRAFT agent (1.7155 g, 4.85 $\times 10^{-4}$ mol), MMA (9.7167 g, 9.70 $\times 10^{-2}$ mol) and AIBN (0.008 g, 4.85 $\times 10^{-5}$ mol) were dissolved in toluene (10 mL) to give [monomer]:[RAFT]:[initiator]=200:1:0.1. The solution was thoroughly degassed in an ice bath for 30 min before being placed in an oil bath at 60 °C for 18h. The polymerization was stopped by placing the solution in an ice bath for 30 min. The final solution was then precipitated in diethyl ether to yield a brittle, pink solid. The monomer conversion was 49% by ^{1}H NMR ($M_{n,th}$ = 13290 g mol^{-1}, $M_{n,sec}$ = 17353 g mol^{-1}(with respect to PMMA standards), $Đ$ = 1.07). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.6 (3*n*H,-COOC**H**$_3$), 1.9 - 1.8 (2*n*H, CH_2 of the main chain), 1 - 0.8 (3*n*H, CH_3 of main chain).

4. SPG membrane emulsification using amphiphilic macroRAFT agent as stabilizer

Inverse emulsions were prepared by SPG membrane emulsification using macroRAFT agent as stabilizer. Hydrophobic membranes with different pore size (0.1, 0.2, 0.8 and 3.0 μm) were used to produce different size droplets. A membrane was wetted with the continuous phase (toluene) before emulsification by ultrasonication. A representative emulsion preparation process is as follows: the macroRAFT was dissolved in toluene (30 g) to create the continuous phase of the emulsion. In a

separate vial, distilled water (1.5 g, 5 wt% rel to toluene) and NaCl or Na_2CO_3 (0.06 g, 4 wt% rel to water) were mixed to create the dispersed phase and then poured into the vessel. For guest molecule encapsulation, SRB or FBSA was dissolved in the dispersed phase as a model compound (0.01 (SRB), 0.05(FBSA) wt% rel to water). By applying pressure with nitrogen gas, the disperse phase was passed through membrane into the continuous phase which contains macroRAFT stabilizer. The emulsion formulations are listed in Table 1. The emulsification pressure was determined by gradually increasing pressure until the water started to permeate for each membrane.

5. Inverse Emulsion Periphery RAFT Polymerization

For a typical crosslinking reaction via inverse emulsion periphery RAFT polymerization, a solution consisting of MMA (monomer; 0.0313 g; 0.31×10^{-3} mol), EGDMA (crosslinker; 0.0077 g; 0.39×10^{-4} mol) and AIBN (initiator; 0.128 mg; 0.78×10^{-6} mol) was added to the mixture after emulsification to give a [M]:[CL]:[RAFT]:[I] ratio of 200:25:1:0.5 (Table S2). The resulting mixture, prepared in accordance with the procedure described above, was degassed by purging with nitrogen in an ice bath for 30 min. Polymerization was carried out at 60 °C for 6 h with constant stirring in an oil bath. After polymerization, 1 mL of the emulsion was collected for gravimetric analysis to obtain overall conversions. DLS and TEM analyses were carried out on the diluted emulsion (0.1 mL of emulsion in 2 mL of solvent).

Table S1. Synthesis parameters and molecular weight data of amphiphilic macroRAFT agents

	$[MMA]_0/[P(DEGMA)]_0/[AIBN]_0$	Time (h)	Conv. (%)	$M_{n,th}$ (g mol^{-1})	$M_{n,sec}$ (g mol^{-1})	PDI	HLB[a)]
$P(DEGMA_{17}$-*b*-$MMA_{97})$ "macroRAFT1"	200:1:0.1	18	49	13,290	17,353	1.07	5.3
$P(DEGMA_{16}$-*b*-$MMA_{82})$ "macroRAFT2"	200:1:0.1	18	41	11,479	14,983	1.07	5.8

All experiments except Exp 2 (Table 1 in main text) were prepared using macroRAFT1. Exp 2 was prepared using macroRAFT2.

[a)] HLB values were obtained from the number-average molecular weight ratio of the hydrophilic segment (M_h) and the total molecular weight (M): HLB = $20 \times \frac{M_h}{M}$

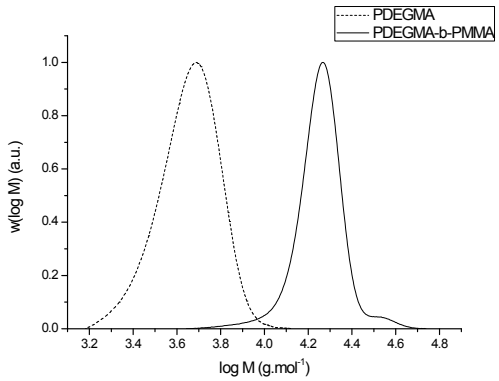

Fig. S1 Molecular weight distributions of poly(DEGMA) (dashed line) and poly(DEGMA-*b*-MMA) (solid line) ("macroRAFT1" as per Table S1).

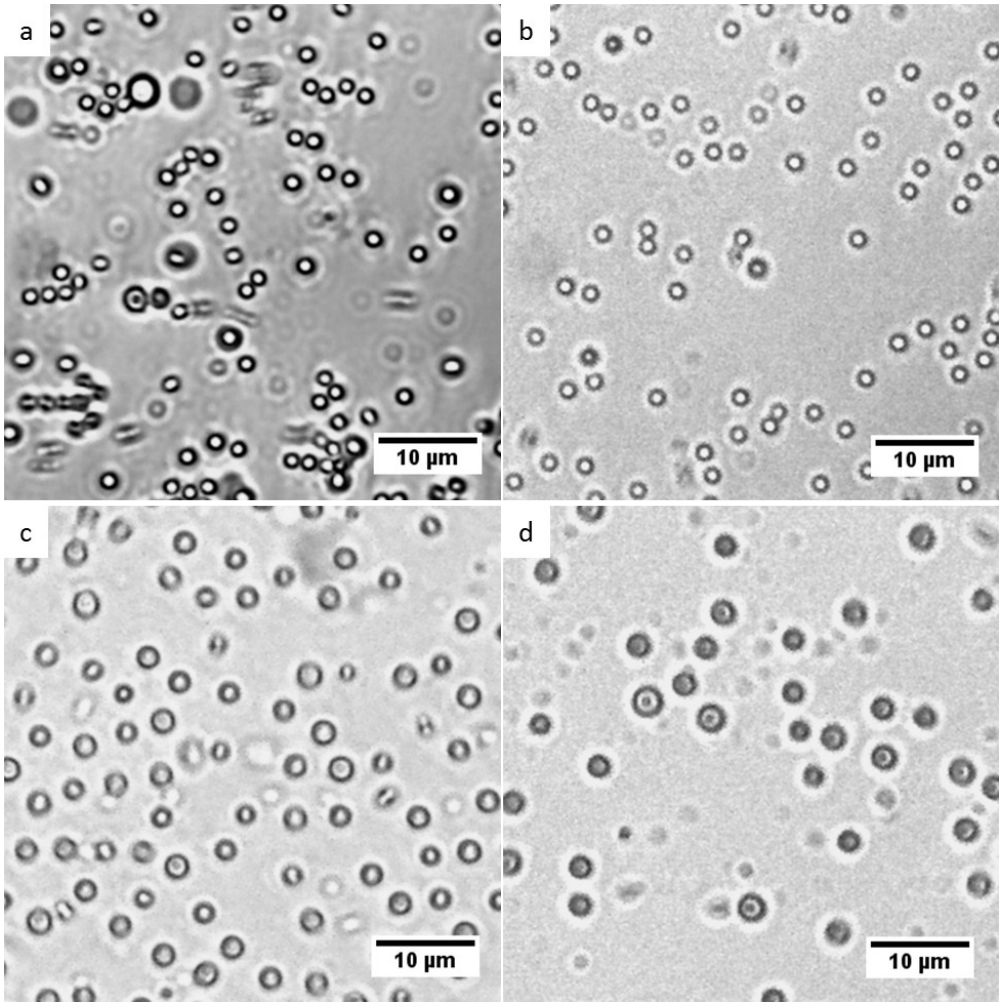

Fig. S2 Optical micrographs of droplets stabilized with different amounts of macroRAFT; (a) 0.05, (b)0.2, (c) 0.5, (d) 1 wt% rel to toluene (pore size 0.8 µm). These droplets were prepared using "macroRAFT2".

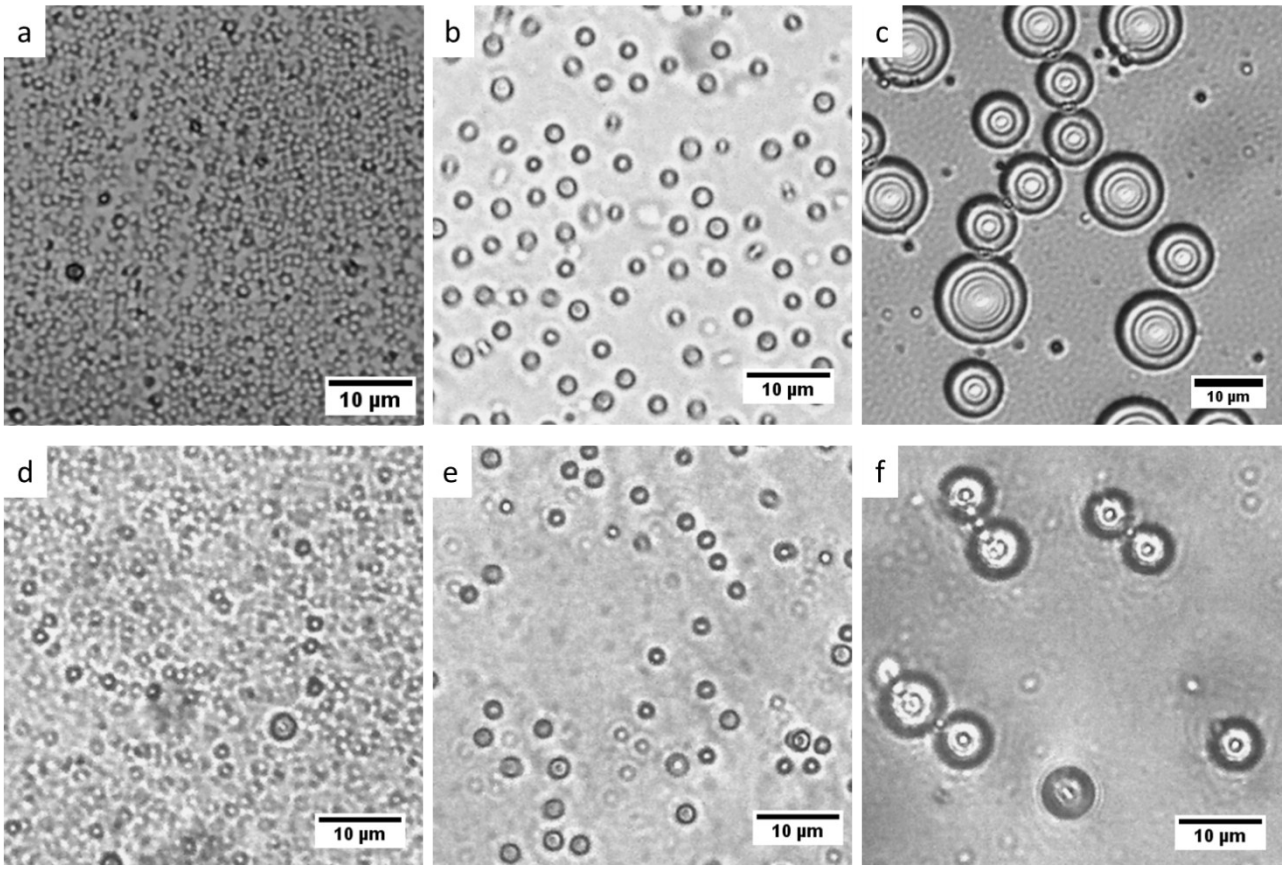

Fig. S3 Optical micrograph of droplets just after emulsification (a-c) and after one month at room temperature (d-f); Pore size =0.2 µm (a, d), 0.8 µm (b, e), 3.0 µm (c, f) ("macroRAFT2").

Table S2. Inverse miniemulsion periphery polymerization recipes

Continuous phase	Dispersed phase	Exp 2	Exp 3	Exp 4	Exp 6
toluene (g)		2.0772	2.0772	2.0772	2.0772
macroRAFT (g)[a]		0.0208	0.0104	0.0104	0.0208
MMA (g)[d]		0.0325	0.0156	0.0156	0.0313
EGDMA (g) [d]		0.0080	0.0039	0.0039	0.0077
	H_2O (g)[b]	0.1039	0.1039	0.1039	0.1039
	NaCl (g)[c]	0.0042	0.0042	0.0042	0.0042

[a] 10 (Exp 2, 6), 5 (Exp 3, 4) wt% rel to H_2O. [b] 5 wt% rel to toluene. [c] 4 wt% rel to H_2O.
[d] $[MMA]_0:[EGDMA]_0:[RAFT]_0:[I]_0$ molar ratio 200:25:1:0.5.
Note that "macroRAFT1" was used for Exp 3-6, while "macroRAFT2" was used for Exp 2 (see Table S1).

Table S3. DLS results

	d_n (nm)	d_i (nm)	d_v (nm)	PDI
Exp 2 –initial droplet	443	492	539	0.19
Exp 2 –crosslinked	395	471	967	0.34
Exp 3 –initial droplet	3964	4204	4579	0.12
Exp 3 –crosslinked	2476	3309	4131	0.30
Exp 4 –initial droplet	4460	4255	4781	0.05
Exp 4 –crosslinked	1997	1616	2762	0.24

Table S4. Laser diffraction results

	D(v,0.1) (μm)	*D(v,0.5)* (μm)	*D(v,0.9)* (μm)	$D_{4,3}$* (μm)
Exp 6 –initial droplet	2.00	4.45	6.76	4.42
Exp 6 –crosslinked	2.24	4.38	7.33	5.35

*volume average diameter

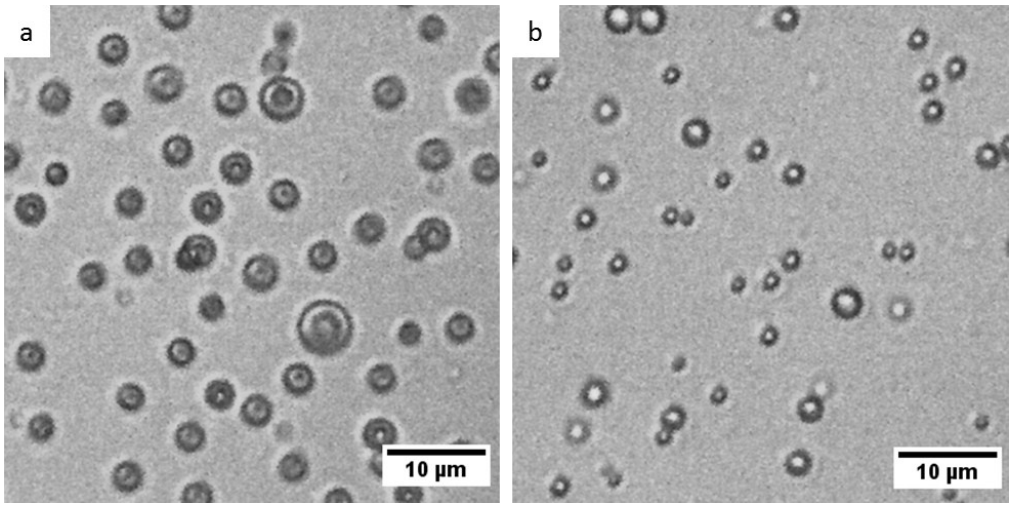

Fig. S4 Optical micrograph of droplets (Exp 3 in Table 1) (a) kept at room temperature and (b) kept at 60°C for 6 h.

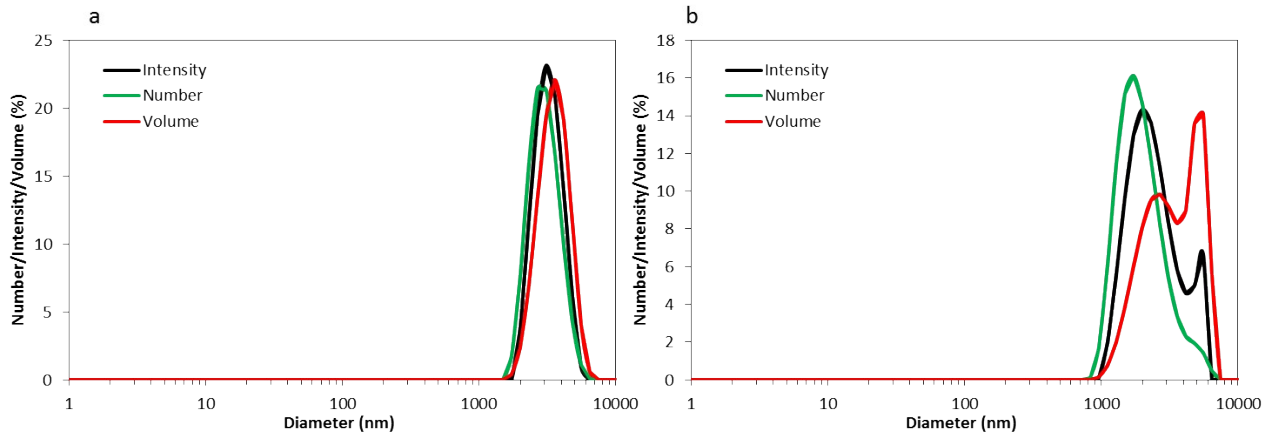

Fig. S5 DLS of droplets (Exp 3 in Table 1) (a) kept at room temperature and (b) kept at 60°C for 6 h.

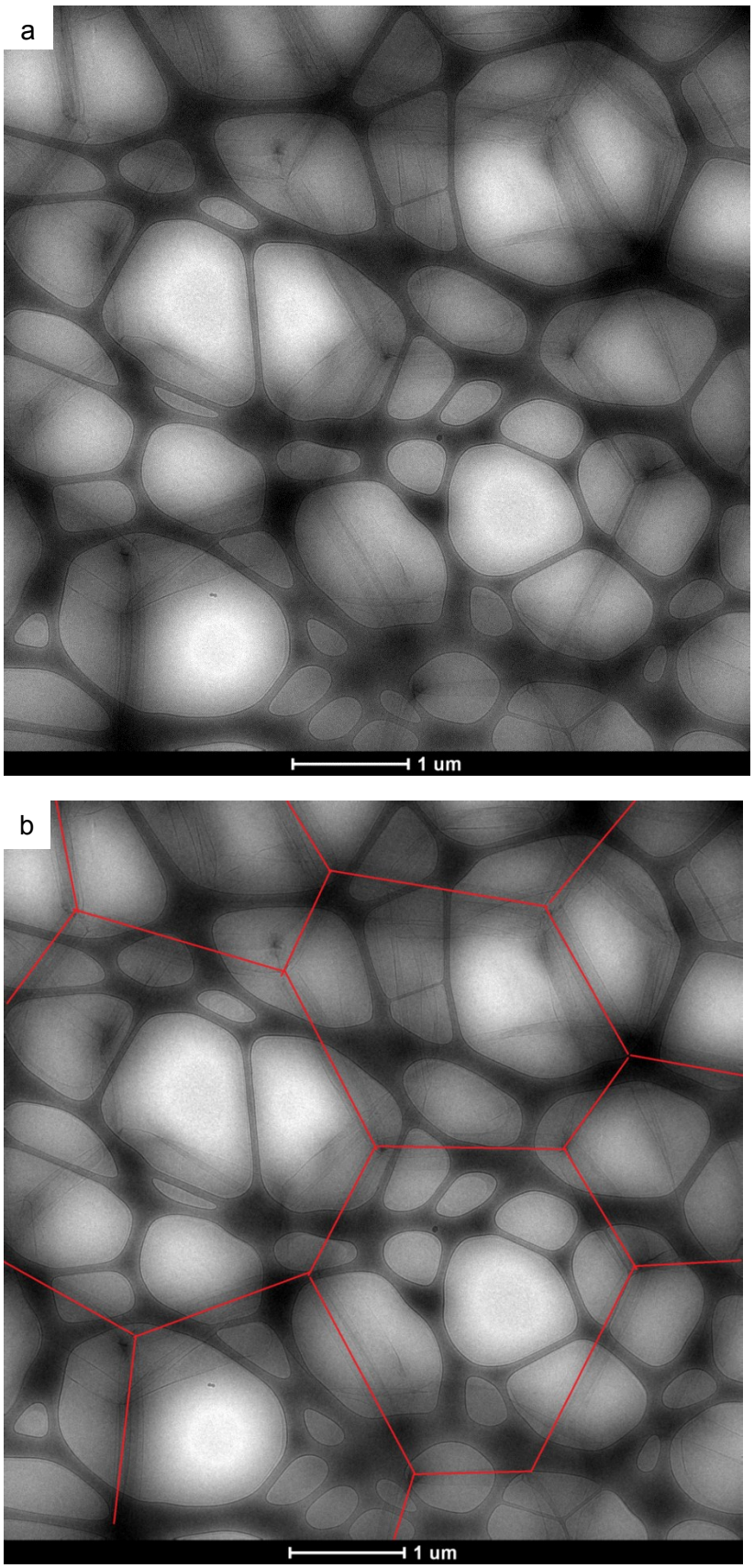

Fig. S6 Cryo-electron micrograph of Exp 3 (a: original, b: with lines added as guide to the eye indicating shells in Fig. S6b). These samples were placed on a lacey grid. The bright areas which have round shape are holes on a support film of grid.

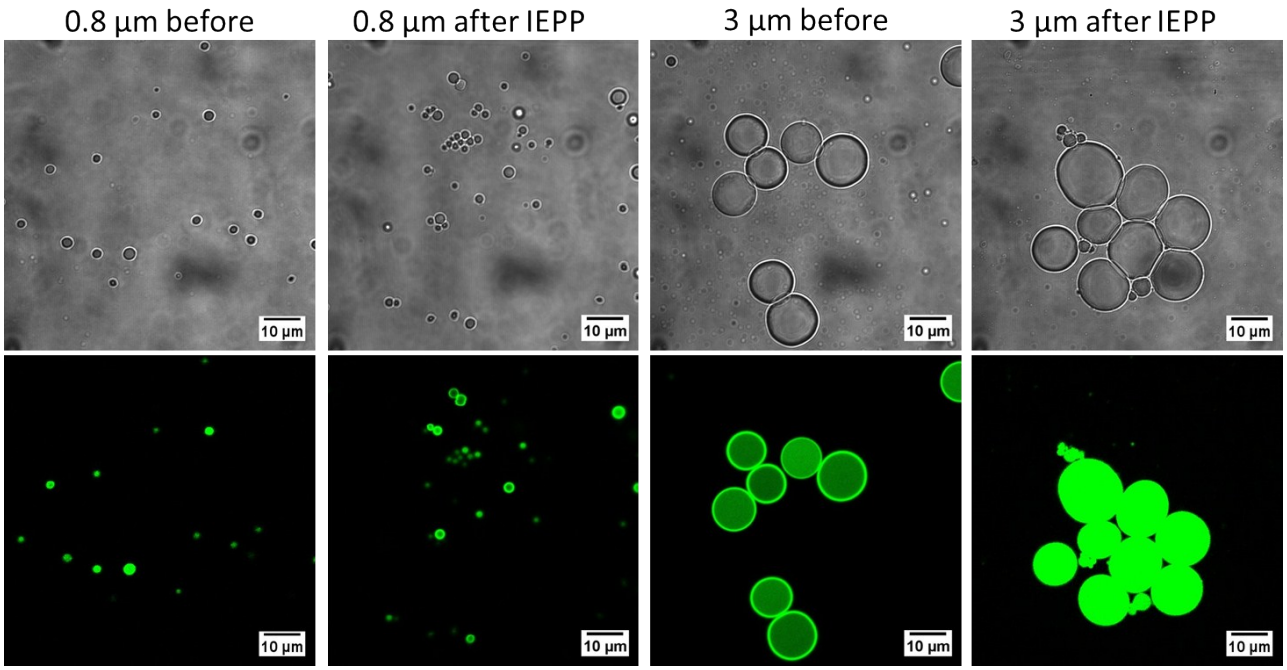

Fig. S7 Laser scanning confocal micrographs of microcapsules encapsulating fluorescein-labelled BSA before and after IEPP (see main text for details).

Table S5. Encapsulation efficiency and loading content of microcapsules

	EE(%)	LC(%)
FBSA (pore size 0.8 µm)	97	0.2
FBSA (pore size 3 µm)	98	0.2
SRB (pore size 0.8 µm)	99	0.01

Reference

1 Y. Mitsukami, M. S. Donovan, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2001, **34**, 2248-2256.