Electronic Supplementary Information

Supramolecular colloidosomes: fabrication, characterization and triggered release of cargo

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S1: MICROFLUIDIC DEVICE AND MICRODROPLET GENERATION

The microfluidic device was fabricated using soft lithography.¹ To make the mould, the design of the microfluidic device was printed as a negative photo-mask and then transferred onto a silicon wafer spincoated with SU-8 photoresist *via* UV photolithography. Poly(dimethylsiloxane) (PDMS) and the cross linker (Sylgard 184 elastomer kit, Dow Corning) in a 10:1 ratio were poured onto the mold and allowed to stand overnight at 70 °C. The PDMS layer, imprinted with the microfluidic channel design, was removed and, using a biopsy punch, inlets and an outlet were formed. The PDMS stamp and a glass slide were exposed to oxygen plasma for 8 s in a Femto Plasma Asher and then pressed together to seal the microfluidic channels. To render these channels hydrophobic, they were flushed with a 0.5% *v/v* solution of trichloro(1H,1H,2H,2H-perfluorooctyl)silane in Fluorinert FC-40 (3M) and finally cured at 120 °C for two hours prior to use.

The device design, as shown in Figure S1A, contains three aqueous inlets that intersect an oil flow at a T-junction. Droplets are generated at this intersection, before passing through a winding channel to aid mixing, and are finally collected onto a glass slide. The diameter of the junction is 40 x 40 μ m with a channel depth of 75 μ m. To generate microdroplets, three aqueous streams (PS-MV + CB[8], *p*-Np and cargo i.e. FD) with a combined flow rate of 120 μ L/h met a perpendicular flow of fluorous oil (220 μ L/h), where the shear forces caused formation of aqueous droplets in oil. The continuous phase comprised of the perfluorinated oil, FC-40 (3M), with 3 wt% surfactant (XL-01-171, Sphere Fluidics). To maintain constant droplet dimensions and component concentrations when comparing between droplets of different compositions, an equivalent flow of deionized water was used as a replacement. The resultant monodisperse microdroplets had a diameter of ~50 μ m, as shown in Figure S1B. It should be noted that while PS-MV does stabilize water-in-oil droplets, droplets with the opposite curvature i.e. oil-in-water droplets were unstable.



Figure S1 A Schematic of the microfluidic chip and B brightfield image of monodisperse microdroplets.

The microdroplets in perfluorinated oil (FC-40) were collected on a glass slide where evaporation of the aqueous phase resulted in the formation of colloidosomes. Droplets can also be collected and stored in a vial with perfluorinated oil. To clean the colloidosomes, the carrier oil is evaporated and subsequently the colloidosomes are washed with volatile fluorous oil (HFE-7500) to remove residual surfactant.

S2: MATERIALS AND INSTRUMENTATION

Commercially available compounds were obtained from Sigma Aldrich and, unless specified, all reagents and solvents were reagent grade or better and were used as supplied without further purification. FC-40 was obtained from 3M. All aqueous solutions were prepared in deionized water treated with a Milli- Q^{TM} reagent system ensuring a resistivity >15 M Ω cm⁻¹.

The surfactant used XL-01-171, was received from Dr. Xin Li, Sphere Fluidics Ltd (N.B. C. Abell is a founder of Sphere Fluidics).

Synthesis of PS-MV nanoparticles

1-Methyl-1'-(4-vinylbenzyl)-[4,4'-bipyridine]-1,1'-diium chloride iodide² (StMV, 0.23 g, 0.50 mmol) was dissolved in water (100 mL) to form a homogeneous solution. This was followed by the dropwise addition of a mixture of styrene (St, 5.208 g, 50.0 mmol) and divinylbenzene (DVB, 0.65 g, 5.0 mmol). This mixture was left to stir for 1 hour under an inert atmosphere, before elevating the temperature to 80 °C. Polymerization was initiated through addition of 2,2'-azobis(2-methylpropionamide) dihydrochloride (AIBA, 0.271 g, 1.0 mmol). After 24 hours, the resultant polymer nanospheres (PS-MV) were purified by dialysis and dispersed in water (100 mL). PS-MV nanoparticles were analyzed by: infrared spectroscopy, transmission electron microscopy, dynamic light scattering and had a zeta potential of +55 mV.



Figure S2 A Transmission electron micrograph and B FT-IR spectrum of the PS-MV nanoparticles.

Synthesis of *p*-Np and CB[8]

Synthesis of the naphthol-functionalised poly(N,N-dimethylacrylamide) polymer (*p*-Np) has been reported previously.² CB[8] was synthesized according to the procedure published by Day³ and Kim⁴ and further isolated and purified.⁵



Figure S3 Structure of the copolymer *p*-Np, where n = 10%.²

Instrumentation

For visualization and imaging of microdroplets a Vision Research monochrome Phantom v7.2 fast camera was used, attached to an Olympus IX71 inverted microscope. Fluorescence imaging was performed using an Olympus IX81 inverted optical microscope coupled with an EM-CCD camera, Andor Technology iXonEM+ DU 897. Scanning electron microscopy (SEM) images were recorded using a Leo 1530 variable pressure SEM with InLens detector. Laser scanning confocal microscope (LSCM) fluorescence measurements were carried out using a Leica TCS SP5 confocal microscope.

FITC-dextran was imaged with excitation at 468-508 nm and detection at 515-550 nm. The PS-MV nanoparticles were fluorescent across a broad range of wavelengths.

S3: VARIATION OF WALL THICKNESS WITH NANOPARTICLE CONCENTRATION

Droplets containing exclusively PS-MV nanoparticles (50, 100, 250, 500 μ M) were prepared as described in S2. Analysis by laser scanning confocal fluorescence microscopy, using the FITC filter preset (Ex: 488 nm) demonstrated that the PS-MV nanoparticles preferentially moved to the interface of the droplet, with corresponding fluorescence observed for all concentrations of nanoparticles. On increasing the concentration of PS-MV inside the droplet, the total fluorescence of the droplet was seen to increase. While initial increases resulted in an increase in fluorescence at the interface, further increases in concentration led to increasing fluorescence throughout the droplet. This is attributed to a saturation of the interface with nanoparticles, offering no further stabilization to PS-MV remaining in suspension within the bulk of the droplet.



Figure S4 Confocal fluorescent micrographs of microdroplets containing PS-MV nanoparticles. The images (top to bottom) are of methyl viologen concentrations of 50 μ M, 100 μ M, 250 μ M and 500 μ M. The fluorescence intensity across the cross-section of the droplet is shown to the right.

S4: CAPSULE AND CARGO



Figure S5 Confocal fluorescent micrographs of droplets containing PS-MV, CB[8], *p*-Np and cargo of FD (40 kDa, 3.3 μ M). **A** Fluorescence of the PS-MV colloidal particles at the droplet interface, **B** fluorescence of FITC-dextran cargo within the droplet and **C** overlay of A and B.

S5: FORMATION OF CORE-SHELL PARTICLES

Higher molecular weight FD (500 kDa, $3.3 \,\mu$ M) was found to inhibit the diffusion of the PS-MV within the microdroplet, preventing the formation of a colloidosome. This led to the formation of solid microparticles, rather than a capsule skin (Figure S6). Lowering the FD(500) concentration to 0.17 μ M within the droplet was found to result in colloidosome formation.



Figure S6 Images of core-shell particles formed on evaporation of droplets containing PS-MV, CB[8], *p*-Np and high MW FD cargo (500 kDa, 3.3 μ M). A Bright-field micrograph of the core-shell particles. **B** Fluorescence of the core-shell particles on exposing them at (562/40 nm) with emission (641/75 nm) and for comparison, **C** fluorescence micrograph of emission from both FD and the colloidal particles on exposure to light (~460 nm) and emission (~480 nm).

S6: Change in morphology of colloidosomes with the amount of fitcdextran encapsulated



Figure S7 SEM images showing the variation in PS-MV \subset CB \subset *p*-Np capsule structure on increasing the amount of cargo encapsulated from **A** empty, to containing **B** 0.3 µM and **C** 3.3 µM of FD cargo (150k Da).

The flexible nature of the polymer-linked colloidal wall results in the collapse of the colloidosome. This is expected and has been observed in similar colloidosomes stabilized with covalent and electrostatic polymer cross-links.⁶ The elastic nature of the colloidosomes can be inferred by the swelling under osmotic pressure when the dextran-loaded colloidosomes are hydrated in water. Our experiments have shown that increasing the concentration of PS-MV only leads to the formation of larger capsules with the same properties (e.g. porosity, S9). Further, increasing the concentration of copolymer (p-Np) to maximize cross-linking still results in collapsed colloidosomes upon evaporation.

S7: COMPARISON OF EVAPORATION AND COLLAPSE OF MICRODROPLETS CONTAINING EXCLUSIVELY PS-MV WITH THOSE CONTAINING PS-MV, CB[8] AND *P*-NP.



Figure S8 Bright-field micrographs of partially and completely dried capsules **A** drying of the Pickering emulsion with 100 μ M of PS-MV and **B** droplets containing PS-MV, CB[8] and *p*-Np.

S8: CARGO RELEASE STUDIES

Determination of fluorescence intensity and percentage cargo release

Dried capsules containing FITC-dextran cargo were collected on a glass slide and placed inside a Petri dish to limit evaporation during cargo retention studies. Hydration was carried out in deionized water (10 μ L) with fluorescence images collected over 60 minutes using a fluorescence microscope. The fluorescence intensity plots were obtained by analyzing the fluorescence images and calculating the mean colloidosome fluorescence intensity, using *ImageJ* (NIH).⁷ To compensate for background fluctuations (e.g. excitation intensity), this was divided by the mean fluorescence of the entire image to give the relative fluorescence intensity and finally normalized against the initial value, t₀.

When a colloidosome was wetted, FD inside the colloidosome began to swell by absorption of water until it filled the entire colloidosome, causing it to inflate. During this initial phase of inflation, there was no release of FD from the colloidosome, with the time required to fill the colloidosome not only dependent on the molecular weight of FD, but also varied between individual colloidosomes. To maintain uniformity this initial wetting period has been omitted from discussions of cargo retention.

After the initial inflation period (where passive release from the colloidosome was not observed) swelling of the colloidosome was found to be negligible and as such was discounted from the fluorescence analysis. Similarly we assumed that the net fluorescence intensity change due to diffusion of FD in and out of the field of view is negligible, resulting in the mean background fluorescence varying only with physical fluctuations. We assumed that the fluorescence quantum efficiency of fluorescein-dextran during the hydration process did not change, with bleaching of FD in the colloidosomes expected to have a corresponding effect on the background, and as such is removed during data processing. The fluorescence intensity plots obtained by this method were found to closely reflect the trends observed in the original series of images. To plot the graphs, an average of three colloidosomes was taken with the uncertainty in fluorescence intensity estimated to be $\pm 10\%$.

Modeling of release rates

Most release models within the literature refer to microparticles, where release from the solid matrix is either diffusion or erosion-limited. However, there are few attempts to model diffusion of cargo from a hollow colloidosome. One notable example modelled the release of fluorescein from sintered latex colloidosomes to determine the diffusion coefficient.⁸ We have attempted to apply their approach to passive release of cargo from our system; however the predicted diffusion coefficient is highly sensitive to the thickness of the colloidosome wall, which we currently do not know with sufficient precision. Furthermore, the fit of the modelled curves are not well matched to the experimental data, indicating this simple diffusion model may not be sufficient.

Cargo release from colloidosomes without cross-links

As discussed above, no release of FD was observed during the initial period after wetting for supramolecular colloidosomes. However, when directly hydrated with 1 mM ADA solution, the disruption of the supramolecular complex resulted in significant, immediate release of FD on wetting. Similarly, in the capsule-like colloidal assembly fabricated from PS-MV and *p*-Np in the absence of CB[8], FD was observed to be released immediately on wetting and continued throughout the swelling process. Both of these cargo release profiles are illustrated in Figure S9.



Figure S9 The release of FD (150 kDa, 3.3 μ M) on (i) hydration of the PS-MV \subset CB \subset *p*-Np capsule using 1 mM of the competitive guest, ADA in water and (ii) hydration of the PS-MV.*p*-Np capsule with water.

S9: CARGO RELEASE AND SIZE CHANGE WITH **PS-MV** CONCENTRATION

On partial evaporation of droplets containing PS-MV, CB[8] and *p*-Np, folds were seen on the surface of the droplet indicating formation of the colloidosome skin (Figure S8). At a given concentration of PS-MV, skin formation occurs at the same droplet diameter across all droplets. Once completely dry, the nature of the collapse of the hollow capsule results in colloidosomes folded in many different ways, giving the appearance of polydispersity. By measuring at the initial point of skin formation during evaporation, the capsule diameter was measured for PS-MV \subset CB \subset p-Np colloidosomes with [MV²⁺] = 17 μ M, 33 μ M, 50 μ M and 67 μ M. CB[8] and *p*-Np concentrations were kept constant at 17 μ M and consistently a cargo of FITC-dextran (FD150, 3.3 μ M) was used. For each concentration, the capsule diameter was measured to be a sphere, the surface area was calculated (Figure S10). The increase in concentration is reflected by an increase in capsule surface area. From the linear plot, there appears to be a certain concentration threshold at the interface for the capsules to be formed. In droplets with higher concentration of colloidal particles this threshold is reached earlier leading to the formation of larger capsules.



Figure S10 Plot of the change in surface area of the PS-MV \subset CB \subset *p*-Np capsules on variation of MV²⁺ concentrations while keeping CB[8] and *p*-Np constant at 17 μ M. [MV²⁺] = 17 μ M, 33 μ M, 50 μ M and 67 μ M.

On rehydrating the same capsules, it was seen that the capsules with $[MV^{2+}] = 67 \mu M$, $[CB[8]] = [Np] = 17 \mu M$. This may be attributed to the decrease in the relative cross-linking in the larger capsule. In comparison, when the ratio between PS-MV, CB[8] and *p*-Np was maintained at 2:1:1 (i.e. maintaining the relative cross-linking), and the concentrations doubled to $[MV^{2+}] = 67 \mu M$, $[CB[8]] = [Np] = 33 \mu M$, the capsules demonstrated a similar ability to retain cargo. The latter capsules were again much larger, due to the higher concentration of PS-MV particles and formed capsules with a diameter close to those formed with $[MV^{2+}] = 67 \mu M$, $[CB[8]] = [Np] = 17 \mu M$. This shows that the additional polymer has almost no effect on size of the capsule. This demonstrates that size of the capsule can be controlled by controlling the amount of material used, and that the uniformity in capsule property is maintained on changing the size of the capsule.

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