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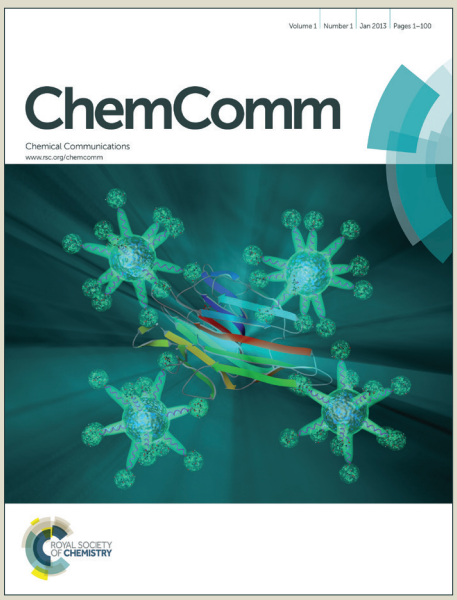

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ARTICLE TYPE

Supramolecular colloidosomes: fabrication, characterisation and triggered release of cargo†

Godwin Stephenson,[a] Richard M. Parker,[a] Yang Lan,[a,b] Ziyi Yu,[a] Oren. A. Scherman[a,b] and Chris Abell*[a]

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We report a one-step method of assembling supramolecular colloidosomes at the interface of microfluidic droplets. The self-assembly process utilises a versatile CB[8] host-guest system to reversibly crosslink polystyrene nanoparticles *via* a polyacrylamide linker. These micrometre-sized hollow structures can be loaded with water-soluble cargo during formation, which can then undergo triggered release.

Colloidosomes are microcapsules formed by locking a layer of amphiphilic colloidal particles at the surface of a micron-scale droplet.[1,2] The capacity to form a colloidosome is dictated by the ability of the component colloidal particles to self-assemble at the interface between two immiscible fluids.[3] Such colloidal assemblies are known as Pickering emulsions,[4] with the ability of the particles to wet both phases and pack densely at the interface key to their reported high stability.[3] Methods of permanently locking together such emulsion-stabilised particle layers include: thermal annealing or sintering of the colloids,[2] the addition of a polyelectrolyte,[5] gelation of the internal phase[6] and the formation of covalent bonds between the particles either directly or *via* a polymer.[7] Passive release of cargo from colloidosomes has been shown to be dependent on the size of pores between colloidal particles.[2, 8] Recently, methods to actively release cargo have been reported, including the use of temperature and pH-sensitive latex particles.[9] However, the inability to release encapsulated cargo on demand *via* milder or more versatile triggers has limited their usefulness.

Recently, we reported the formation of supramolecular microcapsules, self-assembled within microfluidic droplets.[10] The macrocycle cucurbit[8]uril (CB[8], Fig. 1C) was used to crosslink electron-rich naphthol-functionalised polymers and electron-deficient methyl viologen-functionalised gold nanoparticles. The formation of this high affinity supramolecular ternary complex (K_a up to 10^{12} M^{-2})[11] is driven by hydrophobic interactions and the release of high energy water from the CB[8] cavity.[12] Building upon this supramolecular microcapsule platform, here we describe the formation of Pickering emulsions of functionalised polystyrene nanoparticles and their conversion into stable colloidosomes through a supramolecular polymeric crosslink (Fig. 1A, 1B), and demonstrate the ability to encapsulate, retain and subsequently trigger the release of cargo through disassembly of the ternary supramolecular complex.

Aqueous microdroplets were formed from the periodic shearing of an aqueous flow by a perpendicular flow of an immiscible perfluorinated oil using a T-junction geometry within a microfluidic device (Fig. S1, ESI). The monodisperse microdroplets formed were typically 40-50 μm in diameter, dependent on the flow rate. The simultaneous loading of both the colloidosome-forming components and a molecular cargo during droplet generation ensured both high reproducibility and encapsulation efficiency. The microdroplets were transferred within the carrier oil onto a glass slide at room temperature, where evaporation of the inner aqueous phase occurred.

Initially, we focused on the formation of stable Pickering

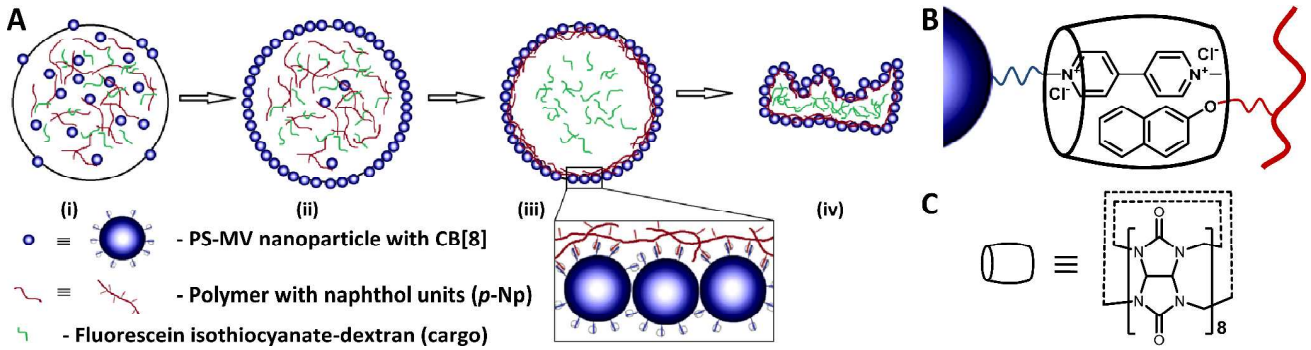

Fig. 1 A Schematic of colloidosome formation: (i) monodisperse aqueous droplets containing the methyl viologen-functionalised polystyrene nanoparticles (PS-MV), naphthol-functionalised polyacrylamide (*p*-Np), CB[8] and cargo as an emulsion in oil, (ii) self-assembly of PS-MV at the water-oil interface, (iii) the PS-MV nanoparticles are crosslinked with *p*-Np, *via* a ternary supramolecular complex with CB[8]; the cargo remains dispersed within the droplet, (iv) evaporation of the aqueous phase leads to further crosslinking at the thin shell wall and collapse of the flexible colloidosome structure. **B** Schematic of the ternary supramolecular complex formed between PS-MV, *p*-Np and CB[8]. **C** The molecular structure of CB[8].

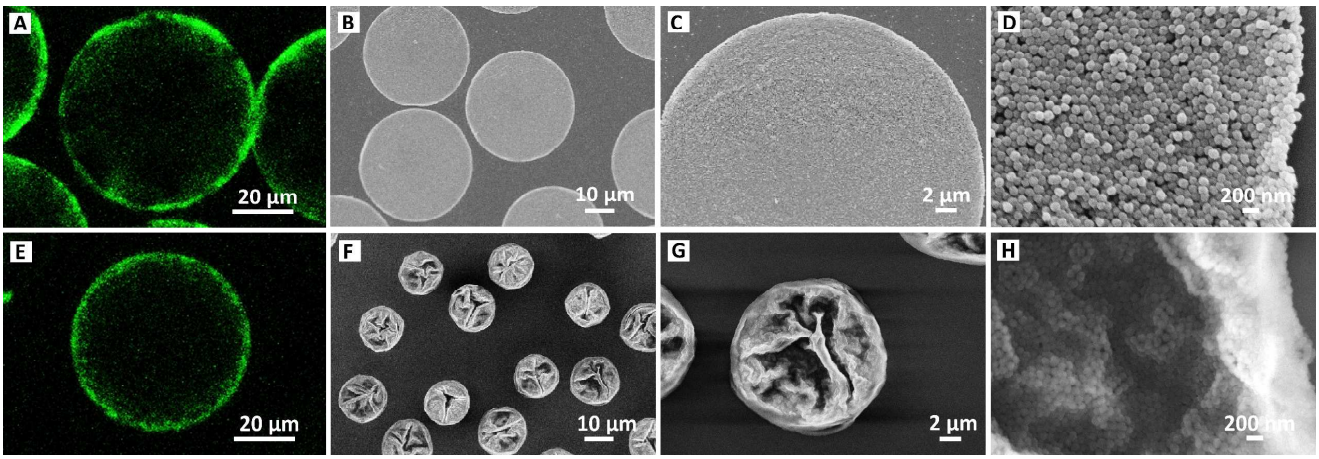

Fig. 2 A-D The colloidal assembly of PS-MV nanoparticles: **A** Laser scanning confocal micrograph of PS-MV assembled at the interface of the microdroplet. **B** Scanning electron micrographs (SEM) of the dried colloidal assembly. **C, D** Expansions illustrating the dense packing of PS-MV nanoparticles. **E-H** The colloidosome formed from interfacial assembly of PS-MV, CB[8] and *p*-Np: **E** Laser scanning confocal micrograph illustrating the localization of PS-MV at the droplet interface. **F** SEM of the dried colloidosomes formed by crosslinking PS-MV with *p*-Np *via* CB[8]. **G** An expansion of a single colloidosome showing the folded skin and **H**, to show the PS-MV nanoparticles embedded within the polymeric skin.

emulsions at the water-oil interface of microdroplets from a suspension of 100 nm methyl viologen-functionalised polystyrene nanoparticles, PS-MV, (zeta potential = +55 mV, for characterization see S2, ESI). These were visualised by laser scanning confocal microscopy, with a clearly-defined fluorescent layer localised at the droplet interface (Fig. 2A). Increasing the concentration of PS-MV within the droplet from $[MV^{2+}]$ = 50 µM to 500 µM led to an increase in the intensity of this layer. At 250 µM and above, fluorescence within the bulk of the droplet was also observed, indicating that saturation of the interface had occurred (Fig. S4, ESI).

Upon droplet evaporation, the accumulation of colloidal particles at the interface resulted in the formation of a capsule-like shell, with complete evaporation resulting in collapse to a flat structure. However, weak interactions between the nanoparticles often led to breakage of this shell. Scanning electron microscopy (SEM) of this structure is shown in Figures 2B and 2C, with the dense packing of PS-MV nanoparticles shown in Figure 2D.

To stabilise the colloidal shell, a copolymer of poly(*N,N*-dimethylacrylamide), comprising 10 mol% of naphthol-modified hydroxyethylacrylamide (*p*-Np, 10.1 kDa, Fig. S3, ESI)[13] was introduced to crosslink the PS-MV particles *via* a supramolecular complex with CB[8]. Microdroplets containing just the copolymer *p*-Np demonstrated a high rate of droplet coalescence. However, stable aqueous microdroplets containing PS-MV, *p*-Np and CB[8] at concentrations of 33, 17 and 17 µM, respectively were formed within the microfluidic device from a pre-mixed aqueous solution. The formation of a Pickering emulsion of PS-MV in the presence of *p*-Np and CB[8] was confirmed by laser scanning confocal microscopy (Fig. 2E). Evaporation of the inner aqueous phase resulted in the formation of a wrinkled skin that on complete evaporation collapsed without evidence of cracking to yield a highly folded colloidosome (PS-MV⊂CB⊂*p*-Np, Fig. 2F, 2G), with a densely packed layer of PS-MV nanoparticles embedded within the polymeric skin (Fig. 2H). This is in contrast with the situation depicted in Figure 2D, where individual PS-MV particles are clearly resolved.

To assess the ability of the PS-MV⊂CB⊂*p*-Np colloidosome to retain macromolecular cargo and to probe the porosity of the colloidal skin, the rate of release of fluorescein isothiocyanate-dextran (FD) upon hydration of the colloidosome was monitored by fluorescence microscopy. It was noted that empty colloidosomes were inherently fluorescent due to the broad spectrum fluorescence of the constituent PS-MV particles and this was accounted for in the following experiments. A series of FD cargoes ranging from 10 – 250 kDa were loaded simultaneously with the colloidosome-forming components in the aqueous flow during droplet generation (as above) to a final concentration of 3.3 µM within the microdroplet. Stable droplets containing FD were readily formed (Fig. S5, ESI), and the cargo was retained during droplet evaporation and corresponding colloidosome formation. Interestingly, higher molecular weight FD (500 kDa, 3.3 µM) was found to inhibit diffusion of PS-MV to the droplet interface, preventing colloidosome formation (Fig. S6, ESI). Empty colloidosomes were almost completely flat once dried, with little change upon addition of water, while those containing cargo exhibit a folded surface (Fig. S7, ESI).

Upon hydration, the FD was observed to slowly swell and spread, filling the interior volume of the colloidosome, with a corresponding increase in colloidosome diameter. As FD was retained within the colloidosome during this initial swelling period, the ability to retain macromolecular cargo over time was investigated only once this process was complete. To study the rate of passive release of FD from the colloidosomes, hydration was carried out using deionised water (Fig. 3A). It was found that colloidosomes containing FD of molecular weight greater than 150 kDa demonstrated markedly different behaviour to those containing smaller FD (10, 40, 70 kDa). FD(10) and FD(40) cargo followed a similar release profile with 50% of cargo released ($t_{50\%}$) in 11 minutes, with almost complete release in 30 minutes. FD(70) initially followed a similar release profile, but after 10 minutes the release rate slowed with a $t_{50\%}$ of 16 minutes. The larger FD(150) was released significantly slower with a $t_{50\%}$ of 40 minutes, with a steady rate observed throughout the experiment. In contrast, colloidosomes containing FD(250) only released 30% of their cargo over this time.

Droplets of PS-MV and *p*-Np prepared in the absence of CB[8] produced colloidosome-like structures upon evaporation.

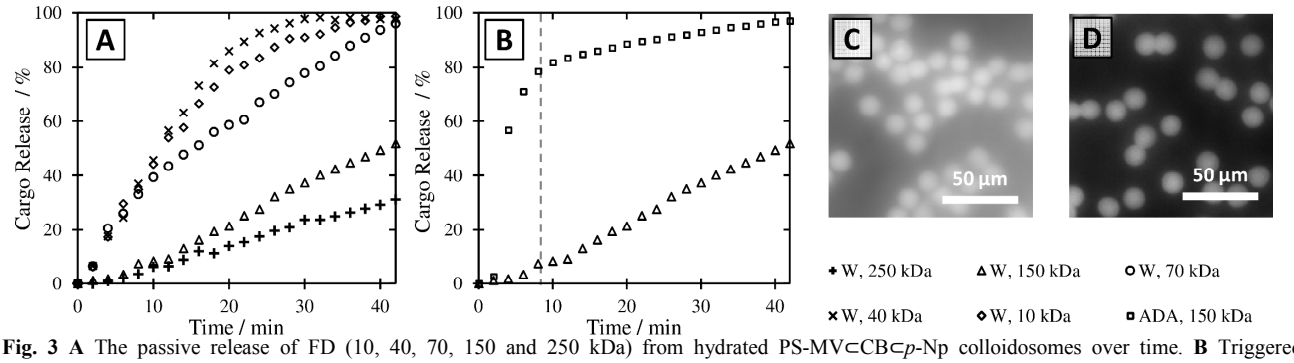

Fig. 3 **A** The passive release of FD (10, 40, 70, 150 and 250 kDa) from hydrated PS-MV⊂CB⊂*p*-Np colloidosomes over time. **B** Triggered disassembly of hydrated colloidosomes with the competitive guest, 1-adamantylamine (ADA), results in rapid release of FD (150 kDa) when compared with passive release over the same period. The difference between these release profiles 8 minutes after the addition of ADA (dashed transect) is illustrated with the fluorescence micrographs: **C** on addition of ADA (82% release) and **D** in deionized water alone (8% release).

However, upon rehydration, FD (150 kDa, 3.3 µM) was immediately released upon wetting, rather than after the initial swelling period. Complete loss to bulk media was observed in under 15 minutes from wetting (Fig. S9, ESI). Similarly, colloidal assemblies formed exclusively from PS-MV did not swell on hydration, but immediately released FD, with complete loss of FD of all molecular weights in under 5 minutes.

To demonstrate active triggered release of cargo from the supramolecular colloidosomes, the competitive guest for CB[8] 1-adamantylamine (ADA) was employed. ADA forms a strong 1:1 complex with CB[8],[14] with displacement of the methyl viologen and naphthol moieties. A 1 mM aqueous solution of ADA was used to ensure a large excess relative to CB[8] in the colloidosomes. Colloidosomes containing FD (150 kDa, 3.3 µM) were rehydrated with water. Once the initial swelling period was complete, ADA was introduced. Immediate, rapid release of FD into the bulk was observed – indicating breakdown of the supramolecular complex, with $t_{50\%}$ less than 1 minute after addition of ADA (Fig. 3B). The difference between triggered and passive release of cargo is illustrated at 8 minutes after addition of ADA (Fig. 3C, 3D). Colloidosomes exposed to ADA released 82% of FD(150) over this time-scale, compared to 8% for those hydrated in water alone. A second study, where dry colloidosomes were wetted directly with a 1 mM ADA solution resulted in release of FD during the initial swelling period (Fig. S9, ESI); with 50% released within 15 minutes of wetting. While slower, this release is analogous to the wetting of colloidosomes formed in the absence of CB[8].

In summary, we have demonstrated a one-step method of fabricating colloidosomes from microfluidic droplets, *via* supramolecular crosslinking. Slow, passive release of macromolecular cargo was observed, with the rate of release dependent on the size of the cargo. It is anticipated that a reduction in the size of the nanoparticle or an increase in the amount of crosslinking may further improve packing at the interface, allowing for improved cargo retention (Fig. S10, ESI). Triggered release, as demonstrated under mild conditions, can be initiated by disassembly of the supramolecular crosslink, with resultant rapid release of cargo. Here, a competitive guest was employed, but a broad range of stimuli can be envisaged for on-demand cargo release. Further studies on these supramolecular colloidosomes will involve the use of functionally-active nanoparticles and the development of a nanoparticle-only system.

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Notes and references

[a] *Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK. Tel: 787865; *E-mail: ca26@cam.ac.uk*
[b] *Melville Laboratory for Polymer Synthesis, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK. Tel: 787865; E-mail: oas23@cam.ac.uk*

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