Supplementary Information for:

Self-assembly of cross-linked β-cyclodextrin nanocapsules

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Contents:

Materials and methods

βCDS was synthesised as described in ref. [9] in the main text.

Dialysis tubing (molecular weight cut off 12.000 a.m.u.) was soaked in distilled water (30 min) and thoroughly rinsed prior to use. Dialysis was performed with magnetic stirring (18 h) against distilled water (4 dm^3) with one change of water after 2 h.

ICP-OES (inductively coupled plasma resonance plasma-optical emission spectroscopy) measurements were performed at the Microanalytical Laboratory, School of Chemistry, The University of Manchester using a Fisons Horizon ICP-OES.

Sulfur elemental analysis was performed at the Microanalytical Laboratory, School of Chemistry, The University of Manchester, using a Carlo Erba EA1108 Elemental Analyzer.

CHN elemental analysis was performed Exeter Analytical Inc., CE440 Elemental Analyzer, calibrated against Acetanilide and checked against S-Benzylthiuronium chloride internal standard.

IR spectra were recorded on a Thermo Nicolet Avatar 370 FT-IR spectrometer. Samples were analysed as KBr disks.

^1H and ^{13}C NMR were recorded on a JEOL-E270 instrument (^1H NMR at 270 MHz and ^{13}C NMR at 67.9 MHz). Spectra were recorded at ambient temperature with the exception of samples dissolved in d_6-DMSO. Samples in d_6-DMSO were recorded at 25ºC.

AFM analysis was performed on a Digital Instruments Nanoscope III Multimode instrument. One micron square scans were collected in air using tapping mode operated at a scan frequency of 1 Hz. Samples were spin coated onto mica and baked for 1 min at 50°C prior to examination by AFM.

UV-Vis spectroscopic analysis was performed using a Hitachi U3000 dual path spectrophotometer.

XPS measurements were performed on a Kratos AXIS HSi instrument equipped with a charge neutraliser and Mg K-alpha X-ray source. Spectra were recorded at normal emission using an analyser pass energy of 20 eV, X-ray power of 159 W and were energy referenced to the valence band and adventitious carbon at 285 eV. Survey scans were recorded at 160 eV pass energy.

Samples for rotary shadowing were suspended in H_2O, sprayed onto mica and allowed to dry. The samples were shadowed with platinum / carbon at an angle of 6º. The sample surface was then coated with carbon at 90º. The shadowed sample was then "floated" off the mica surface and allowed to dry. Samples were then placed on a carbon coated copper grid prior to visualisation using a JEOL 100CX transition electron microscope.

Preparation of βCDS capsules

Preparation of βCDS capsules in water

βCDS (10.0 mg, 8.0×10^{-3} mmol) was vigorously stirred in water (500 ml) in the presence of air (15 h) The solution was concentrated to ~50 ml. The resulting mixture was filtered through a 0.45 μM PTFE filter to separate any insoluble material followed by drying under reduced pressure to leave an off-white material (4.3 mg, 43%).

Preparation of βCDS capsules at high pH

βCDS (50.0 mg, 4.0×10^{-2} mmol) was dissolved in 0.1 M aqueous sodium hydroxide (500 ml). The solution was stirred in the presence of air (24 h) and then neutralized with hydrochloric acid (5 ml, 12 M) resulting in slight precipitation. The solution was concentrated and dialysed against distilled water to remove any inorganic salts. The resulting mixture was filtered through a 0.45 μM PTFE filter to separate any insoluble material followed by drying under reduced pressure to leave an off-white material. (31.5 mg, 63%).

Preparation of βCDS capsules at using hypoiodide as an oxidising agent

βCDS was also prepared using 0.5 equivilents of $[OI]^-$ (per sulfur) as an oxidising agent. βCDS (100 mg, 0.08 mmol, 0.56 mmol sulfur equivalents), iodine (71.2 mg, 0.28 mmol) and potassium iodide (1.5 g) were stirred for 24 h in 0.1 M $NaOH_{aq.}$ (500ml). The reaction was the worked up as for the polymer prepared at high pH. βCDS capsules were isolated (56.6 mg. 57%).

ICP and elemental analysis characterisation of βCDS capsules prepared at pH 13.

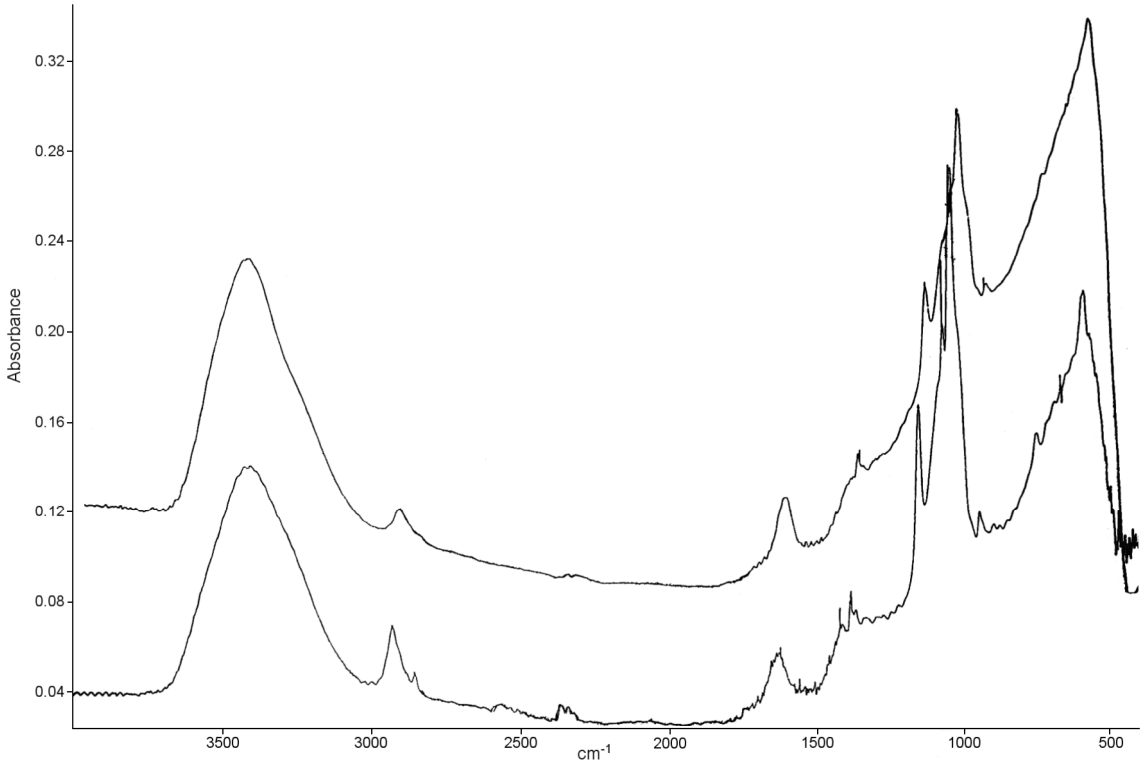
	Elemental analysis			ICP results	
	C, %	H, %	N, %	S, %	Na, %
Found	34.77	4.76	0.26	14.55	3.82
Calculated[a]	34.71	4.96	0.43	14.92	3.07

[a]Calculated for the following mixture: 92.0% βCDS, 2.2% DMF, 5.8% H_2O. βCDS was assumed to contain 2 sulfonic acid groups and 2 Na^+ counterions per molecule.

Comparison of CHN/ICP and XPS elemental analysis results.

Sample	Results from CHN ICP analysis	Results from XPS analysis
	Disulfide bonds per cyclodextrin	Disulfide bonds per cyclodextrin
$(\beta CD\text{-}S)_n$ polymer (1) base	6.2	5.4
$(\beta CD\text{-}S)_n$ polymer (2) hypoiodide	5.8	6.0
$(\beta CD\text{-}S)_n$ polymer (3) H_2O_2 55 min	4.3	3.3
$(\beta CD\text{-}S)_n$ polymer (4) H_2O_2 24 h	3.1	2.1

Figure S1. IR spectra of βCDS (bottom) and βCDS capsules (top)



Assay of thiol concentration during oxidation of βCDS

βCDS was stired in 0.1 M $NaOH_{aq.}$ (~ pH 13) following the standard synthetic procedure for preparation of βCDS polymer. The samples were then assayed using Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid)]. To avoid rapid oxidation of Ellman's reagent at the high pH of the reaction mixture, the assay was carried out in pH 8.2 in tris buffer (0.1M).

Ellman's reagent stock solution 4 mg / ml was made up using degassed pH 8.2 tris buffer (0.1 M) and stored in the dark on ice.

For each assay, Ellman's reagent stock solution (100 μl) was added to degassed buffer (5 ml) followed by addition of the βCD-SH reaction solution (500 μl). A blank solution was made up as above, containing of buffer (500 μl) in place of βCDS and was used to obtain a baseline for UV-Vis spectroscopy. Each solution was incubated for 5 min before the absorbance was measured at 412 nm.

A calibration curve was prepared in the same concentration range as the βCDS reaction mixture. Cysteine dissolved in degassed buffer was used to make up 7 standard solutions (0 – 1.25 mM) and analysed as described above. The absorbance at 412 nm was plotted against concentration to give a gradient which was subsequently used to estimate the thiol concentration of the βCDS reaction mixture.

Figure S2. ^{1}H NMR spectra of βCDS (1) and βCDS nanocapsules prepared in base (2), in the presence of hypoiodite (3) and in the presence of H_2O_2 (24 h) (4)

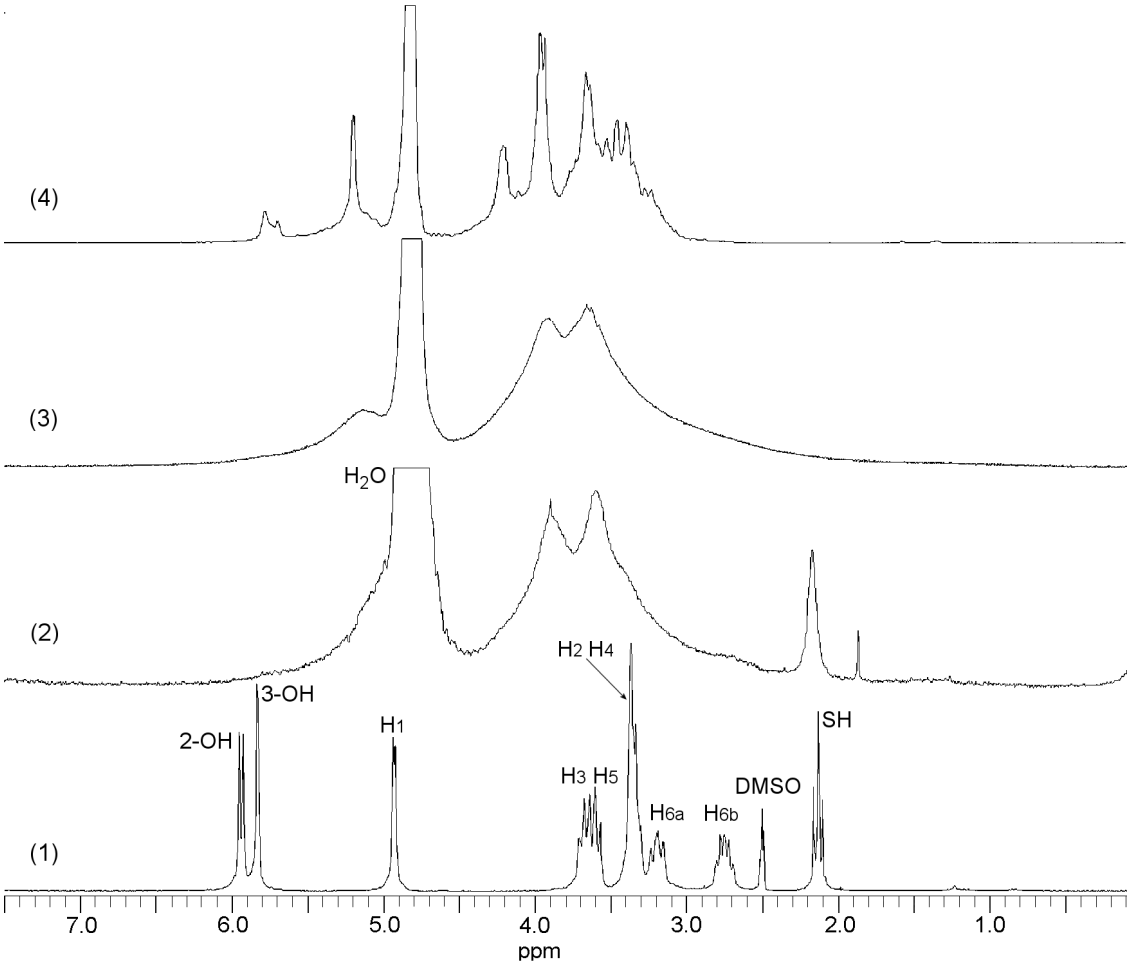


Figure S3. ^{13}C NMR spectra of βCDS (1) and βCDS nanocapsules prepared in the presence of hypoiodite (2) and in the presence of H_2O_2 (24 h) (3)

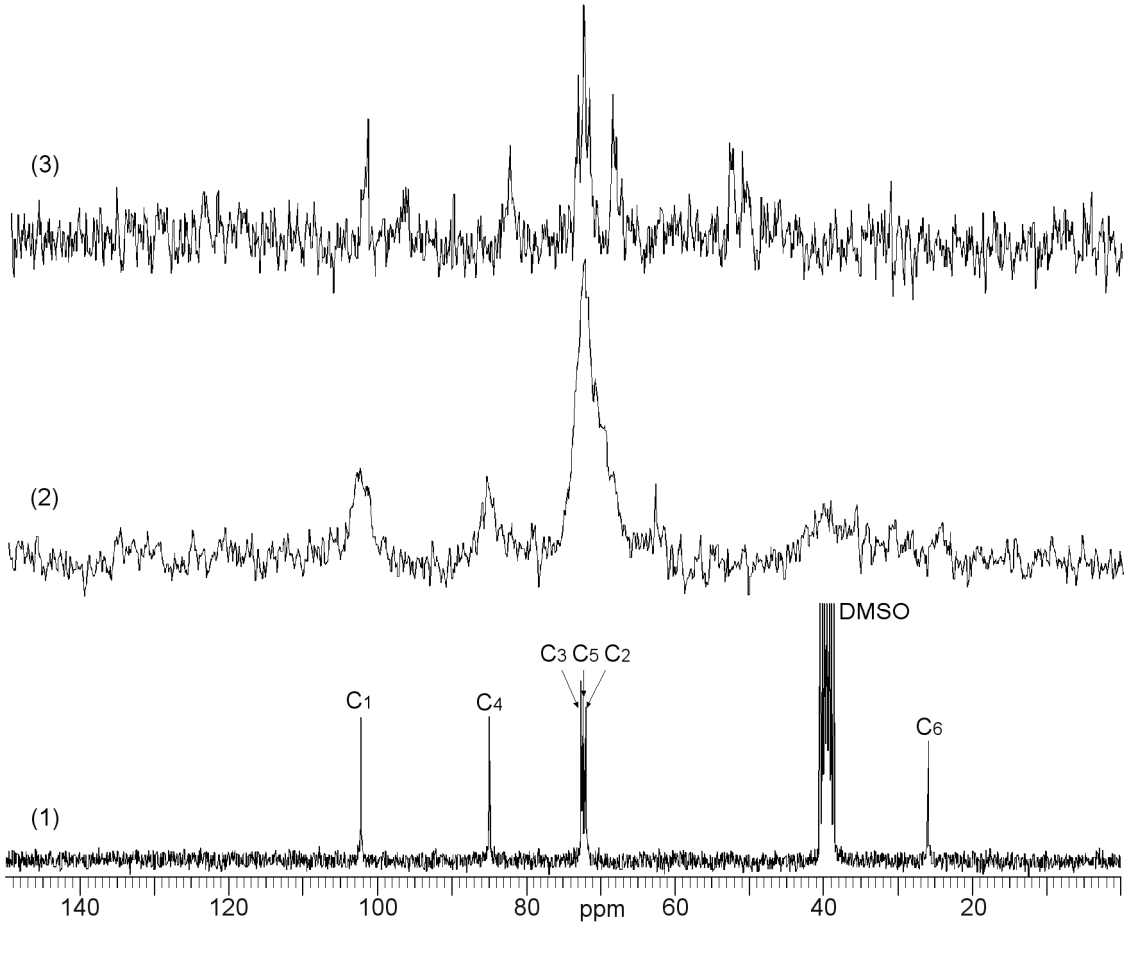


Figure S4 XPS spectra for βCDS (i), $(βCDS)_n$ polymer prepared in base (ii), hypoiodite (iii), H_2O_2 for 55 min (iv) and H_2O_2 for 24 h (v).

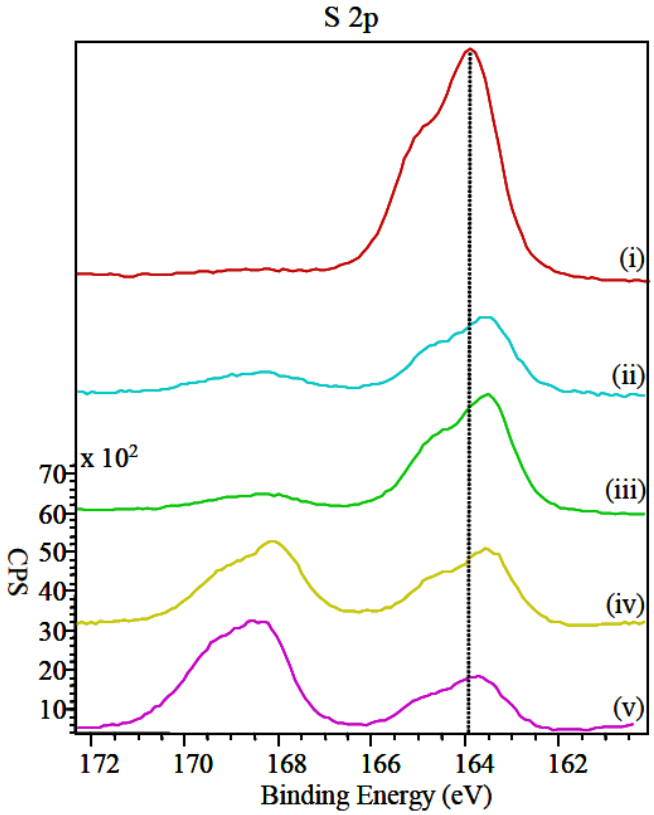
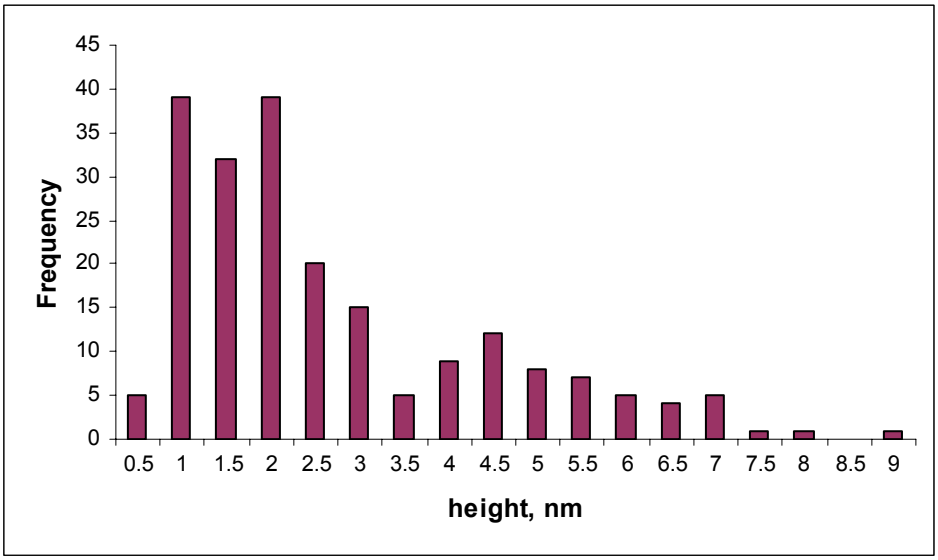
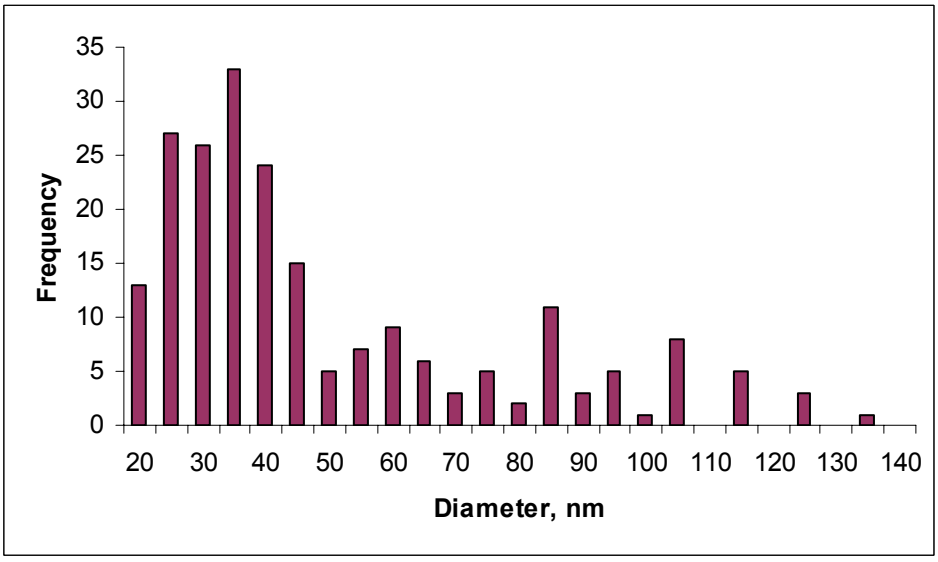


Figure S5. Statistical analysis of AFM images of βCDS nanocapsules

Distribution of capsule heights in the AFM images

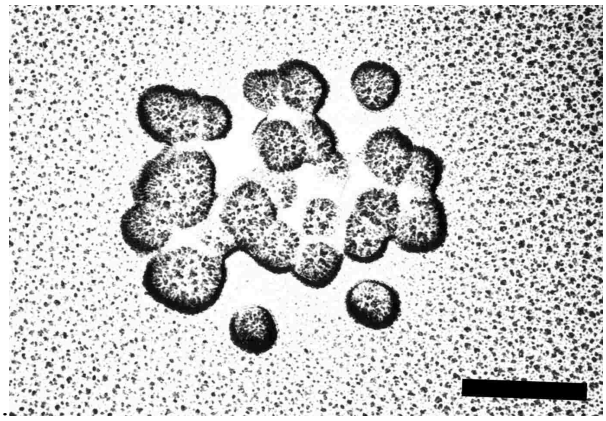


Nanocapsule diameter histogram

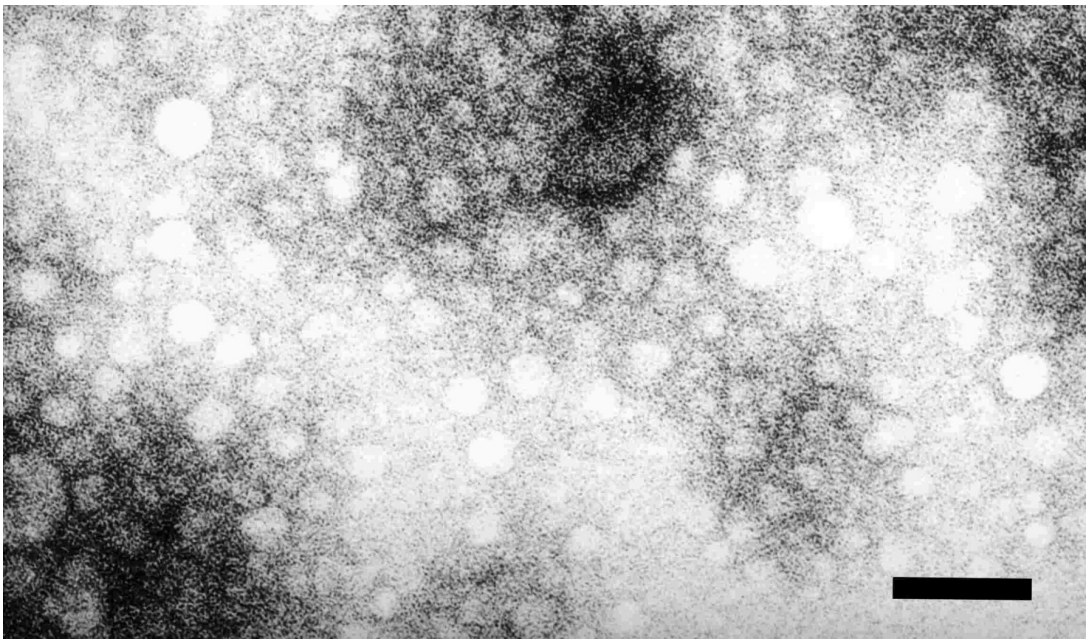


The size of the features was found to be independent of the initial concentration of the sample solution suggesting that the structures are stable and not in dynamic equilibrium with solution-based species.

Figure S6 TEM images of βCDS polymer: rotary shadowing of a freeze fractured sample (a) and negative staining of polymer with uranyl acetate (b). Scale bar is 100 nm in both images. Both images showed spherical features ca. 30±5 nm in diameter.



a



b

Encapsulation of Reichardt's dye in βCDS capsules

A stock solution of Reichardt's dye in acetone (1 mg ml^{-1}) was prepared. In each case an aliquot (1 ml) of the Reichardt's dye stock solution was transferred to a flask and deposited on its walls by flushing with N_2 to evaporate the acetone. NaOH aq. (0.1 M, 50 ml) was added, along with βCDS (10 mg), and the solution was sonicated (5 s) before being rapidly stirred in the presence of air (24 h). Control solutions were prepared in exactly the same manner without βCDS and with β-cyclodextrin (10 mg). After 24 h, an aliquot of each sample 2.5 ml) was filtered through a 0.45 um filter and UV spectrum of each sample was recorded using 0.1 M $NaOH_{aq.}$ as a background. A further portion of each sample (10 ml) was then dialysed against distilled water to remove free Reichardt's dye. The NaOH concentration was corrected by the addition of 5M $NaOH_{aq.}$ and the UV spectra recorded again. The volume of solution was measured before and after dialysis to correct the spectra for dilution.

The remainder of the solution of βCDS polymer formed in the presence of Reichardt's dye was placed under nitrogen and a 250-fold excess of mercaptoethanol was added. The mixture was stirred for 24 h, filtered through a 0.45 μm PTFE syringe filter to remove insoluble material and dialysed prior to UV-Vis analysis.

A control experiment was conducted by adding a 250-fold excess of mercaptoethanol to βCDS polymer (5 mg) in 0.1 M $NaOH_{aq.}$ The solution was stirred under nitrogen (24 h) filtered and dialysed prior to UV-Vis analysis.