Electronic Supplementary Material (ESI) for Chemical Communications

A Supramolecular Route Towards Core-Shell Polymeric Microspheres in Water *via* Cucurbit[8]uril Complexation

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S.1 Synthesis and characterization of C1

As shown in Figure S1, the MV-functionalised polymer microspheres of **C1** was synthesized using post-functionalisation[1]: Firstly, cross-linked core-corona microspheres of **C0** were prepared using soap-free emulsion polymerization.[2] Secondly, **C0** were modified using MV-functionalised isocyanate of MV-Hex-NCO di(hexafluorophosphate).

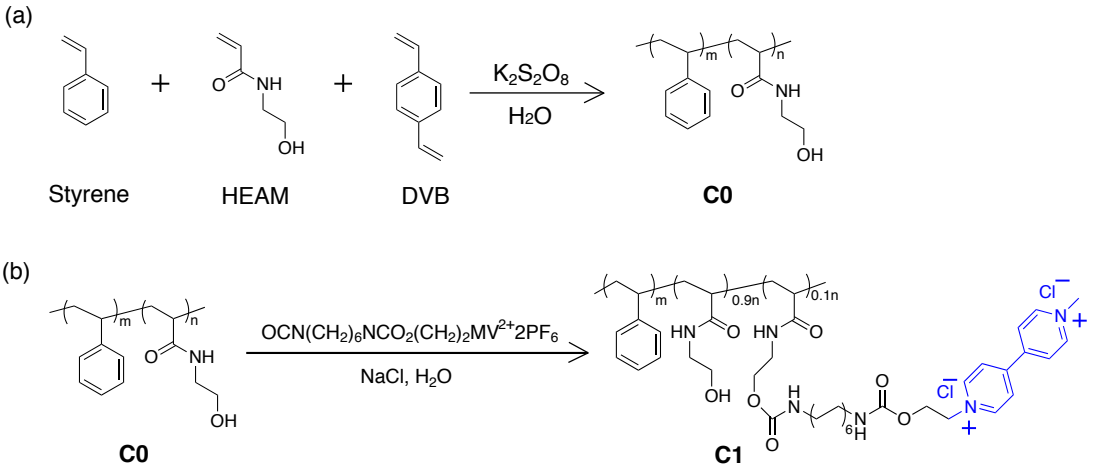


Figure S1: (a) Schematic synthesis of **C0**; (b) Synthesis of **C1**.

Figure S2a shows the TEM image of **C0**. Monodispersed microspheres with the size of 261 $\pm$ 29 nm can be observed from the TEM image. Figure S2b shows the hydrodynamic diameter distribution of **C0** in neutral aqueous solution measured by DLS. The average hydrodynamic diameter (D_h) of **C0** is 338 nm and with a low PDI of 0.13. The D_h of **C0** is larger than the size measured by TEM since **C0** swells in water.

Figure S2c shows the TEM image of **C1** with the size of 249 $\pm$ 38 nm. Figure S2b displays the hydrodynamic diameter distribution of **C1** in neutral water measured by DLS. The D_h of **C1** is 425 nm with a PDI of 0.21. Compared to the D_h of **C0**, the D_h of **C1** is much larger, which might results from the fact that **C1** has charged components of MV.[3]

Figure S2d, the ZP of **C0** in an aqueous suspension (1.0 mg/mL) is -63.0 mV since it is stabilized by negative charges from the dissociation of the initiator of $K_2S_2O_8$ (KPS).[4] The ZP of **C1** at the same condition is +44.9 mV as it is stabilized by the hydrophilic and positively charged MV. The increased zeta potential further confirmed the formation of **C1**.

The synthesis of **C1** was further tracked by FTIR spectrometer. Line a in Figure S3 is the FTIR spectra of **C0**. The characteristic absorption at 1600, 1493, 1452, 757, and 697 cm^{-1} are attributed to the PS segment, and two broad absorption at 3200-2700 cm^{-1} are ascribed to the methylene units and the N-H stretching vibration. The absorption at 1660-1780 cm^{-1} is originated from to the carbonyl groups in the PHEAM segments. Moreover, the wide absorption at 3500-3200 cm^{-1} is ascribed to the stretching vibration of O-H in PHEAM segment. Line b is the FTIR spectra of **C1**. Compared to the FTIR spectra of **C0**, the weakening absorption at O-H stretching vibration absorption region (3500-3200 cm^{-1}) and increasing absorption at carbonyl absorption region (1660-1780 cm^{-1}) confirm the formation of **C1**.

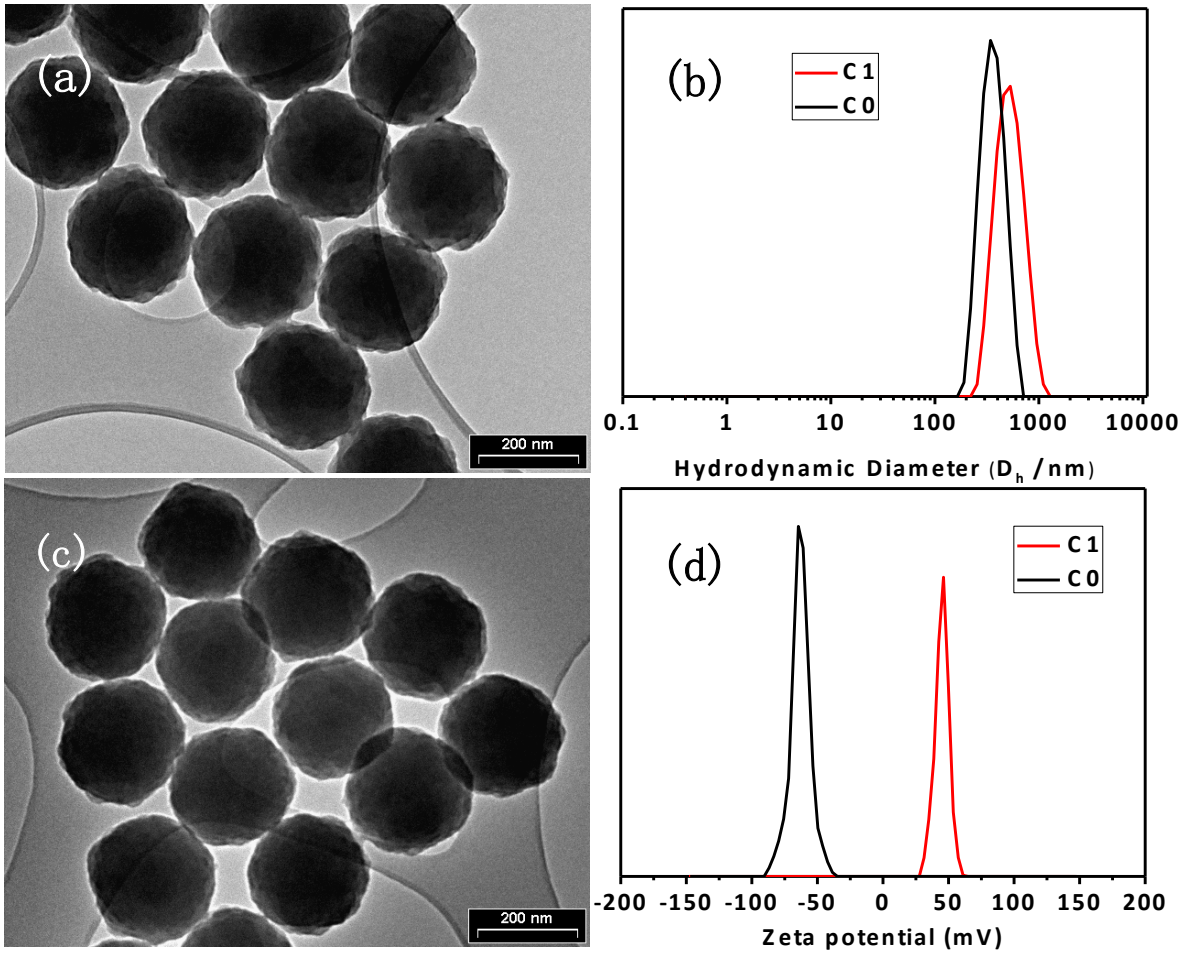


Figure S2: (a) TEM image of **C0**. (b) Hydrodynamic diameter distribution of **C0** and **C1** in water. (c) TEM image of **C1**. (d) Zeta potential of **C0** and **C1** in water.

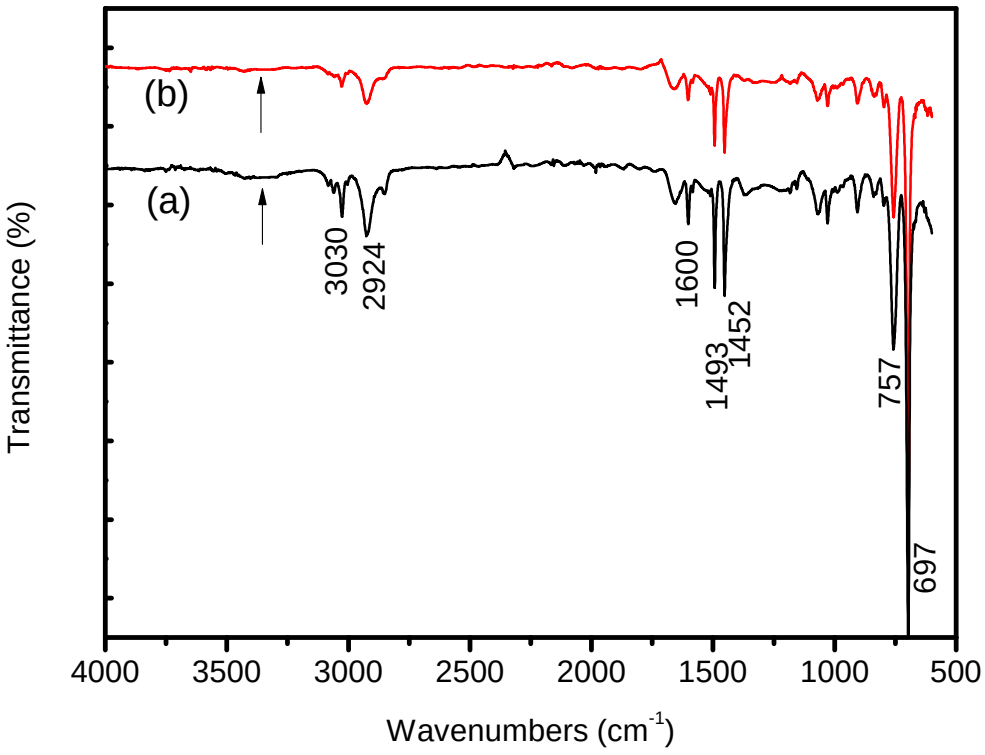


Figure S3: FTIR spectra of (a) **C0** and (b) **C1**.

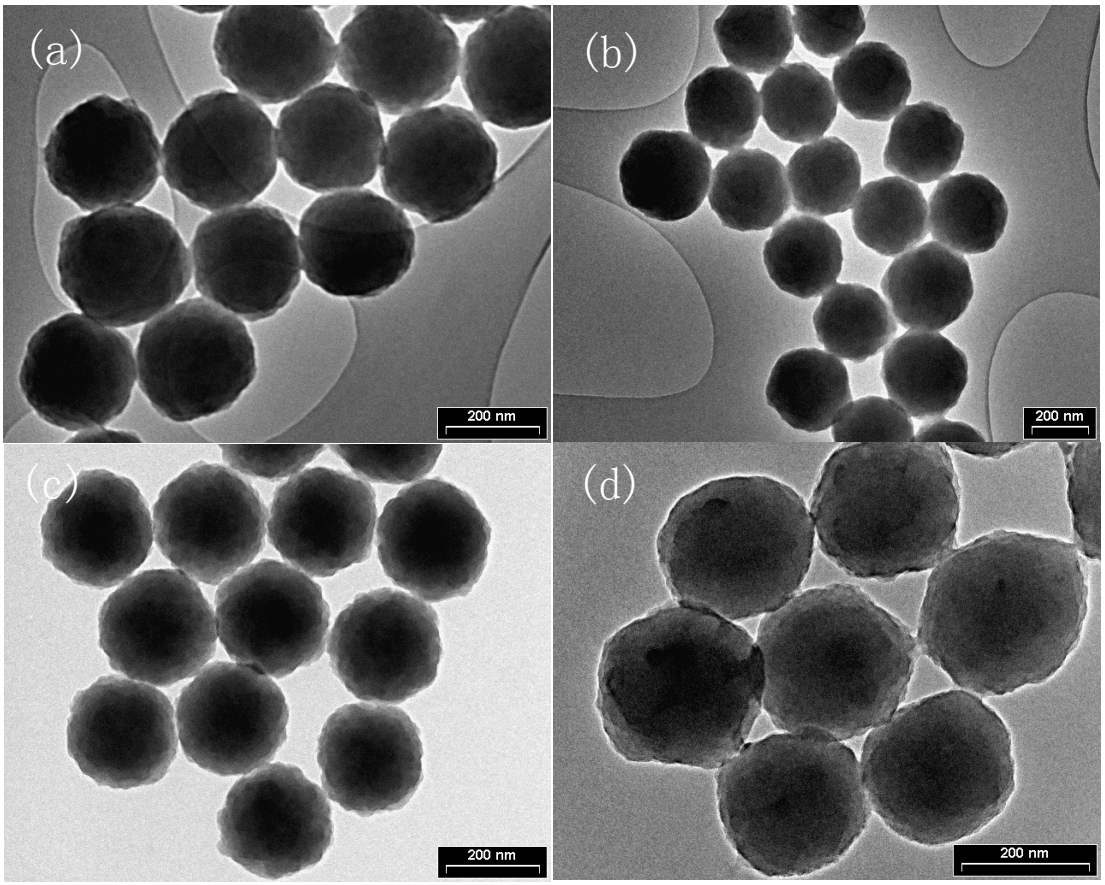


Figure S4: TEM images of (a) **C1**+**P1**, (b) **C1**+CB[7]+**P1**, (c) **C0**+CB[8]+**P1** and (d) **C1**+CB[8].

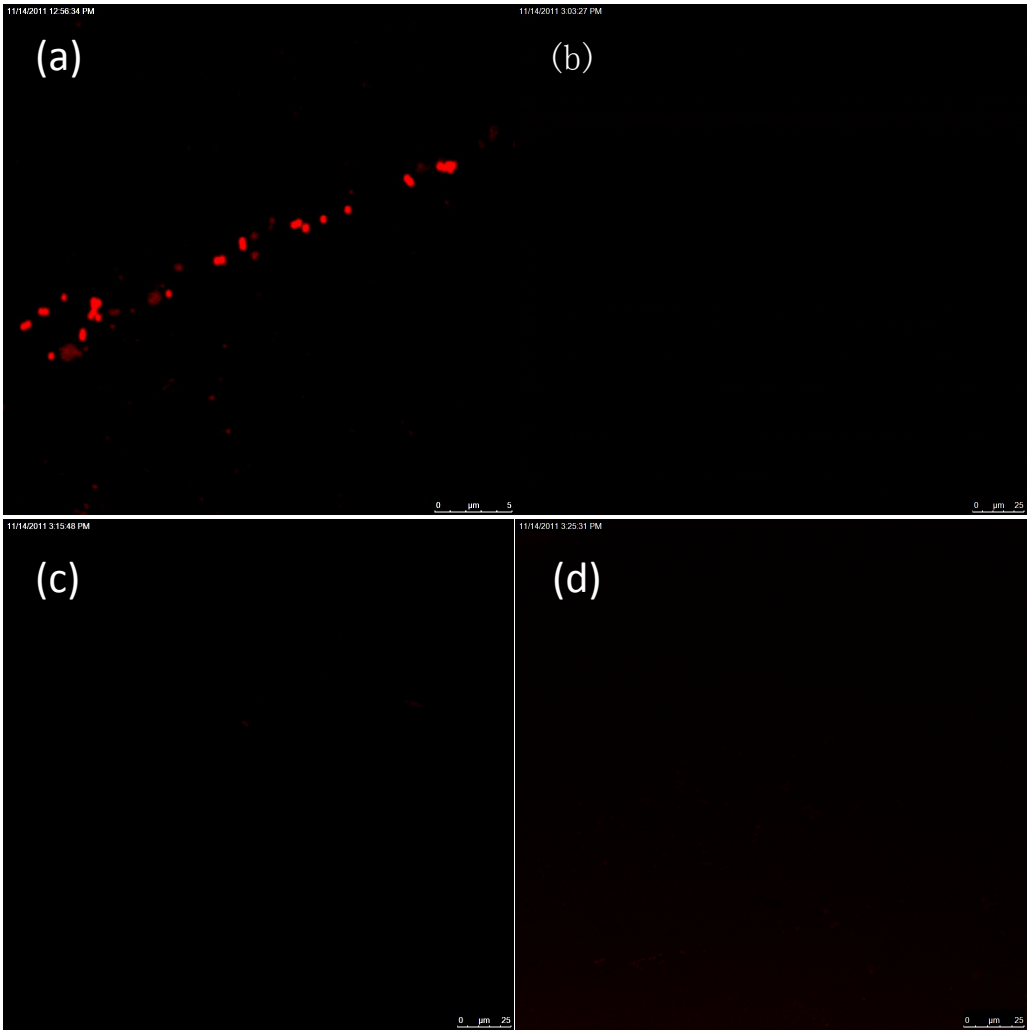


Figure S5: CLSM images of (a) **C1**+CB[8]+**P2**, (b) **C1**+**P2**, (c) **C1**+CB[7]+**P2** and (d) **C1**+CB[8]+**P2**+ADA.

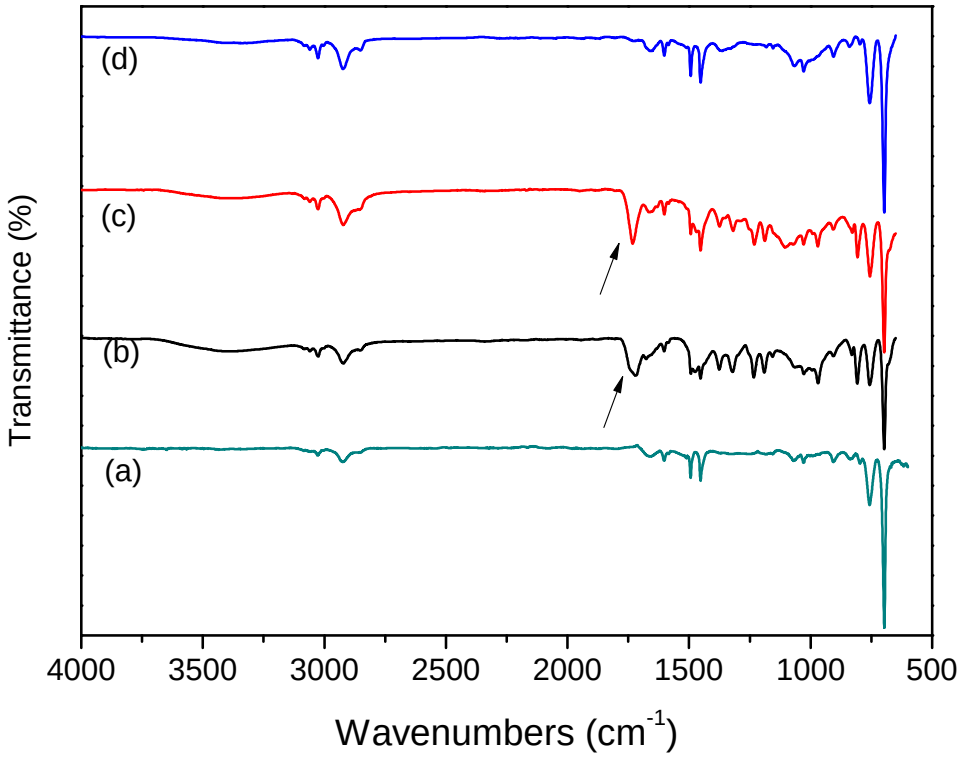


Figure S6: FTIR spectra of (a) **C1**, (b) **C1**+CB[8], (c) core-shell microspheres of **C1**+CB[8]+**P1** and (d) **C1**+CB[8]+**P1**+ADA.

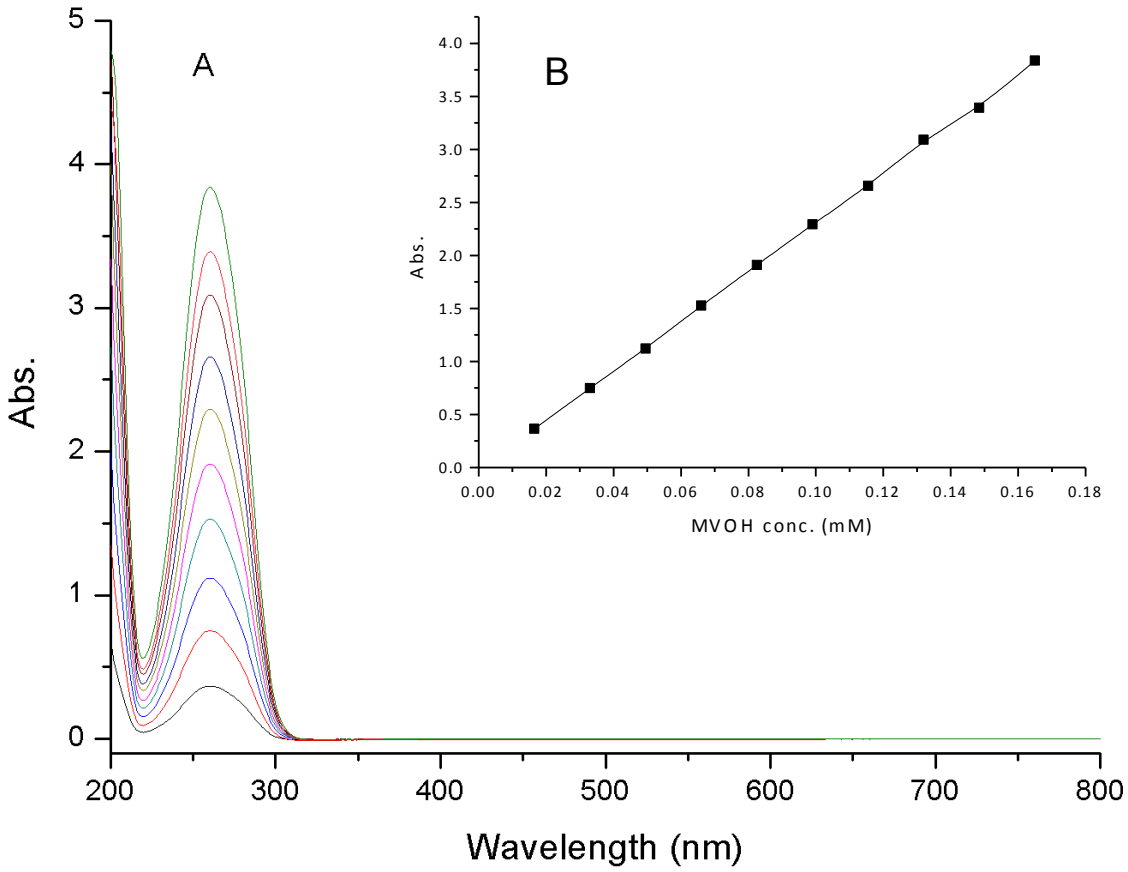


Figure S7: (A) Changes in the absorption spectra of 1-(2-hydroxyethyl)-10-methyl-[4,4'-bipyridine]-1,10-diium dichloride (MVOH) upon standard addition of known amounts of MVOH. (B) The calibration curve for MVOH at 261 nm.

S.2 Experimental

S.2.1 Materials and general methods

All starting materials were purchased from Alfa Aesar and Sigma Aldrich and used as received unless stated otherwise. CB[7] and CB[8] was prepared as documented previously.[5,6]

^{1}H NMR (400 MHz) spectra were recorded using a Bruker Avance QNP 400. ATR FT-IR spectroscopy was performed using a Perkin-Elmer Spectrum 100 series FT-IR spectrometer equipped with a universal ATR sampling accessory. UV-*vis* studies were performed on a Varian Cary 4000 UV-*vis* spectrophotometer. Gel permeation chromatography (GPC) was carried out in dimethylformamide (DMF) 0.1 M LiBr. DMF GPC was performed on two Jordi 5 mm DVB-Glucose columns connected in series with a SPD-M20A prominence diode array detector and refractive index detector (both Shimadzu) calibrated in relation to poly(methyl methacrylate) standards. Transmission electron microscopy (TEM) characterisation was carried out by a JEOL 2000FX TEM under an accelerating voltage of 200 kV. Samples were prepared by applying one drop of the as-synthesised microspheres onto a Holey R carbon coated copper TEM grid (400 mesh) drying overnight. Dynamic light scattering (DLS) and zeta potential (ZP) measurements were performed on Malvern Zeta-sizer NS90 instrument. Fluorescence images were recorded using an EM-CCD camera (Xion+, Andor Technologies) connected to an inverted microscope (IX 71, Olympus).

S.2.2 Synthesis of C0

To a 250 mL flask, N-(hydroxyethyl) acrylamide (HEAM , 0.5757 g, 5.0 mmol) was first dissolved in water (100 mL) to form a homogeneous solution. Subsequently, the mixture of styrene (5.208 g, 50.0 mmol) and divinylbenzene (DVB , 1.432 g, 11.0 mmol) was added. Nitrogen was placed into the mixture for 1 h before elevating the temperature, and the nitrogen blanket was maintained throughout the polymerization. After stabilizing at 80 °C, polymerization was initiated by addition of $K_2S_2O_8$ (0.297 g, 1.10 mmol). After 24 h polymerization, the product of the **C0** were purified by centrifugation and dried in vacuum oven at 50 °C for 4 days.

S.2.3 Synthesis of C1

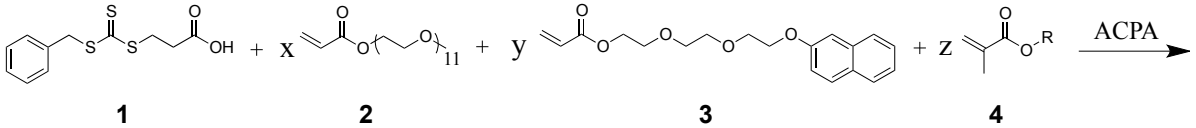
To a solution of MV-Hex-NCO di(hexafluorophosphate)[1] (0.6740 g, 1 mmol) in 50 mL of anhydrous acetonitrile were added dried **C0** (0.48 g, containing 0.3334 mmol OH group) and a drop of dibutyltin dilaurate (TDL). The reaction mixture was stirred for 7 days at room temperature. After the reaction, the product of **C1** is purified by centrifugation, washed with acetonitrile twice and washed with water twice. Counter ion exchange and further purification was achieved by dialysis against 0.5 wt% aqueous sodium chloride solution for 3 day, followed by dialysis against deionized water for 4 days. Finally, the product of **C1** was dispersed in 50 mL water. (containing 0.03 mmol MV, the concentration of MV is 0.60 mM). The density of the MV on **C1** is 2.5 MV molecules per nm^2, calculated by the equation below:

$$d_{MV} = \frac{n_{MV} \times N_A}{4\pi r^2 \times N}, N = \frac{m_o}{\frac{4}{3}\pi r^3 \times \rho}$$

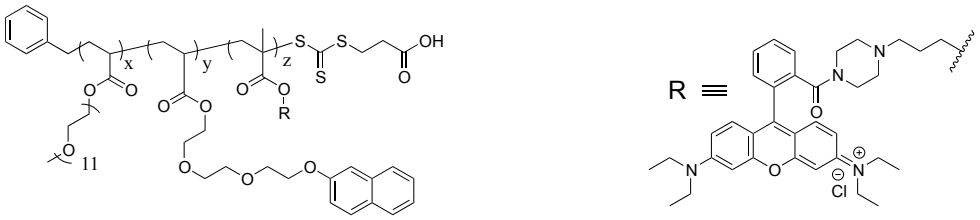
d_{MV}: the density of MV on **C1**; n_{MV}: moles of MV of **C1**; N_A: Avogadro constant; r: average radius of **C1** from DLS; N: number of **C1**; m_o: weight of **C1**; ρ: density of **C1**. Assuming that **C1** is ideal sphere, and ρ = 1 g/cm^3.

To determine the MV percentage of **C1**, 0.3 mL of the **C1** dispersion was added into 2.7 mL concentrated HCl solution. The mixture was stirred for 10 h at 60 °C. The resulted mixture was filtered over 0.22 μm PVDF filters before taking UV-*vis* measurement. The concentration of MV was obtained from fitting to the calibration curve (Figure S7).

S.2.4 Synthesis of Np-polymer



1 **2** **3** **4**



The monomer of **3**, **4** and the Np-polymers of **P1** and **P2** are synthesized according to our recently published paper.[7,8] The typical procedure for **P1** is as follow: the chemical **1** (6.20 mg, 0.025 mmol), **2** (0.9830 g, 1.82 mmol), **3** (0.27 g, 0.78 mmol), 4,4-azobis(4-cyanopentanoic acid) (ACPA, 1.50 mg, 0.0053 mmol), and dioxane (1.2 mL) were added to a Schlenk tube and the mixture was thoroughly degassed. The mixture was subsequently immersed in an oil bath thermostated to 70 °C for 3 h. The polymerization was quenched using liquid nitrogen, after which time the solution was diluted with THF and added dropwise to diethyl ether. The yellow oily product was isolated and dried in a vacuum oven. ^{1}H NMR (D_2O, 500 MHz, 298.5 K) δ = 7.58, 7.28, 6.98, 4.11, 3.60, 3.31, 2.31, 1.81, 1.45 ppm. GPC (DMF): M_n = 44.0 kDa, PDI = 1.20.

The typical procedure for **P2** is as follow: chemical **1** (6.30 mg, 0.026 mmol), **2** (0.50 g, 1.05 mmol), **3** (0.18 g, 0.52 mmol), **4** (34.0 mg, 0.052 mmol), ACPA (1.4 mg, 0.0052 mmol), and dioxane (1.0 mL) were added to a Schlenk tube and the mixture was thoroughly degassed. The mixture was subsequently immersed in an oil bath thermostated to 70 °C for 10 h. The polymerization was quenched using liquid nitrogen, followed by dilution with tetrahydrofuran before the solution was added dropwise to diethyl ether. The polymer was dissolved in water and then placed into a dialysis tubing (nominal molecular weight cut-off 2,000 Da) and was dialyzed against water for more than 48 h with three times of water replacement. The aqueous solution was then lyophilized to give **P2** as a pink oil (0.46 g, 68%). ^{1}H NMR

(D_2O, 500 MHz, 298.5 K) δ = 7.41, 7.18, 6.98, 4.11, 3.60, 3.31, 0.91, 0.35 ppm. GPC (DMF): M_n = 25.7 kDa, PDI = 1.18. Fluorescence spectrum: λ_{ex} max (H_2O) = 566 nm, λ_{em} max (H_2O) = 580 nm.

S.2.5 Reversible preparation of core-shell microspheres

The procedure was carried out in a stepwise fashion. Firstly, CB[8] (3.9870 mg, 3.0×10^{-3} mmol) and 7 mL H_2O were added to 0.5 mL **C1** dispersion (containing 3.0×10^{-4} mmol MV). The formed mixture was sonicated for 10 min. Subsequently, 2.5 mL of the P1 solution (1.76 g/L, containing 3.0×10^{-3} mmol Np) was added into the mixture and stirred for 1 hour. Then the formed core-shell microspheres were washed once with water.

The dissociation of the core-shell polymeric microspheres was achieved by addition of 1-adamantaneamine (ADA). After adding the **P1** solution, 2.27 mg ADA (1.5×10^{-2} mmol) were added in and stirred at room temperature for 1 h. Then the mixture were washed once with water.

All the control experiments were done under the same condition.

DLS samples preparation: for **C1**+CB[8], CB[8] (3.9870 mg, 3.0×10^{-3} mmol) and 9.5 mL H_2O were added to 0.5 mL **C1** dispersion (containing 3.0×10^{-4} mmol MV). The formed mixture was sonicated for 10 min before sample preparation. For **C1**+CB[8]+**P1** and **C1**+CB[8]+**P1**+ADA, the samples was prepared after washing.

S.2.6 Cytotoxicity experiments

HeLa cells were cultivated in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin/ streptomycin. Cells were grown as a monolayer and were passaged upon confluence using trypsin (0.5%, w/v in PBS). The cells were harvested from culture by incubating in trypsin solution for 10 min. The cells were centrifuged and the supernatant was discarded. 3 mL of serum-supplemented DMEM was added to neutralize any residual trypsin. The cells were resuspended in serum-supplemented DMEM at a concentration of 2×10^4 cells mL^{-1}. Cells were cultivated at 37 °C and 5% CO_2.

The toxicities of the polymeric microspheres were assessed by determining their ability to affect the proliferation and viability of HeLa cells cultured in DMEM. The polymeric microspheres were incubated at 37 °C for 24 hours, in 24-well multiplates at 1×10^4 cells per well for 24 h at 37 °C in 500 μL of medium. The different cell viabilities were evaluated using the MTT assay on the HeLa cell lines. Here, 10 mL of sterile filtered MTT stock solution in PBS (5 mg/mL) was added to each well, reaching a final MTT concentration of 0.5 mg/mL. After 5 h, unreacted dye was removed by aspiration. The formazan crystals were dissolved in DMSO (100 mL per well), and the absorbance was measured using a microplate reader at a wavelength of 570 nm. Cell viability (%) = $[A]_{test}/[A]_{control} \times 100\%$, where $[A]_{test}$ is the absorbance of the wells without polymeric microspheres and $[A]_{control}$ is the absorbance of the control wells. All experiments were conducted with six repetitions and averaged. The control group consists of cells incubated without polymers and cultured in DMEM.

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