Electronic Supplementary Information for

Fabrication of dual-responsive micelles based on the supramolecular interaction of cucurbit[8]uril†

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Experimental Section

Materials

The hydrophilic hydroxyl-capped hyperbranched polyphosphate (HPHEEP-OH, M_n=5500, the average number of hydroxyl groups per molecule is 24) was synthesized by self-condensing ring-opening polymerization of cyclic phosphate inimers according to Reference 1. The polymer was dissolved in deionized water, enclosed in a dialysis membrane (MWCO 1.0 kDa), and then purified by dialyzing in deionized water before use. Nile Red (99%, J&K Scientific Ltd.), coumarin 102 (97%, Fluka), N,N-dicyclohexylcarbodiimide (95%, DCC, Sinopharm Chemical Reagent Co., Ltd.) and 4-dimethylaminopyridine (99%, DMAP, Aldrich) were used as received without further purification. Cucurbit[8]uril (CB[8]), stannous octanoate [$Sn(Oct)_2$] and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich and used as received. 4,4'-bipyridine and 3-indolepropionic acid (IPA-COOH) were obtained from Aladdin. 3-bromopropionic acid was purchased from J&K Chemical Inc. Methyl iodide was purchased from Hongda Chemical Inc. $_{D,L}$-Lactide (LA) was purchased from Beijing Yuanrong Technological Co. Ltd. All other reagents and solvents were purchased from the domestic suppliers and used as received.

Characterization

Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra of the samples were recorded in

deuterated-dimethyl sulfoxide (DMSO-d_6) using a Bruker DMX500 spectrometer. Dynamic light scattering (DLS) measurements were conducted at 25 °C on a laser particle size analyzing system (Brookhaven 90 plus, Brookhaven Instruments Corporation). The scattering angle was kept at 90° and the wavelength was set as 658 nm. Number-average hydrodynamic diameter was adopted. Transmission electron microscopy (TEM) measurements were performed on a JEM-1200EX TEM operating at 80 kV in bright field mode. The samples were prepared by drying a drop of a dilute aqueous solution of micelles onto a carbon-coated copper grid. To confirm the complexation, fluorescence measurements were taken at an excitation wavelength of 279 nm and the emission monitored from 280 to 500 nm. Excitation and emission slit widths were both maintained at 4.5 nm and spectra were accumulated with a scan speed of 200 nm min^{-1}.

Synthesis of indole-terminated poly ($_{D,L}$-lactide) (PLA-IPA)

Hydroxyl-terminated poly ($_{D,L}$-lactide) (PLA-OH) was synthesized by ring-opening polymerization of LA in the presence of ethanol using $Sn(Oct)_2$ as the catalyst and toluene as the solvent. PLA-IPA was then synthesized by the coupling of PLA-OH and IPA-COOH. In a dried 50 mL round-bottom flask, IPA-COOH (113 mg, 0.6 mmol), DCC (124 mg, 0.6 mmol), DMAP (24 mg, 0.2 mmol) were first dissolved in 12 mL dichloromethane. In order to activate the carboxyl groups of IPA-COOH, the solution was then stirred at 0 °C for 1 h. Next, PLA-OH (1.06 g, 0.2 mmol) was dissolved in 8 mL dichloromethane and the solution was added dropwise into the flask at 0 °C. After reaction at room temperature for 48 h, the resulting solution was filtrated and then dialyzed over dichloromethane for 3 days. At last, the solvent was removed by distillation and the final product was obtained after drying in vacuum.

Synthesis of 1-carboxylpropyl-1'-methyl-4,4'-bipyridinium (MV-COOH)

Firstly, 1-Methyl-4,4'-bipyridinium iodide was synthesized according to Reference 2. Then, 1-Methyl-4,4'-bipyridinium iodide (3 g, 10 mmol) and 3-bromopropionic acid(15.3 g, 50 mmol) were dissolved in 200 mL acetonitrile, heated to reflux for 24 h. A yellow precipitate was collected by vacuum-filtration, washed with hot acetonitrile. The desired product was

recrystallized from methanol and ethanol, and dried under vacuum overnight.

Synthesis of methyl viologen-containing hyperbranched polyphosphate (HPHEEP-MV)

HPHEEP-MV was synthesized by the esterification reaction of HPHEEP-OH and MV-COOH. In a dried 50 mL round-bottom flask, MV-COOH (180 mg, 0.4 mmol), DCC (99 mg, 0.48 mmol), DMAP (20 mg, 0.16 mmol) were first dissolved in 12 mL DMSO and then stirred for 1 h. Next, HPHEEP-OH (550 mg, 0.1 mmol) was dissolved in 8 mL DMSO and the solution was added dropwise into the flask. After reaction at room temperature for 48 h, the resulting solution was enclosed in dialysis membrane (MWCO 1.0 kDa), and then dialyzed over DMSO for 3 days. After dialysis, the solvent was removed by distillation and the final product was obtained after drying in vacuum.

Preparation of micelles

The supramolecular micelles were prepared by a dialysis method. Firstly, 24.4 mg HPHEEP-MV and 2.7 mg CB[8] were dissolved in 8 mL water under stirring. Secondly, 11 mg PLA-IPA was first dissolved in 8 mL DMSO at room temperature. The HPHEEP-MV and CB[8] complex solution was then added dropwise into the PLA-IPA solution under magnetic stirring. After stirring for another 6 h, the solution was dialyzed against deionized water for 3 days to totally remove the solvent DMSO. The final polymer concentration was adjusted by adding deionized water to 1 mg mL^{-1}.

Determination of the critical micelle concentration (CMC)

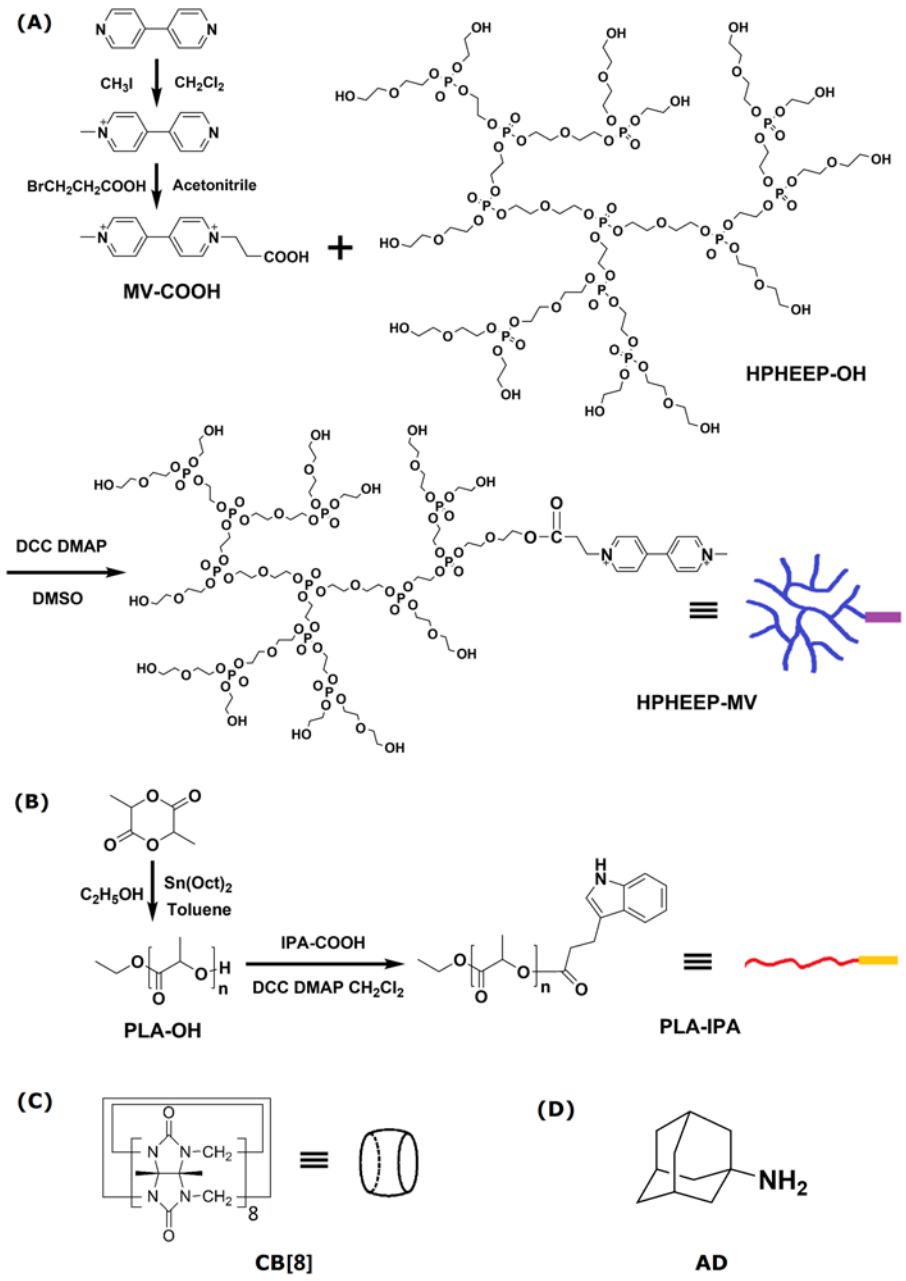
The CMC of the micelles was determined using Nile Red as a fluorescence probe. Fluorescence spectra were obtained at room temperature using a spectrofluorometer (FP-770, Japan Spectroscopic). The concentration of the polymers was varied from 1×10^{-3} mg mL^{-1} to 1 mg mL^{-1}. Nile Red in acetone (0.02 mg mL^{-1}) was then added to each polymer solution and the final concentration of Nile Red in each sample was fixed at 3×10^{-4} mg mL^{-1}. Fluorescence measurements were taken at an excitation wavelength of 550 nm and the emission monitored from 580 to 720 nm. Excitation and emission slit widths were both maintained at 10.0 nm and spectra were accumulated with a scan speed of 100 nm min^{-1}.

***In vitro* cytotoxicity test**

HepG2 (a human liver carcinoma cell line) and HUVEC (Human Umbilical Vein Endothelial Cells) were cultivated in different media, Dulbecco's modified eagle's medium (DMEM) and RPMI-1640 culture medium, and both with 10% fetal bovine serum (FBS), antibiotics penicillin (100 IU mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C under a humidified atmosphere containing 5% CO_2. MTT assay was used to determine cytotoxicity of the micelles. Before determination, the cells were seeded in a 96-well plate at 8×10^3 cells per well in 200 μL of corresponding medium. After 24 h culture, the culture medium was replaced with 200 μL of medium containing different concentrations of micelles (50—250 mg L^{-1}). Phosphate buffered saline (PBS) was chosen as control. The cells were incubated for another 24 h. Followed the culture medium was removed and wells were washed with PBS. Then, 100 μL culture medium and 20 μL of 5 mg mL^{-1} MTT assays stock solution in PBS were added to each well. After incubating the cells for 4 h, the medium containing unreacted MTT was removed carefully. The obtained blue formazan crystals generated by live cells were dissolved in 150 μL DMSO, and the absorbance at a wavelength of 570 nm of each well was measured using a microplate reader. The relative cell viability (%) was determined by comparing the absorbance at 570 nm with control wells.

Encapsulation and triggered release of coumarin 102

Coumarin 102 was selected as hydrophobic model drug to be encapsulated into the micelles and fluorescence spectroscopy was used to investigate release behaviours of the micelles. 2.0 mL coumarin 102 solution in acetone (0.05 mg mL^{-1}) was first added dropwise into 10 mL blank 0.5 mg mL^{-1} micellar solution under vigorous stirring. The samples were then sonicated for 15 min and stirred for 24 h in the dark. After the encapsulation process, the fluorescence spectrum of the micelles was recorded immediately. The micelles were then exposed to no trigger, adamantaneamine and $Na_2S_2O_4$. Fluorescence measurements were taken at an excitation wavelength of 420 nm and the emission monitored from 450 to 650 nm. Excitation and emission slit widths were both maintained at 4.0 nm and spectra were accumulated with a scan speed of 100 nm min^{-1}.



Scheme S1 Synthetic routes for (A) HPHEEP-MV and (B) PLA-IPA, and chemical structures of (C) CB[8] and (D) AD.

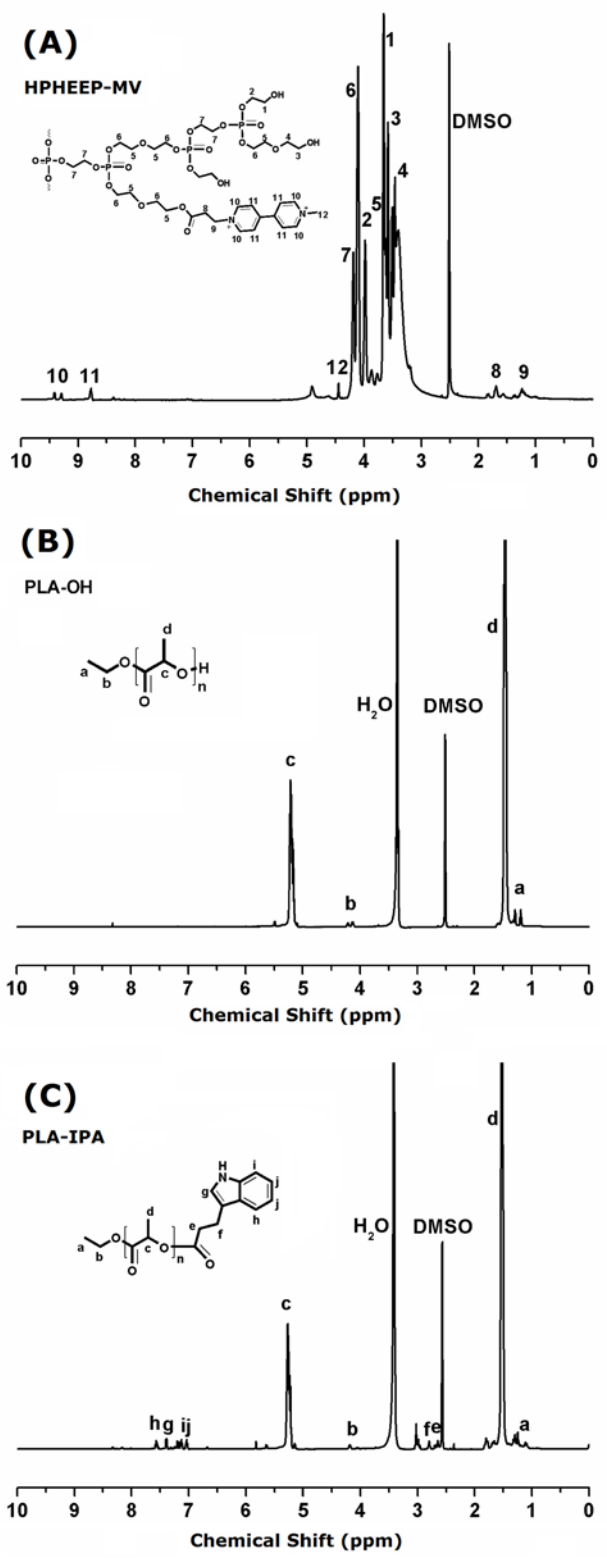


Fig. S1 ^{1}H NMR spectra for HPHEEP-MV (A), PLA-OH(B) and PLA-IPA (C).

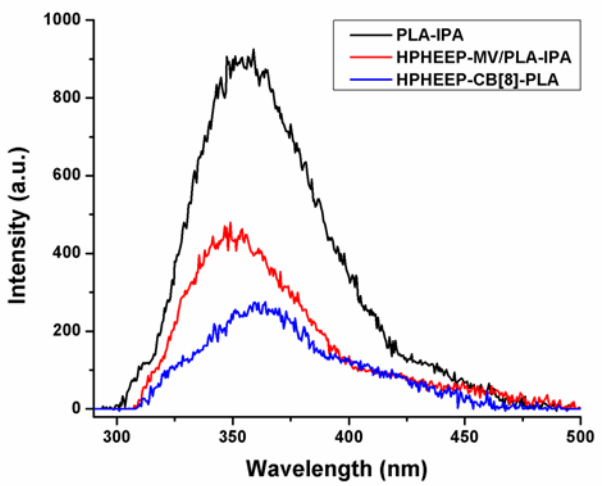


Fig. S2 Fluorescence emission spectra of PLA-IPA (black) and in the presence of 1 equiv of HPHEEP-MV (red) or HPHEEP-CB[8] complex (blue). All spectra were obtained with excitation at 279 nm at room temperature in DMF/H_2O (1:1 v/v). The initial concentration of PLA-IPA in each sample was 0.5 mg/mL.

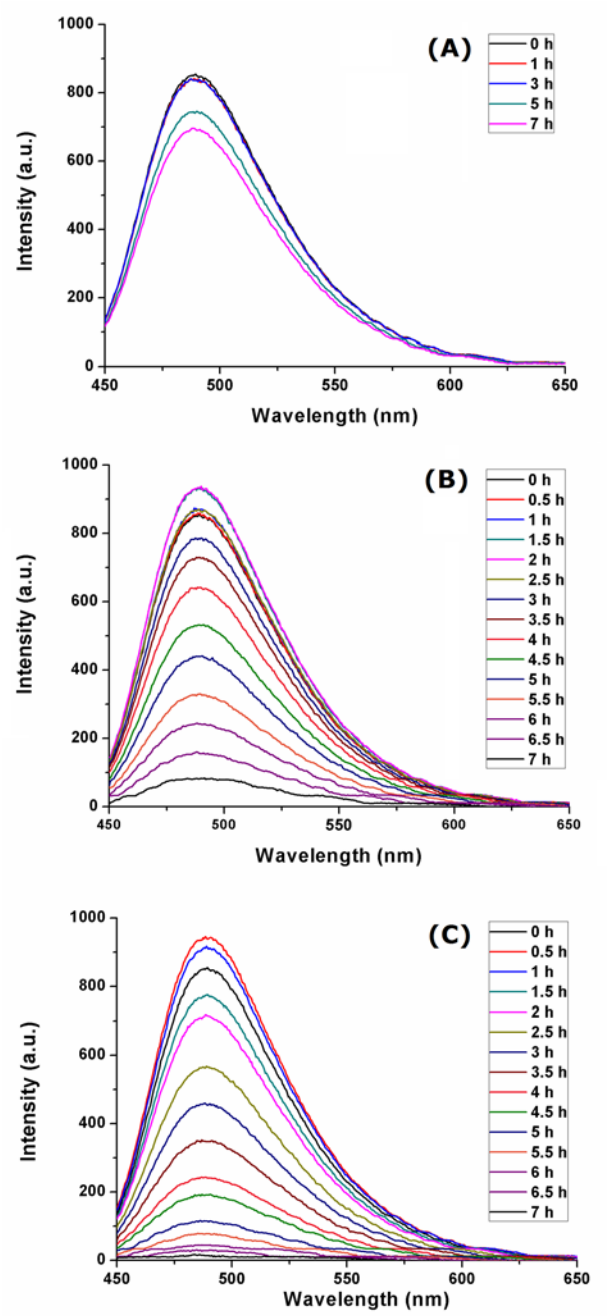


Fig. S3 Fluorescence emission spectra of the supramolecular micelles with encapsulated coumarin 102 after after being exposed to (A) no trigger, (B) adamantaneamine and (C) $Na_2S_2O_4$.

References

1 J. Y. Liu, W. Huang, Y. F. Zhou and D. Y. Yan, *Macromolecules*, 2009, **42**, 4394.

2 H. Yamaguchi and A. Harada, *Biomacromolecules*, 2002, **3**, 1163.