Electronic Supplementary Information for

Biocompatible and biodegradable supramolecular assemblies formed with cucurbit[8]uril as a smart platform for reduction-triggered release of doxorubicin†

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Experimental Section

Materials

Poly(ethylene glycol) methyl ether (PEO-OH, M_n=5000), 3-indolepropionic acid (IPA) and 4,4'-bipyridine were purchased from Aladdin. Hydroxyl-capped PLA (PLA-OH, M_n=2700) and PEO-*b*-PLA (M_n=7200) were synthesized by ring-opening polymerization of $_{D,L}$-Lactide using n-dodecyl alcohol and PEO-OH as the initiator, respectively. $Sn(Oct)_2$ was used as the catalyst for these reactions. 1-methyl-4,4'-bipyridinium iodide (CH_3-bpy) was synthesized by the reaction between 4,4'-bipyridine and methyl iodide. Thionyl bromide, cucurbit[8]uril (CB[8]), n-dodecyl alcohol and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma-Aldrich. Doxorubicin hydrochloride (DOX·HCl, 99%, Zhejiang Hisun Pharmaceutical Co., Ltd.), Nile Red (99%, J&K Scientific Ltd.), N,N-dicyclohexylcarbodiimide (DCC, 95%, Sinopharm Chemical Reagent Co., Ltd.) and 4-dimethylaminopyridine (DMAP, 99%, Aldrich) were used as received without further purification. Methyl iodide was purchased from Zhejiang Hongda Chemical Co., Ltd. Toluene, acetonitrile and triethylamine (Et_3N) were distilled before use. Other reagents and solvents were obtained from domestic suppliers and used as received.

Synthesis of PLA-IPA

PLA-IPA was synthesized by the esterification reaction between PLA-OH and IPA. IPA (56.7 mg, 0.3 mmol), DCC (61.8 mg, 0.3 mmol), DMAP (12.2 mg, 0.1 mmol) were dissolved in 10 mL dichloromethane in a dried 50 mL round-bottom flask. After stirring at 0 ºC for 1 h, 270 mg PLA-OH (0.1 mmol) was dissolved in 10 mL dichloromethane and then added dropwise into the flask. The solution was stirred at room temperature for 3 days. To remove impurities, the obtained solution was filtered and dialyzed against dichloromethane with a regenerated cellulose membrane (MWCO 1.0 kDa) for 3 days. Finally, the solvent was removed by distillation and the product was obtained after drying under vacuum (yield: 74%).

Synthesis of PEO-MV

First, 25 g PEO-OH (5 mmol) was dissolved in 120 mL toluene in a 500 mL round-bottom flask under N_2 atmosphere. Then, 2.5 mL Et_3N (18mmol) and a solution composed of 1 mL Br_2OS (13 mmol) and 50 mL toluene was added sequentially by dropping into the flask. After refluxing at 125 ºC for 3 h, the precipitate in the suspension was removed by filtering, and the remained solution was refluxed in the dark overnight at 125 ºC. After rotary evaporation, the product was precipitated in anhydrous methanol and then dried under vacuum (yield: 93%).

Then, 745 mg 1-methyl-4,4'-bipyridinium iodide (2.5 mmol) was dissolved in 100 mL acetonitrile at 80 ºC followed by adding of 2.5 g PEO-Br (0.5 mmol). After refluxing at 100 ºC for 7 days, the solvent was removed using a rotary evaporator and the crude product was dissolved in dichloromethane and precipitated in dried ether. By further dialysis against deionized water for 3 days and freeze-drying, some light brown powders were obtained (yield: 48%).

Preparation of Assemblies and Critical Aggregation Concentration (CAC)

First, 11.0 mg PEO-MV (2.12 μmol) and 2.8 mg CB[8] (2.12 μmol) were dissolved in 5 mL aqueous solution. Second, 6.1 mg PLA-IPA (2.12 μmol) was dissolved in 3 mL acetone under stirring at room temperature. Then, the prepared solution of PEO-MV/CB[8] complex was added dropwise into the PLA-IPA solution under vigorous stirring. After stirring for another 6 h, the solution was dialyzed against deionized water for 3 days to totally remove the organic solvent. The final polymer concentration was tuned to 2 mg mL^{-1} by adding deionized water.

PEO-*b*-PLA control micelles were prepared by a traditional dialysis method.

To determine the CAC, ternary complex solutions with various concentrations from 1×10^{-4} mg mL^{-1} to 0.8 mg mL^{-1} were prepared. Nile Red in acetone (0.02 mg mL^{-1}) was then added to each solution and the final Nile Red concentration was fixed at 3×10^{-4} mg mL^{-1}. Fluorescence measurements were taken at an excitation wavelength of 550 nm and the emission monitored within 580~720 nm. Excitation and emission slit widths were 6.5 nm and the scanning speed was 100 nm min^{-1}.

Cell Culture and *In Vitro* Cytotoxicity Assay

HepG2 (a human liver carcinoma cell line) and HUVEC (Human Umbilical Vein Endothelial Cells) were respectively cultivated in Dulbecco's modified eagle's medium and RPMI-1640 culture medium, and both with 10% fetal bovine serum, 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 the cytotoxicity of assemblies. Before the test, cells were seeded in a 96-well plate at 8×10^3 cells per well in 200 μL of corresponding medium. After culture for 24 h, the medium was replaced with 200 μL of medium containing assemblies of different concentrations (50~250 μg mL^{-1}). Phosphate buffered saline (PBS, pH 7.4, 10 mM) was chosen as the control. Cells were incubated for another 24 h followed by removing the culture medium and washing wells with PBS. Then, 180 μL culture medium and 20 μL of 5 mg mL^{-1} MTT solution in PBS were added into each well. After incubating for 4 h, the medium containing unreacted MTT was removed carefully. The blue formazan crystals were dissolved by 150 μL dimethyl sulfoxide, and the absorbance at a wavelength of 570 nm of each well was collected. To test the cytotoxicity of DOX-loaded assemblies, HepG2 cells were used.

Loading and Triggered Release of Doxorubicin

4 mg DOX·HCl was dissolved in a mixed solvent of 1 mL tetrahydrofuran and 1 mL methanol and was neutralized by triethylamine to make it hydrophobic DOX. Then, the DOX solution was added dropwise into 10 mL of 2 mg mL^{-1} assemblies under vigorous stirring. After stirring for another 6 h in the dark, the solution was dialyzed against deionized water for

3 days to remove organic solvents. To determine the drug loading content (DLC) and the drug loading efficiency (DLE), DOX-loaded assemblies after freeze-drying were dissolved in dimethylformamide (DMF) and analyzed by measuring the UV absorbance at the wavelength of 480 nm. The calibration curve was obtained with DOX in DMF with various concentrations. The DLC and DLE were calculated according to the following formula:

DLC (%) = (weight of loaded drug) / (weight of loaded drug + weight of polymer) × 100%

DLE (%) = (weight of loaded drug) / (weight of drug in feed) × 100%

The release of DOX was conducted by dialysis against PBS in the dark at 37 °C. 2 mL DOX-loaded assemblies (0.8 mg mL^{-1}) with different concentrations of $Na_2S_2O_4$ were added into a dialysis tube and then were placed into 10 mL PBS. At predetermined time intervals, 2 mL solution outside the dialysis tube was collected and replaced by 2 mL fresh PBS. It should be noted that some $Na_2S_2O_4$ with the same concentration with the solution in the dialysis tube was also added into the PBS. The concentration of DOX was determined by measuring the UV absorbance at 480 nm.

Characterization

Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra were recorded in different deuterated solvents by a Bruker DMX500 spectrometer and scanned in the range of 0~15 ppm. Fluorescence measurements were taken at an excitation wavelength of 279 nm and the emission monitored within 280~600 nm. Excitation and emission slit widths were both set at 2.8 nm and spectra were collected with a scan speed of 50 nm min^{-1}. UV-visible spectra were carried out with a UV-vis Shimadzu UV-2505 spectrometer and the spectra were accumulated within the range of 350~850 nm. Transmission electron microscopy (TEM) measurements were conducted 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 assemblies onto a carbon-coated copper grid. 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.

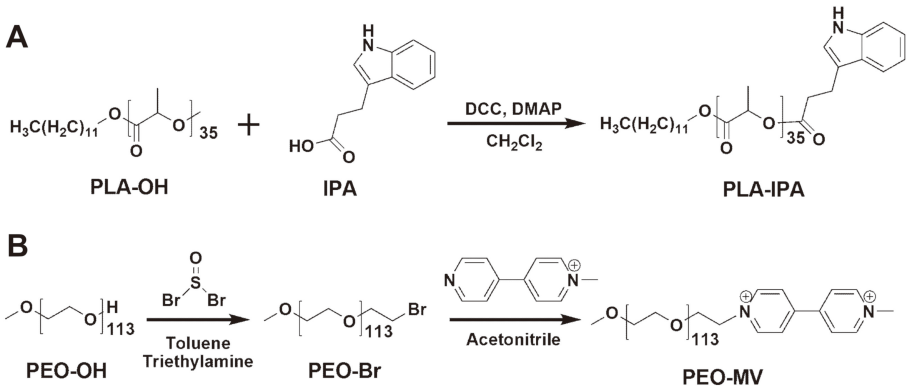

Fig. S1 Synthetic routes for (A) PLA-IPA, and (B) PEO-MV.

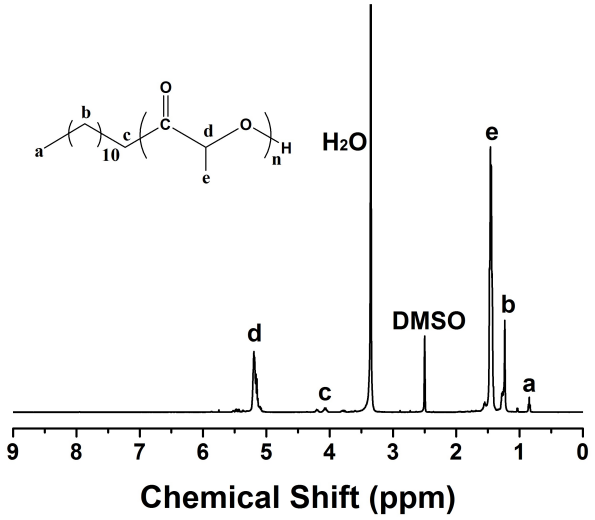

Fig. S2 ^{1}H NMR spectrum of PLA-OH in DMSO-d_6.

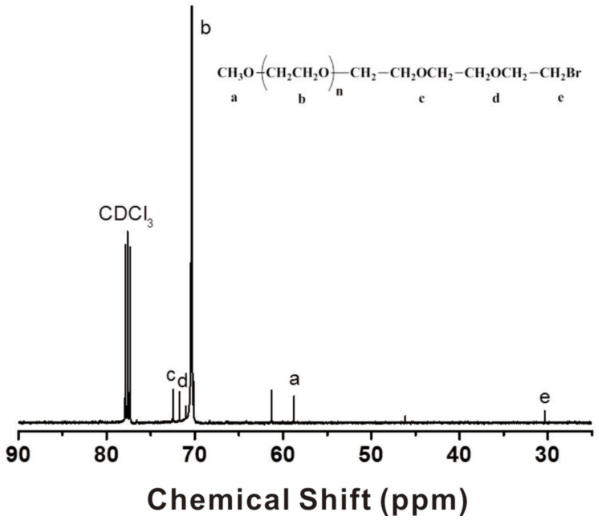

Fig. S3 ^{13}C NMR spectrum of PEO-Br in $CDCl_3$.

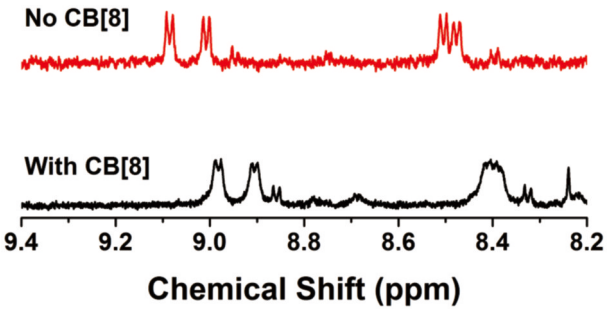

Fig. S4 Partial ^{1}H NMR spectra of PEO-MV and PEO-MV/CB[8]/PLA-IPA ternary complex in DMSO-d_6.

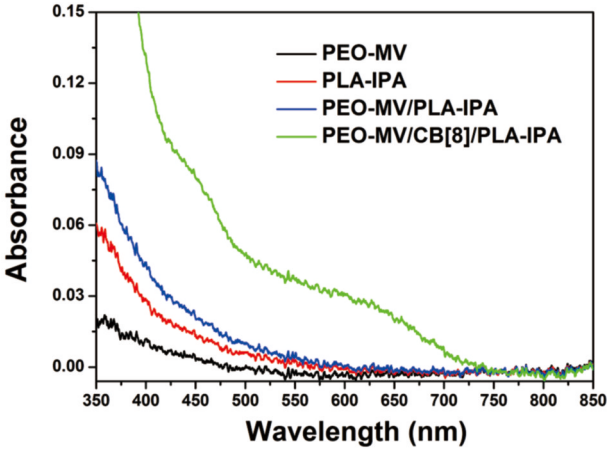

Fig. S5 UV-vis spectra of PEO-MV, PLA-IPA, PEO-MV/PLA-IPA binary complex and PEO-MV/CB[8]/PLA-IPA ternary complex.

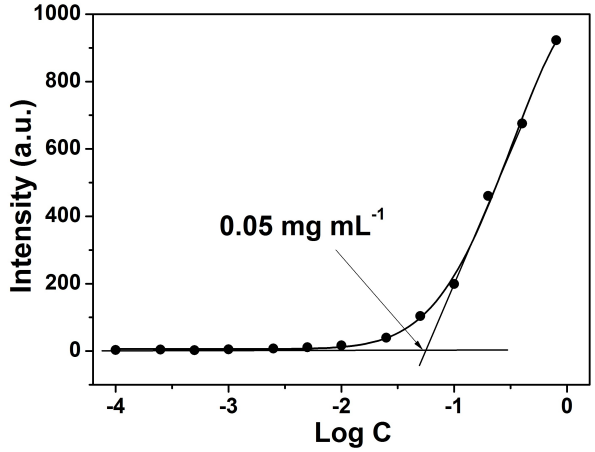

Fig. S6 The plot of the fluorescence intensity at 601 nm *versus* the log of concentration to determine the CAC of CB[8]-based supramolecular assemblies.

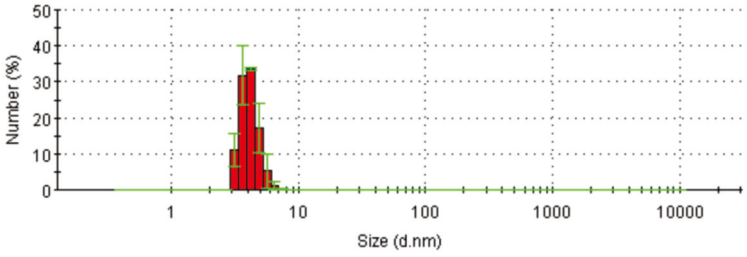

Fig. S7 DLS size distribution of PEO-MV in aqueous solution.

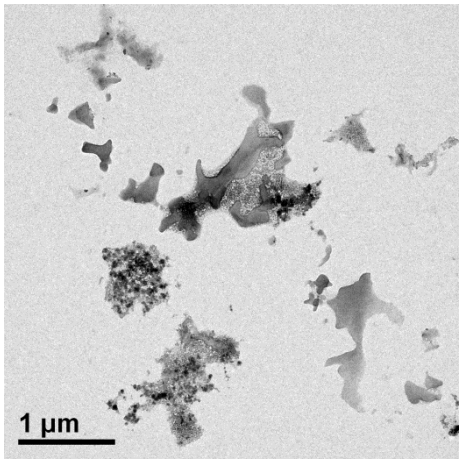

Fig. S8 TEM image of the supramolecular assemblies after being exposed to 5 mg mL^{-1} $Na_2S_2O_4$.

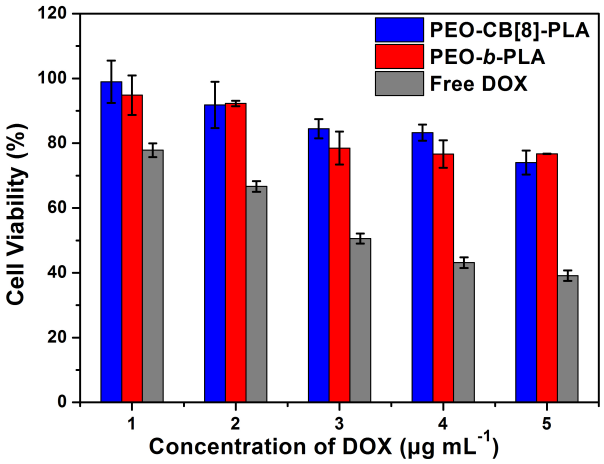

Fig. S9 Cytotoxicity of DOX-loaded supramolecular assemblies (PEO-CB[8]-PLA), control micelles (PEO-*b*-PLA) and free DOX against HepG2 cells determined by MTT for 24 h.

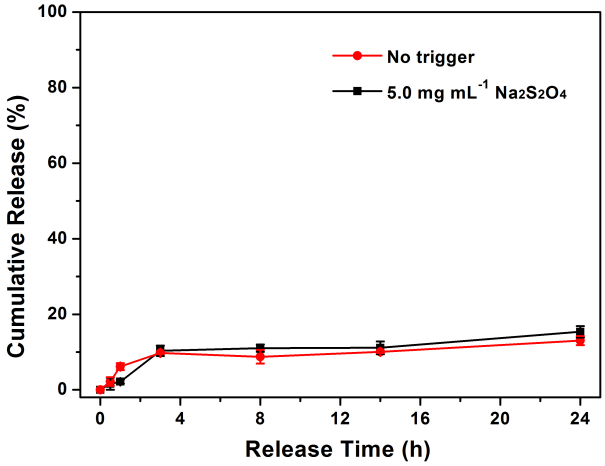

Fig. S10 Drug release profiles of PEO-*b*-PLA micelles in the presence or absence of 5 mg mL^{-1} $Na_2S_2O_4$.