Supporting Information

Stable Amphiphilic Supramolecular Self-assembly Based on Cyclodextrin and Carborane for Efficient Photodynamic Therapy

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Experimental section

Materials. Monomethyloxy poly(ethylene glycol) (average Mn 2000), indocyanine green (ICG), Hoechst 33258, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT) were purchased from Sigma-Aldrich Co. Decaborane ($B_{10}H_{14}$) was purchased from Changchun Randall technology Co., Ltd and used without further purification. 1-(hydroxymethyl)-1,2-dicarba-carbrane (HMCB) was synthesized according to our previous work.[1] 5-(4-hydroxyl phenyl)-10,15,20-triphenyl-porphyrin (TPP) was synthesized in our previous work.[2] β-Cyclodextrin, propargyl bromide, propargyl alcohol, N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA), octanedioic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and dimethylaminopyridine (DMAP) were purchased from Aladdin Chemistry Co. Ltd. Shanghai, China. Cuprous chloride (CuCl) was purified following the prior experimental manipulation. Lyso-Tracker Red was purchased from Beyotime Biotechnology Co., Ltd. (China). All other chemicals were purchased from Sigma-Aldrich and used as received.

Methods. ^{1}H, ^{13}C, and ^{11}B NMR spectra were measured by a Unity-400 NMR spectrometer at room temperature. ^{1}H and ^{13}C chemical shifts are reported in ppm with tetramethylsilane as an internal reference. ^{11}B chemical shifts are reported in ppm relative to an external standard of $BF_3{\cdot}Et_2O$. Fourier Transform Infrared (FTIR) spectra were recorded on a Bruker Vertex 70 spectrometer. The size and size distribution of NP were determined by dynamic light scattering (DLS) with a vertically polarized He-Ne laser (DAWN EOS, Wyatt Technology, USA). ζ potential measurements were determined on a Malvern Zetasizer Nano ZS. The morphology of the polymeric NP was measured by TEM performed on a JEOL JEM-1011 electron microscope. TEM samples were prepared from micellar solution of the NP dropped onto amorphous carbon coated copper grids. X-ray photoelectron spectroscopy (XPS, Thermo ESCALAB 250) was used to measure the surface elements content of NP. UV-vis absorption spectra were monitored with a Shimadzu UV-2450 PC UV/Vis spectrophotometer. The fluorescence intensity tests were obtained using PerkinElmer LS-55Spectrofluorophotometer. Cellular uptake was measured by a Zeiss LSM 700 (Zurich,Switzerland) confocal laser scanning microscopy (CLSM) and a FACS Calibur flow cytometer (BD Biosciences).

Synthesis of di-[2-(1,2-dicarba-closo-dodecaborane)methylene] suberate (CB-C_8-CB). CB-C_8-CB was synthesized in two steps (Scheme S1). Firstly, dipropargyl suberate was synthesized through ester condensation reaction. Octanedioic acid (2 g, 11.5 mmol), EDC (6.613 g, 34.5 mmol), DMAP (1.405 g, 11.5 mmol) were dissolved in 50 mL of dried dichloromethane and stirred in an ice bath for 0.5 h. Subsequently, propargyl alcohol (3.9 g, 68.9 mmol) was added dropwise to the solution. The reaction mixture was stirred at room temperature for 24 h, and then washed with saturated NaCl solution and water. The organic layer was dried over anhydrous MgSO4, filtered, and evaporated to yield 4.8 g of the compound. Yield: 84.2%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) (Fig. S1A): 2.47 (t, 2H, HC≡C-), 4.68 (d, 4H, ≡C-CH_2O-), 2.33 (t, 4H, OCCH_2-), 1.65 (m, 4H, -CH_2-), 1.34 (m, 4H, -CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) (Fig. S2A): 172.67, 77.76, 74.77, 51.70, 33.76, 28.52, 24.49. FT-IR (KBr, cm^{-1}) (Fig. S4A): ν 3295, 2939, 2854, 2132, 1738.

Secondly, CB-C_8-CB was synthesized with a similar method reported in our previous work.[3] In brief, a dried flask containing decaborane $B_{10}H_{14}$ (0.326 g, 2.67 mmol), CH_3CN (14 mL, 267 mmol) and toluene (30 mL) was warmed at reflux for 1 h. After cooling, dipropargyl suberate

(0.264 g, 1.15 mmol) was added and the mixture was kept at 100 ^{o}C for 24 h. The mixture was cooled and the solvent evaporated under reduced pressure. Then the crude mixture was dissolved in CH_2Cl_2, filtered, and the filtrate was purified by chromatography (ethyl acetate: petroleum ether 1:5, Rf = 0.2). A white solid (0.24 g) was obtained after removing the volatiles. Yield: 43%. Its successful synthesis was confirmed by ^{1}H, ^{13}C and ^{11}B NMR, FT-IR, and ESI-MS (Fig. S1–S5). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) (Fig. S1B): 3.79 (s, 2H, $C_{cage}H$), 4.55 (s, 4H, C_{cage}-CH_2O-), 2.38 (t, 4H, $OCCH_2$-), 1.65 (m, 4H, -CH_2-), 1.36 (m, 4H, -CH_2-), 1.5-3.0 (br, 20H, BH).^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) (Fig. S2B):172.22, 71.92, 64.48, 59.32, 33.53, 28.59, 24.33. ^{11}B NMR (400 MHz, $CDCl_3$, δ ppm) (Fig. S3): -1.74， -4.36， -9.33， -11.44，-12.97. FT-IR (KBr, cm^{-1}) (Fig. S4B): ν 3060, 2938, 2938, 2853, 2601, 1729.MS (ESI^+) (Fig. S5): [M + Na^+] calcd for $C_{14}B_{20}H_{38}O_4Na$: 510.5, found: 510.7.

Synthesis of PEG-CD by the "click" reaction. Alkynyl-terminated PEG (PEG-alkynyl) and azido-modified β-cyclodextrin (β-CD-N_3) were firstly prepared according to the references.[4, 5] The "click" reaction was carried out under argon in Schlenk tube. PEG-alkynyl (0.265 g, 0.132 mmol), β-CD-N_3 (0.307 g, 0.264 mmol), PMDETA (0.092 g, 0.528 mmol) and CuCl (0.026 g, 0.264 mmol) were reacted in 10 mL of dried DMF at 60 ^{o}C for 48 h after a deoxidation process. After cooling to room temperature, the product was purified by dialysis against ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) solution and pure water to remove the catalyst and unreacted cyclodextrin. After lyophilization, the final product (PEG-CD) was obtained (0.35 g). Yield: 83.3%.

Isothermal titration calorimetry measurements. The host-guest interaction between PEG-CD and carboane in water was characterized by using a NanoITC apparatus (TA Instruments) at room temperature at a stirring speed of 250 rpm. Since CB-C_8-CB was insoluble in water, we selected water-soluble 1-(hydroxymethyl)-1,2-dicarba-closo-carbrane (HMCB) as the model compounds to investigate the complexation behaviors between PEG-CD and carborane. The sample cell (1.0 mL) was filled with degassed PEG-CD aqueous solution (0.2 mM). The syringe was filled with HMCB solution (4.48 mM, in DMSO/water, 1:19, v/v). The titration experiment was set to 25 injections of 10 μL each with 600 s intervals. Duplicate titrations were performed to ensure reproducibility. The heats of dilution were determined in blank experiments in which the DMSO/water (1:19) solutions were injected into the sample cell containing PEG-CD. The dilution heats were then subtracted to obtain the binding heats. The data was processed using ITCAnalyze software and fitting in an "independent" binding model. As a comparative group, adamantanemethanol in DMSO/water (1:19) was titrated into the PEG-CD aqueous solution. The experimental condition and procedure was the same as mentioned above.

Construction of supramolecular self-assemblies. PEG-CD (12.8 mg, 4 mmol) and CB-C_8-CB (1.9 mg, 4 mmol) were dissolved together in DMF (5 mL), and the solution was stirred for 6 h at room temperature. Subsequently, 2 mL of water was slowly added to the solution at a rate of 0.04 mL/min under stirring. After being stirred overnight, the solution was dialysized against water to remove DMF (MWCO: 3500) and finally freeze-dried. 2PEG-CD/CB-C_8-CB supramolecular NP were obtained by using PEG-CD (25.6 mg, 8 mmol) and CB-C_8-CB (1.9 mg, 4 mmol). The critical aggregation concentrations (CAC) of supromolecular nanopartilces were determined according to reported procedure, employing hydrophobic pyrene as the probe.[6] The stability of PEG-CD/CB-C_8-CB and 2PEG-CD/CB-C_8-CB NP were evaluated in phosphate-buffered saline (PBS, pH 7.4, 0.01 M) at 37 ^{o}C, respectively. At various time points, the solutions were analyzed

by DLS and TEM.

To determine the surface elements content of NP, X-ray photoelectron spectroscopy (XPS) measurements were conducted. As a control group, the physical mixed samples were prepared by uniformly mixing the corresponding PEG-CD and CB-C_8-CB in acetone and removing the solvent in vacuum. The surface boron content of physical mixed samples was considered as the bulk boron content. The boron content on the surface relative to the total boron atom content in the supramolecular NP was calculated using the ratio of the relative boron content on the surface between supramolecular NP and physical mixture.

TPP loading. TPP@PEG-CD/CB-C_8-CB NP was prepared by a solvent evaporation method. Briefly, 1.0 mg of TPP was dissolved in 1.0 mL of acetone and added dropwise to 5 mL of aqueous solution containing PEG-CD/CB-C_8-CB (10 mg). After being stirred overnight, the solution was transferred to a dialysis bag (MW: 3500) and dialyzed for more than 24 h to get rid of free TPP and organic solvent. The samples of TPP NP and TPP/PEG-CD NP were prepared by adding TPP/acetone solutions into blank water or PEG-CD aqueous solution. DLS analysis showed that the NP became larger after the encapsulation of TPP (Dh: 136 nm, PDI: 0.124) (Fig.S13).

To measure the amount of TPP loaded into NP, the resulting solution was dissolved in DMF (1:9, v/v) and the concentration of TPP was determined by UV-vis spectrophotometer, with the help of a standard curve obtained from TPP–H_2O/DMF(1:9, v/v) solutions at a series of TPP concentrations. Drug loading content (DLC) and drug loading efficiency (DLE) was calculated according to the following formula:

DLC (wt %) = (weight of loaded drug/weight of drug loaded micelles) × 100%

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

The mass percentage of TPP in TPP@PEG-CD/CB-C8-CB NP was determined by UV-vis spectrophotometry to be 7.5 ± 0.4 wt% with an encapsulation efficiency of 82.6 ± 3.5%.

Singlet oxygen detection. The amount of $^1O_2$ was measured by the following loss of UV absorbance of ICG in aqueous solutions. TPP@PEG-CD/CB-C8-CB, TPP and TPP/PEG-CD (TPP: 5μg/mL) solutions were mixed with ICG (10 μg/mL), respectively. The solutions were illuminated under a 620 nm light source (16 $mW \cdot cm^{-2}$), and the absorption intensity of ICG at the maximum wavelength of 780 nm was detected at predetermined time.

Stability studies. The stability of TPP@PEG-CD/CB-C8-CB NP were evaluated in phosphate-buffered saline (PBS, pH 7.4, 0.01 M) and 10% fetal bovine serum (FBS) at 37 ^oC, respectively. At various time points, the solutions were analyzed by DLS. In addition, the photostability of TPP-loaded NP in water were examined by irradiation with a 620 nm light source (16 $mW \cdot cm^{-2}$) for 90 min. The UV-vis spectra of the solutions were detected every 15 min.

The leakage of TPP from the supramolecular NP under physiological conditions was evaluated at 37 ^oC in PBS (pH 7.4, 0.01 M). 2 mL of TPP@PEG-CD/CB-C8-CB solutions were introduced into a dialysis bag (MW: 3500). The release experiment was initiated by placing the end-sealed bag in 28 mL of PBS with continuous shake at 100 rpm. At selected time intervals, the release media (1 mL) were taken out and then an equal volume of fresh media were replenished. The amount of released TPP was determined by using fluorescence measurements.

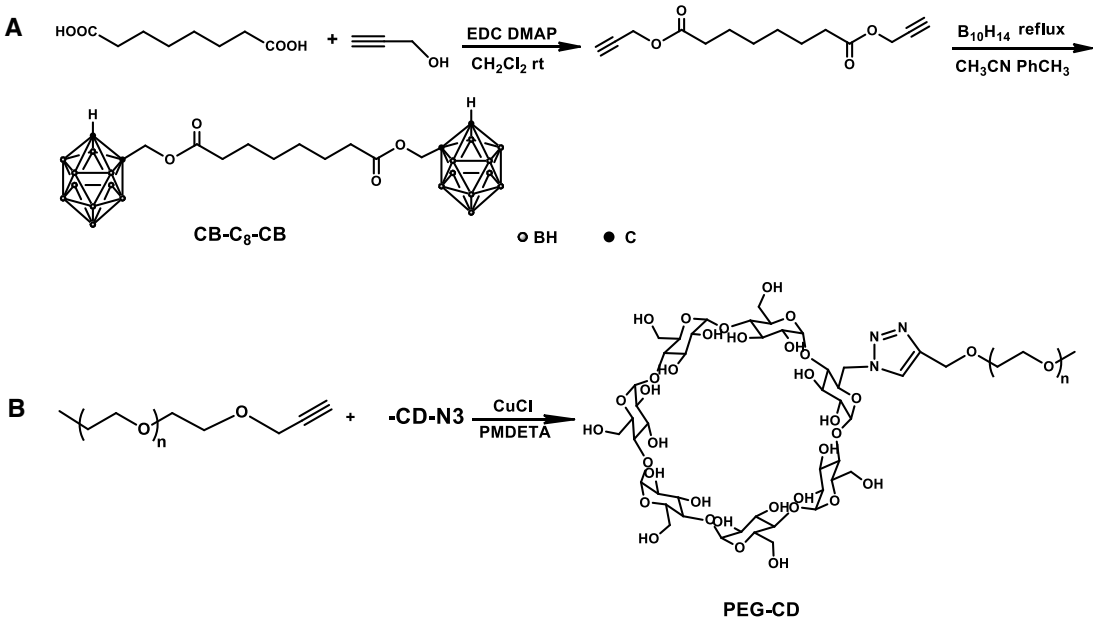
Cell lines. BEAS-2B (human bronchial epithelial cell) and HepG2 (human hepatoma cells) and HeLa (human cervical cancer cell line) were chosen for cell tests and supplied by the Medical Department of Jilin University, China. These cells were cultured in Dulbecco's modified Eagle's

medium (DMEM, GIBCO) supplied with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), 2 mM $_L$-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma).

***In vitro* phototoxicity.** First, the cytotoxicity of TPP@PEG-CD/CB-C_8-CB NP against normal human bronchial epithelial BEAS-2B cells was evaluated *in vitro* because a photodynamic therapeutic agent should have low cytotoxicity and systemic toxicity for clinical application. BEAS-2B cells were seeded at a density of 4×10^3 cells per well in 96-well plates and incubated for 24 h, then treated with TPP, TPP/PEG-CD, and TPP@PEG-CD/CB-C_8-CB NP at various TPP concentrations from 0. 25 to 8 μg/mL at 37 °C for another 24 h in the dark, and finally cell viability was evaluated using the MTT assay. To verify the phototoxicity of TPP@PEG-CD/CB-C_8-CB NP, HepG2 and HeLa cells were seeded at a density of 4×10^3 cells per well in 96-well plates and incubated for 24 h, then treated with free TPP, TPP/PEG-CD, and TPP@PEG-CD/CB-C_8-CB NP at various TPP concentrations from 0.125 to 8 μg/mL at 37 °C for another 6 h in the dark. Subsequently, the cells were washed with sterilized PBS 3 times, and fresh culture medium was added. The cells were irradiated with a 620 nm light source (16 $mW \cdot cm^{-2}$) for 60 min, and then continuously incubated for another 24 h at 37 °C, and finally cell viability was also evaluated using the MTT assay. For dark toxicity of TPP-loaded supramolecular NP, the same procedures were conducted without light irradiation.

Cellular uptake. Cellular uptake by HepG2 cells was examined using confocal laser scanning microscope (CLSM) and flow cytometry analysis. HepG2 cells were seeded in a glass bottomed 6-well plate at 1×10^5 cells per well in 2 mL of culture medium and allowed to adhere for 24 h. Then the cells were treated with free TPP, TPP/PEG-CD, and TPP@PEG-CD/CB-C_8-CB NP (5 μg/mL equivalent TPP concentration). After incubation for 6 h at 37 ^oC, the supernatant was removed，and the cells were washed with ice-cold PBS, followed by adding fresh culture medium. The cells were irradiated with a 620 nm light source (16 $mW \cdot cm^{-2}$) for 15 min and the lysosomal compartments of the cultured HepG2 cells were stained with the probe of Lyso-Tracker red DND-99. After the cells were fixed with 4% formaldehyde and the nucleus being stained with Hoechst 33258, the slides were mounted and imaged with a Zeiss LSM 700 confocal laser scanning microscope imaging (CLSM) system (Germany).

For flow cytometry analysis, the co-incubation of cells and samples was the same as the above procedure. Then a single cell suspension was prepared consecutively by trypsinization, washing with PBS, and filtration through 200 nylon mesh. Finally, 10,000 cells were lifted using a cell stripper (Media Tech. Inc.) and analyzed using a FACS Calibur flow cytometer (BD Biosciences) for TPP. Blank cells with addition of equivalent PBS were analyzed and their fluorescent intensity was designated as the threshold value. Only the fluorescent intensity that exceeded the threshold value can be considered as the uptake signal.



Scheme S1. Synthetic procedure of (A) CB-C_8-CB and (B) PEG-CD.

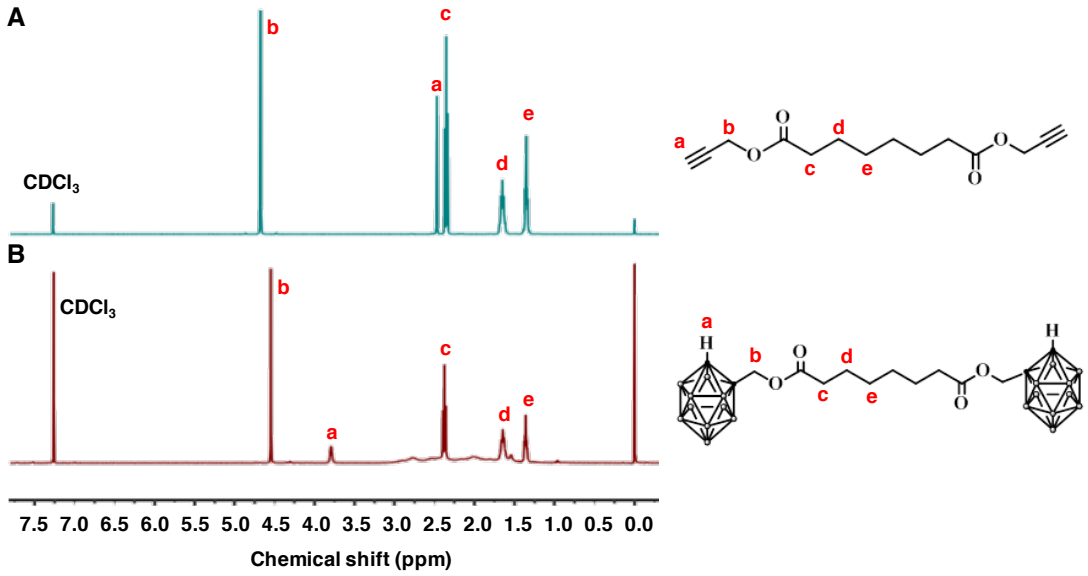


Fig. S1. ^{1}H NMR spectra of (A) dipropargyl suberate and (B) CB-C_8-CB in $CDCl_3$ (400 MHz).

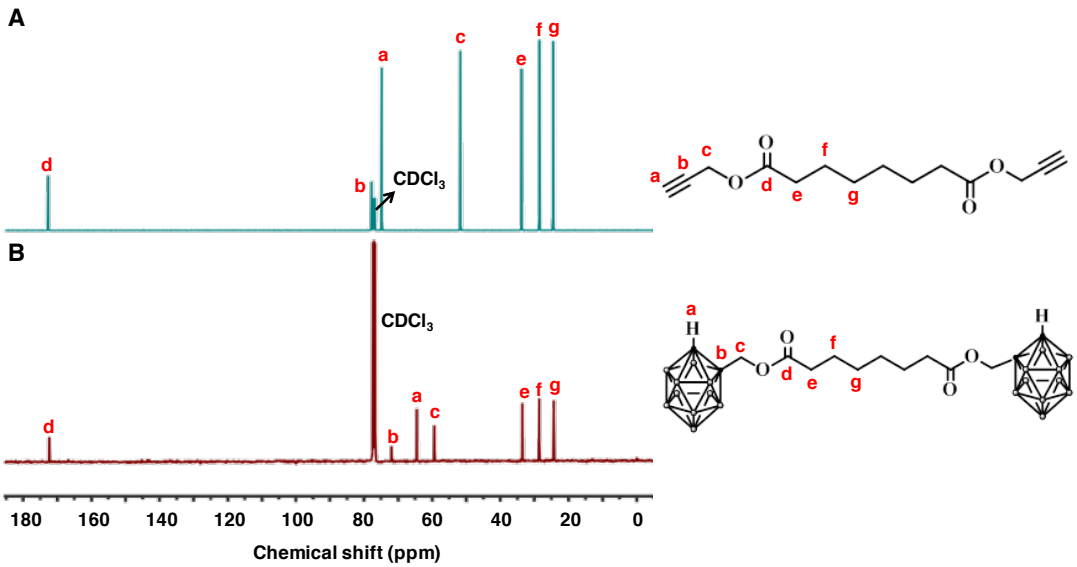


Fig. S2. ^{13}C NMR spectra of (A) dipropargyl suberate and (B) CB-C_8-CB in $CDCl_3$ (100 MHz).

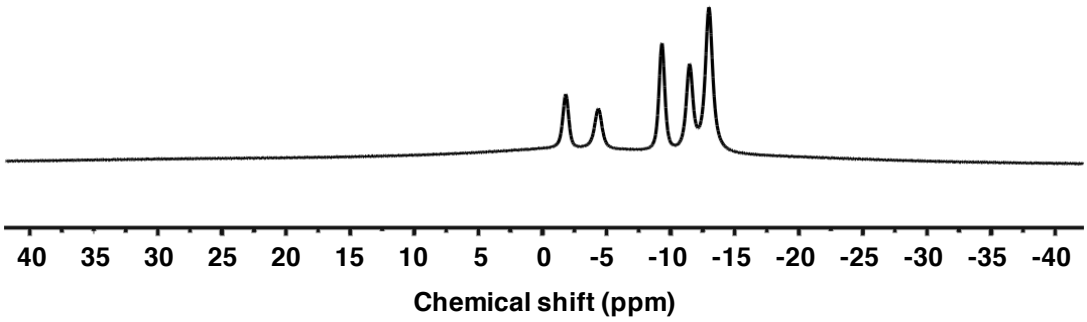


Fig. S3. ^{11}B NMR spectrum of CB-C_8-CB in $CDCl_3$ (400 MHz).

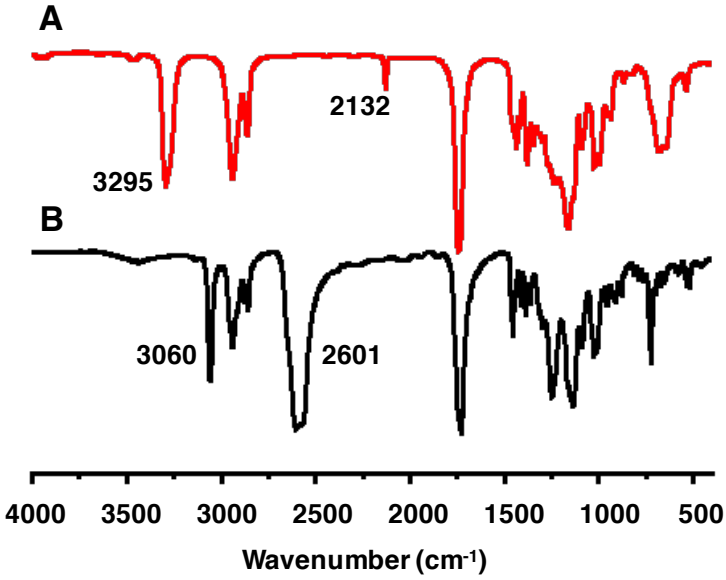


Fig. S4. FT-IR spectra of (A) dipropargyl suberate and (B) CB-C_8-CB.

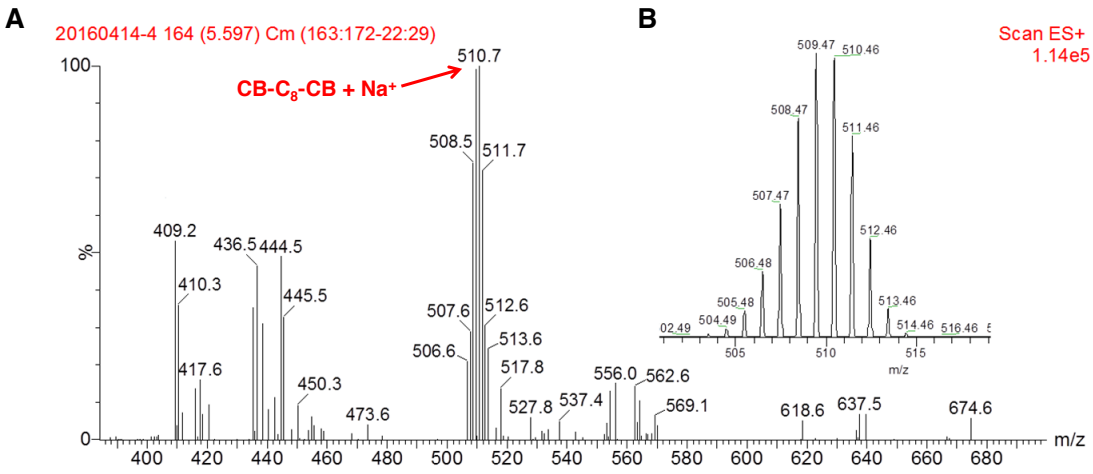


Fig. S5. (A) Measured and (B) theoretical ESI-MS spectra of CB-C_8-CB in positive mode.

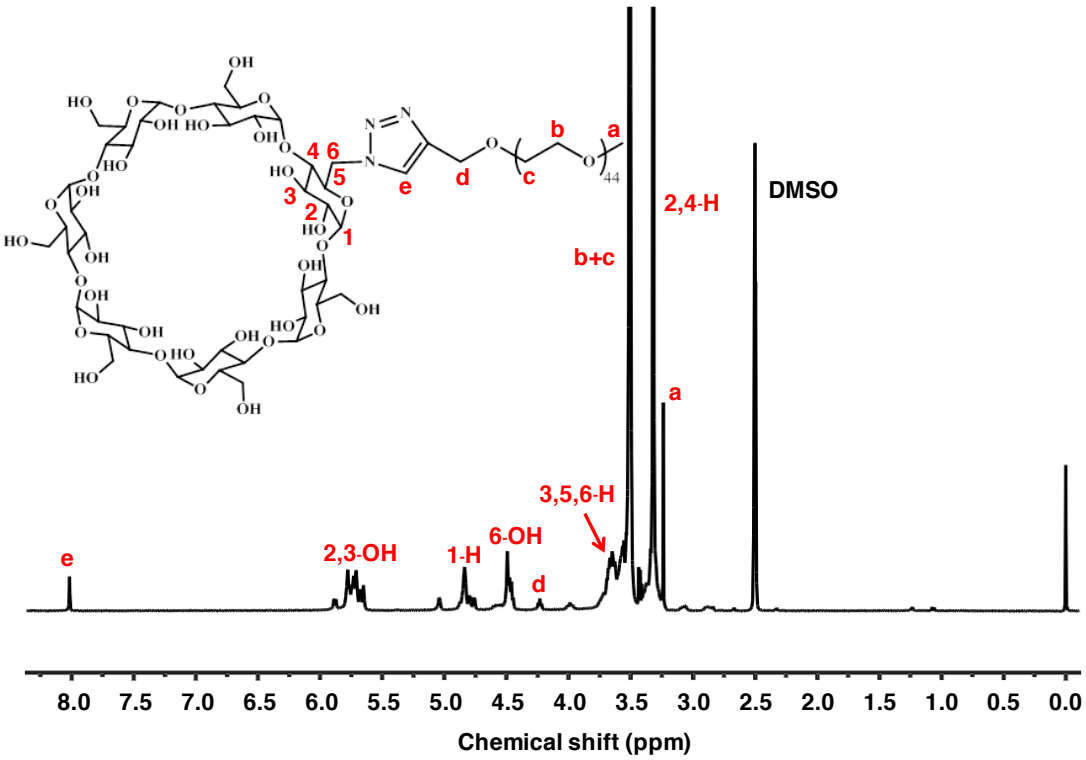


Fig. S6. ^{1}H NMR spectrum of PEG-CD in DMSO-d_6 (400 MHz).

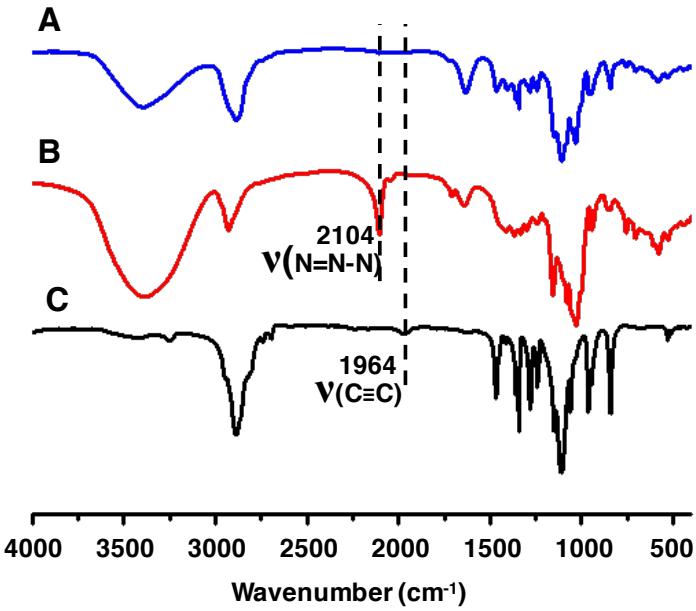


Fig. S7. FT-IR spectra of (A) PEG-CD, (B) β-CD-N_3 and (C) PEG-alkynyl.

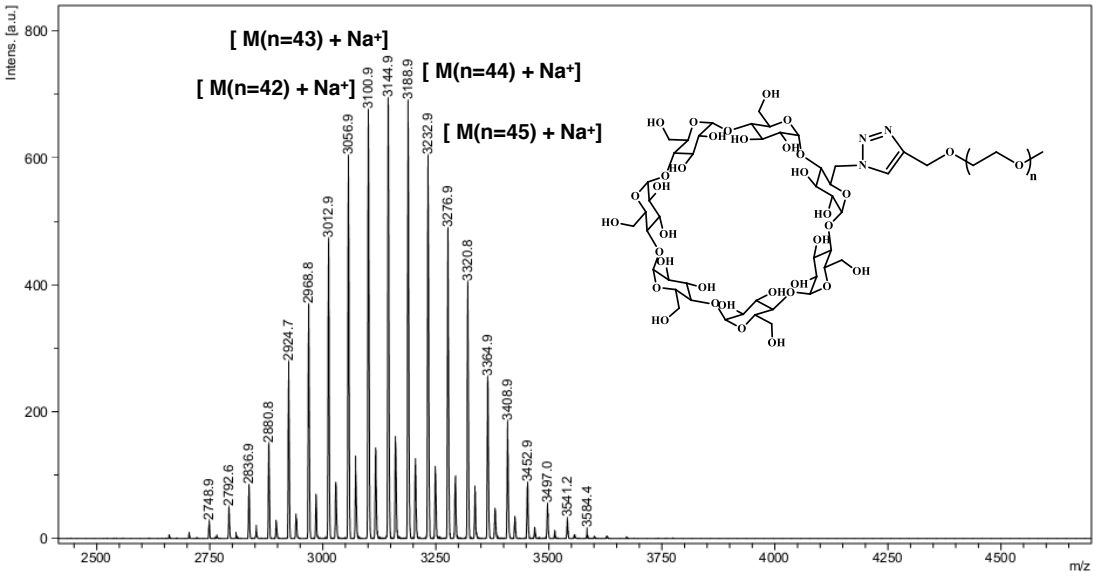


Fig. S8. MALDI-TOF mass spectrum of PEG-CD.

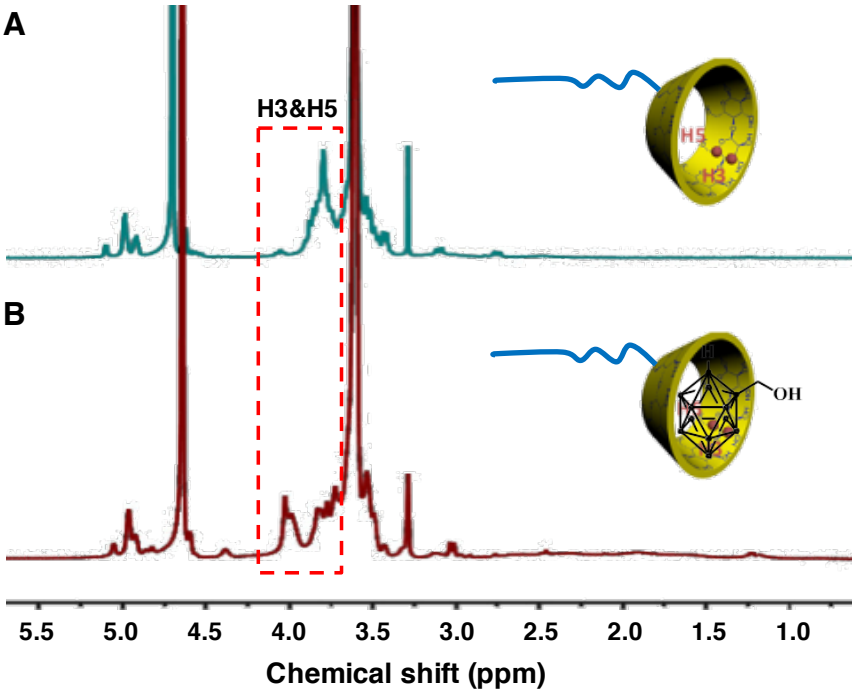


Fig. S9. ^{1}H NMR spectra of (A) PEG-CD and (B) HMCB/PEG-CD in D_2O (400 MHz).

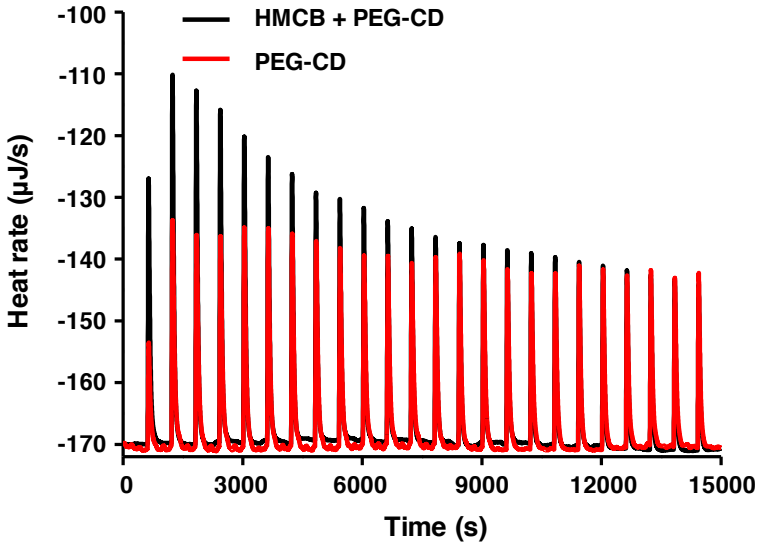


Fig. S10. ITC thermogram changes while HMCB in 5% DMSO aqueous solution or blank 5% DMSO solutions were titrated into PEG-CD aqueous solution.

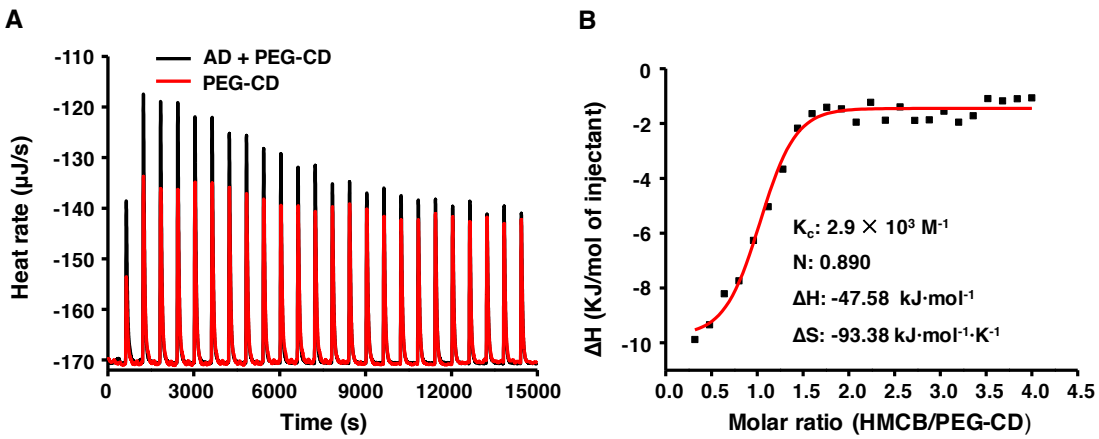


Fig. S11. ITC thermogram changes while adamantanemethanol (AD) in 5% DMSO aqueous solution or blank 5% DMSO solutions were titrated into PEG-CD aqueous solution. (B) The resulting calorimetric curves after subtracting the heat of dilution of PEG-CD solution.

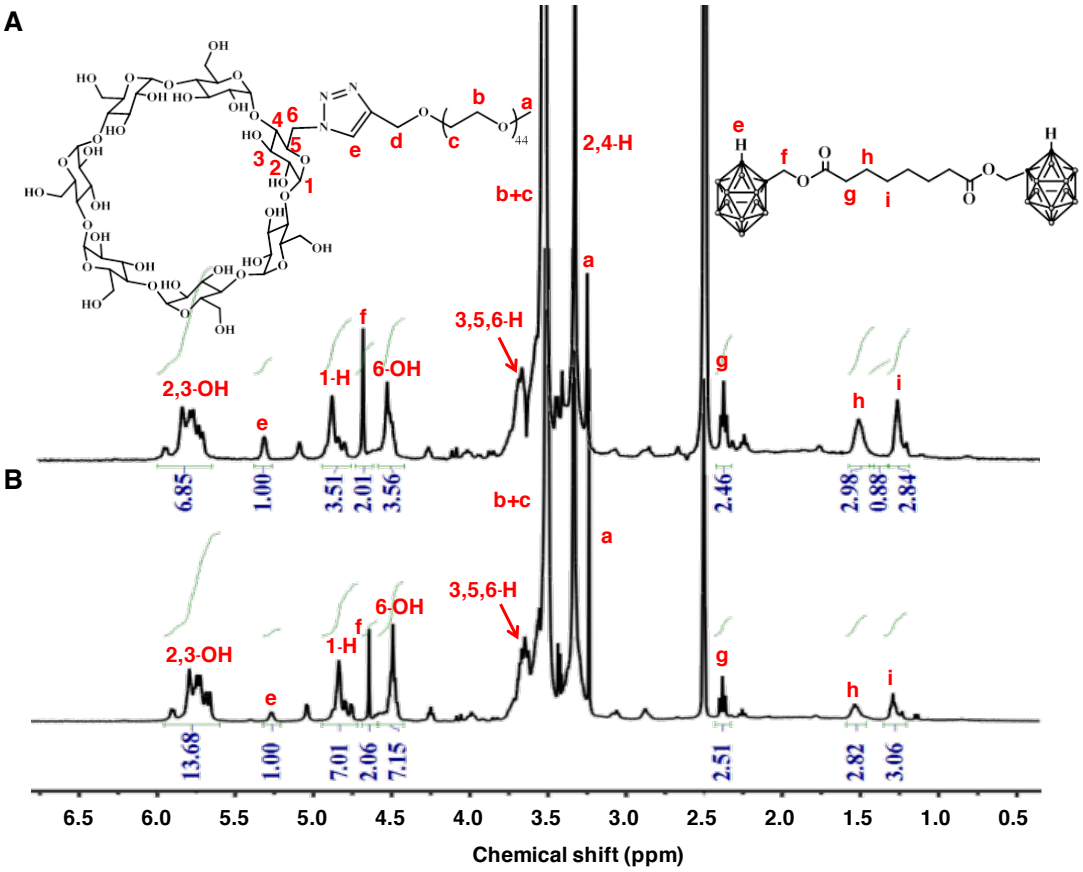


Fig. S12. Magnified ^{1}H NMR spectra of (A) PEG-CD/CB-C_8-CB and (B) 2PEG-CD/CB-C_8-CB in DMSO-d_6 with assignments and integration.

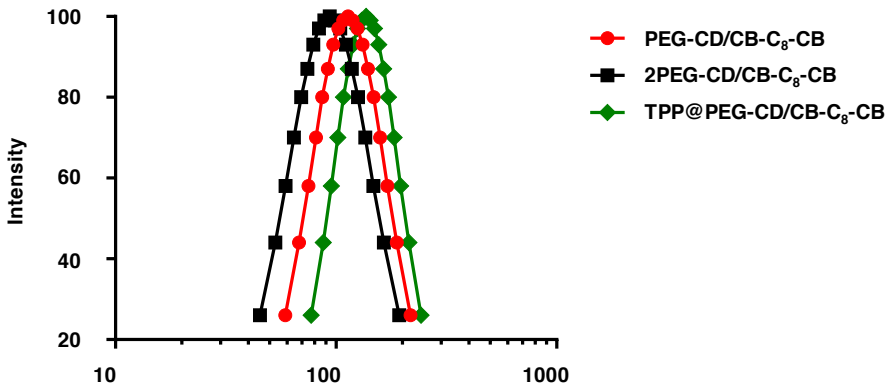


Fig. S13. DLS of PEG-CD/CB-C_8-CB NP, 2PEG-CD/CB-C_8-CB NP and TPP@PEG-CD/CB-C_8-CB NP.

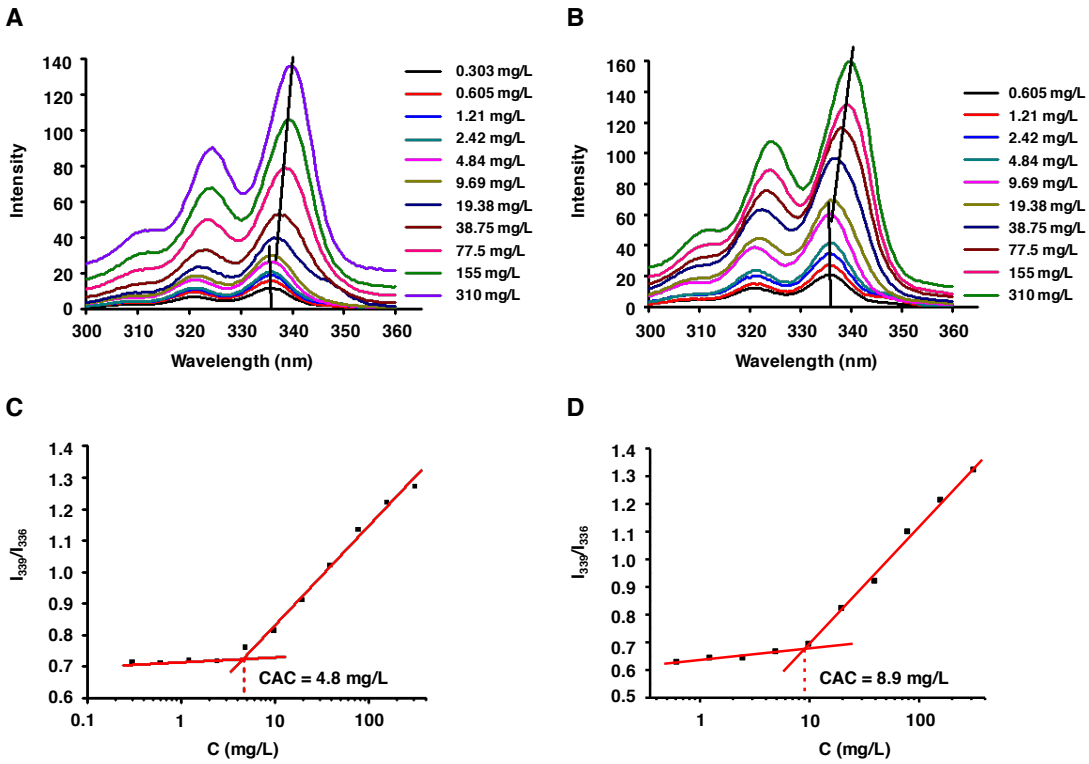


Fig. S14. Fluorescence excitation spectra of pyrene in solutions with different concentrations of (A) PEG-CD/CB-C_8-CB and (B) 2PEG-CD/CB-C_8-CB NP (emission at 391 nm). (C) Plot of the I_{339}/I_{336} ratio vs different concentrations of PEG-CD/CB-C_8-CB NP; (D) Plot of the I_{339}/I_{336} ratio vs different concentrations of 2PEG-CD/CB-C_8-CB NP.

Table S1. Characteristics of supramolecular nanoparticles.

sample	CAC (mg/L)[a]	D_h (nm)[b]	PDI[b]	Avg. Count Rate (Kcps)[c]	ζ (mv)[d]
PEG-CD	-	514	0.259	7.1	-16.78±1.14
PEG-CD/CB-C_8-C8	4.8	113	0.124	157.2	0.55±0.32
2PEG-CD/CB-C_8-CB	8.9	93	0.185	65.2	-18.37±0.91

[a] Critical aggregation concentration. [b,c] Determined by DLS. [c] The reflection of the signal intensity. [d] ζ potential measures in water.

Table S2. The relative contents of elements on the surface of nanoparticles from supramolecular self-assembly and physical mixture samples measured by XPS.

Sample	C%	O%	N%	B%	S/P (%)[c]
PEG-CD + CB-C_8-CB[a]	65.08	24.98	0.76	9.18	10.5
PEG-CD/CB-C_8-CB[b]	75.75	22.83	0.27	0.97	
2PEG-CD + CB-C_8-CB[a]	62.58	31.24	1.17	5.01	53.4
2PEG-CD/CB-C_8-CB[b]	63.79	33.03	0.5	2.68	

[a] Nanoparticles from supramolecular self-assembly of PEG-CD and CB-C_8-CB. [b] Physical mixture samples of PEG-CD and CB-C_8-CB. [c] The ratios of surface boron contents between supramolecular nanopartilces and physical mixtures.

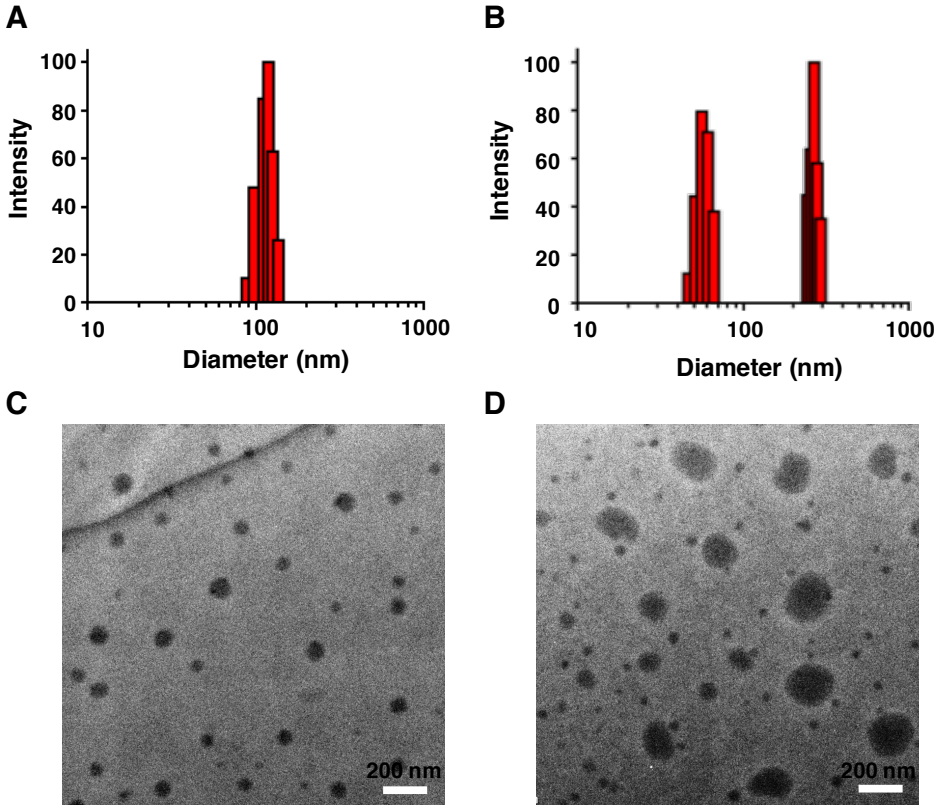


Fig. S15. DLS and TEM images of (A) (C) PEG-CD/CB-C_8-CB NP and (B) (D) PEG-CD/2CB-C_8-CB NP after kept in PBS (pH 7.4, 0.01 M) at 37 ^{o}C for a week, respectively.

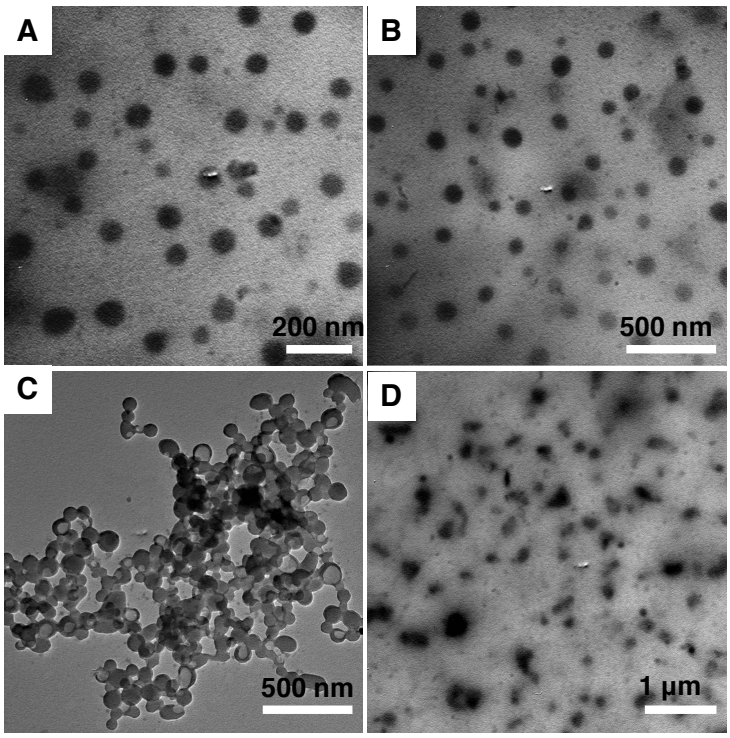


Fig. S16. TEM images of TPP@PEG-CD/CB-C_8-CB NP after kept in PBS (pH 7.4, 0.01 M) at 37 ^{o}C for (A) 0 h or (B) 120 h, and (C) TPP, (D) TPP/PEG-CD in aqueous solution.

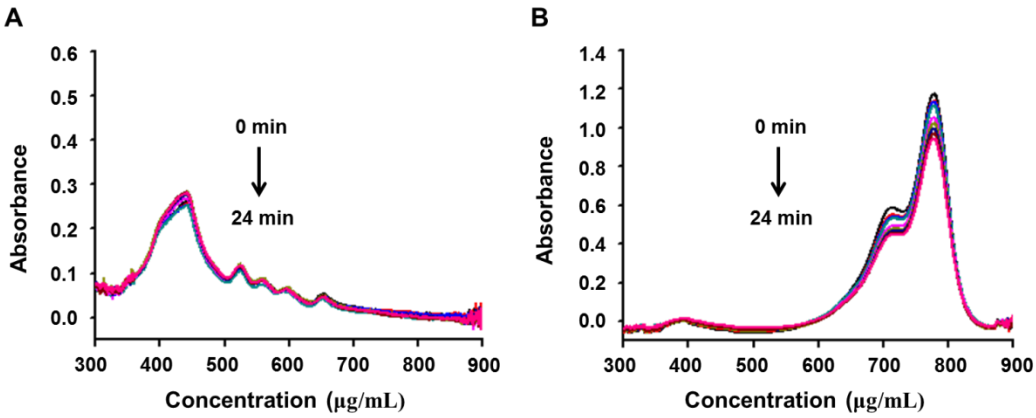


Fig. S17. UV-vis absorbance spectra of (A) TPP, (B) ICG after each two minutes irradiation at 620 nm (16 mW·cm^{-2}, eight times in total).

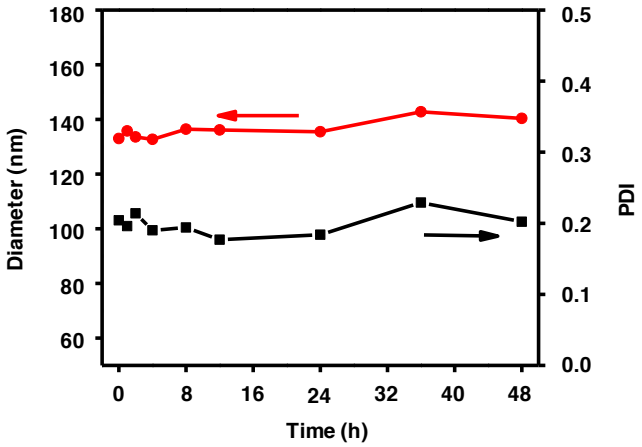


Fig. S18. Size-stability of TPP@PEG-CD/CB-C_8-CB NP 10% fetal bovine serum at 37 °C.

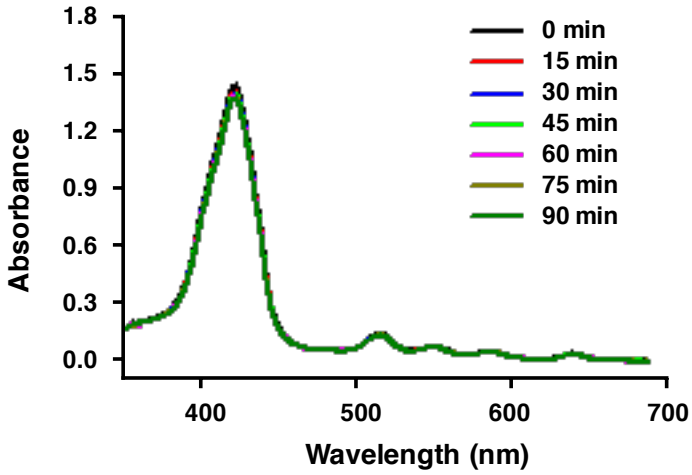


Fig. S19. Photostability of TPP@PEG-CD/CB-C_8-CB nanoparticles under the irradiation (620 nm, 16 $mW \cdot cm^{-2}$).

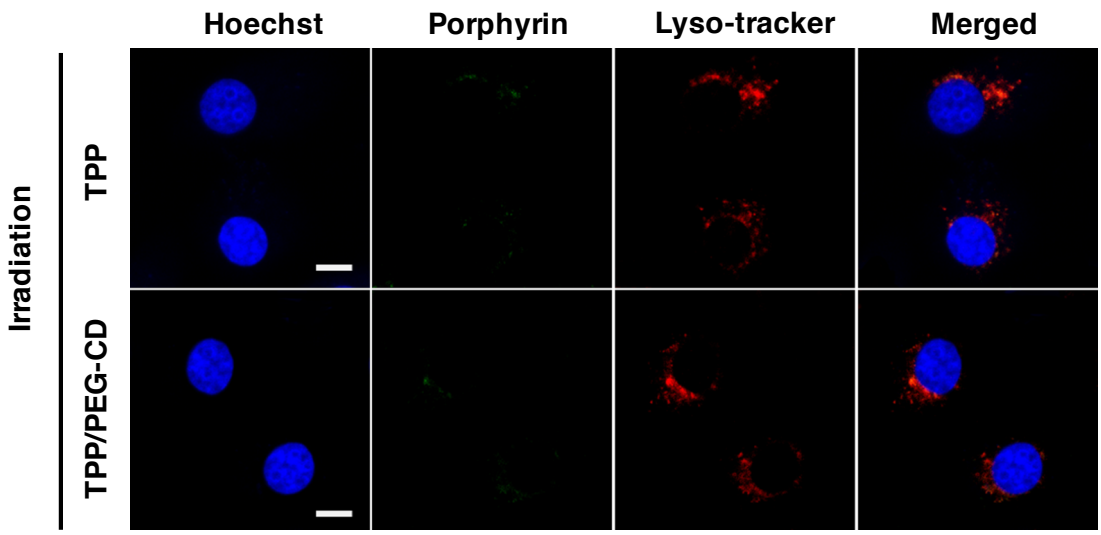


Fig. S20. Confocal images of HepG2 cells incubated with free TPP and TPP/PEG-CD for 6 h with irradiation (620 nm, 15 minutes, 16 $mW \cdot cm^{-2}$). Scale bar: 10 μm.

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