Supporting Information

Supplementary information includes experimental details; Synthesis routes of CD-Peptide-SH and mPEG-PLLMA(-Pep-CD)-PAsp(DBP); ^{1}H NMR spectra of β-cyclodextrin derivates and lysine derivates; size distributions of nanoparticles and drug loading contents of micelles; the cytotoxicity of blank micelles against C6 cells and bEnd3 cells; the statistical apoptosis rate of C6 cells; *in vivo* and *ex vivo* fluorescence imaging of Dir-MC; the weights of mice receiving various treatments; *ex vivo* histological analyses of organs.

Supplementary information accompanies this paper is available free of charge *via* the Internet at http://pubs.acs.org.

Experimental

Synthesis of mono-6-deoxy-6-tosyl-β-cyclodextrin (CD-OTs)

30 g of β-CD (16.5 mmol) was added into NaOH solution (18 g NaOH dissolved in 120 mL water) in ice bath followed by the addition of 6 g p-tolylsulfonyl chloride (6.3 mmol) with vigorous stirring. After 5 h's reaction at 0 °C, the precipitate was removed by filtration. Then 10% HCl was added into the filtrate to adjust the pH value to 7, the mixture was kept in 4 °C overnight. Afterwards, the white solid was recrystallized twice in hot water to obtain 12.5 g of product as a white crystalline powder. (Yield: 39.7%)

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.43 ppm (s, 3H, -ArC***H***$_3$), 3.35-3.15 ppm (m, 42H, ***H***OD shielded, H_2, H_3, H_4, H_5, H_{6a}, H_{6b}), 4.60-4.41 ppm (m, 6H, *6*-O***H***), 4.91-4.75 ppm (m, 7H, H_1), 5.85-5.65 ppm (m, 14H, *2,3*-O***H***), 7.48 ppm (d, 2H, Ar***H***), 7.79 ppm (d, 2H, Ar***H***).

MALDI-TOF MS: m/z calculated for $C_{49}H_{76}O_{37}S$ $[M + H]^+$ 1289.17, found: $[M + H]^+$ 1289.54 and $[M + K]^+$ 1327.36.

Synthesis of mono-6-N-propargylamino-6-deoxy-β-cyclodextrin (CD-Yne)

1.0 g of mono-6-OTs-β-CD was reacted with excess amount of propargylamine (124 mg, 2.25 mmol) in 15 mL of DMF at 70 °C for 24 h under N_2 atmosphere. After the reaction was completed, the mixture was cooled down to room temperature, and then 30 mL of cold acetone was added. The precipitates were re-dissolved in 30 mL of a water-methanol mixture solution (1:2 v:v), and washed with 50 mL of acetone for 3 times for the removal of unreacted propargylamine. Then the crude product was recrystallized twice in water and acetonitrile mixture, followed by drying under vacuum to obtain a white powder. (Yield: 27.3%)

^{1}H NMR (400 MHz, D_2O-d_2, δ ppm): 1.96 ppm (s, 1H, HC≡C-CH_2N***H***-), 2.75 ppm (t, 1H, ***H***C≡C-CH_2-), 3.03 ppm (d, 2H, HC≡C-C***H***$_2$-), 3.94-3.15 ppm (m, H_2, H_3, H_4, H_5, H_{6a}, H_{6b}), 4.91-4.75 ppm (m, 7H, H_1).

MALDI-TOF MS: m/z calculated for $C_{45}H_{73}NO_{34}$ $[M + H]^+$ 1172.05, found: $[M + Na]^+$ 1294.46.

Synthesis of mono-6-thiol-6-deoxy-β-cyclodextrin

5.0 g Mono-6-OTs-β-CD (3.9 mmol) and 5.0 g thiourea (66 mmol) were dissolved in 25 mL of 80% aqueous methanol and refluxed for 72 h at 110 °C. The solvent was evaporated and the obtained solid was re-dispersed in 80 mL methanol and stirred vigorously at room temperature for 1 h, then the mixture was filtered and the residue was dissolved in 100 mL 10% NaOH and stirred at 50 °C for 5 h. After reaction, the solution pH value was adjusted to 2 with 1 M HCl followed by adding 17 mL trichloroethylene, the mixture was stirred overnight at room temperature. The product was collected by filtering, vacuum drying, and recrystallizing in water and ethanol. (Yield: 24.8%)

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.75 ppm (d, 2H, -C***H***$_2$-SH), 3.35-3.15 ppm (m, 42H, ***H***OD shielded, H_2, H_3, H_4, H_5, H_{6a}, H_{6b}), 4.60-4.41 ppm (m, 6H, *6*-O***H***), 4.91-4.75 ppm (m, 7H, H_1), 5.85-5.65 ppm (m, 14H, *2,3*-O***H***).

Synthesis of MMP-2 sensitive peptide-cyclodextrin conjugate

100 mg of CD-Yne (0.085 mmol), 20 mg of sodium ascorbate (0.1 mmol) and 50 mg of azide-terminal peptide (0.06 mmol) were added into a 20 mL Schlenk tube, then 5 mL dry DMF was added and purged air through three freeze-thaw cycles using Ar gas

as an inert atmosphere. Then 2.5 mg of $CuSO_4 \cdot 5H_2O$ (0.01 mmol) was added into the solution and the mixture was stirred at 40 °C for 24 h. After the reaction was terminated by inletting air and adding 200 μL PMDETA, the solution was translated into dialysis bag (MWCO: 500 Da) and dialyzed against deionic water to remove Cu^{2+}. Finally, the product was obtained as an off-white powder after freeze-drying. (Yield: 59.5%).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.80-0.92 ppm (m, C***H***$_3$- of Leu) 1.03-1.59 ppm (m, -C***H***$_2$- and -C***H***- of peptide and polypeptide), 3.80-3.15 ppm (m, ***H***OD shielded, H_2, H_3, H_4, H_5, H_{6a}, H_{6b}), 4.60-4.41 ppm (m, 6H, *6*-O***H***), 4.91-4.75 ppm (m, 7H**,** H_1), 5.85-5.65 ppm (m, 14H, *2,3*-O***H***), 4.0-4.5 ppm（-CONHC***H***-NH）, 7.37 ppm (b, 1H, -C***H***- of triazole).

Synthesis of N-(tert-butoxycarbonyl)-ε-methacryloyllysine (MA-Lysine-Boc)

18.6 g of L-Lysine hydrochloride (0.11 mol) and 12.61 g of $CuSO_4 \cdot 5H_2O$ (0.055 mol) were added into a 500 mL two-neck round bottle containing 200 mL H_2O and refluxed for 2 h at 100 °C. After the solution was cooled to room temperature, the pH value was adjusted to 9 by adding 2 M Na_2CO_3 solution. Then 11.0 g of methacryloyl chloride (0.105 mol) and 100 mL NaOH (1 M) were added slowly to the solution in an ice-cold bath. The reaction was stirred overnight in the dark and then the solid was filtrated, washed with deionized water, methanol and diethyl ether sequentially. The obtained solid was re-dispersed in 500 mL deionized water after drying in vacuum, and then 29 g of 8-hydroxyquinoline (0.2 mol) dissolved in $CHCl_3$ (300 mL) was added under vigorous stirring. The filtrate was extracted with $CHCl_3$ (3 × 200 mL) to remove the excess 8-hydroxyquinoline. The yellowish aqueous solution was rotary evaporated to about 50 mL and then poured into a large amount of acetone (500 mL) to give a white precipitate. The product was obtained as a white solid after filtrating and vacuum drying. (Yield: 80.3%)

10 g of *N*-methacryloyllysine (46.7 mmol) was dissolved in 200 mL 1 M $NaHCO_3$ aqueous solution and then di-tert-butyl di-carbonate $(BOC)_2O$ (13.1 g, 60 mmol) in 20 mL THF was added dropwise. The solution was stirred for 24 h at room temperature and then extracted twice with 50 mL diethyl ether to remove excess $(BOC)_2O$,

followed by adding 0.2 M H_3PO_4 to acidify to pH 4. Then the mixture was washed with 1000 mL ethyl acetate for three times and the organic phase was collected and dried over anhydrous Na_2SO_4, filtrated and concentrated to 50 mL and stored at -40 °C to recrystallize. The final product was obtained as a white needle crystal or crystalline powder. (Yield: 44.5%)

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 12.34 ppm (s, 1H, COO***H***), 7.90 ppm (br, 1H, -CON***H***-), 7.03 ppm (br, 1H, $(CH_3)_3CO$-C(=O)N***H***-), 5.62 ppm (s, 1H, C***H***$_2$=C-), 5.30 ppm (s, 1H, C***H***$_2$=C-), 3.83 ppm (m, 2H, -CONH-C***H***$_2$-), 3.08 ppm (m, 2H, -CONH-C***H***$_2$COOH), 1.84 ppm (s, 3H, CH_2=C(C)-C***H***$_3$), 1.22-1.71 ppm (m, 15H, $(C\boldsymbol{H}_3)_3$CO-C(=O)NH-, -$(C\boldsymbol{H}_2)_3$-).

ESI-MS: m/z calculated for $C_{15}H_{26}N_2O_5$ $[M + H]^+$ 314.18, found $[M + Na]^+$ 338.10.

Synthesis of ε-Methacryloyl-L-Lysine N-Carboxyanhydride (MA-Lysine-NCA)

Under a nitrogen atmosphere, 4.8 g of MA-Lysine-Boc (15.3 mmol) and 1.54 g of triphosgene (5.2 mmol) were added to 150 mL of dry THF and dry ethyl acetate (1:4 v:v) in a 250 mL flame-dried flask and stirred at 40 °C for 1 h. Then, 1.57 g fresh distilled TEA dissolved in 20 mL ethyl acetate was added dropwise and the reaction was kept stirring at 40 °C overnight. Subsequently, the solution was cooled down to -40 °C rapidly to terminate the reaction. The TEA·HCl salt was removed by filtrating and the filtrate was washed sequentially with cold diluted HCl (0.05 M) aqueous solution, saturated brine and saturated $NaHCO_3$ aqueous solution several times. The organic phase was dried over anhydrous $MgSO_4$ and concentrated to a volume of about 20 mL and then poured into 200 mL fresh distilled petroleum ether to give MA-Lysine-NCA as a white solid. (Yield 35.4%).

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.24 and 5.59 ppm (ds, 2H, –$C(CH_3)$=C***H***$_2$), 4.39 ppm (t, 1H, -C***H***(C)NH-), 3.06 ppm (q, 2H, -C***H***$_2$NH-), 1.8 ppm (s, 3H, -C(C***H***$_3$)=CH_2), 1.2-1.75 ppm (dd, m, 6H, -CH-(C***H***$_2$)$_3$-), 7.85 ppm, 9.04 ppm(s, 2H, -CON***H***-).

Synthesis of methoxy-poly(ethylene glycol)-block-poly(ε-methacryloyl-L-lysine) -block-poly(β-benzyl-L-aspartate) (mPEG-PLLMA-PBLA)

Triblock copolymer of *m*PEG-PLLMA-PBLA was synthesized by sequential ring-

opening polymerization of MA-Lysine-NCA and BLA-NCA. Briefly, 1.25 g of $mPEG_{5k}$-NH_2 (0.25 mmol) was added into a 50 mL flame-dried Schlenk tube and vacuum dried at 70 °C for 2 h. After cooling down to room temperature, 1 g of MA-Lysine-NCA (4.2 mmol) dissolved in 10 mL of anhydrous DMF was then added under N_2 atmosphere. The reaction was sealed and stirred at 35 °C for 48 h, then 1.25 g of BLA-NCA (5 mmol) in 10 mL of DMF was added under N_2. The reaction mixture was stirred at 35 °C for another 48 h before precipitated into excess diethyl ether. The precipitate was then collected by centrifugation and dried under vacuum at room temperature to obtain *m*PEG-PLLMA-PBLA. The polymers were purified by self-assembly in water and dialysis (MWCO: 14 kDa) against a large amount of deionized water to remove small molecules. (Yield: 43.2%, Mn = 12000 Da calculated from ^{1}H NMR analysis).

Synthesis of methoxy-poly(ethylene glycol)-block-poly(ε-methacryloyl-L-lysine) -block-poly(N, N-dibutyl-1,3-propanediamine-L-aspartate) [mPEG-PLLMA-PAsp(DBP)]

*m*PEG-PLLMA-PAsp(DBP) was synthesized by aminolysis of mPEG-PLLMA-PBLA with *N, N*-dibutyl-1,3-propanediamine (DBP). Briefly, 200 mg of *m*PEG-PLLMA-PBLA (0.017 mmol) and 0.5 mL DBP (2.2 mmol) were dissolved in 15 mL DMF under N_2. The reaction mixture was stirred at 35 °C for 12 h before precipitated into diethyl ether for three times. The precipitate was then centrifuged, vacuum dried to obtain product as a slightly yellow solid. (Yield: 63.5%, Mn = 13600 Da calculated from ^{1}H NMR analysis)

Synthesis of MMP-2 substrate peptide conjugated β-cyclodextrin grafted triblock polymers (mPEG-PLLMA(-Pep-CD)-PAsp(DBP))

The mono-6-peptide conjugated β-cyclodextrin grafted tri-block polymers *m*PEG-PLLMA(-Pep-CD)-PAsp(DBP) was synthesized by thiol-ene "Michael addition" between CD-Peptide-SH and *m*PEG-PLLMA-PAsp(DBP). Briefly, 590 mg of CD-Peptide-SH (0.3 mmol) and 100 mg of *m*PEG-PLLMA-PAsp(DBP) (8.3 μmol) were dissolved in 10 mL anhydrous DMF in a 50 mL Schlenk tube and 100 μL dimethylphenylphosphine was added under N_2 atmosphere. The mixture was sealed

and stirred at 40 °C for 72 h followed by being precipitated in diethyl ether three times and then centrifuged, vacuum dried. The obtained solid was re-dissolved in DMF and added into H_2O to assemble micelle. The solution was dialyzed (MWCO: 14 kDa) against deionized water, freeze dried to obtain a white powder. (Yield: 33.7%, *M*n = 27600 Da calculated from ^{1}H NMR analysis)

Synthesis of fluorescence labeled β-cyclodextrin grafted tri-block polymers (mPEG-PLLMA(-Pep-CD-Cr)-PAsp(DBP))

The Cr was conjugated to cyclodextrin on the side-chain of polymer. Briefly, under a dry N_2 atmosphere, 5 μL of oxalyl chloride (8 mg, 60 μmol) and 3 mg of 7-(diethylamino) coumarin-3-carboxylic acid (1.3 μmol) were dissolved in 5 mL anhydrous CH_2Cl_2, followed by adding 0.5 μL of DMF as a catalyst. After 12 h reaction at room temperature, the solvent was evaporated to give 7-(diethylamino)coumarin-3-carboxylic chloride as an orange solid. And then the solid was re-dissolved in 2 mL anhydrous DMF and added to a 5 mL DMF solution containing 25 mg of *m*PEG-PLLMA(S-Peptide-CD)-PAsp(DBP) (1.2 μmol) and 1.5 μL TEA (10 μmol), the reaction was stirred at 40 °C for 24 h. The final product of *m*PEG-PLLMA(-Pep-CD-Cr)-PAsp(DBP) was obtained as a red solid after precipitation in diethyl ether for three times, centrifugation and vacuum drying. (Yield 35%)

Polymer and micelle characterizations

^{1}H NMR spectra were carried out on a Bruker 400 MHz spectrometer and using D_2O-d_2 & DMSO-d_6 as solvents. UV-Vis spectral measurements were recorded using Unico UV-2000 UV-Vis spectrophotometer. The fluorescence spectra were measured on a spectrofluorophotometer (Perkin Elmer Ltd., United Kingdom). The molecular weight distribution of polymers was analyzed using a gel permeation chromatography (GPC) system consisting of a Waters 1515 pump, an Ultrahydrogel™ 500 column, an Ultrahydrogel™ 250 column, and a Waters 2417 differential refractive index detector with PEG as a calibration standard. DMF (HPLC grade) containing LiBr (1.0 g/L) was used as an eluent at a flow rate of 1.0 mL/min. Transmission electron microscopy (TEM) analysis was conducted with a Hitachi model JEM-1400 TEM (Hitachi Co.,

Ltd., Tokyo, Japan) operated at 120 kV. Samples were prepared by drying a drop (5 μL, 1 mg/mL) of sample solution on a copper grid coated with amorphous carbon. A small drop of uranyl acetate solution (2 wt% in water) was added to the copper grid to negatively stain the sample for 60 s and then blotted with a filter paper. The grid was finally dried overnight inside a desiccator before TEM observation. The size distribution of micelles was measured by 90Plus/BI-MAS equipment (Brookhaven Instruments Corporation, USA) at 25 °C at a detection angle of 15° and 90°, respectively. The values are the mean of three runs plus standard deviations.

Drug loading content (DLC) determination

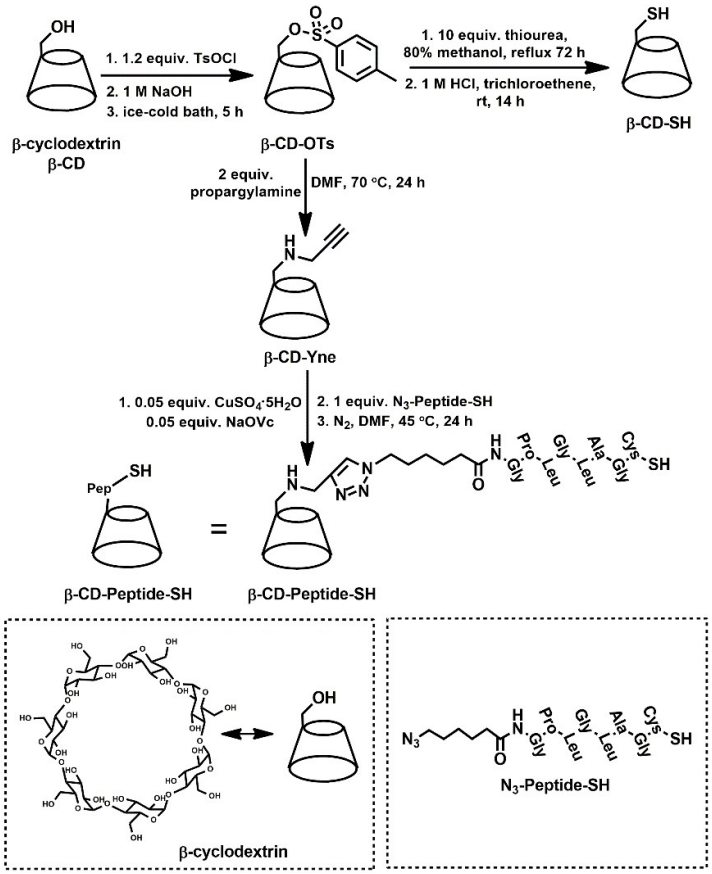
A certain amount of drug-loaded micelle solution was freeze-dried, weighted and then re-dissolved to determine the drug loading content by RP-HPLC analysis. General procedures were as follows, 1 mL of the PTX-loaded micelle solution was lyophilized and re-dissolved in 50 μL DMF (HPLC grade), diluted with 0.95 mL of acetonitrile (HPLC grade), and then analyzed and quantitated by reverse-phase high performance liquid chromatography (RP-HPLC).

The calibration curve was acquired with different concentrations of free PTX and sunitinib and used for the drug quantification. The drug loading content (DLC) was calculated by the following formula:

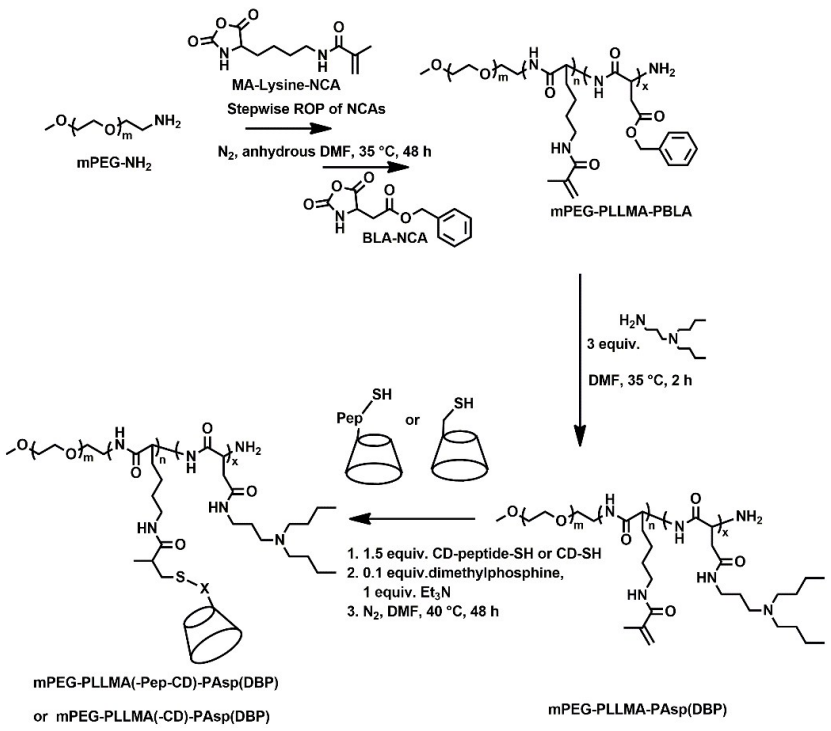
$$Drug\ loading\ content\ (DLC) = \frac{weight\ of\ drug\ in\ micelles}{weight\ of\ drug\ loaded\ micelles} \times 100\ \%$$

The HPLC analysis was achieved on a Waters 1525 HPLC equipped with a Waters 2489 UV-Vis detector. Drug separation was proceeded on a C_{18} Ultimate® Column (150 × 4.6 mm, 5 μm; Welch, Shanghai, China) at 40 °C. For PTX analysis, a mobile phase consisting of acetonitrile, 0.02 M phosphoric acid/ammonium phosphate buffer (pH = 3) (70:30 v:v) at a flow rate of 1.0 mL/min. The effluents were monitored at 227 nm and quantified by pre-determined calibration curve (PTX concentration 0.5 to 255 μg/mL). For sunitinib detection, a mobile phase consisting of acetonitrile and 0.02 M phosphoric acid/ammonium phosphate buffer (pH = 3) (65:35 v:v) at a flow rate of 1.0 mL/min. The effluents were monitored at 270 nm and quantified using the

area under the peak from standard solutions of free sunitinib (0.6 to 18 μg/mL).



Scheme S1. Synthesis routes of CD-SH and CD-Peptide-SH.



Scheme S2. Synthesis of β-cyclodextrin grafted triblock polymers: mPEG-PLLMA(-CD)-PAsp(DBP) and mPEG-PLLMA(-Pep-CD)-PAsp(DBP).

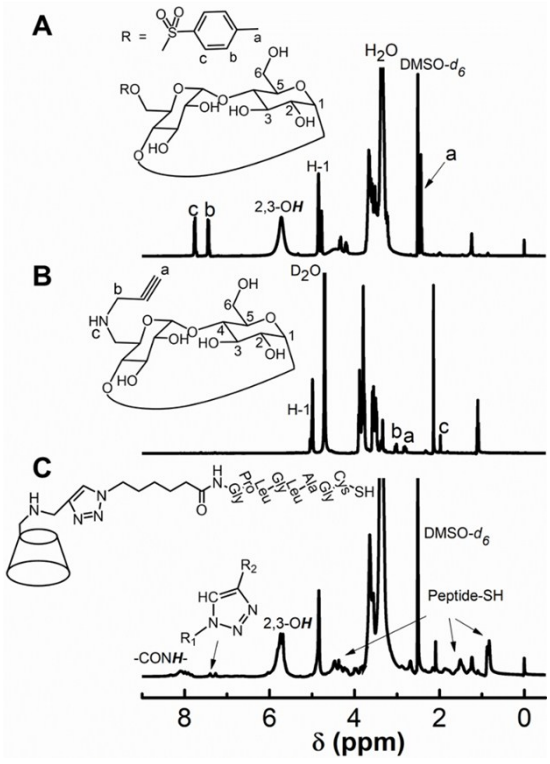


Fig. S1 ^{1}H NMR spectra of β-cyclodextrin derivates: (A) CD-OTs; (B) CD-Yne; (C) CD-Peptide-SH.

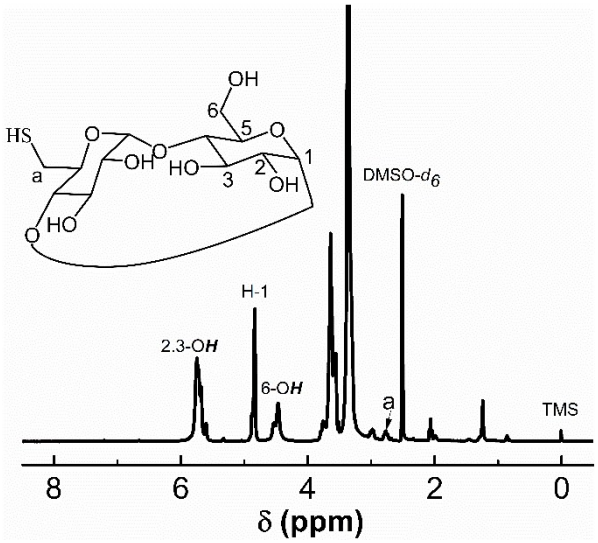


Fig. S2 ^{1}H NMR spectrum of CD-SH.

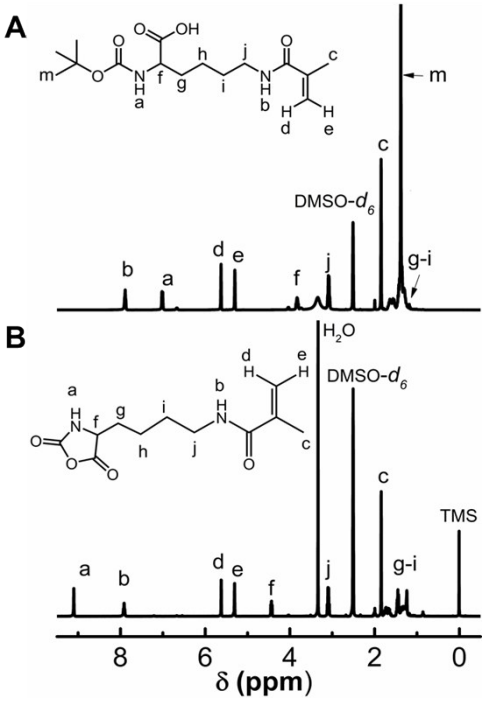


Fig. S3 ^{1}H NMR spectra of lysine derivates: (A) MA-Lysine (BOC); (B) MA-Lysine-NCA. The BOC characteristic peaks at 1.38 ppm disappeared and meanwhile the peak for NCA ring appeared at 9.04 ppm.

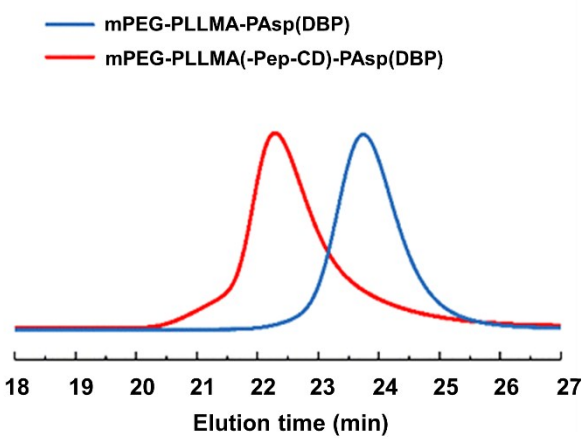


Fig. S4 GPC traces of mPEG-PLLMA-PAsp(DBP) and mPEG-PLLMA(-Pep-CD)-PAsp(DBP). DMF containing 1 g/L LiBr was used as an eluent at a flow rate of 1.0 mL/min.

Table S1. Characteristics of block copolymers.

Polymer	M_n[a] (kDa)	M_n[b] (kDa)	M_w/M_n[b]
mPEG-PLLMA	7.9	19.0	1.20
mPEG-PLLMA-PBLA	12.0	24.0	1.23
mPEG-PLLMA-PAsp(DBP)	13.6	26.9	1.20
mPEG-PLLMA(-Pep-CD)-PAsp(DBP)	27.6	34.5	1.21

[a]calculated by ^{1}H NMR; [b]detected by GPC.

Table S2. Size distributions of nanoparticles

Nanoparticles	Size (determined by DLS)	PDI [a]
psMC	94.4 ± 17.4 nm	0.143
PTX-psMC	101.7 ± 15.2 nm	0.134
Sunit-psMC	123.6 ± 23.5 nm	0.175
PTX-Sunit-psMC	183 ± 34.7 nm	0.242
Sunit-psMC + MMP-2	51.5 ± 8.2 nm	0.407
PTX-Sunit-psMC + MMP-2	60.3 ± 10.1 nm	0.167
MC	130.5 ± 25.5 nm	0.136
PTX-MC	136.0 ± 28.5 nm	0.158
Sunit-MC	150.0 ± 47.5 nm	0.299
PTX-Sunit-MC	157.9 ± 32.1 nm	0.180

a. Polydispersity index.

Table S3. The highest drug loading contents of micelles

Nanoparticles	Sunitinib loading content (%)	PTX loading content (%)
PTX-MC	--	5.1 ± 0.1
Sunit-psMC	1.6 ± 0.1	--
PTX-Sunit-MC	1.27 ± 0.2	5.17 ± 0.1
PTX-Sunit-psMC	1.12 ± 0.1	5.34 ± 0.1

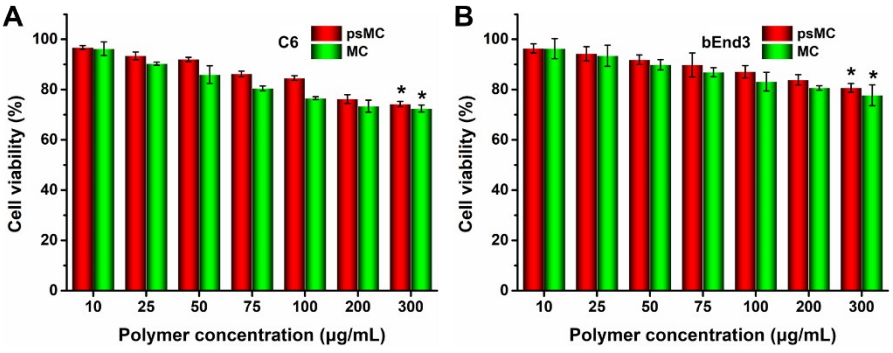


Fig. S5 The cell viabilities of C6 cells (A) and bEnd3 cells (B) after receiving the treatment of psMC and MC at various polymer concentration. *P < 0.05 compared with 10 μg/mL. No significance differences were found between psMC and MC at the same polymer concentration.

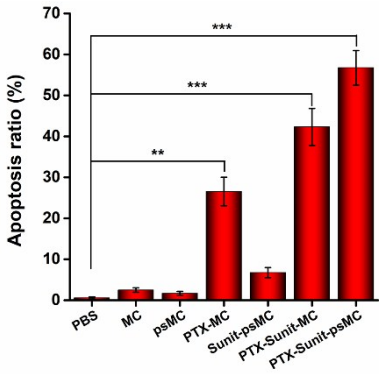


Fig. S6 The statistical analysis of apoptosis rate after C6 cell receiving the treatments of PBS, MC, psMC, Sunit-psMC, PTX-MC, PTX-Sunit-MC and PTX-Sunit-psMC. $^{**}P < 0.01$, $^{***}P < 0.001$.

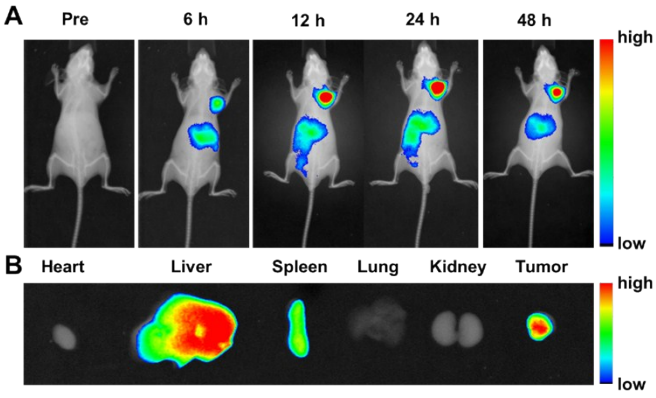


Fig. S7 (A) Typical *in vivo* Dir fluorescence images at different time points after tail vein injection of Dir-MC and (B) *ex vivo* Dir fluorescence images of the organs excised at 48 h post-injection time from the same animal.

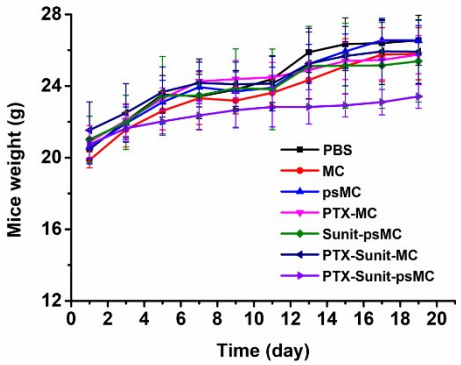


Fig. S8 The weights of mice receiving various treatments. Mice weight were measured every 2 days (n = 6). PTX dose: 500 μg/kg body weight; sunitinib dose: 340

μg/kg body weight.

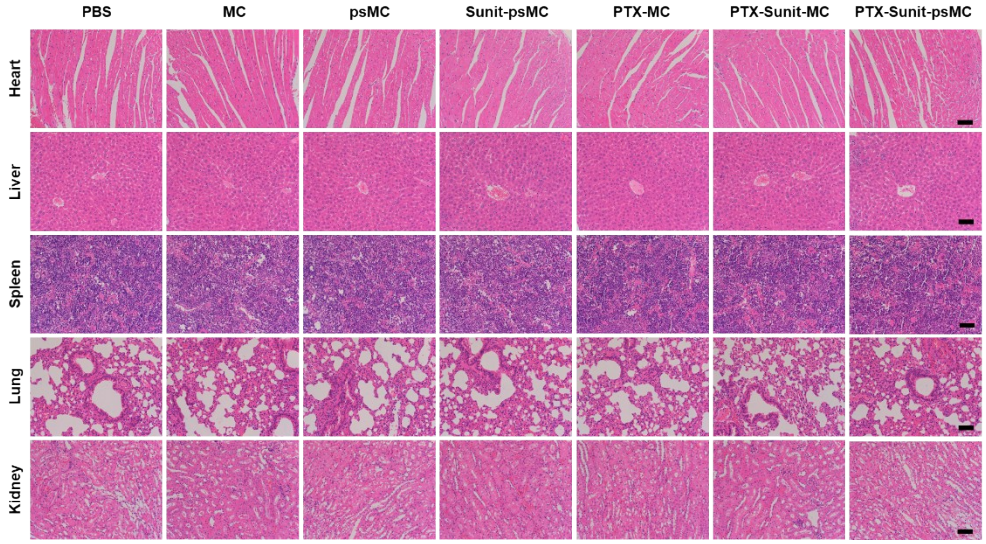


Fig. S9 *Ex vivo* histological analyses of organs excised from animals of different treatment groups at 19 days after the first treatment. Scale bars represented 50 μm.