Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supplementary Information

Carbon dot-modified controllable drug delivery system for sonodynamic/chemotherapy of tumors

Qingxuan Meng [a], Qian Wang [b], Qing Zhang [a], Jia Wang [a], Yuhao Li [b,], Senqiang Zhu [a,*], Rui Liu [a,*], Hongjun Zhu [a]*

[a] School of Chemistry and Molecular Engineering, Nanjing Tech University, Nanjing 211816, China.

[b] School of Materials and Chemistry, Institute of Bismuth Science, Shanghai Collaborative Innovation Center of Energy Therapy for Tumors, University of Shanghai for Science and Technology, Shanghai 200093, China.

Corresponding authors:

Yuhao Li: yhli@usst.edu.cn

Senqiang Zhu: zhusenqiang1993@njtech.edu.cn

Rui Liu: rui.liu@njtech.edu.cn

Experimental section

Materials

Methacrylic acid (MAA) was purchased from Macklin (China). Cystamine dihydrochloride (CD), 1,3-diphenylisobenzofuran (DPBF), folic acid (FA), dicyclohexylcarbodiimide (DCC), acryloyl chloride (AC), and 3-(isobutyryloxy) propyltrimethoxysilane (MPS) were obtained from Aladdin (China). Ammonia (NH_3•H_2O), tetraethyl orthosilicate (TEOS), 2,2-azobisisobutyronitrile (AIBN), and hydrofluoric acid (HF) were supplied by Sinopharm (China). Ethylenediamine was acquired from Ling Feng (China). Sodium hydroxide (NaOH) was procured from Xilong (China). Doxorubicin (DOX) was sourced from Roche (USA). N-hydroxysuccinimide (NHS) was bought from Oridonin (China). Phosphate buffered solution (PBS), triacetonamine hydrochloride (TEMP), and glutathione (GSH) were provided by Yuanye (China). All chemicals were used as received.

Synthesis of N,N-bis(caryloyl)cystamine (BACy)

CD (5.630 g, 25.00 mmol) was added to a 100 mL flask, followed by 40.0 mL of distilled water, and stirred until dissolved. Then, the system was cooled to 0 ℃, and aqueous NaOH (20.0 mL, 5.00 mol L^{-1}) was slowly added via a pressure-equalizing funnel and stirred for 10 min, followed by another pressure-equalizing funnel with dichloromethane solution (10.0 mL, 5.00 mol L^{-1}) of AC. The reaction was kept at 0 ℃ for 0.5 h, then the system temperature was gradually raised to room temperature for another 12 h. The reaction mixture was filtered under reduced pressure, and the solid was recrystallized from petroleum ether/ethyl acetate (V/V = 1/1), yielding a white solid (4.780 g, 73.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (t, J = 5.6 Hz, 1H), 6.23 (dd, J = 17.1, 10.1 Hz, 1H), 6.09 (dd, J = 17.1, 2.3 Hz, 1H), 5.60 (dd, J = 10.0, 2.3 Hz, 1H), 3.47 – 3.38 (m, 2H), 2.83 (t, J = 6.8 Hz, 2H).

Synthesis of MPS@SiO_2

MPS@SiO_2 nanoparticles were synthesized using the Stober method. First, $NH_3 \bullet H_2O$ (1.0 mL) and distilled water (12.5 mL) were mixed well in a 100 mL flask, and then the ethanol solution (52.5 mL) of TEOS (4.0 mL) was rapidly added to the flask. After stirring at the corresponding temperature for 24 h, MPS (2.0 mL) was added to react for another 12 h. At the end of the reaction, a white solid was collected by centrifugation, redispersed in ethanol, and washed three times by centrifugation. The obtained solid was dried in a vacuum oven for 24 h (40 ℃) to afford a white powder (MPS@SiO_2).

Synthesis of CS-PMAA

MAA (0.600 g), BACy (0.075 g), MPS@SiO_2 (0.200 g), AIBN (0.060 g), and CH_3CN (50.0 mL) in a 100 mL single-necked flask were sonicated for 10 min. The mixture was stirred and heated to 83 ℃ for 0.5 h. Then, the solution turned white. The reaction apparatus was changed to a distillation apparatus. Heat the mixture, remove half the solvents by distillation, and get white substances. The white solid was redispersed with CH_3CN, centrifuged, and collected in a vacuum drying oven for 24 h (40 ℃) after repeated three times to obtain a white powder (CS-PMAA).

Synthesis of CDs

Folic acid powder (0.15 g, 0.340 mmol) was added to 100 mL of water. After vigorous stirring, the mixture was added to an autoclave reactor and reacted for 2 h at 180 ℃. Then, the particles were filtered through 0.22 μm Millipore syringe filters before use. A yellow powder (CDs) was obtained by freeze-drying the collected solid under a vacuum for 24 h at −40 ℃.

Synthesis of CDs@CS-PMAA

In a flask, CS-PMAA (0.200 g), NHS (0.575 g, 5.000 mmol), DCC (0.413 g, 2.000 mmol), and CDs (0.10 g) were added along with CH_3CN (20.0 mL). After carboxyl activation at 60 ℃ for 10

h under argon, ethylenediamine (1.0 mL) was added. The reaction was continued for 12 h and then cooled to 25 ℃. The product was centrifuged, and the obtained material was purified by repeated dispersion in distilled water. A pale yellow powder (carbon dots-modified poly(methacrylic acid), CDs@CS-PMAA) was obtained by freeze-drying the collected solid under vacuum for 24 h at −40 ℃.

Synthesis of CDs@HPMAA

CDs@CS-PMAA (0.200 g) was added to a Teflon beaker. Then, CH_3CN (45 mL) was added and sonicated for 10 min. After that, the mixture was stirred slowly, and 5 mL of HF was added dropwise. The reaction continued for 24 h, and the solid was collected by centrifugation and washed with water thrice. The obtained solid was freeze-dried at −40 ℃ under vacuum for 24 h to afford a pale yellow powder (carbon dots-modified hollow poly(methacrylic acid) acid nanoparticles, CDs@HPMAA).

Synthesis of DOX@CDs@HPMAA

Generally, 20 mg of CDs@HPMAA and 12 mg of DOX were dispersed in 20 mL of PBS (pH = 7.4) conditions of gentle stirring for 24 h at 25 ℃. DOX@CDs@HPMAA was then obtained by centrifugation and PBS (pH=7.4) washing three times. The LC and LE of the DOX@CDs@HPMAA were calculated using the standard curve method using the following equations.

$$LC = \frac{Initial\ weight\ of\ DOX - Weight\ of\ DOX\ in\ supernatant}{Weight\ of\ DOX\ loaded\ nanocapsules} \times 100\% \quad (1)$$

$$LE = \frac{Weight\ of\ DOX\ in\ nanocapsules}{Initial\ weight\ of\ DOX} \times 100\% \quad (2)$$

Instrument

Field emission scanning electron microscopy (SEM) was performed on a Quanta Fei FEG-450 instrument. TEM images were obtained on a JEM-2010 transmission electron microscope at an accelerating voltage of 120 kV. The UV–vis absorption and fluorescence spectra were measured by a Puyan TU-1900 and a Hitachi F-4600 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz instrument. Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet IS-5 from Thermo Fisher Scientific. Dynamic light scattering (DLS) was performed on a Malvern S90 instrument. Electron paramagnetic resonance (EPR) spectra were recorded on a Bruker EMX EPR spectrometer. The turbidity measurements were obtained using a WGZ200-AS Nephelometer.

Sonodynamic performance measurements

The mixture solution of 20 μg DPBF and CDs (100 μg mL^{-1}, 5.0 mL) was exposed to different US irradiation (1.0 MHz, 0.5 W cm^{-2}) for 5 min, and the absorbance of DPBF at 423 nm was recorded to measure $^1O_2$ generation. For ESR measurements, the mixture solution of 20 μL TEMP and CDs (100 μg mL^{-1}, 5.0 mL) or CDs@HPMAA (100 μg mL^{-1}, 5.0 mL) was exposed to US irradiation for 5 min, and the signal of $^1O_2$ was detected by ESR spectrometer using TEMP as the trapping agent.

Electrochemical measurements

The CV curves of CDs and CDs@HPMAA were measured based on a three-electrode system using an electrochemical workstation (CHI 760E, China). The reference electrode, counter electrode, and electrolyte were Ag/AgCl, platinum wire, and TBAPF6, respectively. The conductivity type of CDs and CDs@HPMAA was analyzed by impedance spectroscopy. The conduction and valence-band edges of CDs and CDs@HPMAA were determined by a linear potential scan (50 mV s^{-1}).

GSH consumption

The DTNB method measured the GSH content of Control, US, CDs@HPMAA, and CDs@HPMAA + US groups. The concentration of CDs@HPMAA was 200 μg mL^{-1}, and US (1 W cm^{-2}, 10 min). We followed the procedure in the glutathione assay kit instructions (Beyotime, China) for the measurement.

Redox-triggered disassembly of HPMAA

A nephelometer monitored the turbidity change of the CDs@PMAA in response to GSH. Briefly, 3.0 mg CDs@PMAA was dissolved in 10 mL PBS (pH = 7.4), then 10 mM GSH was added. The solution was shaken at 37 ℃. Samples were collected at predetermined intervals, and their scattering light intensities were determined using a nephelometer. The turbidity was calculated by the ratio of the scattering power of the irradiated samples to that of the initial non-degraded model (no GSH added). The molecular weight of the degraded polymers from the HPMAA in the presence of GSH was measured in an aqueous solution by GPC. The degraded polymer solution was filtered through a 0.22 um filter before injected into the GPC.

In vitro DOX release

Typically, DOX@CDs@HPMAA (5.0 mg) was dispersed in 4.0 mL of different buffer solutions (pH = 7.4; pH = 5.0), and the dispersion was divided into four equal parts (pH = 7.4; pH = 5.0; pH = 5.0, with GSH; pH = 5.0, with GSH and US). Each of the DOX@CDs@HPMAA was then transferred into a dialysis bag (molecular weight cut off 3500 Da), which was dialyzed in 10 mL of the corresponding buffer (pH = 7.4 or 5.0) with or without 10 mM GSH and gently shaken at 37 ℃, respectively. The drug release was considered to start as soon as the dialysis bags were immersed into the reservoir. At predetermined intervals, 1 mL of the solution was periodically collected from the reservoir, and the amounts of released DOX from DOX@CDs@HPMAA were

analyzed by measuring the absorption at 481 nm. To maintain a constant volume, 1 mL of fresh buffer medium was replenished in the reservoir after each sampling.

$$Dox\ release\ (\%) = \frac{V\sum_{1}^{i-1} C_i + V_0 C_n}{m} \times 100\% \quad (3)$$

Where V is the sampling volume (mL), V_0 is the total volume of drug release medium (mL), C_i is the concentration of doxorubicin (mol L^{-1}) at the time of sampling i, C_n is the concentration of DOX (mol L^{-1}) at the time of sampling n, m is the total amount of DOX in DOX@CDs@HPMAA, and n is the number of sampling.

Cell culture

Mouse breast cancer (4T1) cells were purchased from the Shanghai Institute of Biochemistry and Cell Biology. 4T1 cells were cultured in RPMI-1640 (Sigma, USA) containing 10% fetal calf serum (FBS, Gibco, ThermoFisher) and 1% penicillin-streptomycin (Gibco, ThermoFisher). All cells were grown in a humid incubator (ThermoFisher, USA) at 37 °C. The gas consists of 5% carbon dioxide and 95% air.

Cell proliferation assay

For dark toxicity, the cell suspension was seeded into a 96-well plate with 5,000 cells in each well and then incubated for 24 h. The RPMI 1640 culture medium (100 μL) containing CDs@HPMAA with the concentration of 0, 25, 50, 75, 100, 150 μg mL^{-1} (0, 28.51, 53.02, 79.53, 106.04, 159.08 μg mL^{-1} for DOX@CDs@HPMAA) were added and cultured in an incubator for 48 h. For sonodynamic toxicity, the concentration of CDs@HPMAA (DOX@CDs@HPMAA) was the same as dark toxicity. After CDs@HPMAA incubated 4T1 cells for 24 h, US with a power density of 0.5 or 0.7 W cm^{-2} for 4 or 6 min was applied. After DOX@CDs@HPMAA incubated 4T1 cells for 24 h, US with a power of 0.5 W cm^{-2} for 6 min was applied. Next, cell viability was determined

by cell counting kit-8 (CCK-8, Beyotime Biotechnology, China). The experiment was carried out according to the manufacturer's instructions. Add 90 μL cell culture medium and 10 μL CCK-8 solution to each well, incubate in the incubator for 1.5 hours, and measure the absorbance at 450 nm using a BioTek microplate reader (Thermo Fisher Scientific, USA). Five replicates were conducted for each concentration to reduce the randomness of the experiment (n = 5). Cell survival rate = [(As−Ab)/(Ac−Ab)] ×100%, where As is the experimental group, Ac is the control group, and Ab is the blank group.

Intracellular ROS level detection

ROS detection kit (Beyotime Biotechnology, China) was used to evaluate the ROS levels in 4T1 cells. 4T1 cells (2×10^4 cells per well) were plated into a 24-well plate and cultured for 12 h. Groups were divided into the control, US, CDs@HPMAA, DOX@CDs@HPMAA, CDs@HPMAA + US, and DOX@CDs@HPMAA + US. The medium was replaced with 300 μL pure fresh RPMI 1640 medium containing either CDs@HPMAA or DOX@CDs@HPMAA at a concentration of 100 μg mL^{-1}. Cells in different groups were cultured for 24 h. Then, some groups were treated by the US. Next, the cells were washed with serum-free RPMI 1640 medium three times according to the instructions of the ROS detection kit and incubated with serum-free RPMI 1640 culture solution containing a 10 μM DCFH-DA probe for 30 min. The cells were then washed with serum-free RPMI 1640 medium for fluorescence imaging (Olympus IX73, Japan).

Calcein-AM/PI staining

2×10^4 cells/well of 4T1 cells were seeded on a 24-well plate for 12 h and then treated with pure fresh or material-containing (100 μg mL^{-1} CDs@HPMAA or DOX@CDs@HPMAA) RPMI 1640 medium (300 μL). Cells in some groups were treated by the US after incubation for another 24 h. The dead and living cells were detected with a Calcein-AM/PI kit (Beyotime, China). Before

staining, the supernatant was discarded, and the cells were washed thoroughly with buffer three times. Then, Calcein-AM solution (2 mM, 5 μL) and PI solution (1.5 mM,15 μL) were added to a 24-well plate and incubated with cells at 37 ℃ for 30 min. Living cells (green fluorescence) and dead cells (red fluorescence) were simultaneously detected by a fluorescence microscope.

Cell migration experiment

4T1 cells were seeded in a six-well plate at 10,000 cells per well. A scratch was made and photographed after the cells adhered to the wall. For different treatment groups, cells were incubated with the medium contained materials (CDs@HPMAA: 150 μg mL^{-1}; DOX@CDs@HPMAA: 159.08 μg mL^{-1}) and incubated for 12 h. Then, some groups were treated by the US. After 24 h, cells were photographed.

Cell apoptosis detection

The Annexin V-FITC/PI apoptosis detection kit (Beyotime Biotechnology, China) was applied to evaluate cell apoptosis. 4T1 cells were seeded in a six-well plate at 10,000 cells per well. After different treatments (CDs@HPMAA: 150 μg mL^{-1}; DOX@CDs@HPMAA: 159.08 μg mL^{-1}), the cells of each group were incubated in the dark with Annexin V-FITC and PI for 20 minutes according to the manufacturer's instructions. The cells were then washed with PBS, and apoptosis was detected by a flow cytometer (Beckman, USA).

Tumor model

The six-week-old Balb/c female mice used for establishing the 4T1 tumor model were obtained from the Laboratory Animal Management Department, Shanghai Family Planning Research Institute authorized by the Shanghai Laboratory Animal Quality Supervision and Inspection Station (SCXK 2018-0006). In detail, the 4T1 cell suspension (2×10^6 cells mL^{-1}, 100 μL) was injected into the axilla of the hind leg of mice. About 7 days later, the 4T1 tumor model was used

for the following studies. All animal experiments were conducted in the Shanghai Ruitaimosi Biotechnology Co., Ltd. authorized by the Shanghai Science and Technology Committee (SYXK 2021-0007) and under the guidelines of the Institutional Animal Care and Use Committee of Shanghai Shidong Hospital (2023-036-01).

In vivo fluorescence imaging

CDs@HPMAA was loaded with NIR fluorescent dye IR780 to enable in vivo fluorescence imaging. Generally, IR780 (1.2 mg) and CDs@HPMAA (1.0 mg mL^{-1}, 6 mL) were thoroughly mixed and under conditions of gentle stirring in the dark for 24 h. CDs@HPMAA-IR780 was then obtained by centrifugation and PBS (pH = 7.4) washing. CDs@HPMAA-IR780 was dispersed in PBS and injected into the tail vein of mice (2 mg mL^{-1}, 200 μL). The fluorescence images of mice at different time points (1, 3, 6, 12, and 24 h) were recorded by a Bruker small animal imaging system from Germany (λ_{ex} = 730 nm, λ_{em} = 830 nm). Then, the mice were euthanized, and their major organs and tumors were collected. Fluorescent images of various organs and tumors were acquired.

Treatment of samples and determination of DOX concentration in blood

Forty-eight six-week-old Balb/c female mice were randomly divided into two groups and fasted for one night. Mice in the two groups were injected with free DOX and DOX@CDs@HPMAA in the tail vein, respectively, and the dose was 5 mg kg^{-1} DOX. After 5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, and 6 h of intravenous administration, about 0.5 mL of orbital blood was taken from each mouse. Then, GSH was added to the blood (10 mM), and pH was adjusted to 5 with hydrochloric acid. Five hours later, the serum was taken as a sample after centrifugation at 4,000 rpm for 10 min. To this end, 200 μL serum was added with 30 μL methanol and mixed for 1 min in a vortex. Subsequently, 2 mL of the mixed solvent ($CHCl_3$/methanol, 4/1, v/v) was added for

10 min vortex uniform mixing. After centrifugation at 3,000 rpm for 10 min, the organic solvent was transferred to a fresh, clean test tube and dried. After redissolution in a 500 μL mixed solvent (methanol/0.06% phosphoric acid in water, 70/30, v/v) and filtration with a 0.22 μm filter membrane, the DOX content in the blood was analyzed by a high-performance liquid chromatography (Shimazu, LC-20A Prominence, Japan). To accurately obtain DOX concentration, DOX was selected as the standard sample by the external standard method, and the concentration was set to 0.5, 1, 2, 4, 6, 8, and 10 μg mL^{-1}. The samples were separated by reverse phase gradient elution using a C18 column (250 × 4.6 mm, 5 μm). The mobile phase was methanol/water (70/30, v/v), the flow rate was 0.9 mL min^{-1}, the detection wavelength was 480 nm, the column temperature was 25 ℃, and the final sample volume was 80 μL for determination.

Synergistic therapy in vivo

Tumor-bearing mice were randomly assigned to six groups (n = 4): (1) control, (2) US, (3) CDs@HPMAA, (4) DOX@CDs@HPMAA, (5) CDs@HPMAA+US, (6) DOX@CDs@HPMAA+US (CDs@HPMAA and DOX@CDs@HPMAA: 2 mg mL^{-1}; US: 0.5 W cm^{-2} for 6 min). Groups (1) and (3) received intravenous injections of PBS. Twelve hours after injection, groups (2), (5), and (6) were subjected to US. The mice underwent two treatments during the 15-day treatment period. After 24 h of US treatment, one mouse from groups (1) and (6) was euthanized, and the tumor was excised for RNA sequencing. They were treated on the first and third days. The mice's tumor volume and body weight were recorded every three days. The tumor volume was calculated as follows: $V = 0.52 \times length \times width^2$. The tumors were removed, captured on camera, and weighed when the mice were put down fifteen days later. The tumors in each group were then divided into tissue slices and stained with H&E, TUNEL, and Ki67 to conduct histological analysis.

Hemolytic test

Mice blood (1 mL) was collected from the mouse eyeball and then diluted with 2 mL PBS. After 1 h, the mixture was centrifuged (1,200 rpm for 5 min). The obtained red blood cells were dispersed in 4 mL PBS. Red cell suspensions were incubated with concentrations of 0, 30, 60, 90, 120, 160 μg mL^{-1} DOX@CDs@HPMAA, PBS, and deionized water. After incubating for five hours, the mixture was centrifuged (13,500 rpm, 10 min) to obtain the supernatant and measure the absorbance values of each group at 570 nm. The hemolysis rate of red blood cells is calculated as follows: Hemolysis rate (%) = $(A_{sample} - A_{PBS})/(A_{water} - A_{PBS}) \times 100\%$.

Hematologic analysis

DOX@CDs@HPMAA (2 mg mL^{-1}, 200 μL) was intravenously injected into Blab/c mice, and blood samples were taken on day 1 and day 21 for various blood indicators. Then, the heart, liver, spleen, lung, and kidney were fixed in formalin solution and sectioned for H&E staining.

Statistical analysis

All data were analyzed using SPSS 21.0 statistical analysis software (IBM SPSS, Armonk, NY, USA). $P < 0.05$ was considered statistically significant. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and **** $p < 0.0001$)

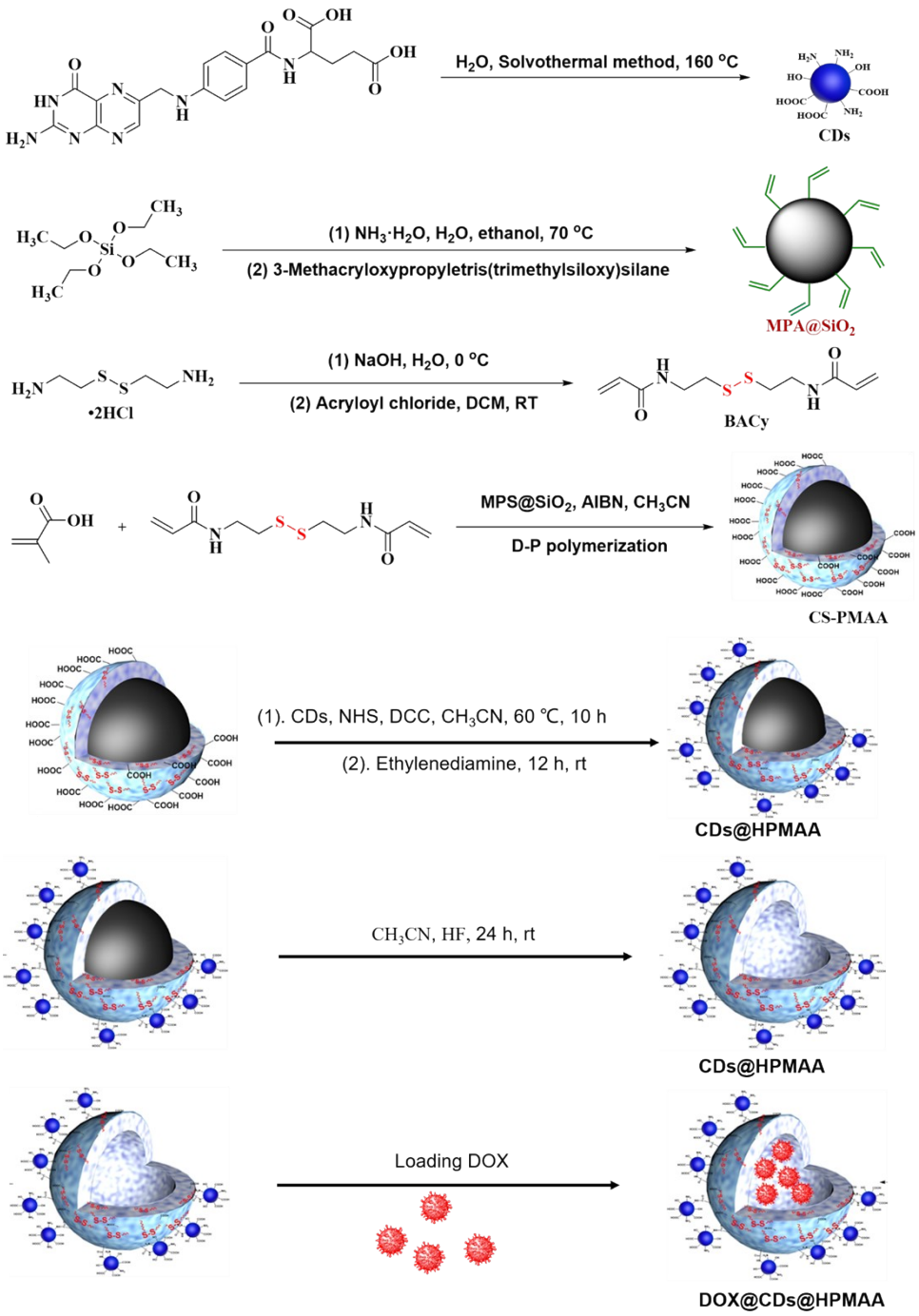

Figure S1. Preparation routes of DOX@CDs@HPMAA

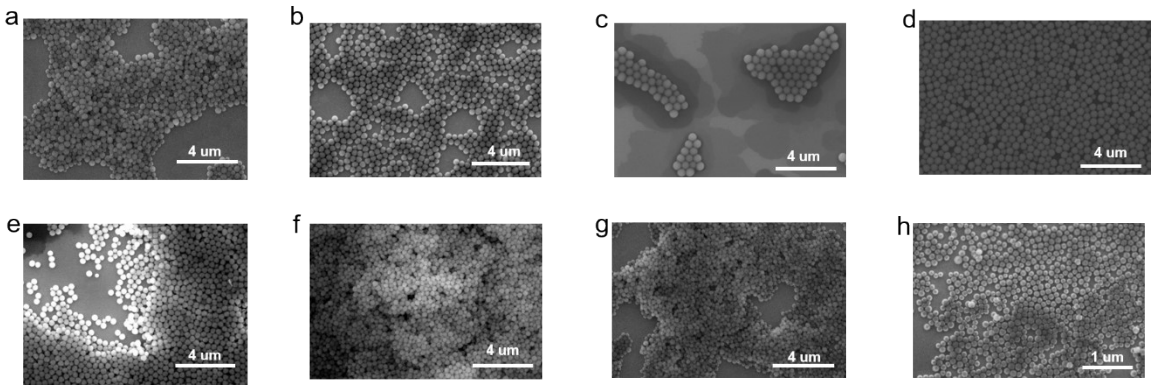

Figure S2. The SEM images of MPS@SiO_2 (a) MPS@SiO_2-1, (b) MPS@SiO_2-2, (c) MPS@SiO_2-3, (d) MPS@SiO_2-4, (e) MPS@SiO_2-5, (f) MPS@SiO_2-6, (g) MPS@SiO_2-7, and (h) MPS@SiO_2 after process exploration (secondary electronic probe, voltage 15 keV).

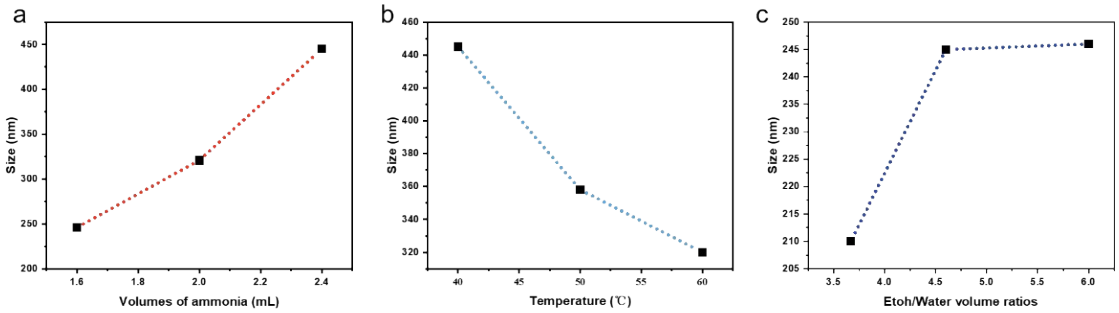

Figure S3. Diameter variation trend of MPS@SiO_2 (a) different volumes of ammonia, (b) different reaction temperatures, (c) different ethanol/water volume ratios.

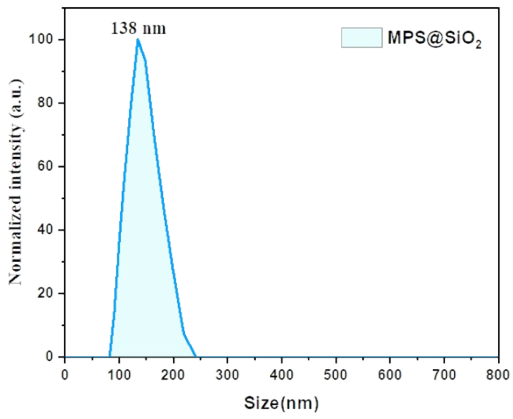

Figure S4. Hydrodynamic diameter distribution of MPS@SiO_2 in ethanol (PDI = 0.022).

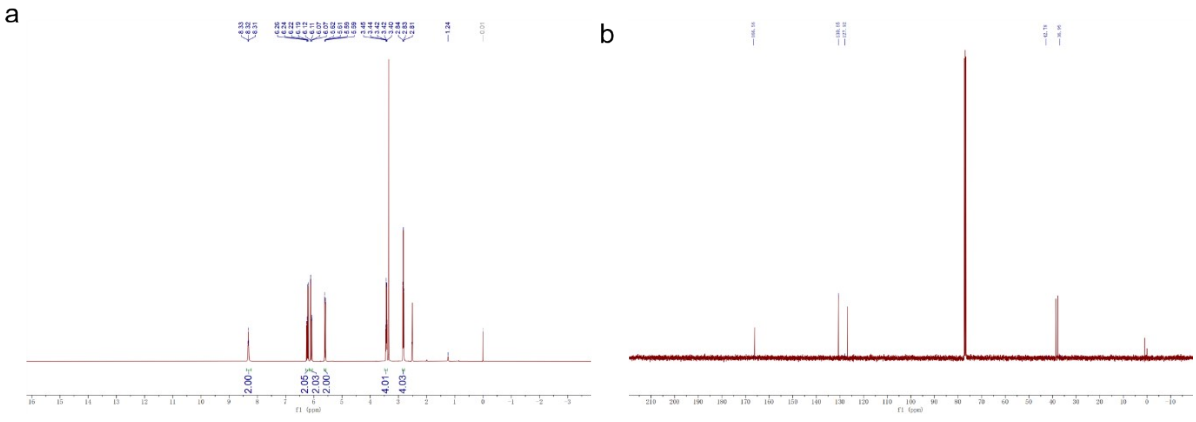

Figure S5. The ^{1}HNMR (a) and ^{13}C (b) spectrum of BACy.

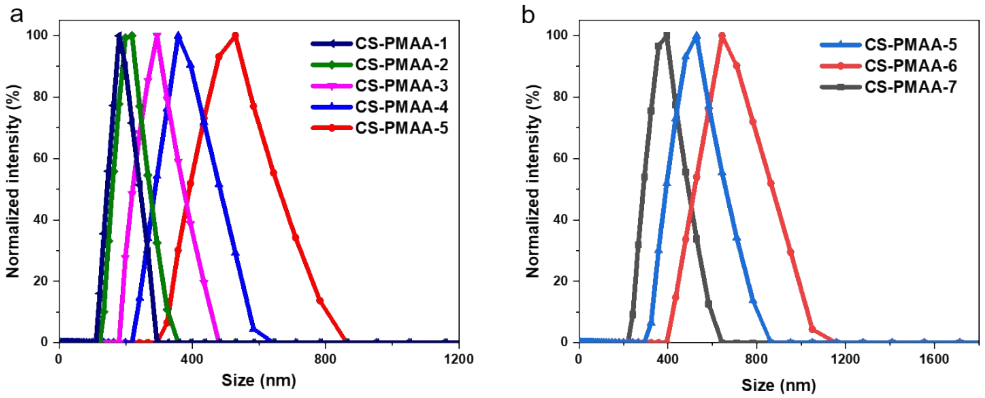

Figure S6. Diameter variation trend of CS-PMAA in PBS (pH = 7.4) (a) different MAA content; (b) MPS@SiO_2 with different sizes.

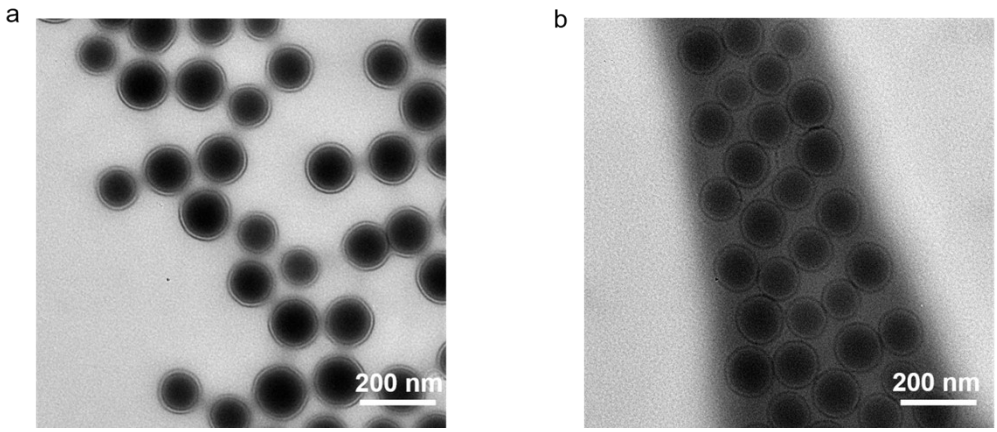

Figure S7. The TEM images of CS-PMAA: (a) CS-PMAA-4, (b) CS-PMAA-5.

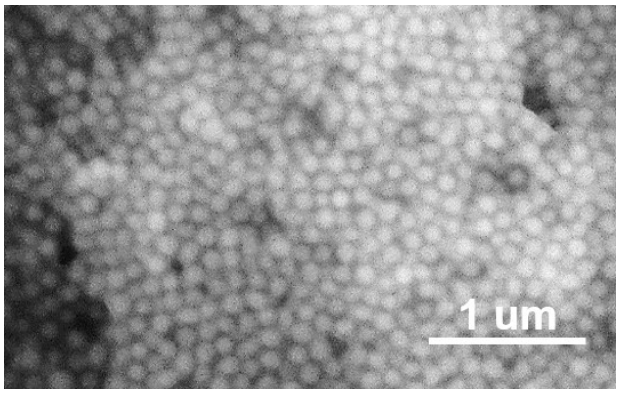

Figure S8. The SEM images of CS-PMAA-5.

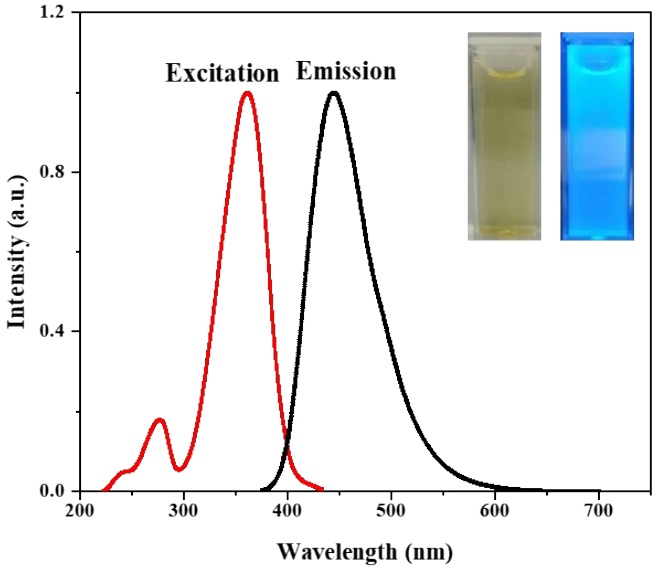

Figure S9. Photoluminescent spectra of CDs (Inset: photographs taken under daylight and 365 nm UV light.)

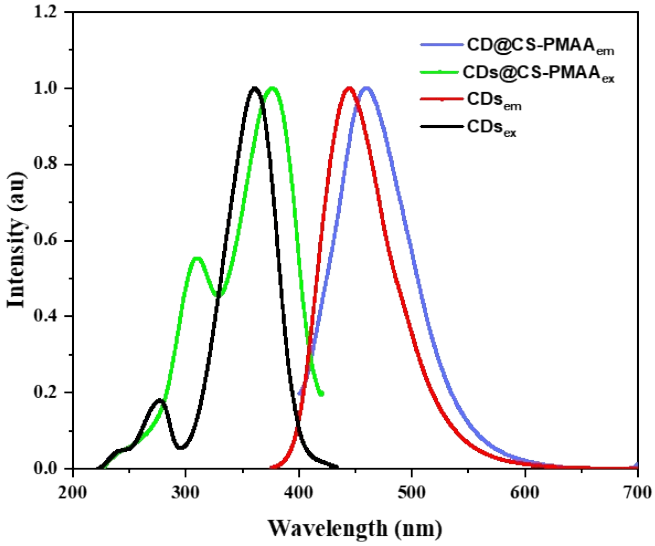

Figure S10. Excitation spectra of CDs, CDs@CS-PMAA, and emission spectra CDs and CDs@CS-PMAA.

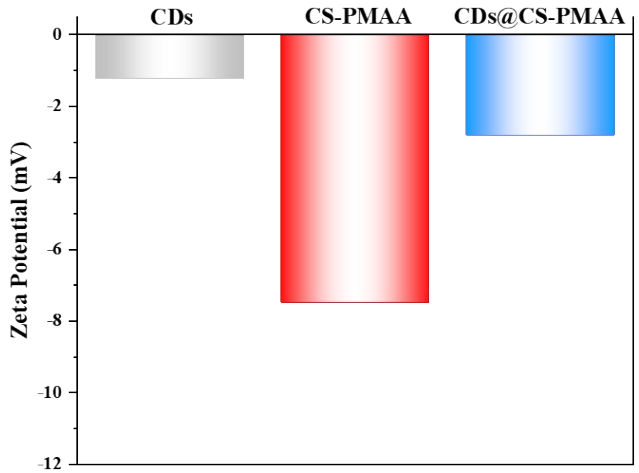

Figure S11. Zeta potential of CDs, CS-PMAA, and CDs@CS-PMAA.

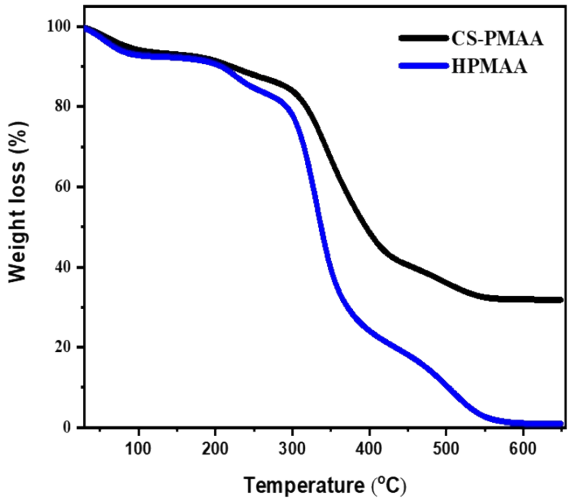

Figure S12. Thermogravimetric curves of CS-PMAA and HPMAA.

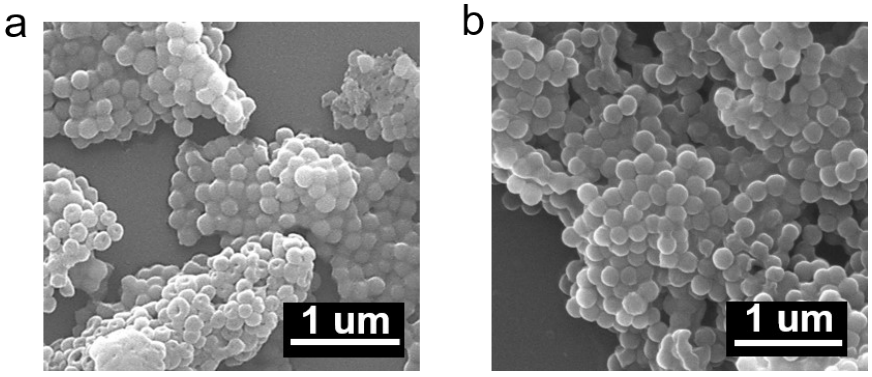

Figure S13. The SEM images of (a) CDs@CS-PMAA and (b) CDs@HPMAA.

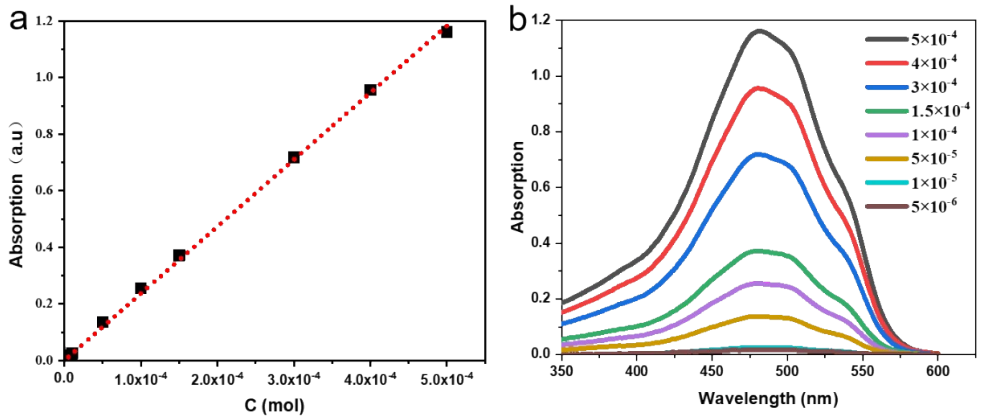

Figure S14. Relationship between the concentration of DOX solution (C, 5×10^{-6} – 5×10^{-4} mol L^{-1}) and the absorption (λ_{ab} = 481 nm, A = 2367.176c, R^2 = 0.99946) b. The UV–vis spectra of DOX solutions at different concentrations).

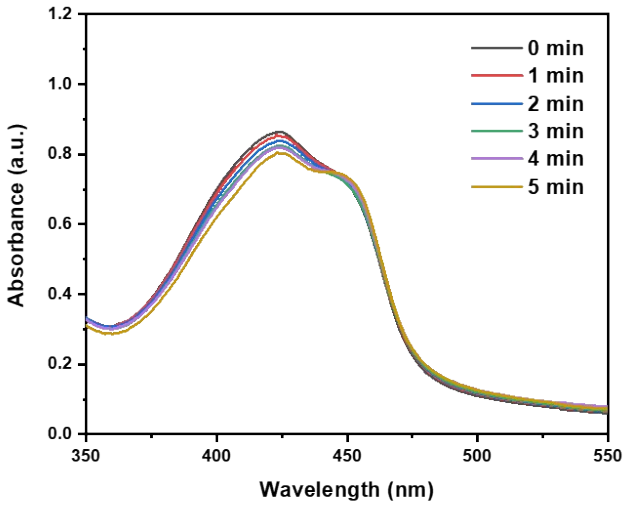

Figure S15. Time-dependent $^1O_2$ generation of H_2O under US irradiation (1.0 MHz, 0.5 W cm^{-2}) for 5 min was detected with the DPBF probe.

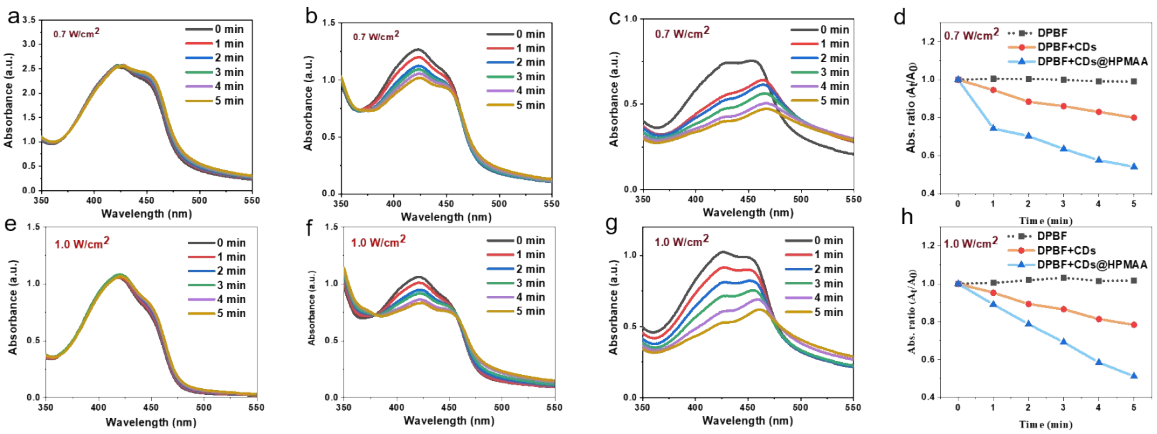

Figure S16. Time-dependent $^1O_2$ generation detection by the DPBF probe under US irradiation (1.0 MHz, 0.7 W cm^{-2} (a-d) or 1.0 W cm^{-2} (e-f)) for 5 min. (a) and (e) control group (H_2O + DPBF), (b) and (f) DPBF + CDs, (c) and (g) DPBF + CDs@HPMAA, (d) and (h) absorption curve overtime at 423 nm.

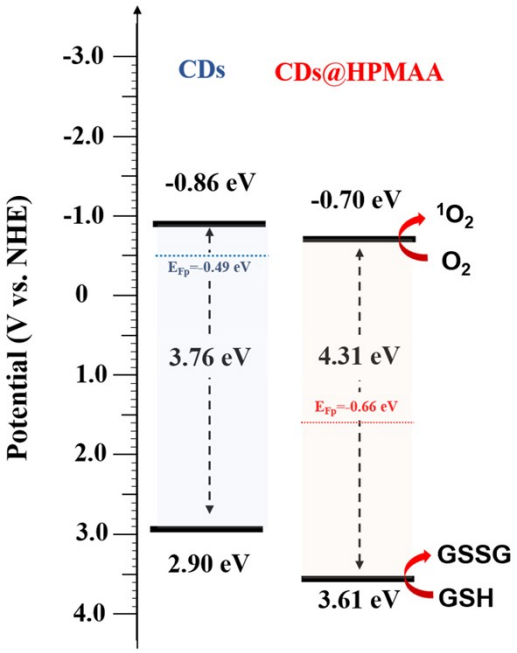

Figure S17. Schematic illustration of the energy-band diagrams of the CDs and CDs@HPMAA.

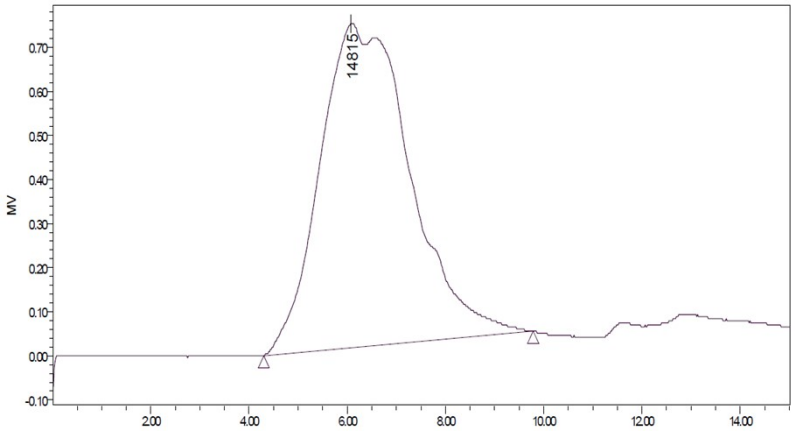

Figure S18. GPC curve of HPMAA after degradation for 3 h by 10 mM GSH.

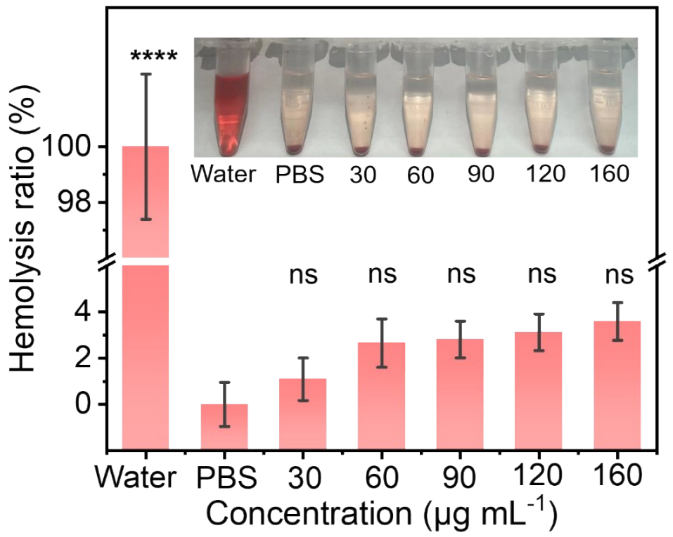

Figure S19. Hemolytic analysis and images of hemolysis of red blood cells after the treatment of DOX@CDs@HPMAA with various concentrations (*: compared with PBS, n = 5, ****p < 0.0001).

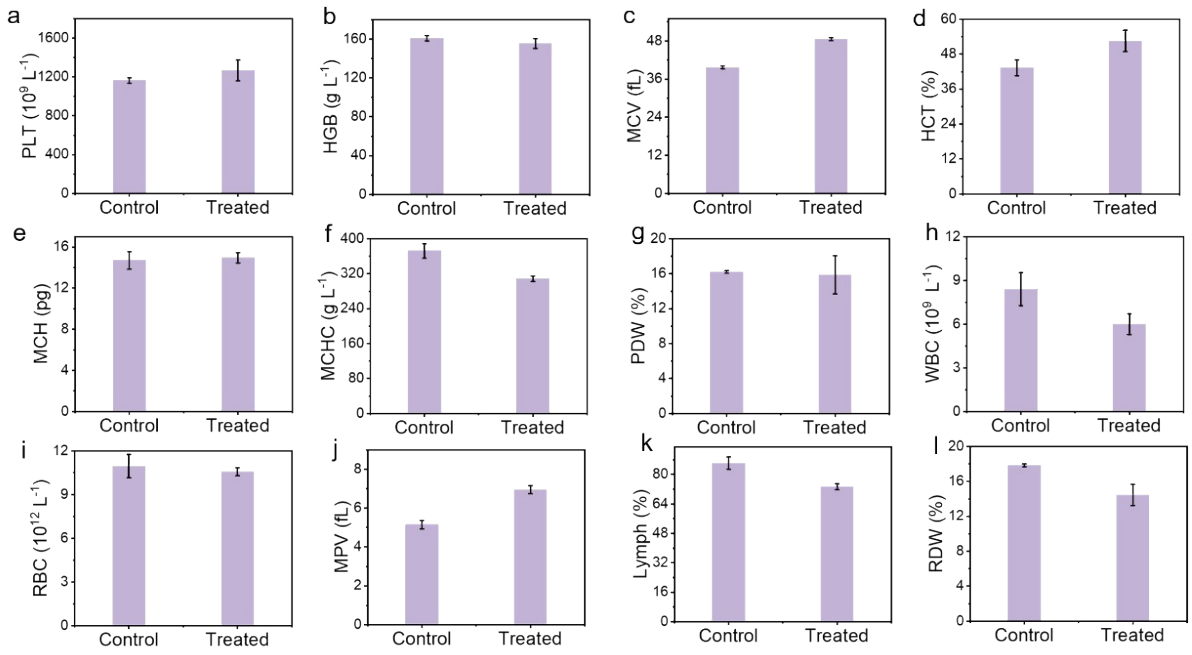

Figure S20. The standard blood routine data of mice treated with DOX@CDs@HPMAA (2 mg mL^{-1}, 200 μL) and PBS (control) by intravenous injection 21 days. (a) Platelets (PLT); (b) Hemoglobin (HGB); (c) Mean corpuscular volume (MCV); (d) Hematocrit (HCT); (e) Mean corpuscular hemoglobin (MCH); (f) Mean corpuscular hemoglobin concentration (MCHC); (g) Platelet distribution width (PDW); (h) White blood cell (WBC); (i) Red blood cell (RBC); (j) Mean platelet volume (MPV); (k) Lymphocyte (Lymph); (l) Red blood cell distribution (RDW).

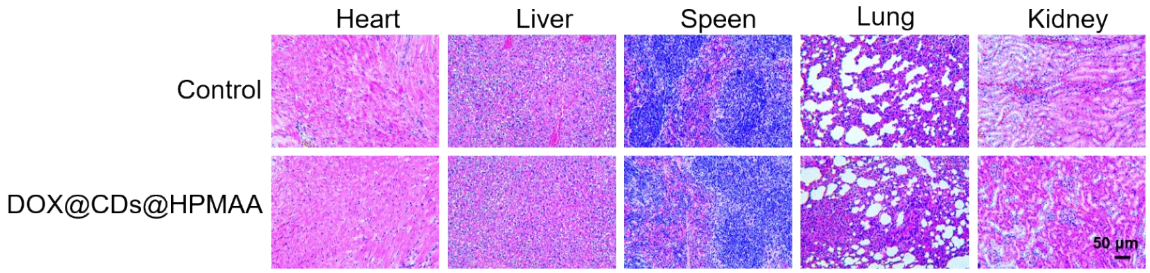

Figure S21. In various treatment groups, H&E-stained sections of mice organs including heart, liver, spleen, lung, and kidney tissues.

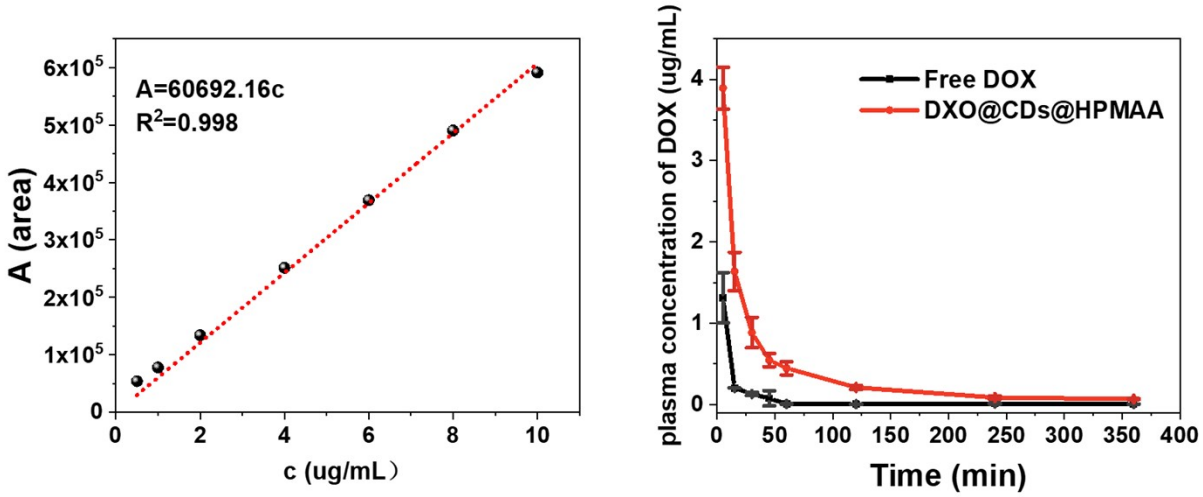

Figure S22. (A) Standard curve of the area under the curve with DOX concentration. (B) Pharmacokinetic characteristics of free DOX and DOX@CDs@HPMAA after intravenous administration of 5mg kg^{-1} in Balb/c female mice (mean ± standard deviation, n = 3).

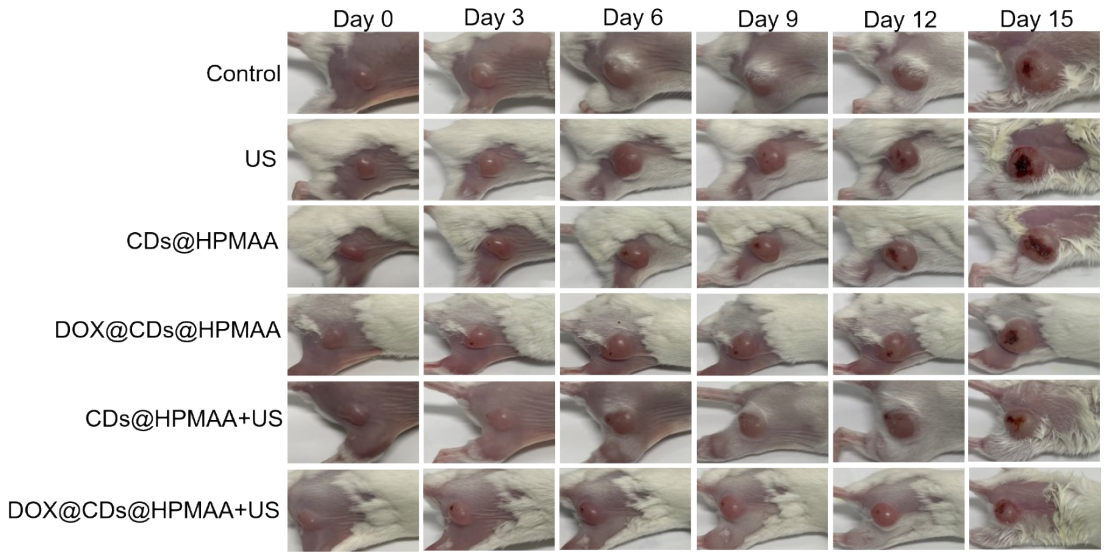

Figure S23. Photographs of tumors in mice during treatment.

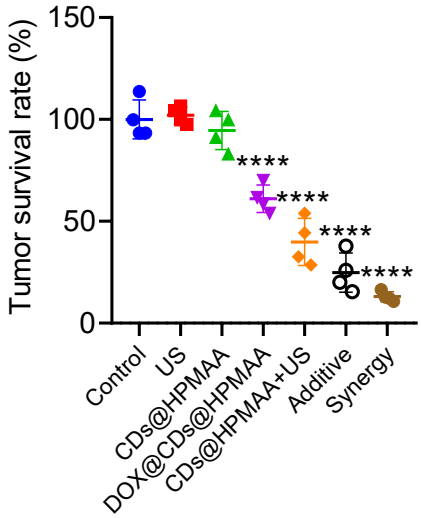

Figure S24. Tumor survival of mice in different groups and synergistic and additive effects of the single treatment group (compared with the control group, **** $p < 0.0001$) ($n = 4$).

Table S1: The recipes of MPS@SiO_2 with different ratios.

Sample	TEOS (mL)	MPS (mL)	EtOH (mL)	H_2O (mL)	$NH_3 \cdot H_2O$ (mL)	T (°C)	Diam. (nm)
MPS@SiO_2-1	4.0	2.0	60.0	10.0	1.6	60	246
MPS@SiO_2-2	4.0	2.0	60.0	10.0	2.0	60	321
MPS@SiO_2-3	4.0	2.0	60.0	10.0	2.	40	445
MPS@SiO_2-4	4.0	2.0	60.0	10.0	2.0	50	358
MPS@SiO_2-5	4.0	2.0	60.0	10.0	2.0	60	320
MPS@SiO_2-6	4.0	2.0	57.5	12.5	1.6	60	245
MPS@SiO_2-7	4.0	2.0	55.0	15.0	1.6	60	210

Table S2: The recipes of CS-PMAA with different ratios.

Sample	MAA (g)	BACy (g)	MPS@SiO_2 (g)	AIBN (g)	CH_3CN (mL)	Diam (nm)
CS-PMAA-1	0.200	0.025	0.200	0.060	50.0	182.5
CS-PMAA-2	0.300	0.038	0.200	0.060	50.0	206.8
CS-PMAA-3	0.400	0.050	0.200	0.060	50.0	270.1
CS-PMAA-4	0.500	0.063	0.200	0.060	50.0	374.6
CS-PMAA-5	0.600	0.075	0.200	0.060	50.0	492.0
CS-PMAA-6	0.700	0.075	0.200	0.060	50.0	665.9
CS-PMAA-7	0.800	0.075	0.200	0.060	50.0	372.5