High drug loading polymer micelle@ZIF-8 hybrid core–shell nanoparticles through donor–receptor coordination interaction for pH/H_2O_2-responsive drug release

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Supplementary materials

Materials

2-(Dimethylamino) ethyl methacrylate (DMAEMA, 99%), 3-aminobenzeneboronic acid (≥ 98%), acrylylchloride (96%), $Zn(CH_3COO)_2$ (99%) and 2-methylimidazole (2-Mim, 98%) were purchased from Aladdin. *N*,*N*-Dimethylformamide (DMF) and dichloromethane (DCM) were dried over CaH_2 and distilled under reduced pressure. Oxalyl chloride ($(COCl)_2$, 98%, Aladdin) was freshly distilled before use. RAFT agent, S-1-dodecyldodecyl-S-(R,R′-dimethyl dimethyl-R″-acetic acid) trithiocarbonate (DDMAT) was purchased from Aladdin without further purification. 2,2-Azobis (isobutyronitrile) (AIBN > 99%, Aladdin) was recrystallized from ethanol for three times before use. PEG_{45}-OH was obtained from Macklin. The Michigan Cancer Foundation-7 (MCF-7), 3T3 mouse fibroblast cells and fetal bovine serum (FBS) were offered by Shanghai cell bank of Chinese Academy of Sciences. Cell Counting Kit-8 (CCK-8) and 4,6-diamidino-2-phenylindole (DAPI) were obtained from Beyotime Institute of Biotechnology (Shanghai, China). The other commercially available agents were used without further purification.

Synthesis of functional monomer 3-acrylamidophenylboronic acid (AAPBA)

The synthetic route of AAPBA was shown in Fig. S1. Typically, 3-aminophenylboronic acid (4.11 g, 0.03 mol) was added into a 250 mL three-necked flask and dissolved in 100 mL of a mixed solvent (*V*(THF):*V*(H_2O) = 1:1) under nitrogen flow. Subsequently, 5.04 g of sodium bicarbonate was added into the flask. Acryloyl chloride (5.43 g, 0.06 mol) was slowly dropped into above solution immersed ice water bath and reacted at room temperature for 24 h. Then solvent was removed by rotary evaporation at 25 °C. The crude solid product was obtained by filtration and then dissolved in ethyl acetate and the organic layer was washed for three times with saturated sodium bicarbonate and saturated sodium chloride solution, respectively. Finally, the organic layer was concentrated by rotary evaporation and white solid product was obtained under vacuum for 24 h. The obtained monomer was characterized by ^{1}H NMR, indicating the successful preparation of the monomer (Fig. S2).

Synthesis of PEG_{45} macro-chain transfer agent

mPEG_{45}-OH macro-chain transfer agent (PEG_{45}-CTA for short) was synthesized by following procedures as shown in Fig. S3. Briefly, 1.7 mL of $(COCl)_2$ was slowly added to DDMAT (0.75 g,

2 mmol) in DCM solution and reacted at room temperature for 24 h. The excess of $(COCl)_2$ was removed by rotary evaporation. Subsequently, PEG_{45}-OH (400 mg, 0.2 mmol) was added into the above mixture solution and reacted for 24 h at room temperature. The product was collected by precipitation three times in cold n-hexane, filtered and dried in vacuum at room temperature for another 1 d. The obtained PEG_{45}-CTA was characterized by ^{1}H NMR, indicating the successful preparation of the macro CTA (Fig. S4).

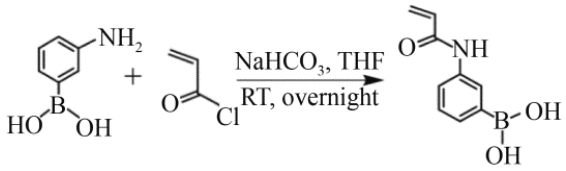

Fig. S1 The synthetic route of functional monomer AAPBA.

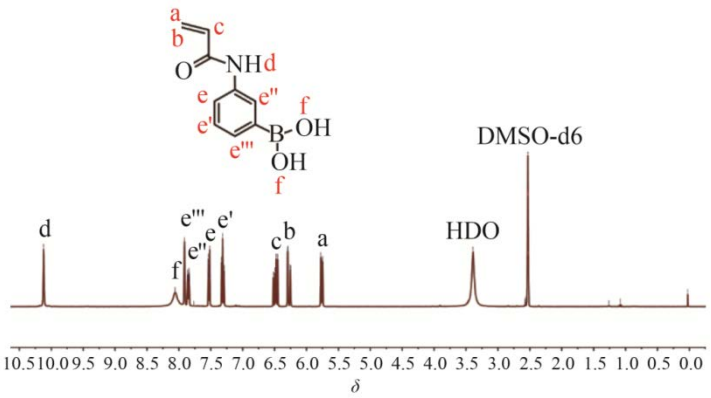

Fig. S2 ^{1}H NMR spectrum of 3-acrylamidophenylboronic acid (AAPBA) in DMSO-*d*6.

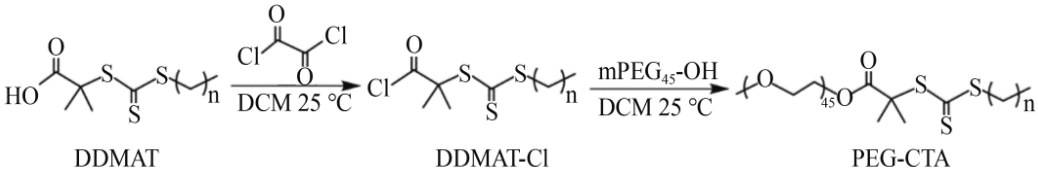

Fig. S3 The synthetic route of macro-PEG_{45} chain transfer agent.

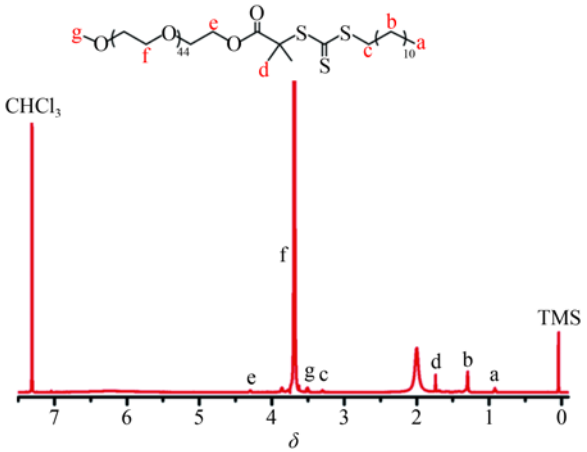

Fig. S4 ^{1}H NMR spectrum of PEG-CTA in $CDCl_3$.

Synthesis of PEG-*b*-PAPBA diblock copolymer

The typical synthesis procedure of PEG-*b*-PAPBA was described as Fig. S5. The macro agent PEG_{45}-CTA (23.46 mg, 0.01 mmol), AAPBA (100 mg, 0.52 mmol) and AIBN (89 μg, 0.53 μmol) were added into the 50 mL schlenk flask. Then, the mixture was degassed by three freeze-evacuate-thaw cycles. The solution was immersed in a preheated oil bath afterwards at 70 °C for 24 h. The crude product was collected by freeze-drying after dialysis in DMF for 1 d and in deionized water for another 3 d. Finally, the sample was then freeze-dried for 2 d to yield the product, PEG_{45}-*b*-$PAPBA_{32}$ (Composition was calculated from ^{1}H-NMR and the subscript denotes the polymerization degree, Fig. S6).

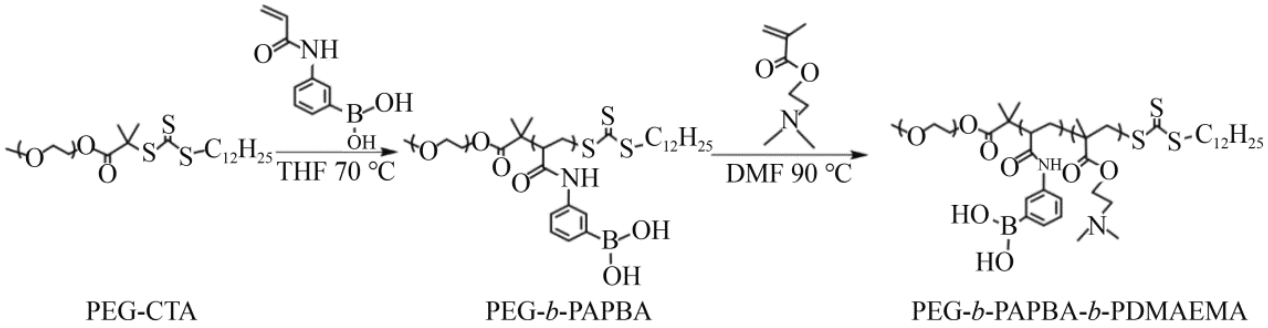

Fig. S5 Synthetic route of triblock copolymers with pendant phenylboronic acid.

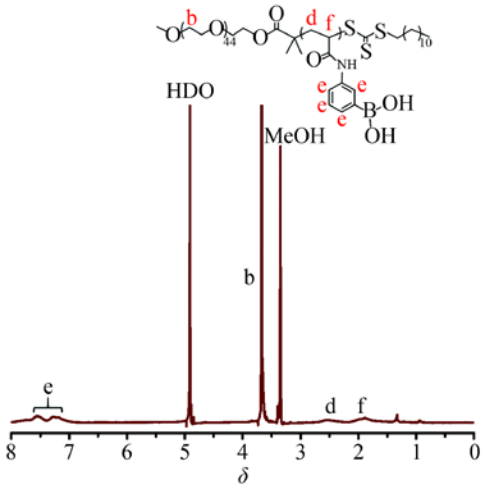

Fig. S6 ^{1}H NMR spectrum of PEG_{45}-*b*-$PAPBA_{32}$ in MeOH-*d*4.

Synthesis of PEG-*b*-PAPBA-*b*-PDMAEMA triblock copolymer

Subsequently, a similar process was applied to synthesize the PEG-*b*-PAPBA-*b*-PDMAEMA triblock copolymer as shown in Fig. S5. In briefly, 82 mg PEG_{45}-*b*-$PAPBA_{32}$ (0.01 mmol), 16 μL DMAEMA (0.1 mmol) and 16.75 μg AIBN (0.1 μmol) were dispersed in 5 mL of fresh DMF and added into the schlenk flask. Then, the mixture solution was placed into the in a preheated oil bath at 60 °C for 24 h after three freeze–thaw–ventilate cycles. The solution was dialyzed against deionized water for 2 d to remove organic solvent DMF and the excess of monomers. Finally, the product was obtained by freeze-dried for another 2 d. The polymerization degree of PDMAEMA block was calculated from ^{1}H-NMR spectrum, yielding the product of PEG_{45}-*b*-$PAPBA_{32}$-*b*-$PDMAEMA_{25}$ (Fig. S7).

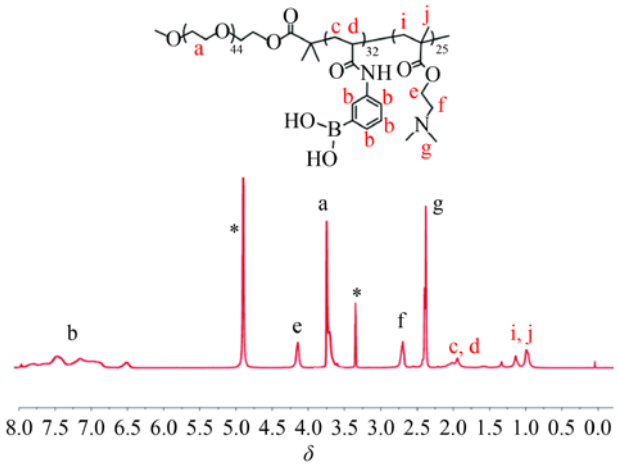

Fig. S7 ^{1}H NMR spectrum of PEG_{45}-*b*-$PAPBA_{32}$-*b*-$PDMAEMA_{25}$ in MeOH-*d*4.

Preparation of high DOX-loaded BCP micelles (DOX-loaded BCP)

The typical nano-precipitation method was utilized to prepare high DOX-loaded BCP micelles (BCP: PEG_{45}-*b*-$PAPBA_{32}$-*b*-$PDMAEMA_{25}$). First, a certain amount of PEG_{45}-*b*-$PAPBA_{32}$-*b*-$PDMAEMA_{25}$ BCP was dropped to DOX solution (10 mg·mL^{-1}, 2 mL) under mild sonication. Afterwards, the mixture was maintained at 37 °C for 6 h in the dark and then dropwise added into deionized water under vigorous stirring. The mixture was stirred in the dark for another 6 h to ensure the complete micellization. Methanol and excess DOX were removed by dialysis (MWCO 3500 Da) against deionized water for 2 d. The DOX-loaded BCP micelles were obtained through a freeze-drying method for another 2 d. Finally, the absorbance at 480 nm of the UV-Vis spectrometer was utilized to determine the free DOX in solution by a standard DOX solution. And the drug loading capacity (DLC) and the drug encapsulated efficiency (EE) were calculated according to the following equations:

$$\mathrm{DLC/wt.\%} = \frac{\text{Weight of loaded drug}}{\text{Weight of drug loaded micelles}} \times 100 \quad \text{(S1)}$$

$$\mathrm{EE/wt.\%} = \frac{\text{Weight of loaded drug}}{\text{Weight of drug initially added}} \times 100 \quad \text{(S2)}$$

Preparation of DOX-loaded BCP micelle@ZIF-8 nanocomposites

$Zn(CH_3COO)_2$ (1.10 mg, 6.0 μmol) was added into the prepared DOX-loaded BCP micelles (0.2 mg·mL^{-1}). Subsequently, 5 mL of 2-methylimidazole (98.6 mg, 1.20 mmol) was dropwise added into above solution and the resultant mixture was kept still at room temperature for 2 h. The resulting white products were collected by centrifugation at a rate of 10000 r·min^{-1} for 10 min and washed thrice with deionized water. Finally, the solid DOX-loaded BCP@ZIF-8 nanocomposites were obtained under vacuum at room temperature overnight.

H_2O_2-triggered disassociation of BCP micelles

The prepared BCP micelles (8 mg) was first dispersed in D_2O (0.5 mL) by sonication, then H_2O_2 (20 μL) was added into above suspension and incubated at 37 °C. The ^{1}H NMR spectra of the solution was recorded at the time of 24 and 48 h.

Morphological change upon external stimuli

The disassembly behavior of DOX-loaded BCP micelles and DOX-loaded BCP@ZIF-8 was also investigated in PBS (pH 7.4) solution containing 200 μmol·L^{-1} of H_2O_2 at 37 °C and pH 5.0. The morphological change of the particles was monitored by TEM.

DOX release behavior from BCP@ZIF-8 nanocomposites *in vitro*

The stimuli-responsive drug release behavior was investigated in PBS solution (pH 7.4) at 37 °C containing different concentrations of HCl or/and H_2O_2. Briefly, DOX-loaded BCP@ZIF-8 nanocomposites were placed in a dialysis bag (MWCO 3500 Da), and immersed in 200 mL of dialysis medium. At a preset time interval, 4 mL of released medium was taken out and replaced by an equal amount of fresh medium. DOX in the release medium was measured by UV-Vis spectrophotometer at λ = 480 nm.

***In vitro* anti-cancer efficacy**

To evaluate the anti-cancer efficacy of DOX-loaded BCP@ZIF-8, MCF-7 and 3T3 cells were treated with free DOX and DOX-loaded BCP@ZIF-8 at various concentration of DOX, respectively. The *in vitro* cytotoxicity was evaluated by the CCK-8 assay. Briefly, MCF-7 and 3T3 cells were seeded onto 96-well plates (100 μL medium)/5000 cells per well and incubated at 37 °C for 24 h. Different concentrations of fresh Dulbecco's Modified Eagle Medium (DMEM, 200 μL) containing DOX-loaded BCP@ZIF-8 solutions and free DOX were replaced the previous culture solution. After incubation for 24 h, the CCK-8 agent (20 μL) was added into the medium and incubated for another 1 h. The enzyme-linked immune detector (Multiskan MK3, Thermo Electron Corporation) was utilized to determined cell viability at 450 nm. Cell viability of each group was calculated by following equation:

$$\text{Cell viability}/\% = \frac{\text{OD}_\text{i}}{\text{OD}_\text{c}} \times 100 \qquad \text{(S3)}$$

where OD_i is the absorbance of surviving cells treated with blank nanoparticles, OD_c is the absorbance of surviving cells of the control group (without blank nanoparticles). The results are measure as the mean ± the standard deviation (SD).

Confocal laser scanning microscopy (CLSM) study

The cellular uptake behavior of DOX-loaded BCP@ZIF-8 was investigated in MCF-7 cells. First, MCF-7 cells were seeded on glass bottom cell culture dish at density of 2×10^5 cells per well and incubated at 37 °C for 12 h. Subsequently, the medium was replaced with 2 mL of fresh medium containing 0.5 mL of DOX-loaded BCP@ZIF-8. Then the medium was continued to incubate for 1, 2 and 4 h at 37 °C. Finally, MCF-7 cells were fixed with 4% paraformaldehyde and stained for nuclei with DAPI. The drug distribution in fixed cells was observed by CLSM (Olympus FV1200, Japan).

Characterizations

^{1}H NMR spectra were recorded on a Bruker DMX-400. The concentration of DOX was determined by Hitachi U-3010 UV-Vis spectrophotometer at 480 nm. Particle size measurements were carried out by dynamic light scattering (DLS) in aqueous solution using a HORIBA Zeta sizer apparatus (LB-550 V) equipped with a 5.0 mW laser diode operating at 650 nm at room temperature. Fourier transform infrared ray (FT-IR) analysis was obtained on a Nicolet 5700 system using diffuse

reflectance sampling accessories. Powder X-ray diffraction (XRD) patterns was characterized on a Bruker D8 Discover X-ray diffractometer (D/max-2500 with Cu-Ka radiation (λ = 1.5406 Å) at a scanning rate of 10(°)·min^{-1}. Transmission election microscopy (TEM) was performed using a JEM-1230 TEM operated at an accelerating voltage of 80 kV. The cell uptake of pH-responsive nanoparticles was evaluated by confocal laser scanning microscope (Olympus FV1200, Japan). Energy dispersive spectrometer (EDS) was performed to evaluate the element content of the micelles and nanoparticles.

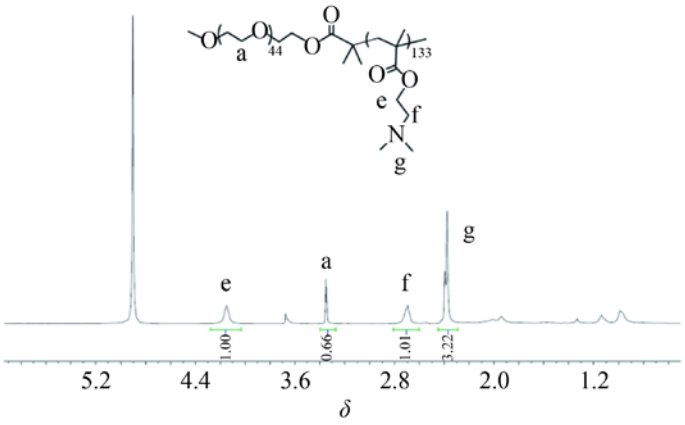

Fig. S8 ^{1}H-NMR spectrum of PEG$_{45}$-*b*-PDMAEMA$_{133}$ in MeOH-*d*4.

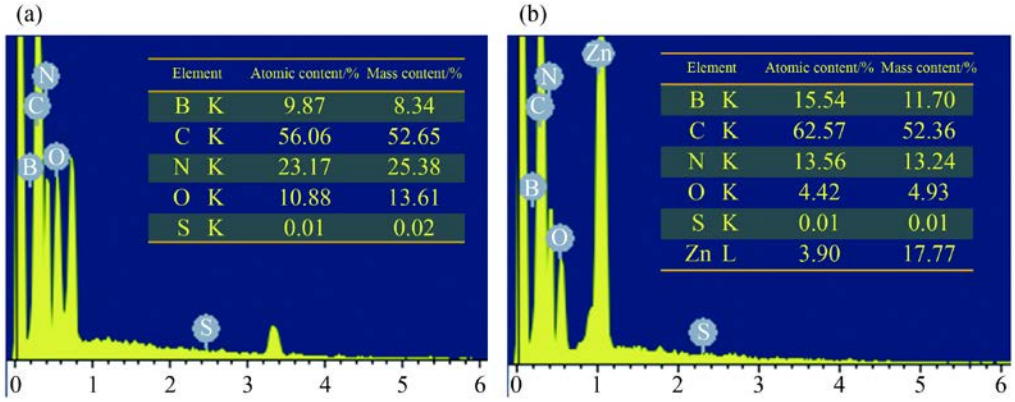

Fig. S9 EDS results of **(a)** DOX-loaded BCP micelles and **(b)** DOX-loaded BCP@ZIF-8 nanocomposites.

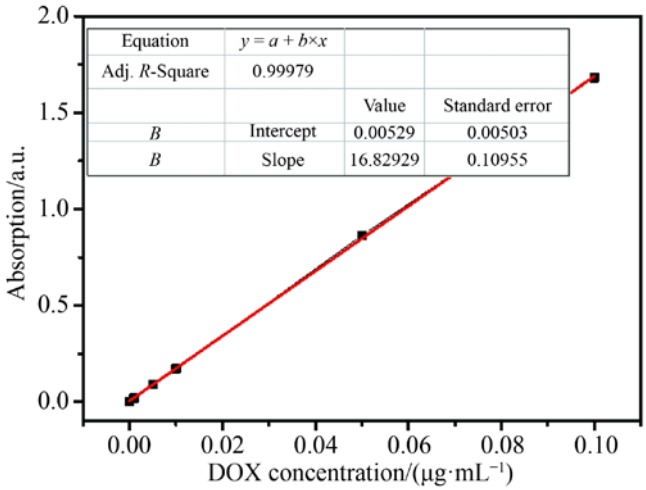

Fig. S10 The standard curve for DOX solutions detected at 480 nm by UV-vis spectrophotometer.