Supporting Information

Reduction-sensitive polymeric carrier for the targeted delivery of a quinazoline derivative for enhanced generation of reactive oxygen species against cancer

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Synthesis of N-carboxyanhydride of β-benzyl-L-aspartate (BLA-NCA)

The method of synthesis of BLA-NCA from β-aspartate benzyl ester was reported and improved. Briefly, 6.00 g of β-benzyl-L-aspartate (0.027 mol，223.23 g/mol) was dissolved in 120 mL of anhydrous ethyl acetate under N_2 atmosphere, and placed in a three-neck flask at 90 ℃ for heating and condensation reflux for 30 min. 4.80 g of bis(trichloromethyl)carbonate (0.016 mol, 296.73 g/mol) was dissolved in 60 mL of anhydrous ethyl acetate, placed in a constant pressure funnel and slowly added to a three-neck flask for reflux reaction at 90 ℃ for 1.5 h. After the reaction, the reactant was clarified from turbidity, and the three-neck flask was sealed and placed in the refrigerator to cool rapidly for 1 h. The cooled solution was washed three times with pre-cooled saturated $NaHCO_3$ solution to remove excess bis(trichloromethyl)carbonate, and after pre-cooled saturated NaCl was washed once for demulsification, the organic phase was placed in an eggplant type flask and dried with excess magnesium sulfate anhydrous for 1 h. The filtrate was condensed and precipitated in the anhydrous petroleum ether. Then the white powder was recrystallized to obtain the white needle crystal solid end product BLA-NCA. Yield: 81.7%.

Synthesis of *m*PEG-PBLA

Poly(ethylene glycol)-block-poly(N-carboxyanhydride of β-benzyl-L-aspartate) (*m*PEG-PBLA) was synthesized by the ring-opening polymerization of N-carboxyanhydride of N-carboxyanhydride of β-benzyl-L-aspartate (BLA-NCA) initiated by *m*PEG-NH_2. Briefly, 0.379 g of *m*PEG-NH_2 (2000 g/mol, 0.190 mmol) was dissolved in 20 mL of anhydrous $CHCl_2$, and 2.0 g of BLA-NCA (249.22 g/mol, 8.025 mmol) dissolved in 2 mL of anhydrous DMF was added into the flask under N_2 atmosphere. The reaction was kept stirring for 48 h at 35 ℃. After the reaction was conducted for another 48 h at 35 ℃, the mixture solution was precipitated into excess cool diethyl ether. The precipitation was collected by centrifugation, washed with diethyl ether, and dried in vacuum until a constant weight to obtain *m*PEG-PBLA. (Mn = 11635 Da, calculated from ^{1}H NMR spectrum). Yield: 86.7%.

Synthesis of *m*PEG-PAsp(DIP/MEA)

Methods of *m*PEG-PAsp(DIP/MEA) was prepared by the mixed amolysis of *m*PEG-

PBLA with N,N-diisopropylethylenediamine (DIP) and mercapto-ethylamine (MEA). Specific operations are as follows, 1.20 g of *m*PEG-PBLA (11600 g/mol, 0.103 mmol) was dissolved in 25 mL of anhydrous DMSO, and 0.527 mL of DIP (144.26 g/mol, 2.917 mmol, 0.798 g/mL) was added into the flask under N_2 atmosphere. The reaction was kept stirring for 12 h at 35 ℃. Then 1.51 g of MEA (77.15 g/mol, 19.596 mmol) was added into the flask under N_2 atmosphere. The reaction was kept stirring for 24 h at 35 ℃. Afterwards, the above solution was dialyzed against methanol (MWCO: 500 Da) and deionized water for 12 h and 24 h, respectively, and then freeze-dried to obtain *m*PEG-PAsp(DIP/MEA). (M_n = 12100 Da, calculated from ^{1}H NMR spectrum). Yield: 80.7%.

The major ^{1}H-NMR spectra of the copolymer *m*PEG-PBLA are as follows: 3.28 ppm (C***H*****$_3$**-, a), 3.5 ppm (-OC***H***$_2$C***H*****$_2$**-, c), 4.62 ppm (-C***H***($CH_2COOCH_2C_6H_5$)-, d), 5.02 ppm (-COOC***H*****$_2$**C_6H_5, f), 7.3 ppm (-CH_2C_6***H***$_5$, g), 8.02 ppm (-N***H***-CO-, h), 2.82 ppm (-C***H*****$_2$**$COOCH_2C_6H_5$, e) (Figure S2). According the integration of the broad peak at 3.5 ppm and the peak at 7.3 ppm, the degree of polymerization for PBLA was calculated to be 47.

Characterization of the nanoparticles

^{1}H NMR spectra were obtained using DMSO-d_6 as solvent on a Bruker Advance 300 MHz spectrometer. FT-IR spectra were recorded on a FTIR spectrometer (Spectrum One, Perkin Elmer, USA) using KBr plates. The particle size and zeta potential were measured by dynamic light scattering (DLS) at 25 ℃ (Brookhaven Omni). The particle size and zeta potential data were collected on an autocorrelator with a scattering light detection angle of 173 °. The intermolecular disulfide bonds formed in the subsurface of the complex were verified by Fourier transform Raman spectroscopy. The morphology of the nanoparticles were observed by transmission electron microscopy (JEM 1400Flash). The preparation method of the samples was as follows: 10 μL of the sample solution sample with a concentration of about 1.0 mg/mL was dropped on the TEM copper net, stained with acetyl uranyl dioxyl (1.0 wt %) for 1 min, and the stain was sucked dry. The loading content of Qd04 was measured by UV-vis method.

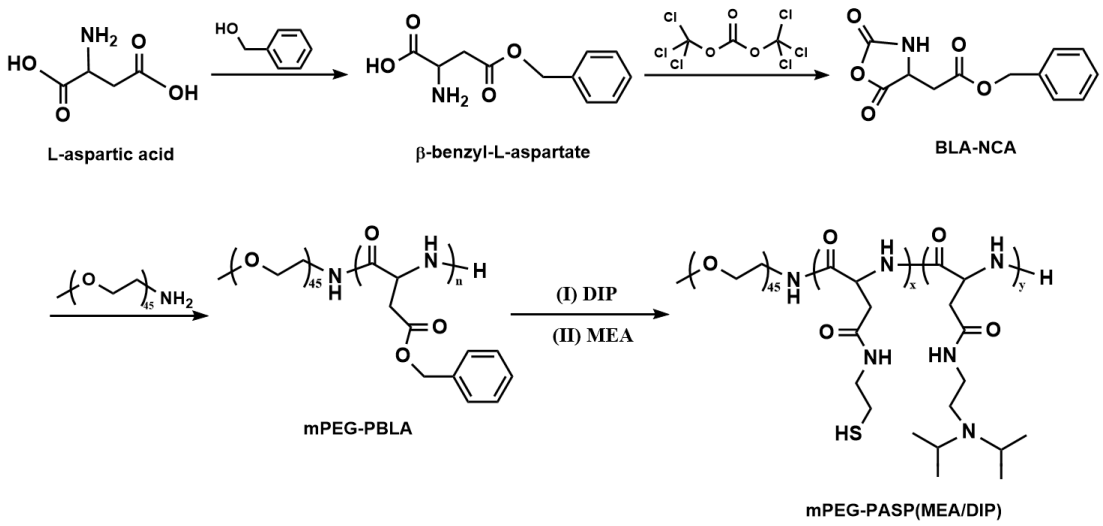

Figure S1. Synthesis of *m*PEG-PAsp(DIP/MEA)

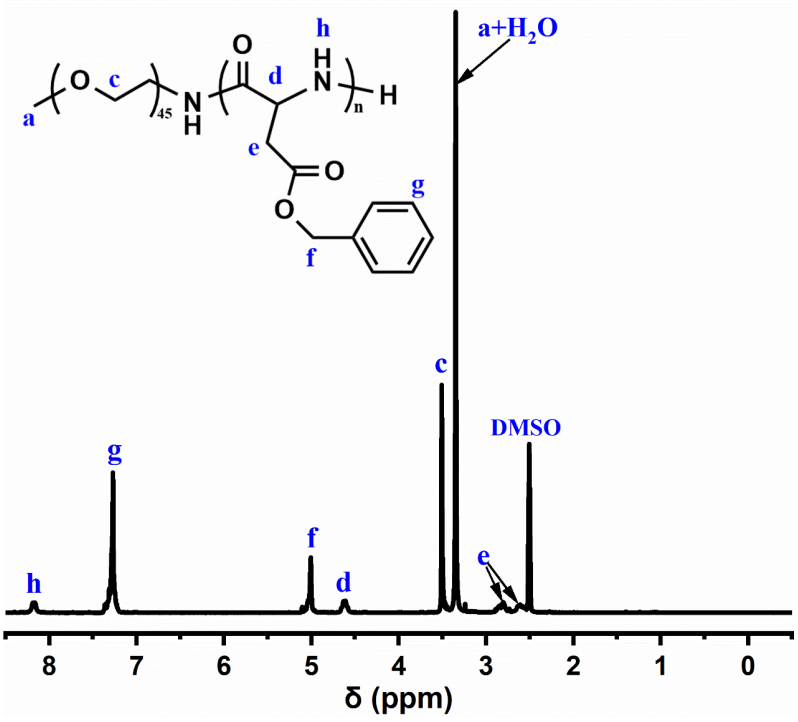

Figure S2. ^{1}H NMR spectrum of *m*PEG-PBLA in DMSO-d6.

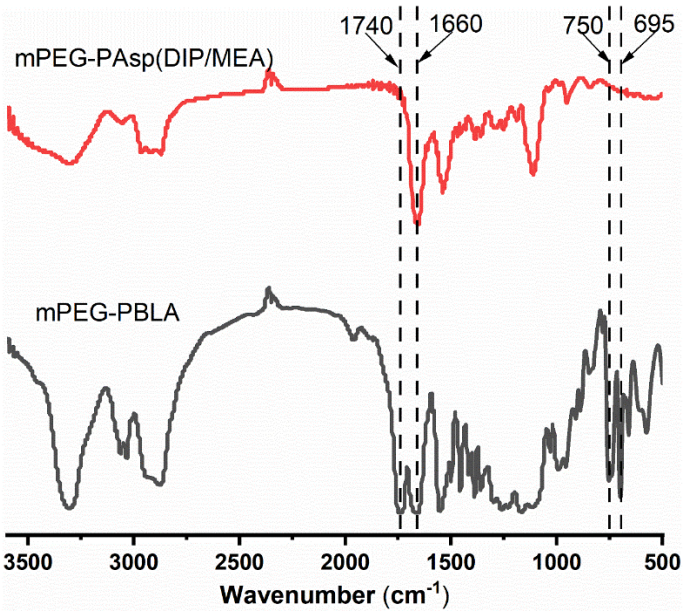

Figure S3. FTIR spectra of *m*PEG-PAsp(DIP/MEA) and its prepolymers.

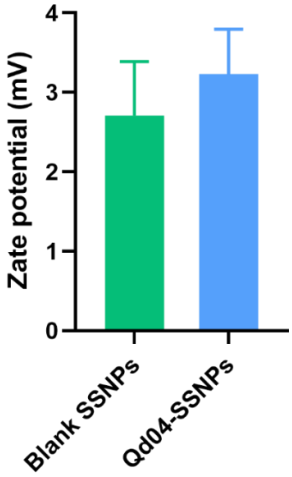

Figure S4. Zeta potentials of nanoparticles. (Mean ± SD; n = 3)

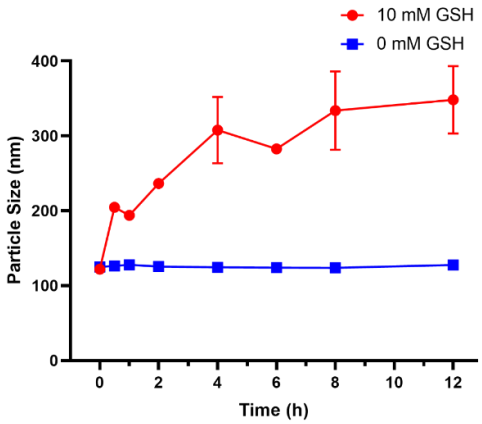

Figure S5. Reduction sensitivity testing of nanodrugs Qd04-SSNPs. (Mean ± SD; n = 3)

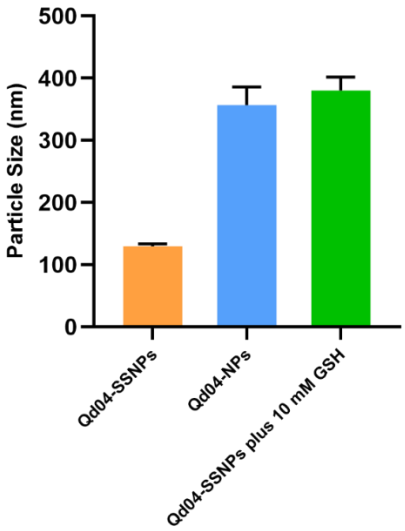

Figure S6. Particle sizes of various nanoparticles at pH 7.4 (Mean ± SD; n = 3).

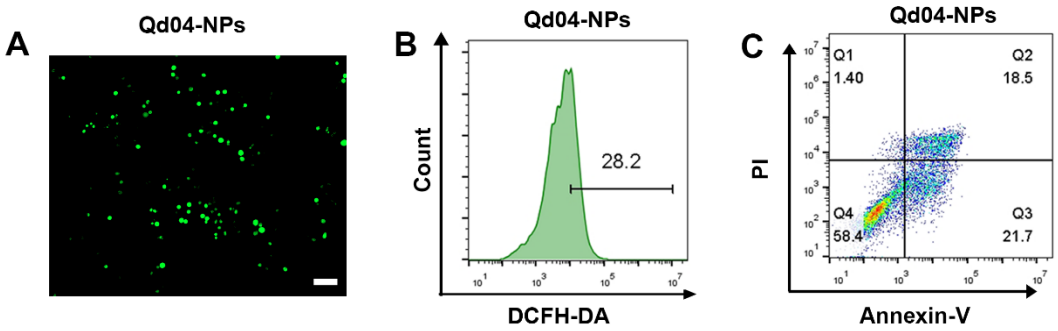

Figure S7. *In vitro* ROS generation and apoptosis of Bel-7402 cells after incubating with Qd04-NPs. (A) ROS fluorescent image in Bel-7402 cells after Qd04-NPs treatment for 24 h. ROS levels are indicated by dichlorofluorescein (DCF) fluorescence. Scale bars: 100 μm. (B) Flow cytometric analysis of ROS in Bel-7402 cells. (C) Flow cytometry analysis shows the apoptosis of Bel-7402 cells after receiving Qd04-NPs treatment or 48 h. Qd04 concentration: 125 nM.

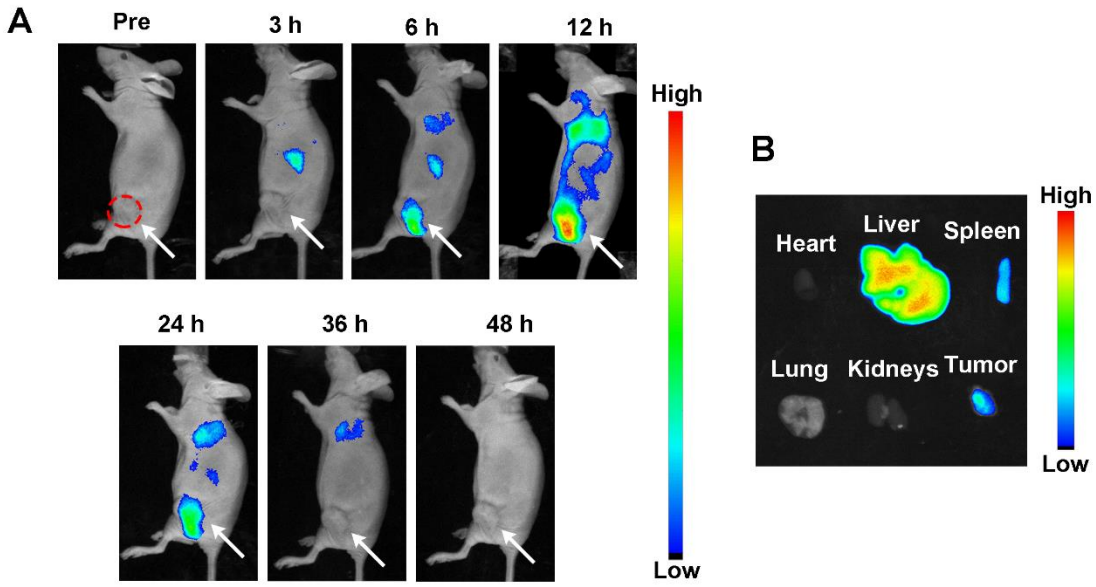

Figure S8. Tumor accumulation and bio-distribution of DiR in HCC tumor-bearing models. (A) *In vivo* DiR fluorescence imaging of mice bearing Bel-7402 tumor at different times after tail vein injection of DiR dissolved in the mixture of physiological saline, DMSO, Cremophor EL, and anhydrous ethanol (Ex = 720 nm, Em = 790 nm). The tumor sites are indicated by white arrows. (B) *Ex vivo* fluorescence imaging of the excised tumors and major organs 48 h post injection.

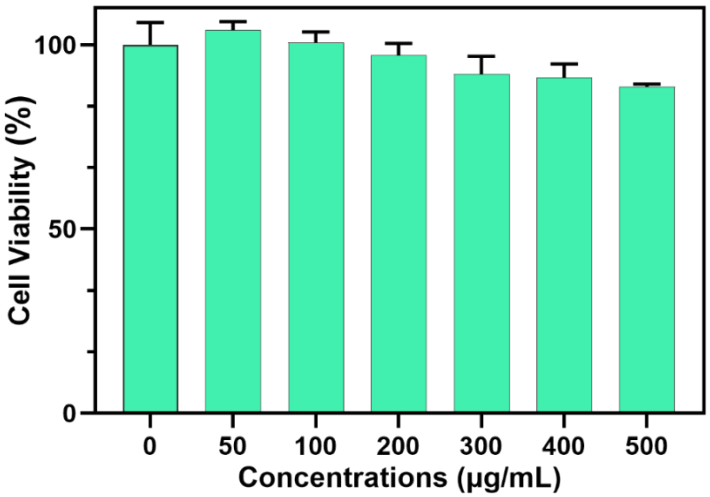

Figure S9. Cytotoxicity of blank polymeric micelles. (Mean ± SD; n = 3)

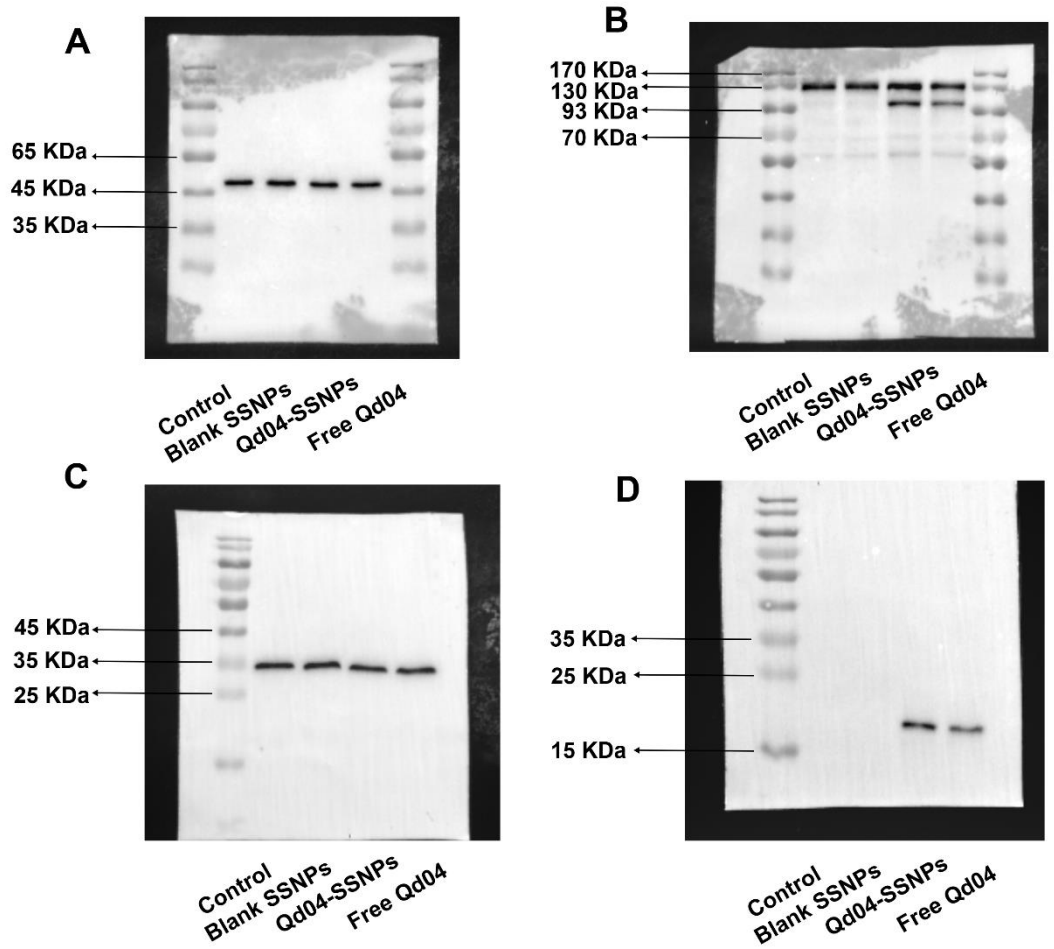

Figure S10. The raw data of western blot experiment. (A) β-actin. (B) PARP. (C) pro-Caspase3. (D) c-Caspase3.