Electronic Supplementary Information for：

Complete Degradable Backbone-Type Hydrogen Peroxide Responsive Curcumin Copolymer: Synthesis and Synergistic Anticancer Investigation

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Instruments

The ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using a Bruker 600 MHz spectrometer operated in the Fourier Transform mode. Chemical shifts are reported in delta (δ) units and expressed in parts per million (ppm) downfield from tetramethylsilane using the residual proton solvent as an internal standard. Molecular weights and molecular weight distributions were determined using a size exclusion chromatograms (SEC) equipped with a Waters 1515 pump and a Waters 2414 differential refractive index detector (set at 40 °C). A series of three linear Styragel columns (HR0.5, HR2, and HR4; 3.6 × 300 mm) was used at a temperature of 40 °C. The eluent used was THF at a flow rate of 0.3 mL/min. FT-IR spectra were recorded on Perkin-Elmer Spectrum BX FT-IR system using KBr pellets at 25 ^{o}C. UV-vis spectra were performed on UNIC 4802 UV/vis double beam spectrophotometers, quartz cells with 1.0 mm lengths were used in UV-vis measurements. Fluorescence spectra were recorded using a RF-5301/PC (Shimadzu) spectrofluorometer. The temperature of the water-jacketed cell holder was controlled by a programmable circulation bath. The slit widths were set at 5.0 nm for both excitation and emission. Transmission electron microscopy (TEM) observations were conducted on a JEM-2100F electron microscope operating at an acceleration voltage of 100 kV. The samples for TEM observation were prepared by casting the corresponding solutions of polymers onto copper mesh grids and drying in air at room temperature. Dynamic light scattering (DLS) measurements were carried on a Nano-ZS90 Zetasizer of Malvern (UK) instrument, all data were averaged over three time measurements. Samples for atomic force microscopy (AFM) measurements were prepared by drop casting solutions of polymers onto pre-cleaned silicon wafers and drying in air at room temperature. AFM images were obtained in tapping mode with a Digital Instruments Dimension 3100 Scanning Probe Microscope, performed at room temperature in air using standard silicon cantilevers with a nominal spring constant of 50 N/m and resonance frequency of ~300 kHz.

Materials

All solvents were obtained from Sinopharm. Co. Ltd. and were purified by the standard procedures before use. THF was further dried over sodium benzophenone ketyl and distilled onto $LiAlH_4$ under nitrogen just before use. Polyethylene glycol (PEG; average $M_n \approx 300$ g/moL), curcumin, and oxalyl chloride were purchased from Aladdin and Sigma-Aldrich and used as received without further purification. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.0 MΩ cm.

Sample Preparation

The synthetic routes employed for the preparation of polymer was shown in Scheme 1a, the reaction was performed in a similar way to the previously reported reference with little modification.[1] The typical procedures were shown as follows:

Synthesis of Poly(curcumin-co-oxalate) Copolymers. In a typical run, a 100 mL of round-bottom flask was charged with PEG (0.41 g, 1.36 mmol; $M_n \approx 300$), curcumin (0.5 g, 1.36 mmol), 20 mL of purified THF, and triethylamine (0.62 g, 6.11 mmol). Under nitrogen protection, oxalyl chloride (0.43 g, 3.4 mmol) in 5 mL of purified THF was added dropwise into the mixture at 0 °C for 30 min. The reaction mixture was allowed to stir at nitrogen atmosphere at room temperature for another 7 h, and quenched with deionized water and extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude poly(curcumin-*co*-oxalate) copolymer was obtained and weighted about 0.75 g (yielding: ~60%). Then, the resultant copolymer was further fractionated by successive dissolution-precipitation cycles in a mixture of THF and methanol (2/3, v/v) at 35 ^{o}C. Several fractions were obtained, and the fourth fraction (60 mg) was selected for further study because the GPC trace showed a monomodal elution peak. Size exclusion chromatograms, ^{1}H NMR, and FT-IR spectra were shown in Figures 1a, 1b, and S1. The M_n was determined to be ~26.4 KDa with a polydispersity (M_w/M_n) of 1.26 by SEC using polystyrene standards.

Determination of Curcumin Content in Poly(curcumin-co-oxalate) Copolymer. Firstly,

a series of THF solutions containing different amounts of curcumin (0.025-0.33 mg) were prepared in advance. Then, the concentration dependent absorption intensity was recorded and a standard calibration curve of Abs vs. Curcumin content was established. THF solution (3.0 mL) of poly(curcumin-*co*-oxalate) copolymer with a concentration of 0.2 g/L was subjected to take an UV-vis detection, the absorption maximum was well located on the standard calibration curve. The final average content of curcumin in copolymer could be calculated to be ~47.5 wt%.

Self-Assembly of Poly(curcumin-co-oxalate) Copolymer. The self-assembly of amphiphilic copolymers was performed through a solvent selective process. Typically, 10.0 mg of poly(curcumin-*co*-oxalate) copolymers was dissolved in 1.0 mL of THF, the solution was stirred and maintained for 30 min at room temperature. Under vigorous stirring, 1.0 mL polymer solution was added via a syringe pump to a dry screw bottle containing 19 mL of DI water at a flow rate of 0.1 mL/min. After the addition was completed, the dispersion was left stirring for another 4 h. THF was then removed by dialysis (MWCO 3.5 kDa) against pure water for 24 h. Fresh water was replaced approximately every 6 h. The obtained dispersion with a characteristic of colloidal aggregates did not exhibit any macroscopic phase separation upon standing at room temperature for more than 7 days, suggesting the formation of stable assemblies.

Preparation of Near Infrared Dyes (IR780) and Anti-Cancer Drug (CPT) Loaded Polymeric Micelles. Hydrophobic IR780 and CPT molecules could be loaded into the hydrophobic region during the co-solvent self-assembly process. Typically, the THF solutions of poly(curcumin-*co*-oxalate) copolymers (1.0 g/L), IR780 (0.15 g/L), and CPT (0.2 g/L) were prepared in advance and then mixed together. Under vigorous stirring, 1.0 mL mixed solution was added via a syringe pump to a dry screw bottle containing 9.0 mL of DI water at a flow rate of 0.1 mL/min. After the addition was completed, the dispersion was left stirring for another 4 h. THF was then removed by dialysis (MWCO 3.5 kDa) against pure water for 24 h. Fresh water was replaced approximately every 6 h. The obtained dispersion with a characteristic of colloidal aggregates did not exhibit any macroscopic phase separation upon standing at room

temperature for more than one week, suggesting the formation of stable IR780/CPT@poly(curcumin-*co*-oxalate) aggregates. Free CPT and IR780 were removed by passing through a 0.22 μm Millipore filter. The final micellar dispersion was diluted with phosphate buffer solution (PBS; pH 7.4) for further use. Following the similar procedural, CPT loaded CPT@poly(curcumin-*co*-oxalate) polymeric micelles were also prepared and served as a control sample.

In Vitro Cargo Release Profile. The cargo release from polymeric micelles was measured by the dialysis method. Briefly, the nanoassembly dispersion (1.0 g/L; 10.0 mL) was placed in a dialysis tube (MWCO 3.5 kDa) and then immersed into 500 mL of water with Tween 20 (1.0% total volume) under gentle stirring at 37 ^{o}C. Then, the system was treated by H_2O_2 (50 mM) and 808 nm NIR light in turn or simultaneously according to the need. At different time intervals, 20 mL external water solution was removed and replaced with equal volume of fresh water. The separated solution was lyophilized and then dissolved in DMSO, the cargo concentration was quantified by measuring the absorbance against a standard calibration curve.

Cell Culture and in Vitro Cytotoxicity Assessment. HeLa cells (5×10^3 cells/well) in Dulbecco's modified Eagle's medium (DMEM) complete medium were plated into a 96-well plate and incubated overnight. Then, the cells were exposed to different micelles with different concentrations at 37 °C for up to 30 h in DMEM complete medium. Then, cells were rinsed with PBS buffer and DMEM complete medium. Cytotoxicity was assessed by adding 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) for another 4h. Cells incubated with blank polymeric micelles were served as positive control.

REFERENCES

(1) Lee, Dongwon; Khaja, Sirajud; Velasquez-Castano, Junan C; Dasari, Madhuri; Sun, Carrie; Petros, John; Tayloe W. Robert; Murthy Niren. In vivo imaging of hydrogen peroxide with chemiluminescent nanoparticles. *Nat. Mater.* **2007**, *6*, 765-769.

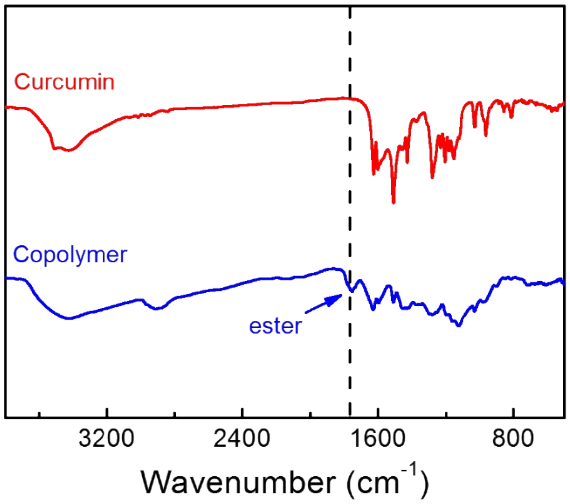

Figure S1. FT-IR spectra obtained for curcumin and poly(curcumin-*co*-oxalate) copolymers at 25 °C using KBr pellets.

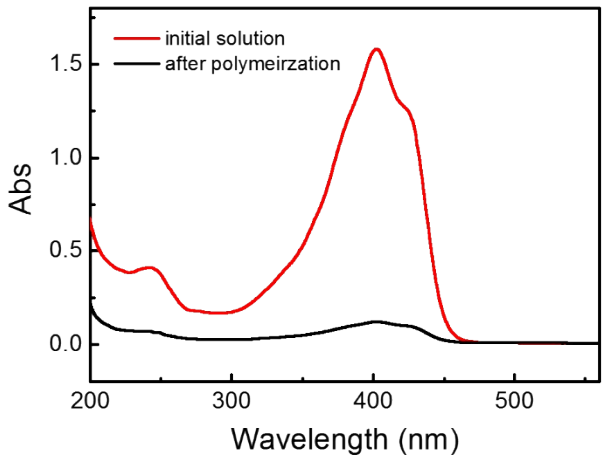

Figure S2. UV-vis spectra recorded for the mixture solution before and after polymerization, respectively.

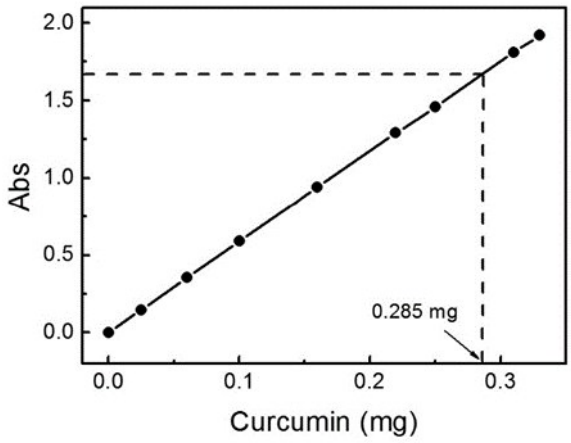

Figure S3. Concentration dependent absorption intensity of curcumin molecules in THF solution.

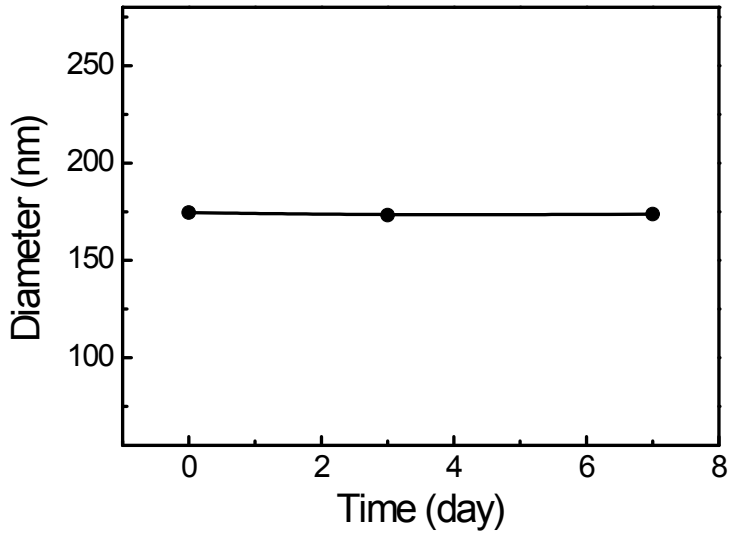

Figure S4. The time dependent size change of poly(curcumin-*co*-oxalate) nanoparticles measured in vitro by DLS in PBS buffer (pH 7.4) at 37 ^{o}C.

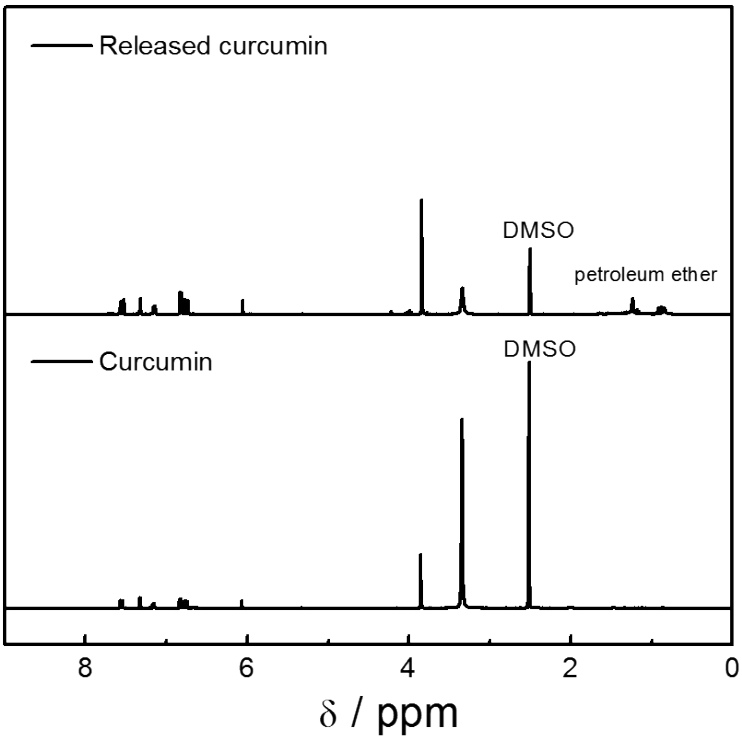

Figure S5. ^{1}H NMR spectra obtained in DMSO for curcumin molecules before polymerization (down) and after H_2O_2 triggered release from micelles (top).

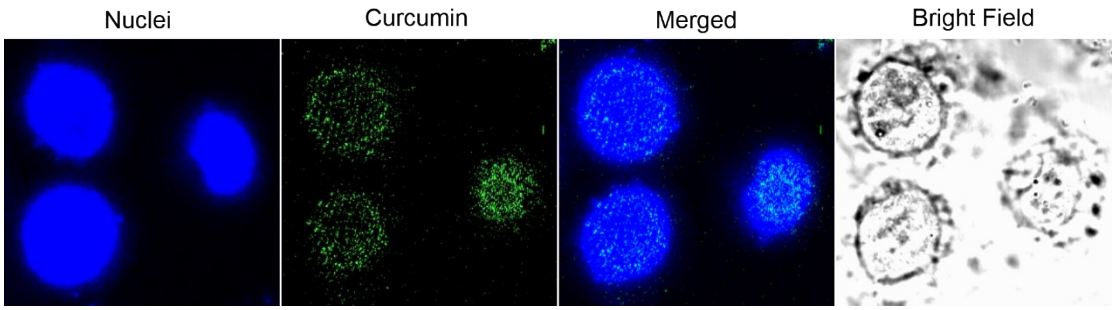

Figure S6. CLSM images obtained for live HeLa cells after incubating with poly(curcumin-*co*-oxalate) micelles (0.2 g/L) for 10 h at 37 °C. Nuclei were stained blue.