Supporting Information

Anti-inflammatory unimolecular micelles of redox-responsive hyperbranched polycurcumin amphiphiles with enhanced anti-inflammatory efficacy in vitro and in vivo

Da Huang,[a,b] Qiang Tang,[a] Minglang Zou,[a] Yongming Wang,[a] Fang Luo,[a,b] Jinyi Mu,[a] Yuanzi Wu,[a] Zuquan Weng,*[a,b] Zhenyu Lin[b]*

[a]College of Biological Science and Engineering, Fuzhou University, Fuzhou, Fujian 350108, China.

[b]Ministry of Education Key Laboratory for Analytical Science of Food Safety and Biology, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, College of Chemistry, Fuzhou University, Fuzhou, Fujian 350108, China.

* Corresponding authors:

Da Huang, E-mail: huangda@fzu.edu.cn
Zuquan Weng, E-mail: zqweng@fzu.edu.cn

Materials

CUR (95%), 1-Propanethiol (99%) ,4-dimethylamino pyridine (DMAP, 99%), acryloyl chloride, *N*-ethyl-*N′*-(*3*-(dimethylamino) propyl) carbodiimide hydrochloride (EDC, 98%), *N,N*-Diisopropylethylamine (DIPEA, 97%), paraformaldehyde (99%), *4′,6*-diamidino-*2*-phenylindole dihydrochloride (DAPI, 98%), dithiothreitol (DTT), and lipopolysaccharide (LPS) were obtained from Aladdin Industrial Corporation (Shanghai, China) and used as received. Oligo (ethylene glycol) monomethyl ether methacrylate (OEGMA, M_n = 475 g/mol, mean degree of polymerization is 5-6) purchased from Aladdin Industrial Corporation was passed through a neutral alumina column to remove the inhibitor. *2,2′*-Azobis (isobutyronitrile) (AIBN) was recrystallized twice from ethanol. Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. All other reagents including triethylamine (TEA), dichloromethane (DCM) and tetrahydrofuran (THF) were analytical grade and used directly.

Characterizations

All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III 500 NMR spectrometer (resonance frequency of 500 MHz for 1H) operated in the Fourier transform mode. $CDCl_3$ or DMSO-d6 were used as the solvent. Gel permeation chromatography (GPC) measurements were performed on GPCmax VE-2001 (Malvern Viscotek) with a Visotek TriSEC Model 302 triple detector array using two I-3078 polar organic columns. THF was used as the eluent at a flow rate of 1.0 mL/min. Molecular weights and polydispersity index (PDI) were determined using the workstation software equipped with the system by a working curve of polystyrene standards. Transmission electron microscopy (TEM) images were obtained using a Hitachi HT7700 TEM after the samples were negatively stained by 2 wt% sodium phosphotungstate. The hydrodynamic average sizes and zeta potentials were assessed by dynamic light scattering (DLS) using a zetasizer (Nano-ZS, Malvern, UK)) with a 632.8 nm laser light set at a scattering angle of 173°. Confocal laser scanning microscope (CLSM)

images were obtained using a Nikon A1 CLSM system and the green FITC channel was used for CUR and DCFH-DA (Excitation: 488 nm, Emission: 520±20 nm) while the blue channel was used for DAPI (Excitation: 405 nm, Emission: 450±20 nm). UV absorbances were measured using a BioTek Synergy H1 microplate reader. High performance liquid chromatography (HPLC) analysis was performed on a Thermo UltiMate 3000 system (Thermo, USA) equipped with a C-18 column (4.6 mm × 250 mm, 5 μm) with UV detection at 420 nm. A mixture of acetonitrile and ultrapure water (7:3, v/v) was used as mobile phase at a flow rate of 1.00 mL/min.

Methods

Synthesis of 4-Cyano-4-(propylsulfanylthiocarbonyl)sulfanylpentanoic Acid (CPP)

CPP was synthesized according to the literature.[1] ^{1}H NMR was shown in Figure S1. CPP: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.35 (t, J = 7.2 Hz, 2H), 2.79 – 2.65 (m, 2H), 2.61 – 2.33 (m, 2H), 2.01 – 1.83 (m, 3H), 1.74 (dt, J = 26.3, 6.1 Hz, 2H), 1.05 (t, J = 7.3 Hz, 3H).

Synthesis of 2-((2-hydroxyethyl)disulfanyl)ethyl acrylate (HSEA)

2'2-dithiodiethanola (7.4 g, 48 mmol) and triethylamine (4,85 g, 48 mmol) were dissolved anhydrous DCM (50 mL) in a 150 mL one-neck round-bottom flask equipped with a magnetic stirring bar, cooled to 0℃ in an ice-water bath. Then, acryloyl chloride (3.25 mL, 40 mmol) in anhydrous DCM (20 mL) was added dropwise through a syringe with vigorous stirring. After the addition, the reaction mixture was stirred at room temperature overnight. The mixture was thrice washed with water (50 mL), and the organic layer was collected and dried over anhydrous Na_2SO_4, filtered and concentrated by rotatory evaporator. The crude product was purified by column chromatography on silica gel with ethyl acetate/hexane (1:4) to obtain HSEMA (3.2431 g, 32.46%). ^{1}H NMR was shown in Figure S2. HSEA: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17 (s, 1H), 5.63 (s, 1H), 5.33 (s, 1H), 4.48 (dt, J = 13.2, 6.5 Hz, 2H), 4.00 – 3.86 (m, 2H), 3.10 – 2.97 (m, 2H), 2.92 (t, J = 5.6 Hz, 2H), 1.98 (s, 3H).

Synthesis of acrylate-SS-CPP (ACPP)

HSEA (0.75 g, 3.6 mmol), EDC (0.828 g, 4.32 mmol) and DMAP (0.04 g, 0.36 mmol) were dissolved anhydrous DCM (40 mL), cooled to 0℃ in an ice-water bath. CPP (1.0 g, 3.6 mmol) in anhydrous DCM (10 mL) was added dropwise through a syringe with vigorous stirring. After the addition, the reaction mixture was stirred at room temperature overnight. The mixture was thrice washed with water (50 mL), and the organic layer was collected and dried over anhydrous Na_2SO_4, filtered and concentrated by rotatory evaporator. The crude product was purified by column chromatography on silica gel with ethyl acetate/hexane (1:4) to obtain ACPP (0.92 g, 54.76%). ^{1}H NMR was shown in Figure S3. ACPP: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (d, J = 17.3 Hz, 1H), 6.16 (dd, J = 17.3, 10.4 Hz, 1H), 5.90 (d, J = 10.4 Hz, 1H), 4.43 (dt, J = 19.4, 6.4 Hz, 4H), 3.35 (t, J = 7.2 Hz, 2H), 2.98 (dt, J = 13.3, 6.5 Hz, 4H), 2.77 – 2.61 (m, 2H), 2.60 – 2.32 (m, 2H), 1.91 (s, 3H), 1.76 (dt, J = 14.6, 7.3 Hz, 2H), 1.05 (t, J = 7.3 Hz, 3H).

Synthesis of 2-((2-hydroxyethyl)disulfanyl)ethyl methacrylate (HSEMA)

First, 2'2-dithiodiethanola (7.4 g, 48 mmol) and triethylamine (4,85 g, 48 mmol) were dissolved anhydrous DCM (50 mL) in a 150 mL one-neck round-bottom flask equipped with a magnetic stirring bar, cooled to 0℃ in an ice-water bath. Then, methacryloyl chloride (3.87 mL, 40 mmol) in anhydrous DCM (20 mL) was added dropwise through a syringe with vigorous stirring. After the addition, the reaction mixture was stirred at room temperature overnight. The mixture was thrice washed with water (50 mL), and the organic layer was collected and dried over anhydrous Na_2SO_4, filtered and concentrated by rotatory evaporator. The crude product was purified by column chromatography on silica gel with ethyl acetate/hexane (1:4) to obtain HSEMA (4.31g, 48.57%). ^{1}H NMR was shown in Figure S4. HSEMA: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17 (s, 1H), 5.63 (s, 1H), 5.33 (s, 1H), 4.48 (dt, J = 13.2, 6.5 Hz, 2H), 4.00 – 3.86 (m, 2H), 3.10 – 2.97 (m, 2H), 2.92 (t, J = 5.6 Hz, 2H), 1.98 (s, 3H).

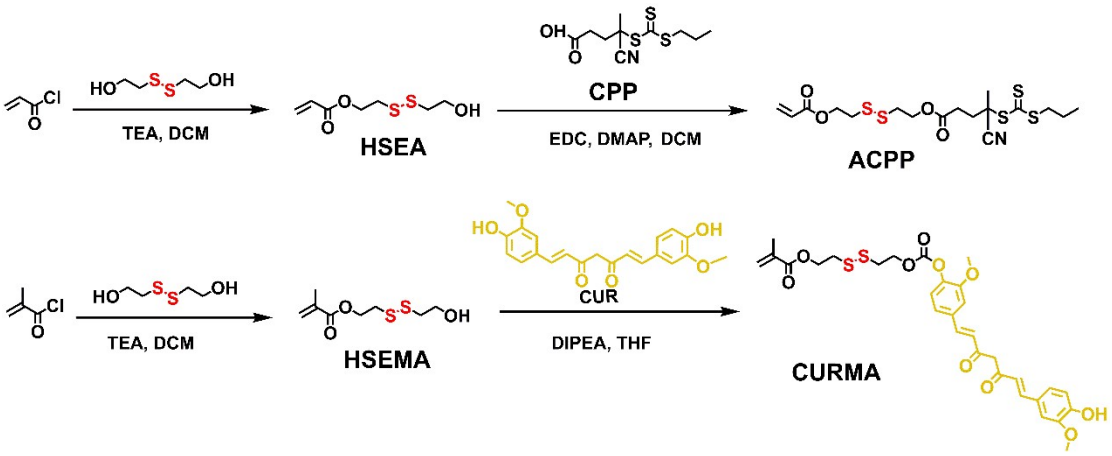
Synthesis of CUR-SS- methacrylate (CURMA)

HSEMA (444 mg, 2 mmol) and DIPEA (0.7 mL, 4 mmol) were dissolved in 50 mL of anhydrous THF, cooled to 0℃ in an ice-water bath. Triphosgene (297 mg, 1 mmol) in

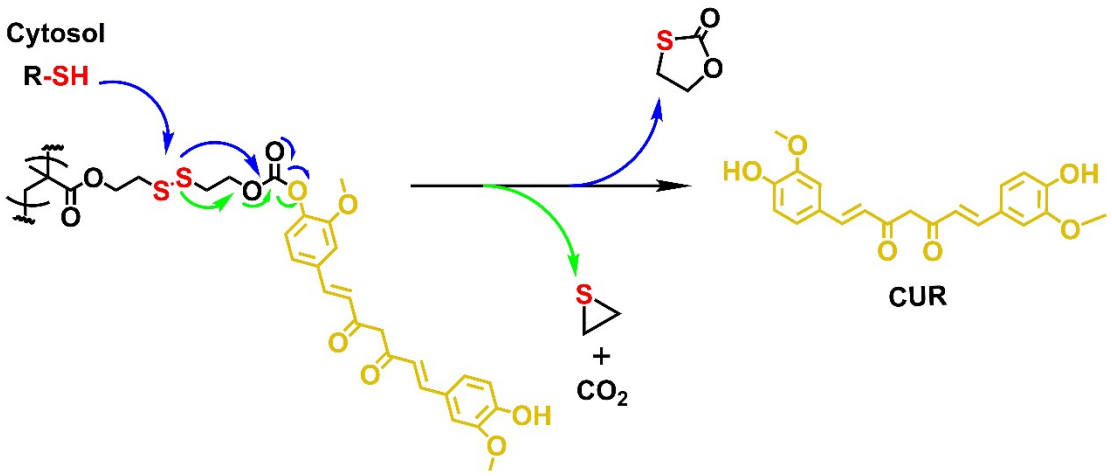
5 mL of dry THF was added dropwise through a syringe with vigorous stirring. And the reaction mixture was stirred at room temperature overnight. After filtration, the reaction mixture was slowly added with a syringe to the solution that CUR (736 mg, 2 mmol) and DIPEA (0.7 mL, 4 eq) were dissolved in 10 mL of anhydrous THF. The reaction mixture was stirred at room temperature overnight. After filtration and evaporation of all of the solvents, the residues were diluted with ethyl acetate, washed with 0.1M HCl (3×50 mL) and water (3×50 mL), and the organic layer was collected and dried over anhydrous Na_2SO_4. After filtered and concentrated by rotatory evaporator, the crude product was purified by column chromatography on silica gel with trichloromethane/ethyl acetate (1:4) to obtain CURMA (473.6 mg, 38.41%). ^{1}H NMR was shown in Figure S5. CURMA: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 7.62 (dd, J = 36.7, 20.7 Hz, 3H), 7.42 – 7.14 (m, 4H), 6.99 (d, J = 15.8 Hz, 1H), 6.90 – 6.68 (m, 2H), 6.11 (d, J = 32.4 Hz, 2H), 5.72 (s, 1H), 4.42 (dt, J = 12.4, 5.9 Hz, 4H), 3.87 (d, J = 12.1 Hz, 6H), 3.09 (dd, J = 14.0, 6.5 Hz, 4H), 1.90 (s, 3H).

Reference

[1] Xu, X.; Smith, A. E.; Kirkland, S. E.; McCormick, C. L. Aqueous RAFT Synthesis of pH-Responsive Triblock Copolymer mPEO-PAPMA-PDPAEMA and Formation of Shell Cross-Linked Micelles. *Macromolecules* **2008,** *41*, 8429-8435.



Scheme S1 Synthetic routes of ACPP and CURMA.



Scheme S2 The self-immolative release of intact CUR from the polymers in response to cytosol redox potential.

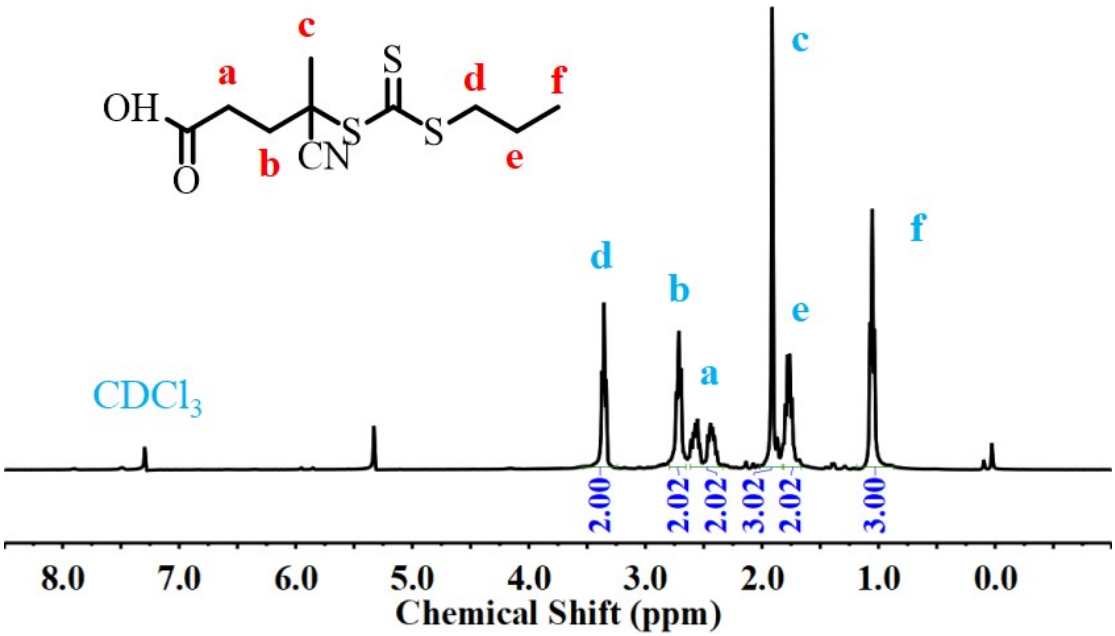


Fig. S1 ^{1}H NMR spectrum of CPP.

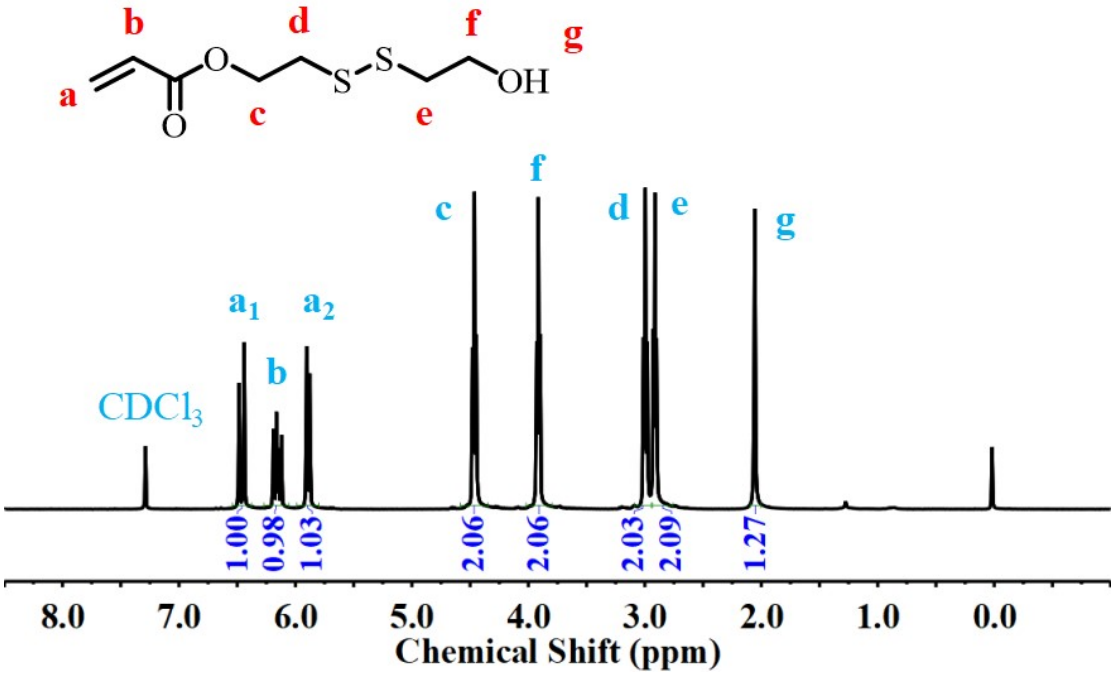


Fig. S2 ^{1}H NMR spectrum of HSEA

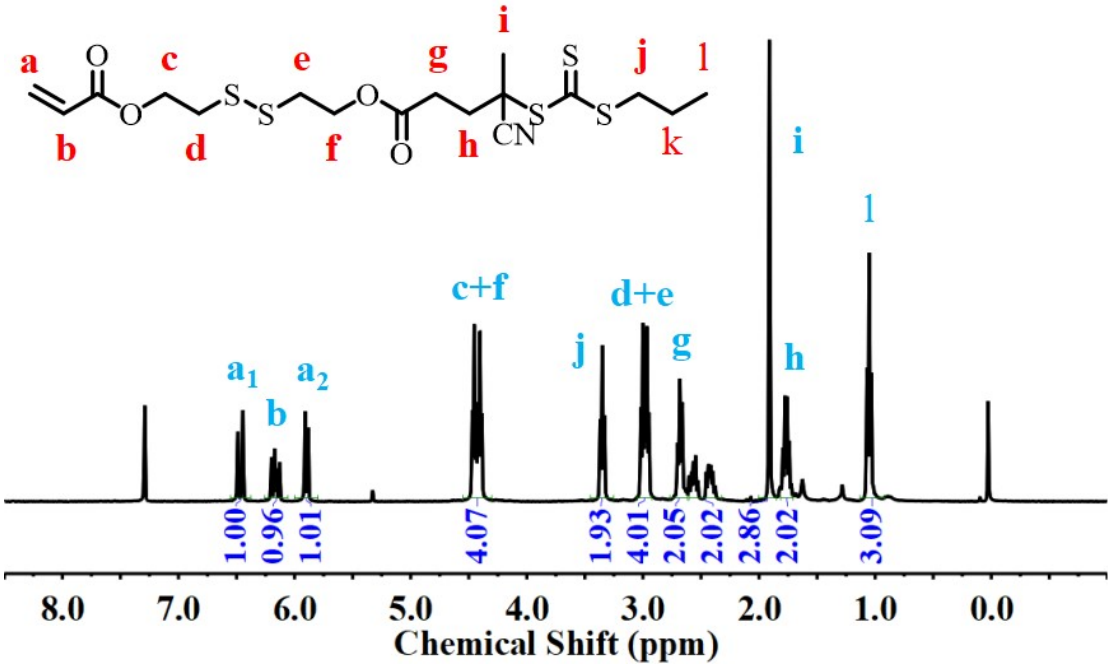


Fig. S3 ^{1}H NMR spectrum of ACPP.

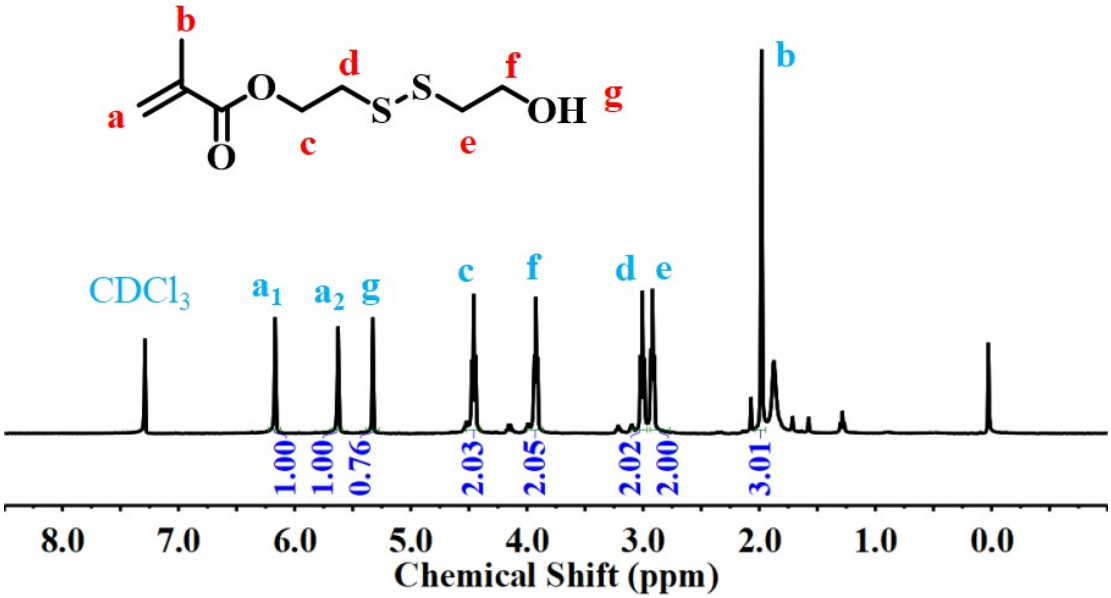


Fig. S4 ^{1}H NMR spectrum of HSEMA.

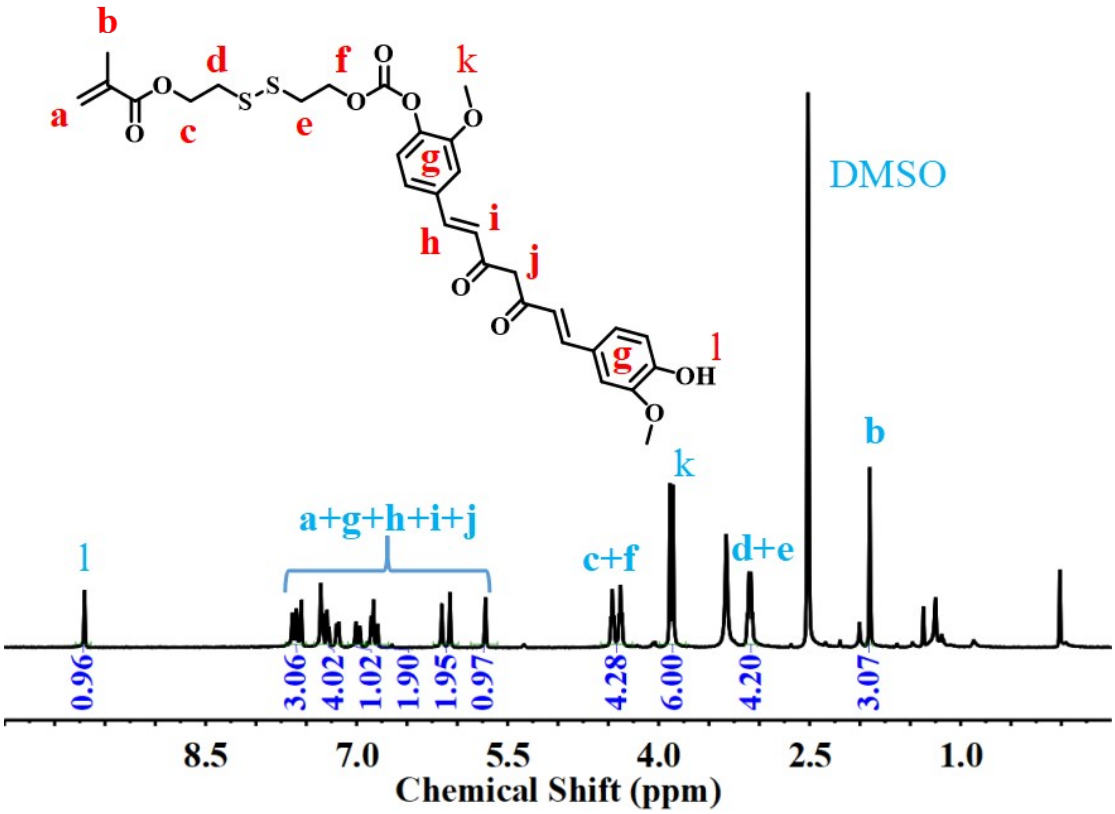


Fig. S5 ^{1}H NMR spectrum of CURMA.

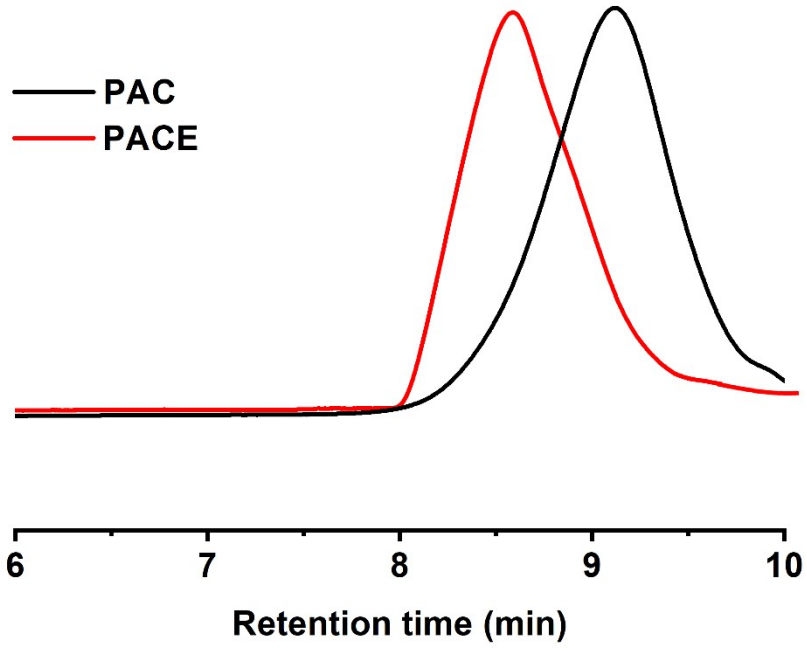


Fig. S6 GPC traces of PAC and PACE.

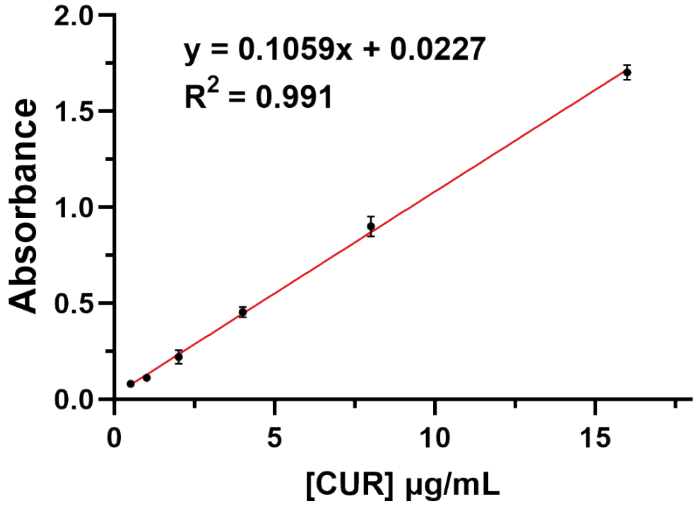


Fig. S7 Calibration curve based on UV-Vis absorbance at 420 nm for the calculation of CUR loading content in PACE.

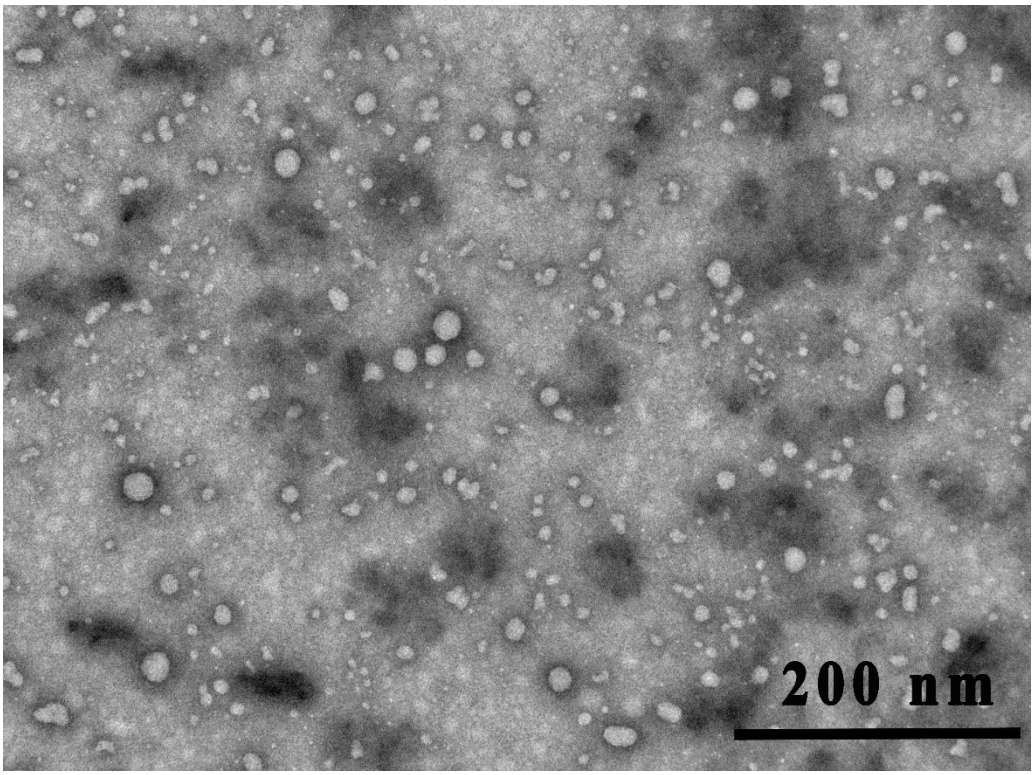


Fig. S8 TEM image of the degradation products after the PACE unimolecular micelles were incubated with 10 mM DTT for 24 hours.

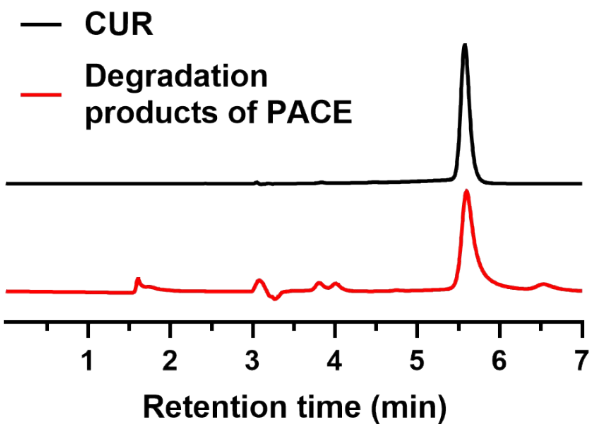


Fig. S9 HPLC analysis of free CUR and degradation products of PACE.

Table S1 Structural parameters of the PAC and PACE

Samples	M_n (kDa)[a]	M_w (kDa)[a]	PDI[a]	DLC (%)[b]
PAC	18.1	23.5	1.30	—
PACE	148.9	189.2	1.27	5.8

[a]Molecular weights and PDIs were determined by GPC.

[b]The loading content of CUR was calculated according to the UV-vis absorbance of CUR using a standard curve.