Supporting Information

Synthesis of Multi-responsive Polymeric Nanocarriers for Controlled Release of Bioactive Agents

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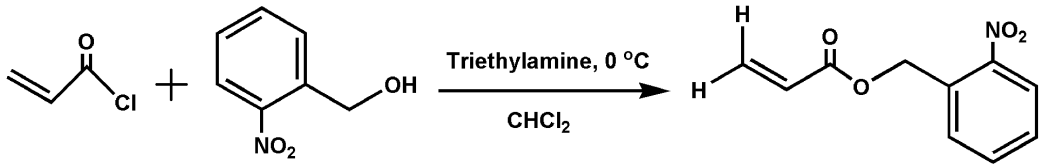
Experiment

Materials

S-1-Dodecyl-S'-(α,α'-dimethyl-α''-acetic acid) trithiocarbonate (DMP) were synthesized according to previously published procedures.[1] Azobisisobutyronitrile (AIBN, 98%) was purchased from East China Chemical Co., Ltd. (Shanghai, China) and purified by recrystallization twice from ethanol and dried in vacuum prior to use. Triethylamine (99.5%), acryloyl chloride (96%), $CHCl_2$, NaOH, $NaSO_4$, 2-(dimethylamino) ethyl methacrylate (DMAEMA) were supplied by Aladdin Reagent CO., Ltd. and used as received. 2-Nitrobenzyl alcohol was purchased from J&K CO., Ltd. Tetrahydrofuran (THF) was dried by heating and refluxing with potassium. MilliQ water was used in all experiments. CO_2 (99.998%) and N_2 (99.998%) were purchased from East China Chemical Co., Ltd. (Shanghai, China) and used as received.

Synthesis of 2-Nitrobenzyl Acrylate

Preparation of 2-nitrobenzyl acrylate was consistent with the procedure previously reported,[2,3] as shown in Scheme 1. 2-Nitrobenzyl alcohol (2.105g, 13.75mmol) and triethylamine (3.84mL) were dissolved in 15mL $CHCl_2$ in a flask prior to be placed in ice bath. After flowing with N_2 for 10 min, a solution of acryloyl chloride (2.105g, 23.25mmol) in $CHCl_2$ (5mL) was added dropwise in to the flask using an additional funnel. 12h later, the mixture was washed with NaOH solution (15mL 1.0mg/mL). The organic layer was collected, and then dried with Na_2SO_4 for 12h. Product was obtained as yellow liquid by evaporating the solvents at room temperature in vacuum after filtering. ^{1}H NMR ($CDCl_3$): δ (ppm): 5.62 (s, 2H, O-C***H***$_2$-C-), 5.91 and 6.52 (d, 2H, ***H***$_2$C=CH-), 6.22 (t, H, CH_2=C***H***-COO-), 7.68-7.46 (m, 3H, C-(C***H***)$_3$-CH), 8.09 (d, H, C-(CH)$_3$-C***H***-CH-NO_2)



Scheme S1. Synthesis of 2-nitrobenzyl acrylate

Synthesis of PDMAEMA

PDMAEMA was prepared via RAFT polymerization (Scheme 1). Briefly, DMP (0.183g, 0.5 mmol), AIBN (0.016g, 0.1 mmol) and DMAEMA (5.39 mL, 0.03mol) were added into a flask with a stirrer. After three times of being vacuumed and pumped with N_2, the flask was kept stirring at 80 ^{o}C for 12h. The resultant product with M_n=1.28×10^4 g mol^{-1} (GPC) was obtained at >95% yield by lyophilization. ^{1}H NMR ($CDCl_3$, ppm): δ 0.8-0.9 (3 H,-C*H*$_3$); 1.8-1.9 (2 H, -C*H*$_2$-); 2.20-2.30 (6 H, -N(C*H*$_3$)$_2$); 2.50-2.60 (2 H, -C*H*$_2$-N); 4.00-4.20 (2 H, -C*H*$_2$-CH$_2$-)

Synthesis of PDMAEMA-*b*-PNBM

At first, PDMAEMA was prepared *via* conventional RAFT polymerization procedure. Briefly, DMP (0.0182 g, 0.05 mmol), AIBN (0.0008 g, 0.005 mmol), DMAEMA (0.535 mL, 3.17 mmol) and DMF (8 mL, 0.1 mol) were added into a flask and kept stirring at 70 ^{o}C for 24 h after three cycles of freeze-pump-thaw procedure. Solid product was obtained by precipitate the mixture in petroleum ether and dried at 25 ^{o}C under vacuum. Then, PDMAEMA was used as macro-RAFT agent to polymerize with 2-nitrobenzyl acrylate. The process can be descript as follows, PDMAEMA (0.12g), 2-nitrobenzyl acrylate (0.5g), AIBN(0.0035g) and THF (5mL) were added into a flask and treated with three cycles of freeze-pump-thaw procedure. The flask was sealed under vacuum and then placed in 70 ^{o}C oil bath with stirring for 24h. Yellow product was precipitate in petroleum ether and dried at room temperature under vacuum. The resultant product with M_n=1.72×10^4 g mol^{-1} (GPC) was obtained by lyophilization.

Preparation of multi-responsive micelles

Multi-responsive micelles were prepared via self-assembly. Briefly, 0.05g

PDMAEMA-*b*-PNBM was dissolved in 20 mL THF, and then dialyzed against DI-water using a 3500 Mw dialysis bag at 25 ^{o}C with shaking.

Drug loading and release

Indomethacin was used as a model drug in this experiment. Drug loaded micelles were prepared by dissolving 0.05g PDMAEMA-*b*-PNBM and 0.005g indomethacin in 20 mL THF before they were put into a dialysis bag, which was placed in water with constant shaking at 25 ^{o}C.

The release experiment was conducted in phosphate buffer solution (PBS) at different pH, temperature, CO_2 concentration, and UV/vis light. Drug loaded micelles was dissolved in 5mL PBS before the solution was put into a dialysis bag and immersed in a beaker containing 50 mL PBS. At predetermined time, 5 mL of liquid was sampled from the outer solution, and then replaced by the same volume of release medium. The drug content was detected by measuring UV-*vis* absorbance spectra at 320 nm.

Characterization

General Characterization and Instrumentation

The ^{1}H NMR spectra were recorded on an AVANCE AV 400MHz Digital FT-NMR spectrometer operating at 400 MHz using deuterated DMSO-d6 and D_2O as the solvent. Gel permeation chromatographic (GPC) analysis was carried out usinga Waters 1525 pumping system (USA) at the flow rate of 0.5 mL min^{-1} with an Ultrahydrogel 500 column (Waters). The eluent was THF. Fourier transform infared (FT-IR) spectra were recorded on a Nicolet 5700 spectrophotometer using an ATR cell or KBr pellets for samples.The sizes and morphologies of the resultant samples were characterized by JSM-2100 transmission electron microscopy (TEM) at an accelerating voltage of 200 kV, whereby a small drop of sample solution was deposited onto a carbon-coated copper EM grid (200 mesh) and dried at room temperature at atmospheric pressure. Dynamic light scattering (DLS) measurements were performed in aqueous solution using a HORIBA Zetasizer apparatus (LB-550 V) equipped with a 5.0 mW laserdiode operating at 650 nm at room temperature.

UV-visible spectra were obtained using a Hitachi U-3010 spectrophotometer.

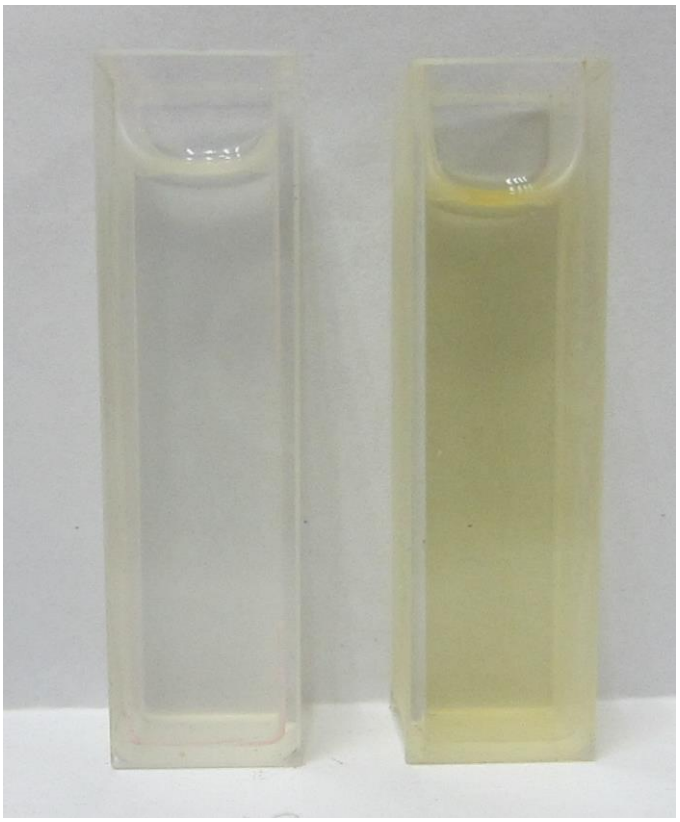


Figure S1. Photograph of micelles dispersed in water before (left) and after (right) UV irradiation.

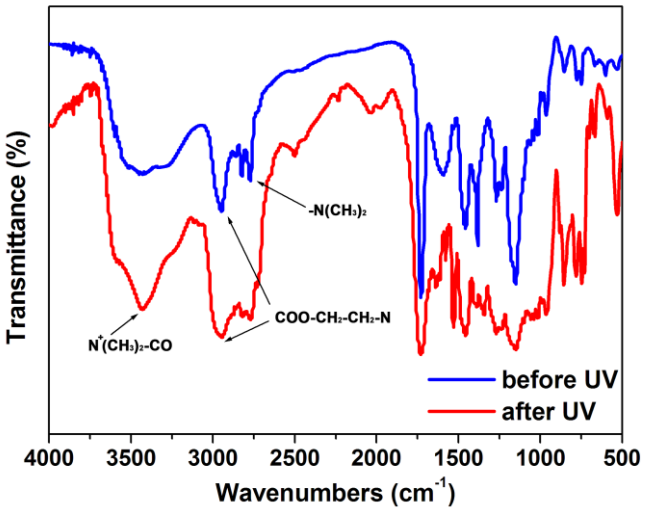


Figure S2. FTIR spectra of multi-stimuli sensitive micelles before and after UV irradiation.

The reaction between the amine groups and carboxylic groups after UV irradiation was confirmed by FT-IR spectra. 4 mL THF solution of micelles was

placed in a quartz cuvette and exposed to UV light (365 nm, 75 Mw/cm^2) for 1 h. Then the solvent evaporated and the aggregates were dried at room temperature before FTIR measurements. As shown in Fig. S1, the peak appears at 3400 cm^{-1} and the peak decreases at 2800 cm^{-1} are caused by the reaction between the amine groups and the carboxylic acid groups after UV irradiation.

results in control release of drugs from micelles.

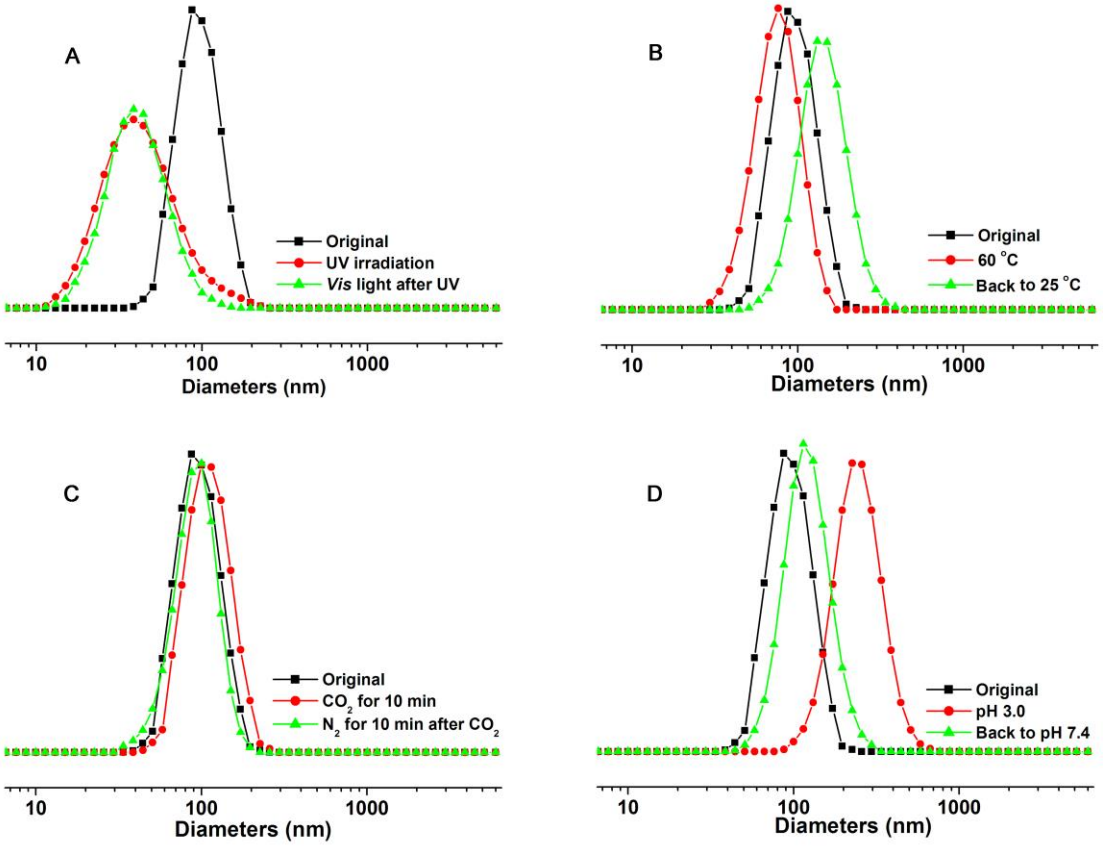


Figure S3. DLS analysis of micelles under *Vis* light after UV irradiation (A), cooled to 25 ^{o}C after heating to 60 ^{o}C (B), bubbled with N_2 for 10min after CO_2 bubbling (C), adjusted pH from 3.0 to 7.4 with NaOH (D).

The reversibility of multi-responses was investigated with assistance of DLS measurement. As shown in Fig. S3, the micelles were able to revert to original states except those treated with UV irradiation. Because the chain of PNBA have been converted into poly(acrylic acid) after UV irradiation, which could not convert into poly(2-nitrobenzyl acrylate) only by avoiding UV irradiation. The micelles shrink as temperature gets higher than their LCST (44^{o}C), and swell as temperature gets lower than their LCST, results in reversible response to temperature. The response to CO_2 is

reversible, because the protonation/de-protonation can be reversibly adjusted by introducing/removing CO_2. And the reversible response to pH shares the same mechanism with CO_2.

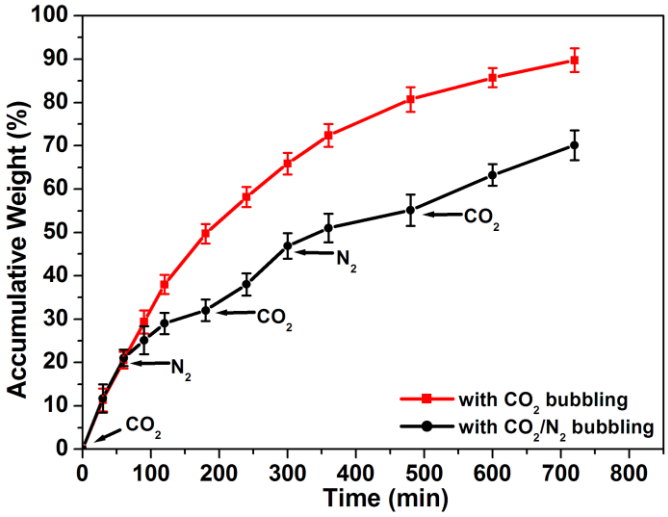


Figure S4. CO_2/N_2 controlled release of indomethacin from multi-stimuli sensitive micelles. Data are presented as average standard deviation (n=3).

When CO_2 were removed from PBS, the release rate of drug from micelles was delayed as compared with CO_2 bubbling throughout the whole process (Fig S4), which is caused by de-protonation of the shell of micelles. Micelles become to be aggregated and shrunk as CO_2 being removed by N_2, retarding the release of drugs. The protonation/de-protonation can be reversibly adjusted by introducing/removing CO_2,

References

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