Supporting Information

CuS nanocrystal@microgel nanocomposites for light-regulated release of dual-drugs and chemo-photothermal synergistic therapy *in vitro*

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‡ These authors have the equivalent contribution to this work.

Part S1: Preparation of citrate-stabilized CuS NCs

Citrate-stabilized CuS NCs were prepared by a slightly modified method reported previously (*ACS Nano*, 2015, **9**, 3926). In a typical experement, 20 mL of $Cu(CH_3COO)_2$ aqueous solution (1.7 mg mL^{-1}) and 20 mL of sodium citrate (2.0 mg mL^{-1}) were added in 50 mL of distilled water. The mixture solution was stirred for 30 min at room temperature. Then, 10 mL of Na_2S (3.4 mg mL^{-1}) was added to the mixture solution and stirred for additional 5 min before transferring to a 90 ^oC water bath. The preparation reaction was continuously conducted for 20 min. After that, the reaction solution was cooled to room tempereature. The as-prepared aqueous suspension of CuS NCs was concentrated by circumrotate evaporation, precipitated with 2-propanol and collected by centrifugation. Colloidal precipitates were dried in vacuum at 60 ^oC, and re-dispersed in aqueous solution for subsequent experiments.

Part S2: Determination method of NO concentration

To detect NO released from Dox/RBS-loaded CuS@PNIPAM-*g*-CS nanocomposites in water-

soluble mediums (PBS, 10 mM, pH6.5), the method of colorimetric Griess reaction was utilized to measure nitrite or nitrate contents in PBS of nanocomposites. The details are available as follows. Upon irradiation of 365 nm light (0.5 W, 0~60 min), the aliquots of PBS aqueous suspension of nanocomposites (0.1 mg mL^{-1}, 5 mL) were taken and then stirred in a centrifugal tube at room temperature. At defined time intervals, the solution was centrifuged for 10 min and the supernatant (0.5 mL) was extracted and replenished with PBS, followed by the combination with the Griess reagent **(I)** (0.1 wt.% of *β*-naphthylethylenediamine dihydrochloride aqueous solution, 1 mL) and the Griess reagent **(II)** (1 wt.% of sulfanilamide in 5 wt.% of phosphoric acid aqueous solution, 1 mL). The resultant mixed solution was incubated for 15 min at room temperature and protected from light (in the dark). A purple-magenta color appeared immediately. The maximum absorbance was recorded (at 540 nm) using UV-vis spectrophotometer. The standard curve was determined by measuring sodium nitrite (0-100 μM) in PBS. The total release of NO was calculated by using the following equations:

$R_1 = C_1 \times 0.005$;

$R_2 = C_2 \times 0.005 + C_1 \times 0.001$;

$R_3 = C_3 \times 0.005 + (C_2 + C_1) \times 0.001$;

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$R_n = C_n \times 0.005 + (C_{n-1} + C_{n-2} + \ldots\ldots + C_1) \times 0.001$

where R_n is the release amount of NO measured at each time point, and C_n is the concentration of NO.

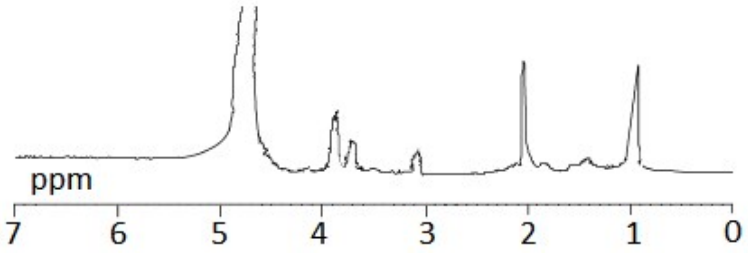

Fig. S1 ^{1}H-NMR spectrum of PNIPAM-*g*-CS microgels.

^{1}H-NMR spectrum indicates the achievement of PNIPAM-*g*-CS microgels as below. ^{1}H-NMR spectrum indicates a sharp proton peak (-CH-CH_2-) at 2.0 ppm, a weak peak (-NH-CH<) at 3.2 ppm and a strong methyl group peak at 1.0 ppm, which are derived from PNIPAM. The strong and broad spectrum band at 4.9 ppm and a weak peak at 3.1 ppm correspond to the proton on the anomeric carbon and on the carbon bearing amino (partially acetamido) groups from CS. The middle-strong peak (RO–CH) at 3.9 ppm is similar to that of CS. The above results demonstrate that the graft reaction had been performed. Because the C_6–OH and C_2–NH_2 of chitosan are more active groups (R. A. A. Muzzarelli, *Carbohydr. Polym.*, 1988, **8**, 1), the grafting copolymers are reacted more easily on the positions.

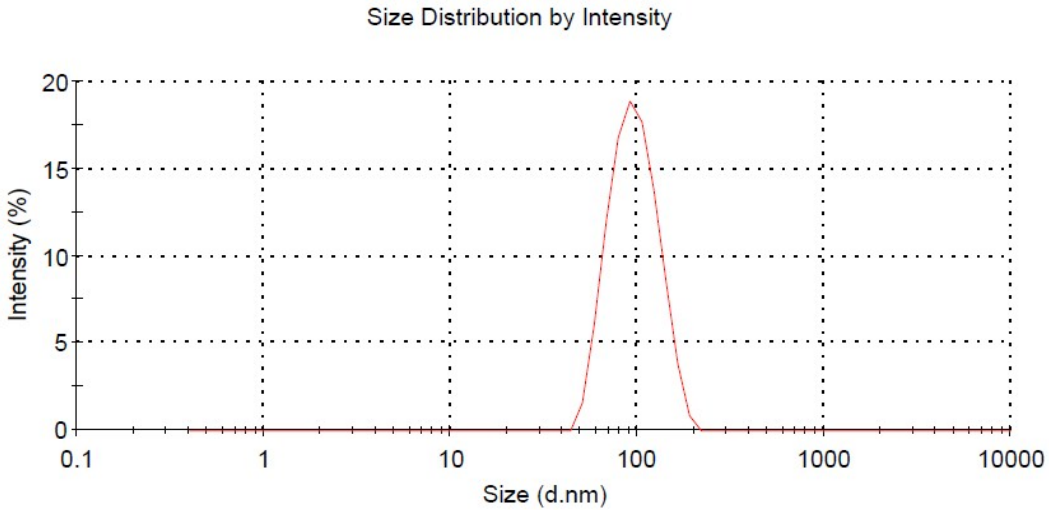

Fig. S2 Size distribution of CuS@PNIPAM-*g*-CS nanocomposites with NIR-light irradiation (980 nm, 0.5W) for 0~120s, determined by DLS and showed an average size of 91.5 nm.

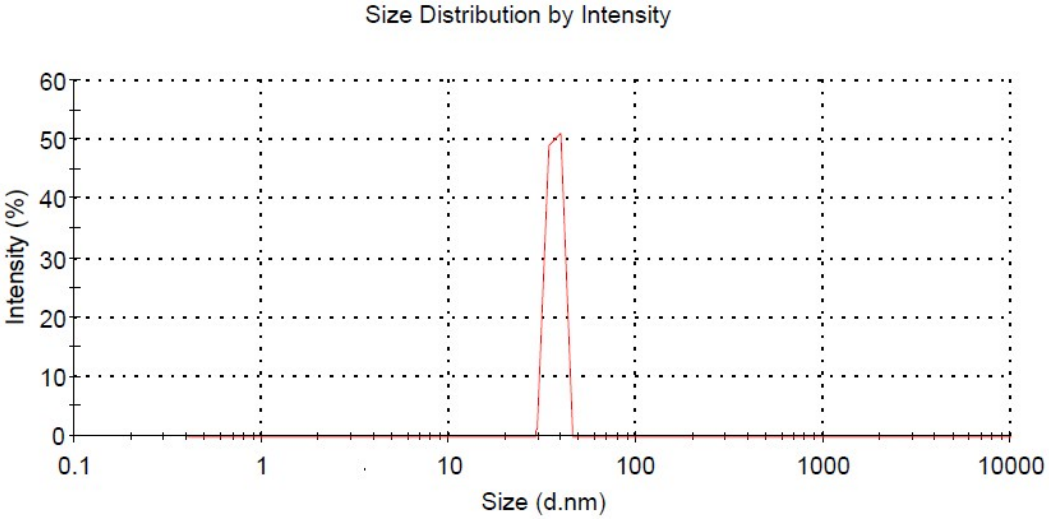

Fig. S3 Size distribution of CuS@PNIPAM-*g*-CS nanocomposites with NIR-light irradiation (980 nm, 0.5W) for 120~210s, determined by DLS and showed an average size of 39.9 nm.

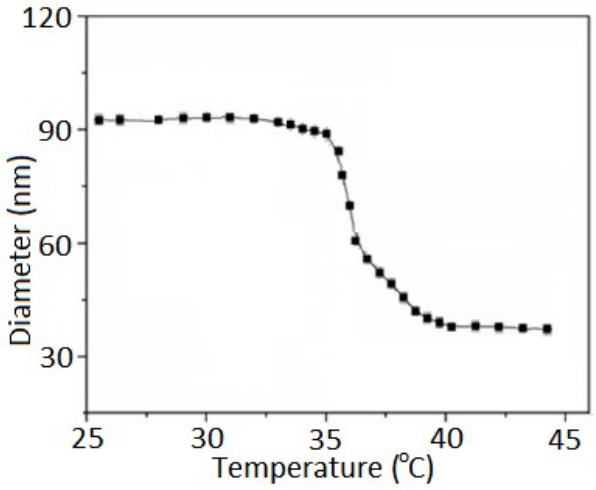

Fig. S4 Hydrodynamic diameters of CuS@PNIPAM-*g*-CS nanocomposites, placed in a water bath and treated by increasing temperature of water bath.

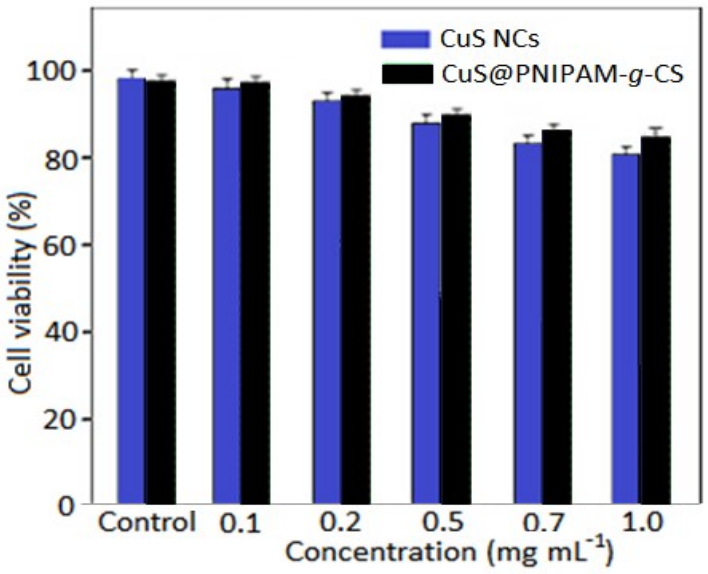

Fig. S5 *In vitro* cell viabilities after incubation with different dosages (0-1 mg mL^{-1}) of CuS NCs and CuS@PNIPAM-*g*-CS nanocomposites.

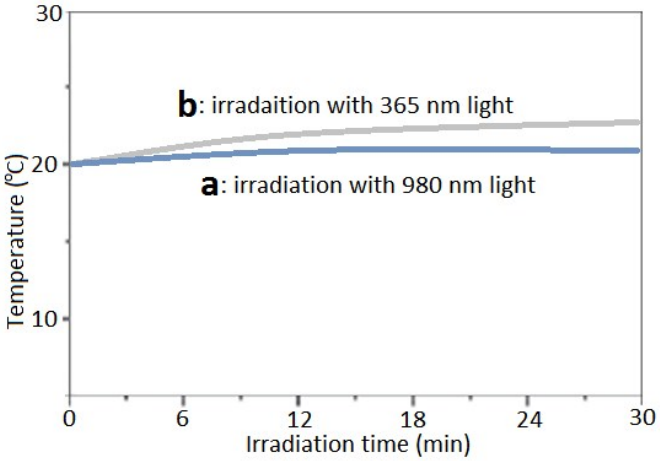

Fig. S6 Temperature elevation in the microenvironment of HeLa cells directly treated with 0.5 W of light irradiation at 980 nm (a) and 365 nm (b) for 0-30 min, without the incubation with CuS@PNIPAM-*g*-CS nanocomposites.