Supplementary Information

$Cu_{2-x}Se/Bi_2Se_3$@PEG Z-Scheme Heterostructure: A Multimode Bioimaging Guided Theranostic Agent with Enhanced Photo/Chemodynamic and Photothermal Therapy

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EXPERIMENTAL SECTION

Materials

Unless specified otherwise, all of the chemicals used were analytical grade and used without further purification. Se powder, CuCl, H_2O_2, Dichlorofluorescein diacetate (DCFH-DA) and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Aladdin Co. Ltd. Propidium iodide (PI) were purchased from Alfa Aesar Co. Ltd.. Isopropanol (IPA) was obtained from Sinopharm Chemical Reagent Co. Ltd.. Calcein acetoxymethyl ester (calcein AM) was supplied by KeyGEN BioTCH. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-Htetrazolium bromide (MTT) was from Sigma Aldrich.

Preparation of Cu Precursor

Briefly, 0.01 mol of CuCl was dispersed in 4 mL of oleylamine (OAm) and 5 mL of oleic acid (OA). Under argon atmosphere, the above mixture was stirred vigorously for 10 min at 130 °C, and a dark blue viscous liquid formed eventually.

Preparation of ODE-Se Solution and $Cu_{2-x}Se$ Nanoparticle

In order to prepare the ODE-Se solution with different activities, the selenium powder (10 mmol) were dispersed in 1-octadecene (ODE, 40 mL) and stirred at 200 °C under argon atmosphere for 1, 2 or 3 h, respectively. Then, the temperatures of all Se solutions were dropped to 180 °C and the as-prepared Cu precursor (9 mL) were rapidly injected into them and kept for 10 min. After cooling to room temperature, three as-prepared products were washed by using 1:1 of ethanol/hexamethylene. The

products that with different react time (1, 2 and 3 h) are named as C-1, C-2 and C-3, respectively.

Bismuth Ion Exchange to Prepare $Cu_{2-x}Se/Bi_2Se_3$ Nanocomposite

50 mg of prepared C-3 nanoparticle was dispersed in 5 mL of 1-octadecene (ODE) and degassed for 30 min at 120 °C. Subsequently, the mixture was stirred vigorously and heated to 140 °C under argon atmosphere. After injecting 0.5 mL of OAm containing 0.125 mmol of bismuth neodecanoate, the above mixture was further stirred for another 30 min at 140 °C. The product was also washed by using 1:1 of ethanol/hexamethylene and named CB0. To investigate the effect of temperature on ion exchange, the reaction temperature was further raised to 180 and 240 °C. In addition, trioctylphosphine (TOP) was introduced to accelerate the exchange of Bi, then 0.02, 0.05, 0.10 and 0.20 mL of TOP were added into the reaction system, and the corresponding products are named as CB1, CB2, CB3 and CB4, respectively.

Photodynamic Property

1 mL of PBS dispersion (pH 7.4) containing 500 μg of as-prepared samples (CB0@PEG, CB1@PEG, CB2@PEG, CB3@PEG, CB4@PEG and C-3@PEG) and 600 μg dichlorofluorescein diacetate (DCFH-DA) was irradiated by 808-nm laser with different illumination times (0 ~ 20 min). After centrifugation, the obtained supernatant was detected and analyzed via fluorescence.

Photothermal Property

Firstly, the as-prepared samples (CB0@PEG, CB1@PEG, CB2@PEG, CB3@PEG, CB4@PEG and C-3@PEG) were dispersed into 1 mL of DI water, respectively. Then, the above aqueous dispersion with different concentrations were

illuminated by 808-nm laser with different power densities (1.0 ~ 3.0 W cm^{-2}), and their temperature at each interval was recorded by using FLIR infrared thermal imager E8. The photothermal conversion efficiencies (η) of C-3@PEG and CB3@PEG were calculated according to the previous reported papers [1].

Chemodynamic Property

The chemodynamic performance of CB3@PEG was investigated by using methylene blue (MB) as the selective ·OH captor. Here, the aqueous dispersion (pH 5.0 and 7.4) containing 10 μg mL^{-1} MB, 10 mM H_2O_2, and 1 mg mL^{-1} CB3@PEG was prepared. At each interval, the supernatant of mixture was obtained by centrifugation and analyzed by UV-vis spectrum. Similarly, the mixture containning 0.5 mg mL^{-1} CB3@PEG, 40 μg mL^{-1} 3,3',5,5'-tetramethylbenzidine (TMB) and 10 mM H_2O_2 with different pH values (pH 5.0 and 7.4) and stored for 3 h. Then, the obtained and measured methods of corresponding supernatant are the same as above.

Synthesis Bi_2Se_3 nanoparticles

The synthetic method of Bi_2Se_3 is same as that of $Cu_{2-x}Se$. Concretely, 10 mmol of Se powder was dispersed in 40 mL ODE, and then the mixture was heated to 200 °C under Ar atmosphere to completely dissolve the selenium powder. Then, the temperature of mixture was dropped to 180 °C and 6.7 mmol bismuth neodecanoate was injected above mixture and kept for another 15 min. After cooling to room temperature, the product was collected and washed by 1:1 of ethanol/hexamethylene.

PEGylation

To improve the biocompatibility of the CB3 nanoparticles, C18PMH-PEG synthesized by the standard protocol was used to modify the as-synthesized materials.[2] Briefly, 50 mg of C18PMH-PEG was dissolved in 4 mL of chloroform. Then, 10 mg of CB3, which was dispersed in 1 mL of chloroform, was dropwise

added to the previous solution under sonication. After sonication for 10 min, the solution was placed under stirring for 2 h. Finally, N_2 was used to dry the solution, and the final solid sample was dispersed in deionized water and stored at 4 °C for future use.

Cell culture

HepG2 (hepatoma cell line) cells were cultured in a monolayer in Dulbecco's Modified Eagle's Medium (DMEM, hyclone) that includes 10% (v/v) fetal bovine serum (FBS, Tianhang Bioreagent Co., Zhejiang) and penicillin/streptomycin (100 U mL^{-1} and 100 mg mL^{-1}, respectively, energy chemical) in a humidified 5% CO_2 atmosphere at 37 °C.

MTT assay

The vitro cytotoxicity of as-prepared sample was assessed by MTT assay. In MTT assay, HepG2 cells were seeded into 96-well plates for 24 h. Then, the cells were incubated with different concentrations of CB3@PEG and CB3@PEG + GSH (GSH 10 mM) nanocomposites for extra 12 h. Among them, the groups which need illuminating by 808-nm laser were disposed of the extra irradiation by 808-nm laser for 20 min. Afterwards, cells were incubated in a medium containing 0.5 mg mL^{-1} MTT for another 4 h. The final medium was then replaced with 150 μL of dimethyl sulfoxide (DMSO) per well and the absorbance was monitored using a microplate reader (WD-2102A) at the wavelength of 492 nm. The cytotoxicity was expressed as the percentage of cell viability compared to untreated control cells.

Fluorescence imaging

To check the synergy therapy effect, the HepG2 cells were incubated with CB3@PEG nanocomposites (1 mL, 60 μg mL^{-1}) for 12 h. After incubation, the cells were stained with calcein-AM and PI for 30 min. After staining, all the cells were

washed by PBS for 3 times and imaged by using Leica DFC450 C Microsystems Ltd..

Intracellular ROS

DCFH-DA was also used as the indicator in the detection of intracellular ROS. Similarly, the HepG2 and BEAS-2B cells were treated with CB3@PEG (60 μg mL^{-1}, 1 mL) for 12 h and then washed three times by using PBS. Subsequently, the cells were incubated with DCFH-DA (10 μmol L^{-1}) for 50 min and irradiated by 808-nm laser (1.5 W cm^{-2}) for 5 min. Ultimately, the fluorescence of DCF was imaged by using Leica DFC450 C Microsystems Ltd..

***In vitro* and *vivo* X-ray CT imaging**

To assess CT contrast efficiency in vitro, the dispersions of CB3@PEG nanoparticle with various concentrations (0, 1.875, 3.75, 7.5, 15.0, and 30.0 mg/mL) were placed into centrifuge tubes to obtain the CT images. For the CT imaging of CB3@PEG nanoparticle *in vivo*, the tumor-bearing mice were anesthetized and then the mice were injected with 100 μL of CB3@PEG dispersions (1 mg mL^{-1}). Both the *in vitro* and *in vivo* CT imaging were acquired on a Philips 64-slice CT imaging system.

***In vitro* and *in vivo* T_1-weighted MR imaging**

The *in vitro* and *in vivo* MR imaging experiments were carried out in a 3.0 T MRI magnet (American GE Discovery MR750 3.0 T magnetic resonance imaging system). *In vitro*, CB3@PEG composites with various Cu concentrations (0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4 and 8 mM) were placed in 1.5 mL of tubes. After scanning, the r_2 relaxivity values were acquired by the curve fitting of $1/T_1$ relaxation time (s^{-1}) versus the Cu concentrations (mM). *In vivo*, the 0.1 mL of CB3@PEG composite suspension with 8 mM of Cu concentration was injected to the tumor of mice. MR scan images were also taken with saline injection as control group.

***In vivo* toxicity**

To obtain the transplanted tumor on the mice, H22 cells were implanted in the left armpit of each female Kunming mouse by subcutaneous injection. All the mouse experiments were performed in compliance with the criteria of The National Regulation of China for Care and Use of Laboratory Animals. All animal procedures were approved by the Animal Ethics Committee of Harbin Normal University. When the tumor diameter is about 7 mm, the mice were divided into three groups (n = 5 group^{-1}) randomly. The mice in control and only laser irriadiation groups were injected with 100 μL of saline. And other groups were treated with CB3@PEG (300 μg mL^{-1}) and CB3@PEG+NIR (808-nm laser irradiation, 1.5 W cm^{-2}, 20 min). All the treatments were carried out every four days for 14 days. The body weights and the tumor size were measured and recorded every two days.

Histological examination

After 14 days of treatment, the organs of the heart, liver, spleen, lung, kidney, and tumor tissues of the mice in control and with CB3@PEG+NIR groups were excised for histological analysis. Then the excised tissues were dehydrated using buffered formalin, various concentrations of ethanol and xylene. Subsequently, the dehydrated tissues were encased by liquid paraffin, which sliced and stained with hematoxylin and eosin (H&E). After staining, the slices were observed by optical microscope.

Characterization

Transmission electron microscopy (TEM) images of samples were tested by Hitachi H-8100 at an accelerating voltage of 20 kV. Powder X-ray patterns (XRD) were tested on Rigaku Ultima IV diffractometer with Cu Ka radiation (40 kV, 20 mA). UV-Vis spectra were recorded on SHIMADZU UV2550 spectrophotometer. X-ray

photoelectron spectroscopy (XPS, VG ESCALAB 220I-XL) were detected with. The fluorescence spectra were surveyed by HORIBA FL-3. Electron paramagnetic resonance (EPR) spectra were recorded on Burker A200 spectrometer with a modulation frequency of 100 kHz.

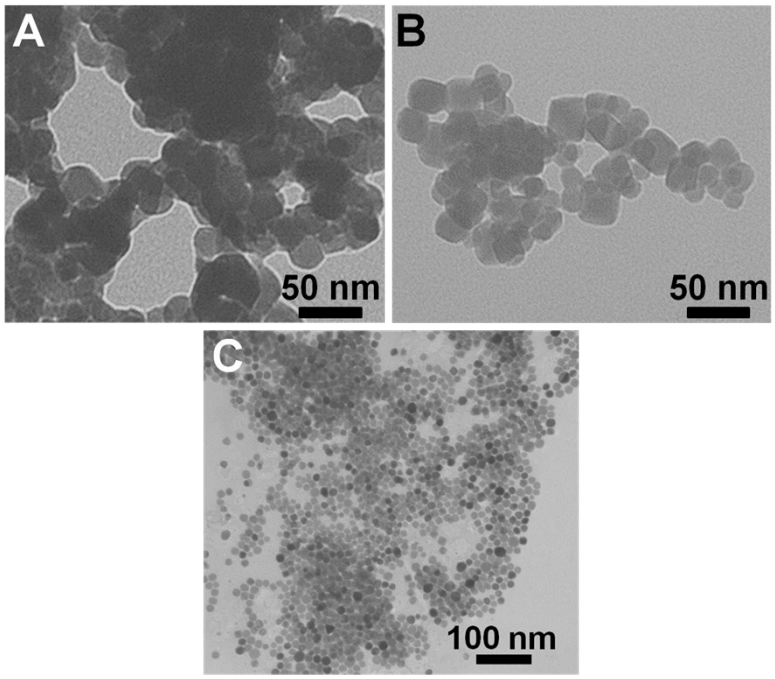

Figure S1. TEM images of (A) C-1, (B) C-2 and (C) C-3.

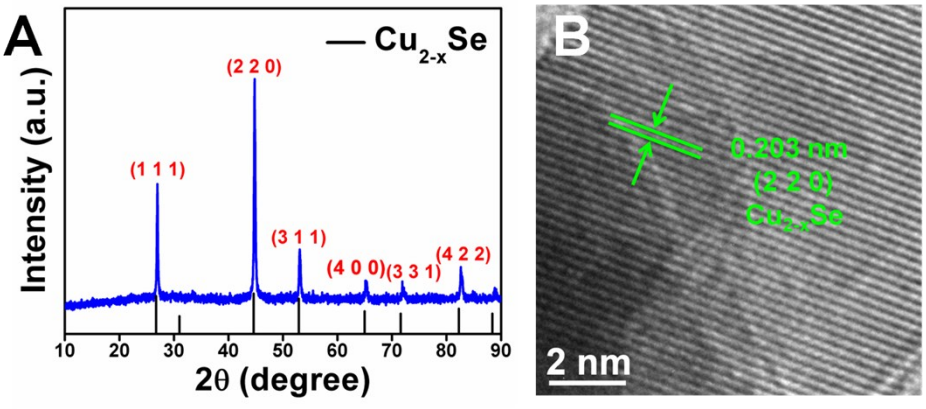

Figure S2. (A) XRD and (B) HRTEM image of C-3.

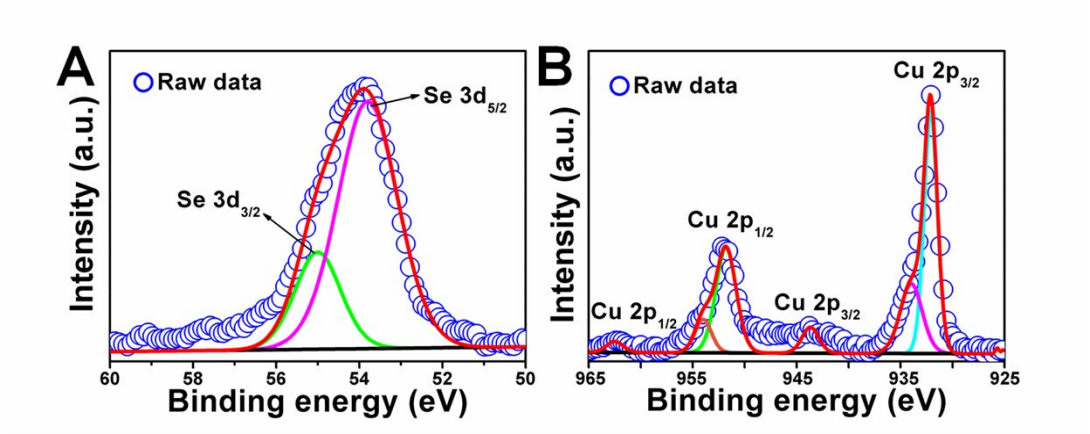

Figure S3. High-resolution XPS spectra of the (A) Se 3d and (B) Cu 2p regions of C-3.

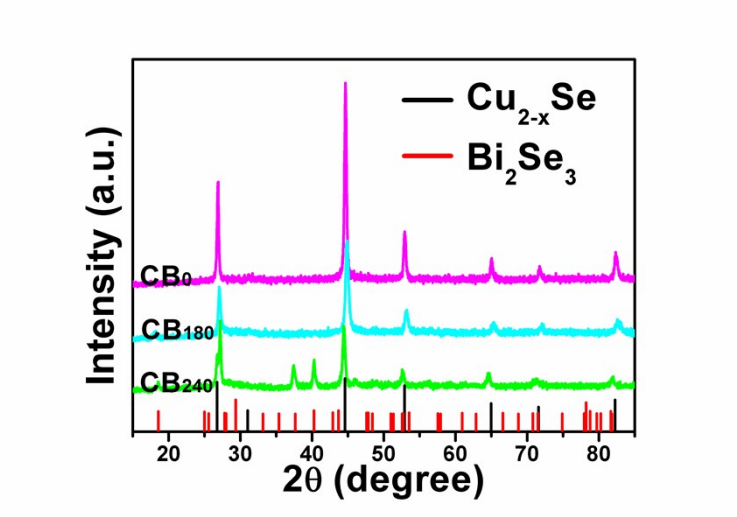

Figure S4. XRD images of CBs with different reaction temperature (140, 180, 240 ^{o}C)

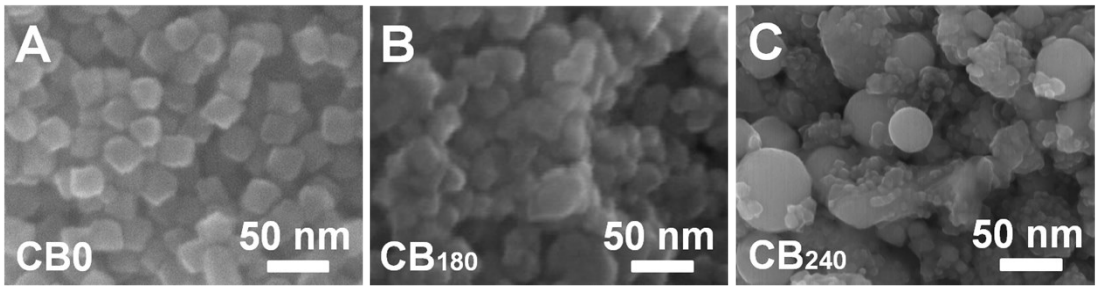

Figure S5. SEM images of the three samples with different reaction temperature (140, 180, 240 ^{o}C).

	Cu	Bi
CB0	1	0.07
CB1	1	0.12
CB2	1	0.19
CB3	1	0.30
CB4	1	0.42

Table S1. Atomic (Cu and Bi) ratio of the five samples derived from ICP data.

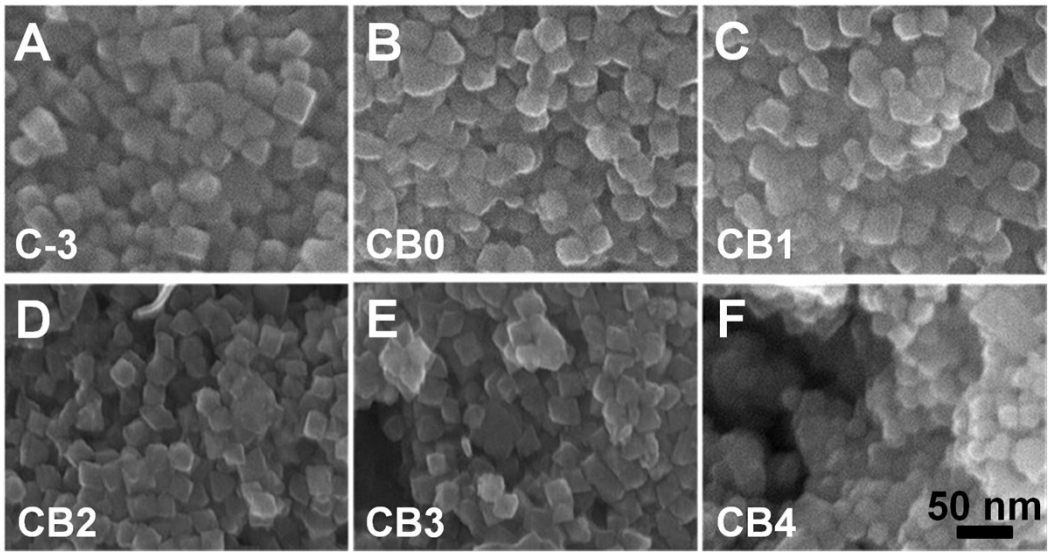

Figure S6. SEM images of the pure $Cu_{2-x}Se$ (A) and *$Cu_{2-x}Se/Bi_2Se_3$* with different of TOP amount (B-F).

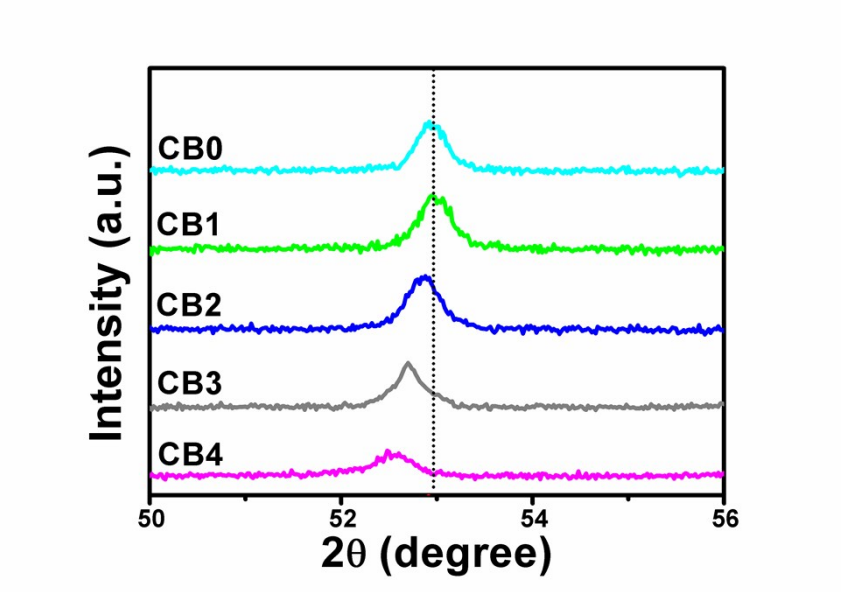

Figure S7. XRD spectra of the the pure $Cu_{2-x}Se$ (CB0) and *$Cu_{2-x}Se/Bi_2Se_3$* with different of TOP amount (CB1-CB4).

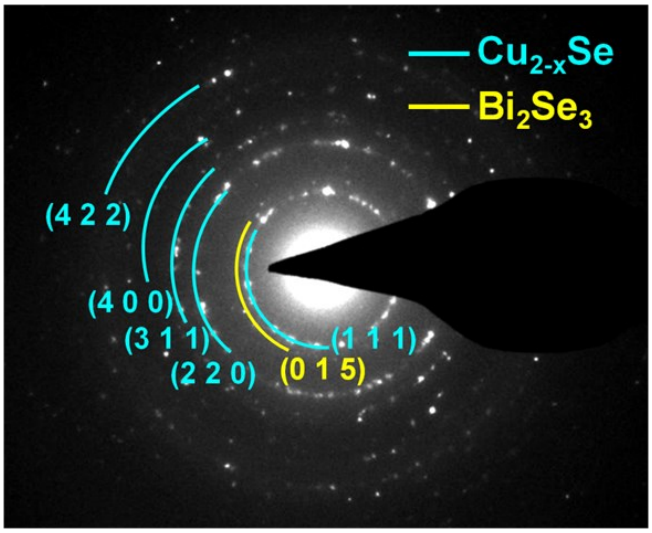

Figure S8. SAED image of CB3.

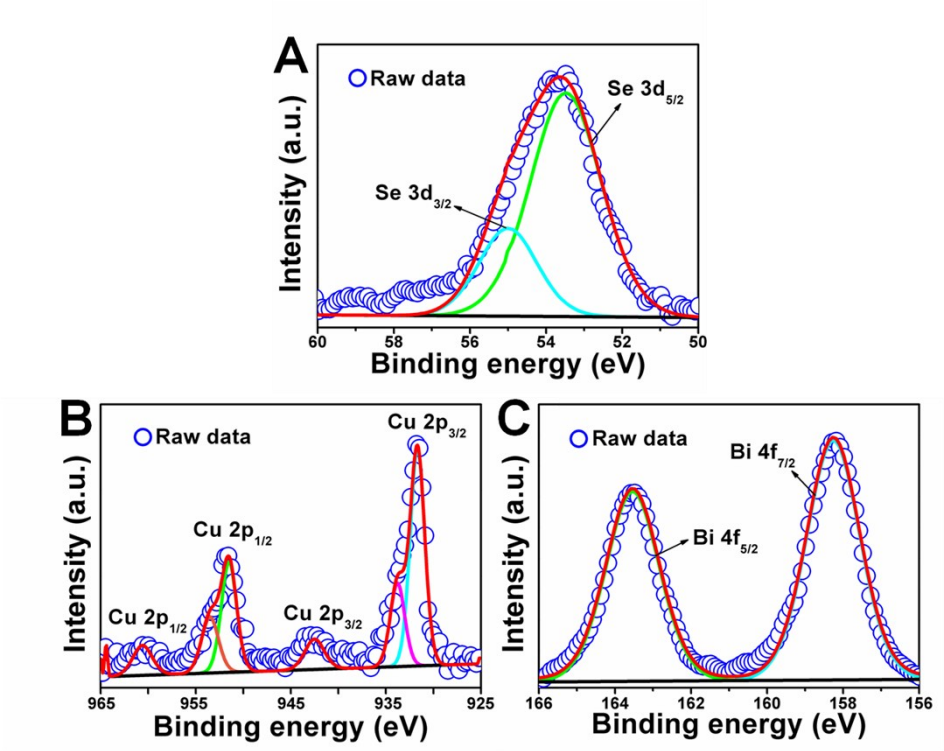

Figure S9. High-resolution XPS spectra of the (A) Se 3d, (B) Cu 2p and (C) Bi 4f regions of CB3.

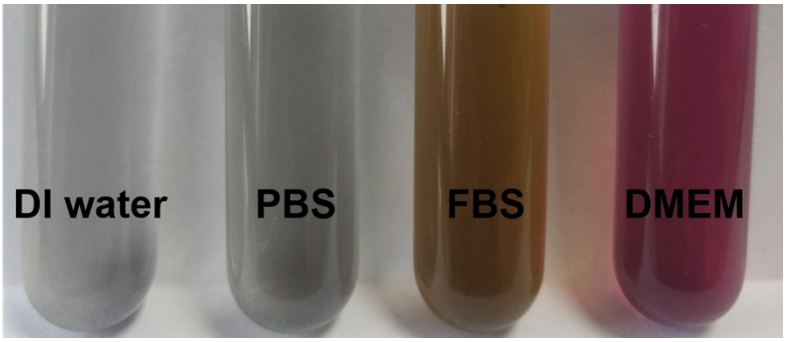

Figure S10. Pictures of CB3@PEG suepension (100 μg mL^{-1}) in different physiological solutions.

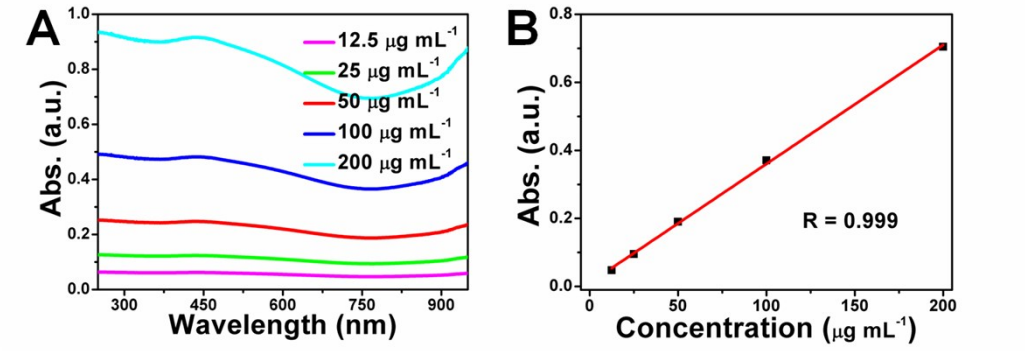

Figure S11. (A) UV-Vis spectrum of CB3@PEG with different concentrations. (B) The linear curve between UV-Vis absorption intensities and concentrations of CB3@PEG.

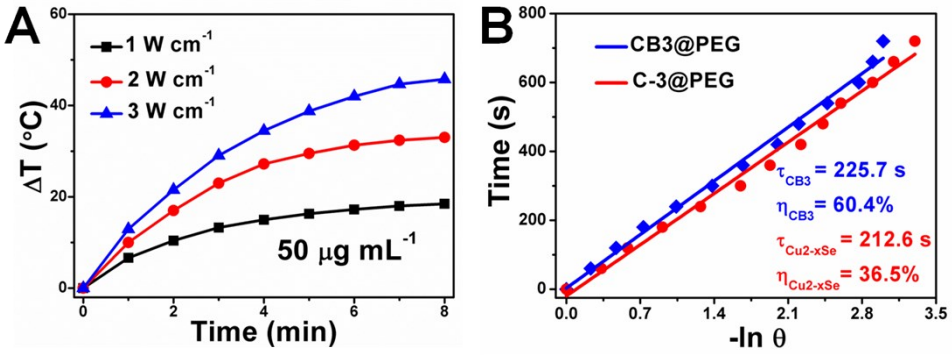

Figure S12. (A) Photothermal heating curves of aqueous dispersions of CB3@PEG irradiated by 808-nm laser with different power densities (1, 2 and 3 W cm^{-2}). (B) Thermal equilibrium time constants of the two systems determined by fitting the time data versus the negative natural logarithm of the driving force temperature from the cooling period.

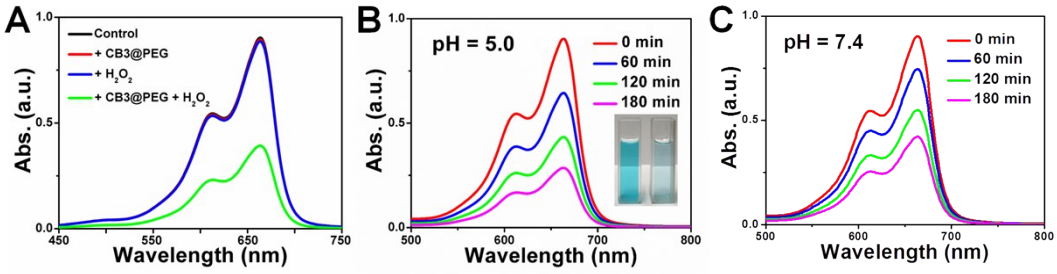

Figure S13. (A) The absorption of MB aqueous after 180 min degradation under different conditions. (B, C) The absorption of MB solution treated with CB3@PEG+H_2O_2 at pH 5.0 and 7.4.

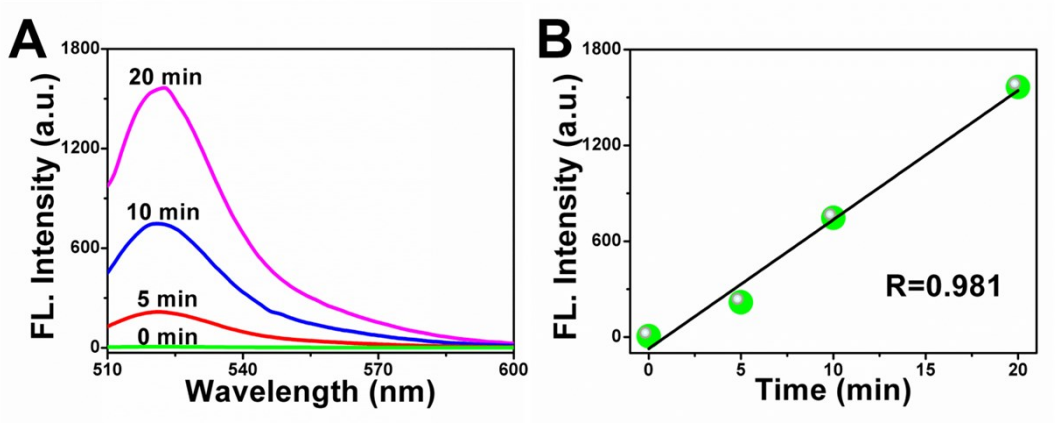

Figure S14. (A) Fluorescence spectra of DCFH and CB3@PEG supernatant after irradiation by 808-nm laser for different times and (B) the linear curve between DCF fluorescence intensities and irradiation time.

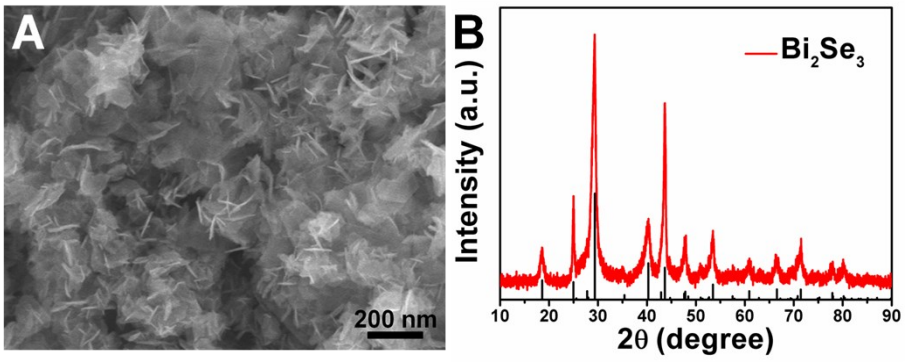

Figure S15. SEM and XRD images of prepared Bi_2Se_3.

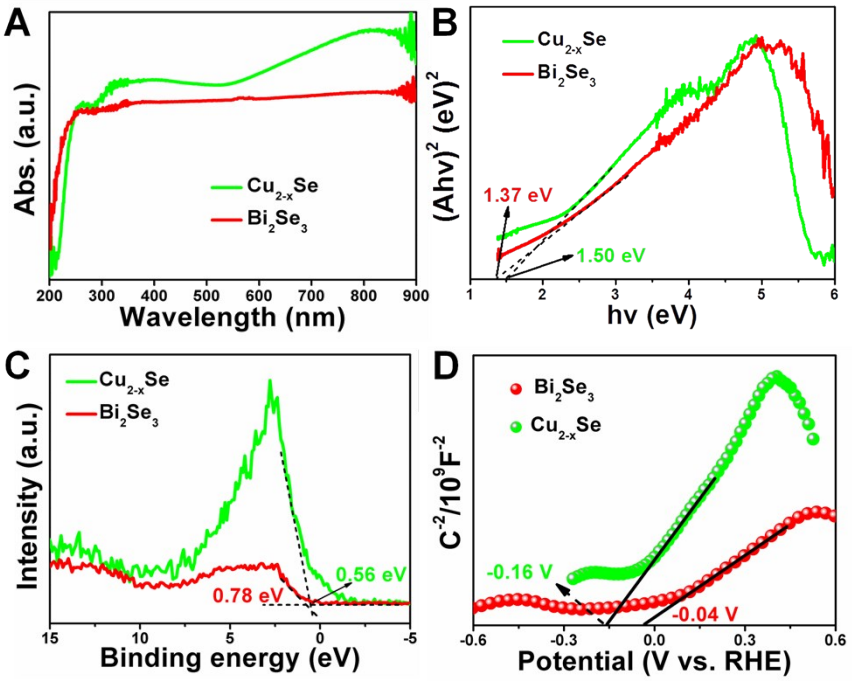

Figure S16. (A) The diffuse reflection spectrum and (B) the corresponding $(Ahν)^2$-hν curves of $Cu_{2-x}Se$ and Bi_2Se_3. (C) The valence-band XPS spectra and (D) Mott-Schottky (MS) curves of $Cu_{2-x}Se$ and Bi_2Se_3.

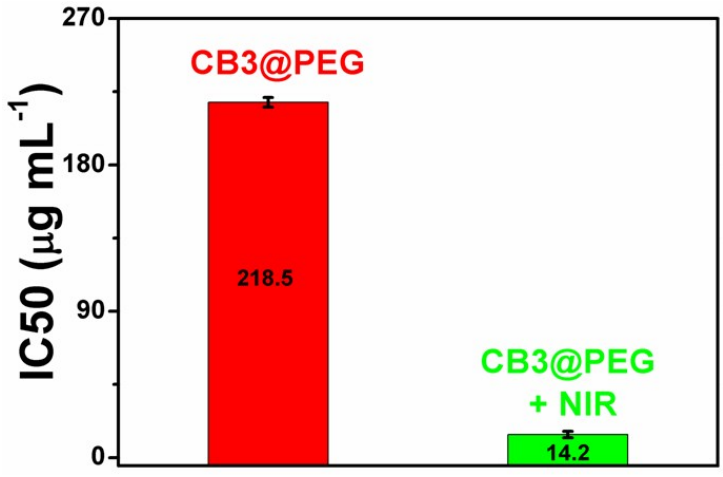

Figure S17. IC50 of CB3@PEG and CB3@PEG+NIR for cancer cells.

Reference

1. Y. Wang, F. Zhang, Q. Wang, P. Yang, H. Lin and F. Qu, *Nanoscale*, 2018, **10**, 14534-14545.
2. C. Wang, L. Cheng and Z. Liu, *Biomaterials*, 2011, **32**, 1110-1120.