

Supporting Information

Reverse Microemulsion-Mediated Synthesis of $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$ for Dual Modal CT/ Fluorescence Imaging *in vitro* and *in vivo*

Jun Chen⁺, Xiao-Quan Yang⁺, Yuan-Zheng Meng, Meng-Yao Qin, Dong-Mei Yan, Yuan Qian, Guo-Qiang Xu, Yong Yu, Zhi-Ya Ma and Yuan-Di Zhao *

Experimental Section.

The reverse microemulsion synthesis of $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$ in situ: Multifunction probe $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$ was synthesized by using the reverse microemulsion method according to literature procedures.^[1] First, W/O microemulsion I was prepared by mixing 1.77 g Triton X-100 (Aldrich), 1.8 mL 1-hexanol, 7.5 mL cyclohexane and 0.48 mL of 1.2 mM bismuth citrate solution (Aldrich, 99.99%) for 15 min, W/O microemulsion II was obtained by mixing 0.48 mL of 1.8 mM sodium sulfide in Triton X-100, 1-hexanol and cyclohexane similarly. The Bi_2S_3 microemulsion reaction was completed by mixing the microemulsion I and the microemulsion II for 2 h. With an interval of 30 min, 0.2 mL CdSe/ZnS, 0.2 mL TEOS (tetraethylorthosilicate) and NH_4OH (0.12 mL, 28 w%) were added successively, and the mixture was stirred at room temperature for 24 h. The final surface layer incorporating PEG was formed by adding 0.1 mL of TEOS for 30 min, and adding 0.020 mL PEG-silane followed by 24 h stirring. The microemulsion system was destabilized by adding ethanol, and the nanoparticles were centrifuged and washed 4 to 5 times with ethanol and 3 times with DI water. Each centrifugation step was followed by vortexing and sonicating to redisperse the sedimented material before the next centrifugation step.

The resulting nanoparticles were dispersed in PBS (pH=7.2).

X-ray CT and fluorescence imaging with Bi₂S₃-QD@SiO₂-PEG: Various concentrations of Bi₂S₃-QD@SiO₂-PEG (2.75, 5.0, 8.25, 11.00, 13.75 and 16.5 mg of Bi/mL) dispersed in deionized water were prepared in 1.5 mL microtubes. A high-resolution CT system was constructed and applied in imaging, the imaging parameters were as follows: the spatial resolution is 100µm, the tube voltage is 50 kV, and the tube current is 0.800 mA.^[2] Fluorescence images of Bi₂S₃-QD@DSPE were acquired with a filter set (excitation filter: 469/35 nm; emission filter: 655/40 nm) and calibrated with an auto-fluorescent background filter set (excitation filter: 396/40 nm; emission filter: 655/40 nm). The CT and fluorescence imaging systems were both self-built.

In vitro cytotoxicity (MTT assay) of Bi₂S₃-QD@SiO₂-PEG: RAW264.7 (murine macrophage cell line) was grown in monolayer in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and penicillin/streptomycin (100 U/mL and 100 µg/mL, respectively) in a humidified 5% CO₂ atmosphere at 37 °C. The cytotoxicity *in vitro* was measured using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. RAW264.7 cells were seeded into a 96-well plate at 2×10⁴/well in 200 µL of media and then incubated overnight at 37 °C under 5% CO₂. 100 µL of new culture medium containing with various concentrations of Bi₂S₃-QD@SiO₂-PEG (0, 0.085, 0.17, 0.34, 0.68, 1.35 and 2.70 mg of Bi/mL) were added, then cells were incubated for another 24 h. Following incubation, cells were incubated in media containing 20 µL of MTT (5 mg/mL) for 4 h. Thereafter, MTT solution was removed, and precipitated violet crystals were dissolved in 100 µL of DMSO with slow shaking for 10 min. The absorbance was measured at 490 nm using an Elx-808 enzyme-linked immunosorbant assay reader (EIA, BIO-TEK, USA).

Confocal fluorescence imaging in vitro: 2×10^4 RAW 264.7 cells per well were cultured in an 8-well cover glass bottom chambers and incubated with $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$ (1 mg of Bi/mL) at 37°C under 5% CO_2 . After 24 h, the cells were washed with PBS, fixed with 4% paraformaldehyde, and stained with 4',6-diamidino-2-phenylindole (DAPI, 1 $\mu\text{g/mL}$ in PBS). Fluorescent signals of cells were imaged using Olympus FV1000 laser confocal scanning microscopy with $\times 60$ oil objective and excitation wavelength of 488 nm (exciting QDs), while DAPI was excited with 405 nm light. Luminescence signals were detected in the wavelength regions of 440-500 nm and 580-680 nm.

Cell preparation for transmission electron microscopy (TEM): RAW 264.7 cells were removed in 6-well plates and every plate 1 mL and incubated at 37 °C under 5% CO_2 for 24 h. 1 mL of new culture medium containing $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$ (1 mg of Bi/mL) was added, while PBS as the control, then the cells were incubated for another 24 h. They were fixed with 2.5% glutaraldehyde for 30 min at 4 °C, post-fixed with OsO_4 and dehydrated in graded concentrations of ethanol (50%, 70%, 90%, 95%, 100%), then embedded in Epon 812 for 48 h at 60 °C. Ultra-thin sections were cut (60-80 nm) and observed with a Tecnai G220 transmission electron microscopy (TEM) at 200 kV. The experimental steps were consulted from reported literature.^[3]

In vitro CT and fluorescence imaging: RAW264.7 cells were seeded in 6-well plates at a density of 1×10^6 cells per plate and grown overnight at 37 °C under 5% CO_2 . Subsequently, various concentrations of $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$ (0, 0.17, 0.34, 0.68, 1.35 and 2.70 mg Bi/mL) were added. After 24 h, the cells were washed three times with PBS to remove free probes and detached by the addition of 1 mL of trypsin. After centrifugation at 1000 rpm for 8 min, cells were dispersed in 1 mL of culture media and transferred to a 1.5 mL microtube. Cell pellets were

prepared by centrifugation at 1000 rpm for 10 min and were detected in self-built CT and fluorescence imaging system.

In vivo CT and fluorescence imaging: All animal studies were approved by the Animal Experimentation Ethics Committee of Huazhong University of Science and Technology. Balb/C mice with average age of 6-8 weeks were purchased from Hubei Medical Laboratory Animal Center. Balb/C mouse (male) was anesthetized using 2% chloral hydrate and 10% urethane. Subsequently, Bi₂S₃-QD@SiO₂-PEG (0.6 mg Bi/g body weight) was injected through the tail vein into the mouse and performed for CT imaging at appropriate time points (pre-injection, 30 min, 1 h, 2 h, 4 h and 24 h) after tail vein injection. After that, the mouse was perfused and cleared by PBS for fluorescence imaging.

Characterization: XRD patterns were collected on a XD-3AX (Japan) using Cu K (0.15406 nm) radiation. TEM images were taken by using a Tecnai G20 U-Twin transmission electron microscopy (USA). Nanoparticles spectra were collected on 2550 UV-visible Spectrophotometer (Japan), LS-55 spectrophotometer (USA) and VERTEX 70 Fourier Transform Infrared Spectrometer (Germany). Cell images were performed on a FV1000 confocal laser scanning microscopy (Japan). The hydrodynamic size of the nanoemulsion was measured by a nano-ZS90 Dynamic light scattering (UK).

- [1]. (a) X. Zhao, R. P. Bagwe and W. Tan, *Adv. Mater.*, 2004, **16**, 173; (b) R. P. Bagwe, C. Yang, L. R. Hilliard and W. Tan, *Langmuir*, 2004, **20**, 8336.
- [2]. X. Q. Yang, Y. Z. Meng, Q. M. Luo and H. Gong, *J. X-Ray. Sci. Technol.*, 2010, **18**, 381.
- [3]. M. Z. Zhang, R. N. Yu, J. Chen, Z. Y. Ma and Y. D. Zhao, *Nanotechnology*, 2012, **23**, 485104.

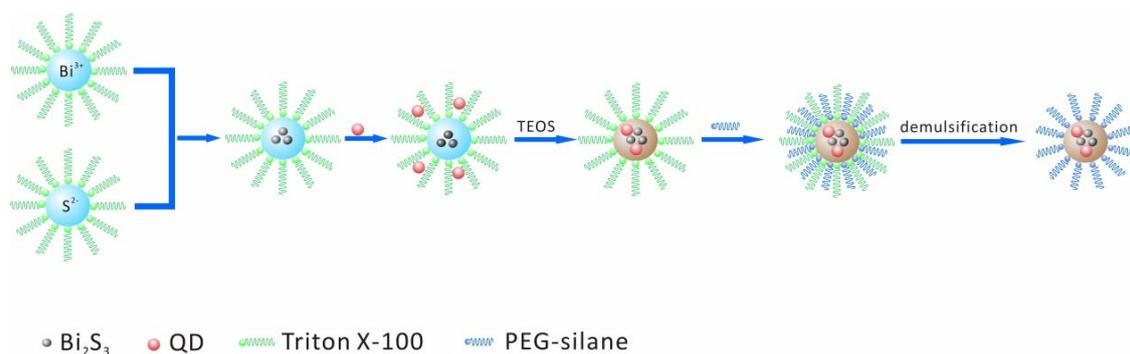


Figure S1. Schematic illustration of the synthetic procedures of multifunction probe $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$.

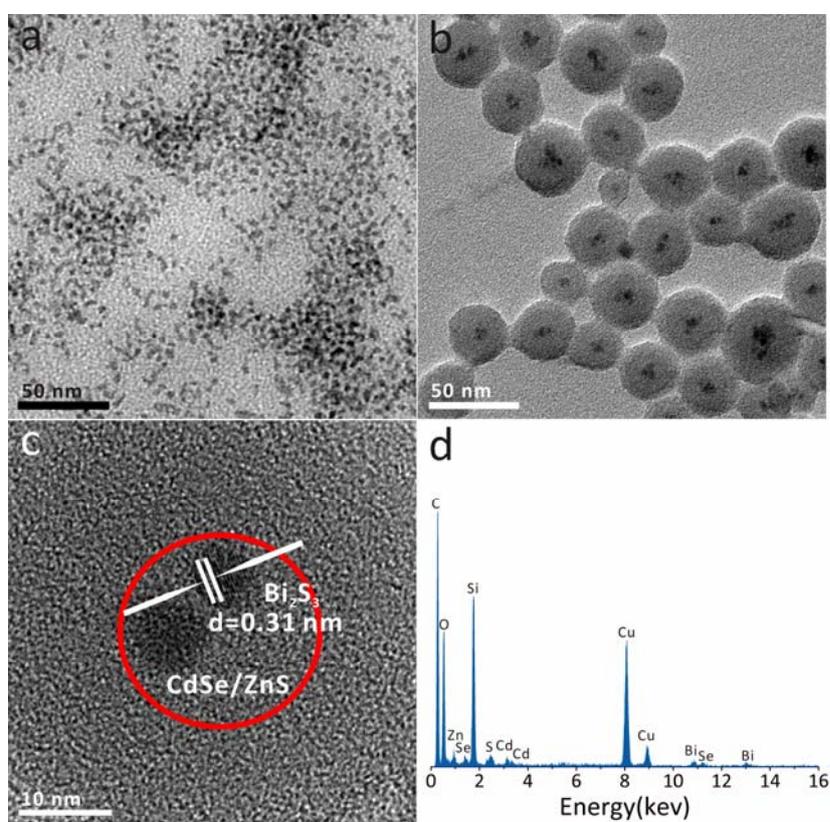


Figure S2. a) TEM image of Bi_2S_3 . b) TEM image of $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$. c) and d) High resolution TEM image and EDS date of the marked $\text{Bi}_2\text{S}_3\text{-QD@SiO}_2\text{-PEG}$ nanoparticle in Figure 1.c).

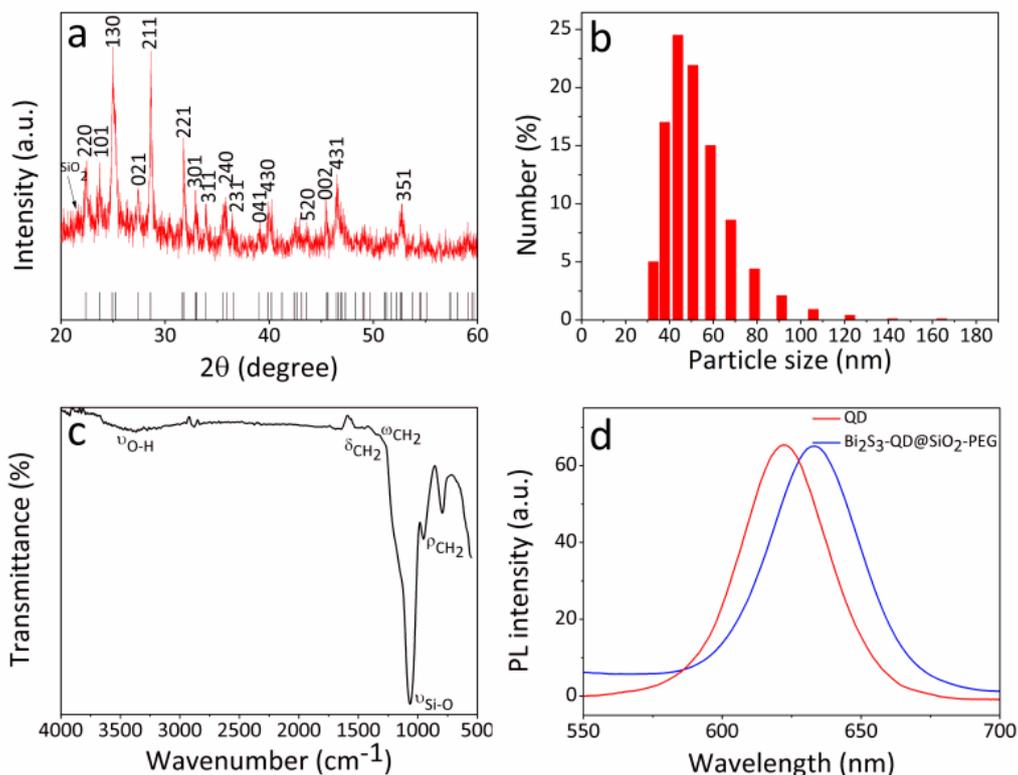


Figure S3. a) XRD pattern, b) Size-distributions and c) FT-IR spectra of the Bi₂S₃-QD@SiO₂-PEG nanoparticle. d) Fluorescence spectra of QD and Bi₂S₃-QD@SiO₂-PEG nanoparticle.

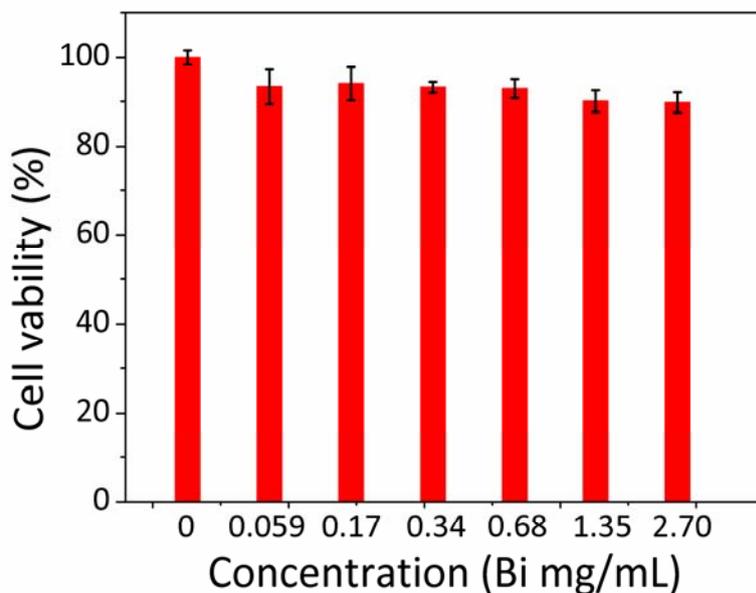


Figure S4. RAW264.7 cells (murine macrophages) were cultured with the nanoparticles of various concentrations. Cytotoxicity of the nanoparticles was determined by MTT assay.

Table S1: CT values of the heart, kidney, liver and spleen of mouse before the injection (previous) and at indicated time intervals after the injection.

	Previous	5min	1h	2h	4h	24h
heart	84.2	90.6	92.4	93.3	74.2	77.0
kidney	61.8	68.8	73.5	76.2	71.6	75.9
liver	68.0	75.2	76.1	77.3	75.6	78.8
spleen	67.2	72.4	76.4	74.6	73.6	65.3