

Electronic Supplementary Information (ESI) for Chemical Science

**Ultrafast synthesis of ultrasmall polyethylenimine-protected
AgBiS₂ nanodots by “rookie method” for *in vivo* dual-modal
CT/PA imaging and simultaneous photothermal therapy**

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Experimental Section

Materials: Polyethyleneimine (PEI) (molecular weight = 10,000 g mol⁻¹, 99%), Bismuth nitrate pentahydrate (Bi(NO₃)₃·5H₂O, 99%) and Thioacetamide (C₂H₅NS, 99%) were purchased from Aladdin Reagents (Shanghai, China). Silver nitrate (AgNO₃, 99%) was purchased from Alfa Aesar (Ward Hill, Massachusetts, USA). Ethylene glycol (EG) was obtained from Beijing Chemical Reagents (Beijing, China). All the above chemicals were used directly without further purification.

Preparation of PEI-AgBiS₂ Nanodots: The PEI-AgBiS₂ nanodots were synthesized at a mass ratio of PEI:AgNO₃:Bi(NO₃)₃·5H₂O:C₂H₅NS of 100:30.3:10.6:9.4 in the starting reaction solution. Typically, 100 mg of PEI was dissolved in a mixture of 3 mL of water and 14 mL of ethanol at room temperature under vigorous stirring. A C₂H₅NS aqueous solution (1 mL, 9.4 mg mL⁻¹) was then added to the above solution. Then, 30.3 mg of Bi(NO₃)₃ and 10.6 mg of AgNO₃ were dissolved in a 2 mL mixture solution of water and EG (water:EG = 1:1) were added to the above reaction mixture and stirred for only 1 min. After the reaction, the resulting nanodots were dialysed (molecular weight cut off = 20,000) against deionized water for 24 h.

Characterization: The crystal structures and phase purities of the products were investigated by powder X-ray diffraction (XRD) with a D8 Focus diffractometer (Bruker) with Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) with an operation voltage and current maintained at 40 kV and 40 mA. X-ray photoelectron spectroscopy (XPS) measurements were conducted with a VG ESCALAB MKII spectrometer. The composition of the samples were studied using a field-emission scanning electron

microscope (FE-SEM, S-4800, Hitachi) equipped with an energy-dispersive X-ray (EDX) spectrometer. Low-/high-resolution transmission electron microscopy (TEM) were carried out on a FEI Tecnai G2S-Twin instrument with a field-emission gun operating at 200 kV. Fourier transform infrared spectroscopy (FT-IR) was recorded on a PerkinElmer 580B IR spectrophotometer using the KBr pellet technique. The absorption spectra were recorded on Shimadzu UV-3600 spectrophotometer. Inductively-coupled plasma optical emission spectrometer (ICP-OES) was taken on an iCAP 6000 of Thermo Scientific. Inductively-coupled plasma mass spectrometry (ICP-MS) was carried on the Thermo Fisher X series^{II}.

Measurement of Photothermal Performance: To evaluate the photothermal effect, 0.2 mL of PEI-AgBiS₂ nanodots aqueous solutions at concentrations of 0, 12.5, 25, 50, 100, 200 $\mu\text{g mL}^{-1}$ were irradiated by 808 nm laser at a power density of 1.2 W cm^{-2} (10 min). Moreover, PEI-AgBiS₂ nanodots (200 $\mu\text{g mL}^{-1}$) aqueous solution were exposed 808 nm laser (10 min) with different power density (0.4, 0.6, 0.8, and 1.0 W cm^{-2}). Afterward, we further repeated six laser on/off cycles for temperature monitoring. A thermocouple microprobe was used to measure the solution temperature every 10 s.

In Vitro Cytotoxicity Assay of PEI-AgBiS₂ Nanodots: HepG2 cells and L929 were chosen for evaluating cytotoxicity of PEI-AgBiS₂ nanodots. In detail, HepG2 and L929 cells were cultured in a 96-well culture plate at a density of 10^5 cells per well and then were maintained at 37 °C and 5% CO₂ in DMEM supplemented with 10% FBS. After 24 h incubation, the cells were treated with different concentrations of

PEI-AgBiS₂ nanodots (0, 18.75, 37.5, 75, 150, 300 μg mL⁻¹) and incubated for another 24 h. Then, 20 μL methyl thiazolyl tetrazolium (MTT) solution in PBS (5 mg mL⁻¹) was added to each well and further incubated for 4 h. Subsequently, 150 μL of dimethyl sulfoxide (DMSO) were added to each well to dissolve the MTT formazan crystals formed. Finally, the absorbance of formazan product was measured at 490 nm by a microplate reader.

In Vitro and in Vivo X-Ray CT Imaging: *In vitro* and *in vivo* CT images were performed on a Philips 256-slice CT scanner. Imaging parameters were as follows: 120 kVp, 300 mA; thickness, 0.9 mm; pitch, 0.99; field of view, 350 mm; gantry rotation time, 0.5 s; table speed, 158.9 mm s⁻¹. PEI-AgBiS₂ nanodots and iobitridol were dispersed in deionized water with different concentrations (0, 1.56, 3.13, 6.25, 12.5, 25, 50 mM) of Bi and I, respectively. For *in vivo* CT imaging, the tumor-bearing kunming mice first anesthetized with 10% chloral hydrate (100 μL) by intraperitoneal injection. Subsequently, PEI-AgBiS₂ nanodots were intratumorally (6.25 mM, 50 μL) and intravenously (40 mM, 80 μL) injected for CT imaging, respectively.

In Vitro and in Vivo PA Imaging: A preclinical photoacoustic computed tomography scanner (Endra Nexus 128, Ann Arbor, MI, USA) was used to obtain PA imaging. For *in vitro* PA imaging, the PEI-AgBiS₂ nanodots suspensions at different concentrations (0, 18.75, 37.5, 75, 150, 300 μg mL⁻¹) were loaded into agar gel cylinders and then the PA signal were measured. For *in vivo* PA imaging, the tumor-bearing kunming mice were intravenously injected with PEI-AgBiS₂ nanodots solution and a series of sequential PA images at specific time (0, 1 h, 2 h, and 24 h)

were acquired to cover the tumor regions. Regions of interests (ROIs) were selected and the PA signal was analyzed using ViewMOST™ software. During the imaging, the mouse body temperature was maintained at 37.5 °C by using a water heating system.

In Vivo Blood Circulation: The PEI-AgBiS₂ nanodots (20 mg kg⁻¹) were injected into healthy mice *via* the tail vein. Then, the blood samples were collected at the designed time points (5 min, 10 min, 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h) after intravenous injection and further weighted and solubilized them with HNO₃ and H₂O₂ (v/v = 1:1) at 60 °C. The content of Bi in blood were measured by ICP-MS.

In Vivo Biodistribution and Metabolism of PEI-AgBiS₂ Nanodots: The tumor-bearing kunming mice intravenous injection PEI-AgBiS₂ nanodots (20 mg kg⁻¹). At 1 h, 2 h, 24 h, 3 d, 7 d, and 14 d post-administration, the mice were sacrificed and then main organs (heart, liver, spleen, lung, and kidney) and tumors were dissected and weighed. Subsequently, these organs and tumors were solubilized by HNO₃ and H₂O₂ (v/v = 1:1) at 60 °C for ICP-MS measurement to determine Bi contents. In addition, the feces of mice were collected after 24 h intravenous injection for further detection of PEI-AgBiS₂ nanodots with TEM.

In Vitro Photothermal Therapy Assays: To study the photothermal therapy effect of PEI-AgBiS₂ nanodots, HepG2 cells were incubated with PEI-AgBiS₂ nanodots at different concentrations (0, 37.5, 75, 150, 300 µg mL⁻¹ in culture medium) for 12 h and then irradiated by 808 nm laser with a power density of 1.2 W cm⁻² for 6 min. Cell viability was measured using the MTT reduction assay. Moreover, the cells were

stained with calcein acetoxymethyl ester and propidium iodide after NIR laser exposure and observed using confocal scanning microscopy.

In Vivo Photothermal Therapy Assays: When the tumor sizes reached approximately 100 mm³, The tumor-bearing mice were randomly divided into four groups with six mice in each group: (1) PBS; (2) PBS+NIR laser; (3) PEI-AgBiS₂ nanodots; (4) PEI-AgBiS₂ nanodots+NIR laser. The mice in group (1) and (2) were intravenous injection PBS solution, and group (3) and (4) were intravenous injection PEI-AgBiS₂ nanodots (20 mg kg⁻¹). After 24 h, the mice in group (2) and (4) were anesthetized and the tumor site were treated with laser irradiation (808 nm, 1.2 W cm⁻², 6 min). During the treatment, the tumor temperature change were monitored by an infrared thermal camera. Moreover, the body weights and tumor size of each mouse were recorded every 2 days using an electronic balance and a caliper, respectively. The tumor volume V (mm³) was calculated by the formula: $V = (\text{length} \times \text{width}^2)/2$. The relative tumor volume was calculated as V/V_0 , where the V_0 was the corresponding tumor volume before the treatment. After various treatments, the mice were sacrificed. The tumors were harvested and weighed to further evaluate the therapeutic efficacy.

In Vivo Blood Panels Examinations: Healthy Kunming mice were intravenous injected with PEI-AgBiS₂ nanodots (20 mg kg⁻¹). The mice were sacrificed at day(s) 1, 7, 14, and 30 after administration and the blood samples were drawn from eye socket. The untreated mice were used as the control. The serum was obtained by centrifuged

for 10 min. Subsequently, the serum biochemistry and a complete blood panel examination were carried out.

Histology Analysis: For the *in vivo* toxicity studies, histology analysis was carried out at the 30th day after intravenous injection PEI-AgBiS₂ nanodots (20 mg kg⁻¹). The mice without any treatment were used as the blank control. The mice were sacrificed and the major organs (heart, liver, spleen, lung, and kidney) from those mice were harvested, fixed in 4% paraformaldehyde solution. Tissue samples were then embedded in paraffin, sliced, and stained using hematoxylin and eosin (H&E). The images of histological sections were collected using an optical microscope.

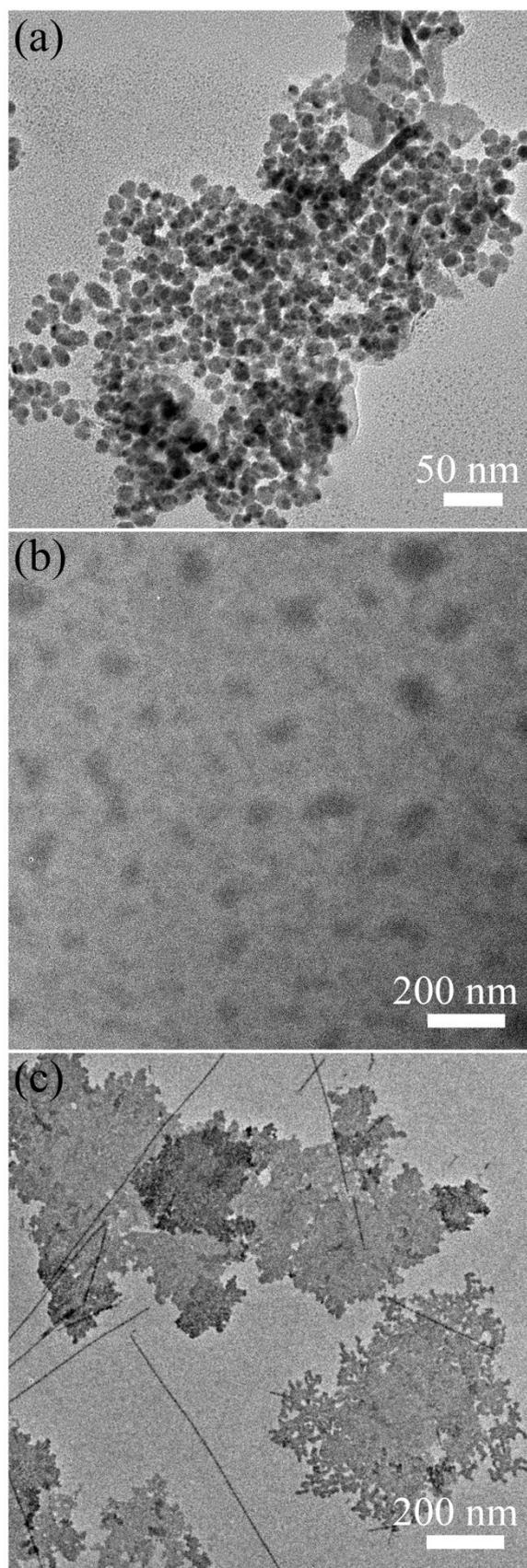


Fig. S1 TEM image of (a) $C_2H_5NS+(Bi^{3+}+Ag^+)$, (b) $PEI+C_2H_5NS$, and (c) $PEI+(Bi^{3+}+Ag^+)$.

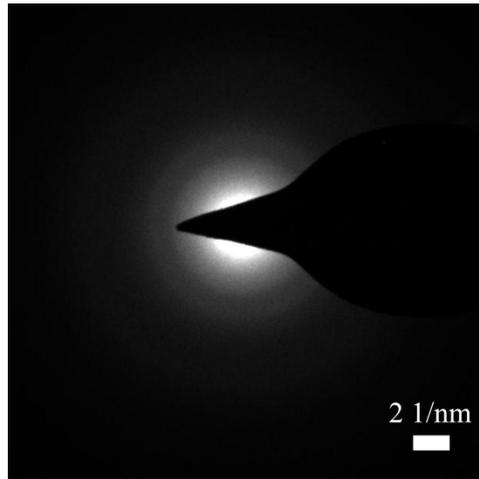


Fig. S2 The corresponding selected area electron diffraction (SAED) pattern of PEI-AgBiS₂ nanodots.

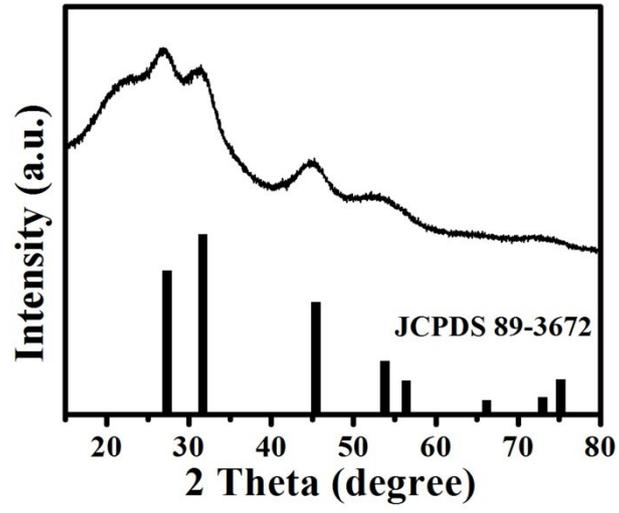


Fig. S3 XRD pattern of PEI-AgBiS₂ nanodots.

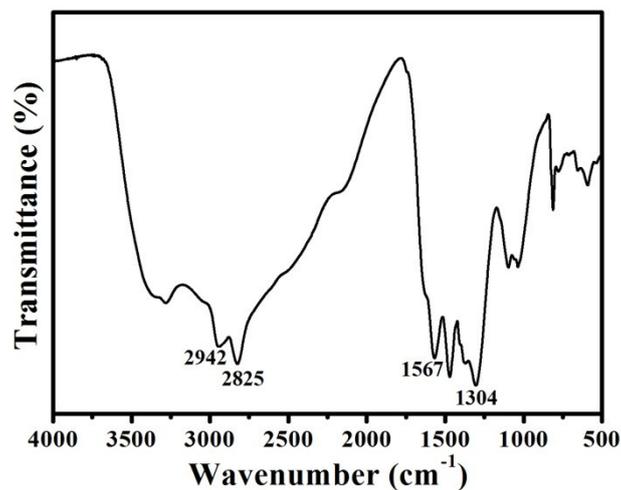


Fig. S4 Fourier transform infrared (FT-IR) spectroscopy of PEI-AgBiS₂ nanodots.

As shown in Fig. S4, as-prepared nanodots show the characteristic peaks of PEI, such as C-H (2939 cm⁻¹ and 2831 cm⁻¹), N-H (1568 cm⁻¹), and C-N (1304 cm⁻¹), suggesting that the PEI molecules were successfully capped on the surface of AgBiS₂ nanodots.

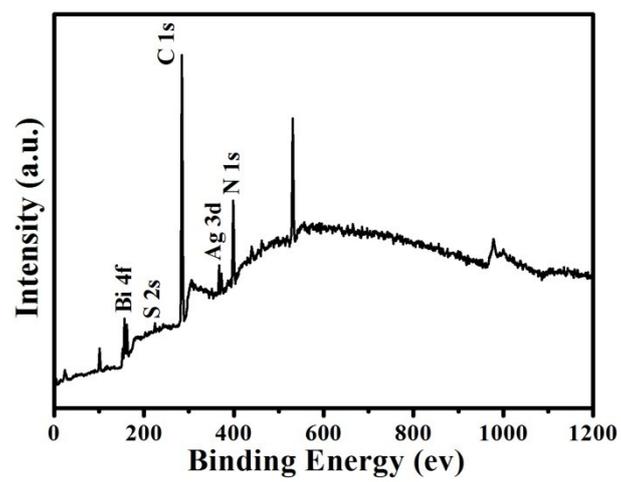


Fig. S5 XPS survey spectrum of PEI-AgBiS₂ nanodots.

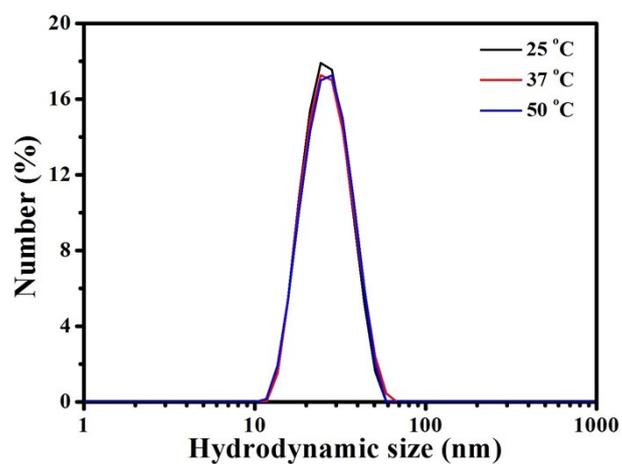


Fig. S6 The size distribution of PEI-AgBiS₂ nanodots aqueous solution at different temperature. The corresponding zeta potential are +24.9 mv (25 °C), +26.1 mv (37 °C), and +25.4 mv (50 °C), respectively.

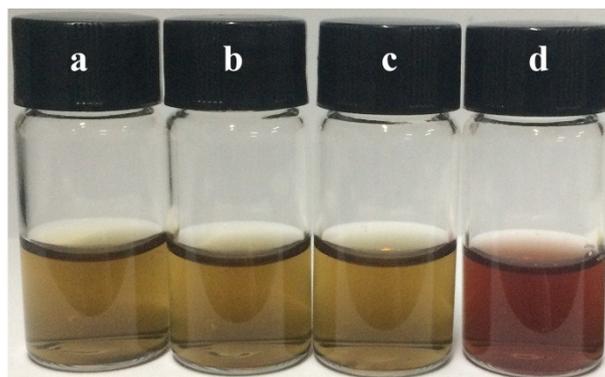


Fig. S7 Photographs of PEI-AgBiS₂ nanodots dispersed in different media. (a) Water, (b) PBS, (c) 0.9% NaCl solution, and (d) cell medium.

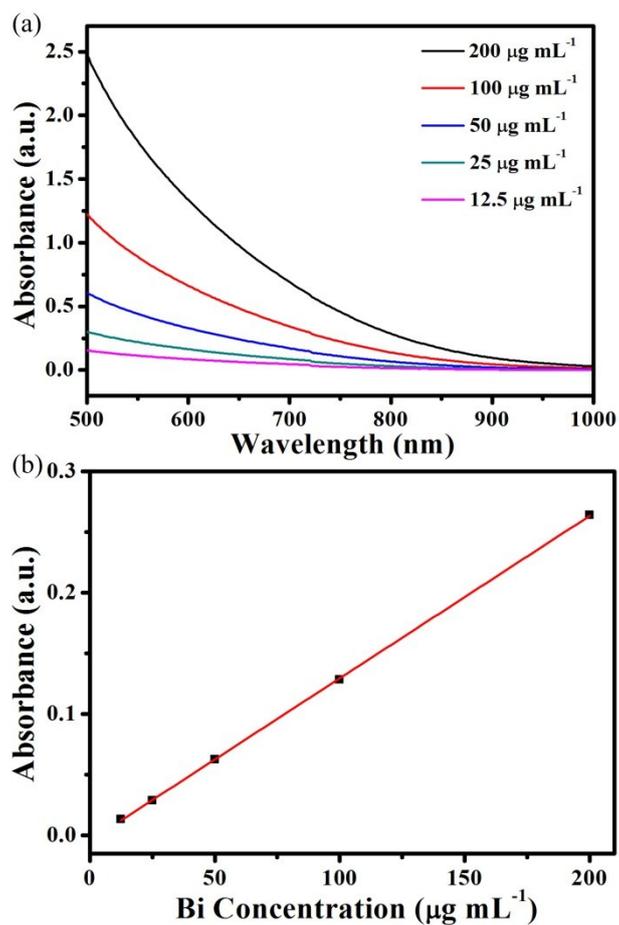


Fig. S8 (a) The absorption spectra of PEI-AgBiS₂ nanodots aqueous solutions with different concentrations. (b) A linear relationship for the optical absorbance at 808 nm as a function of the concentration of PEI-AgBiS₂ nanodots.



Fig. S9 The digital photograph of PEI-AgBiS₂ nanodots solution with different concentrations (From left to right: 200, 100, 50, 25, 12.5 µg mL⁻¹, and water).

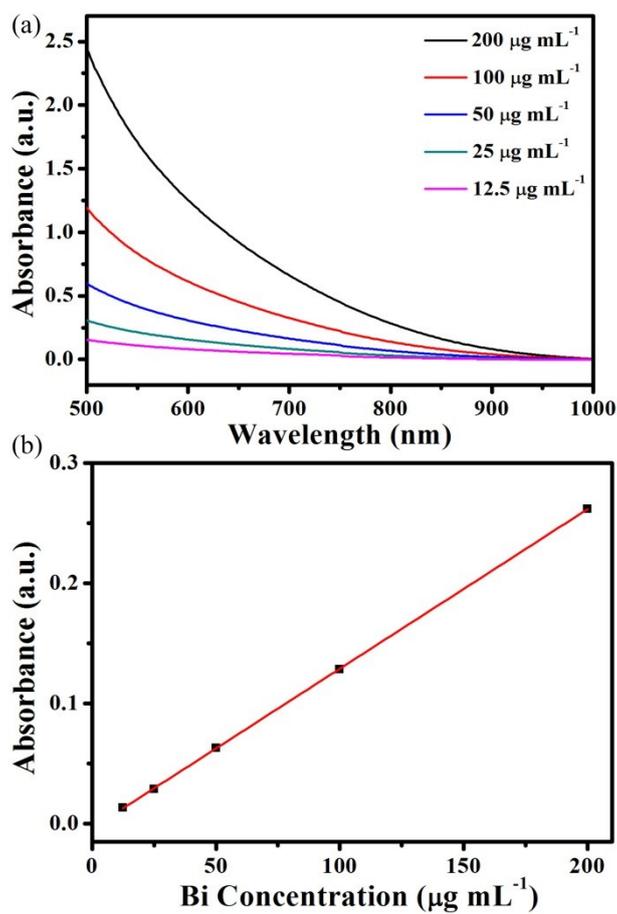


Fig. S10 (a) The absorption spectra of PEI-AgBiS₂ nanodots in PBS solutions with different concentrations. (b) A linear relationship for the optical absorbance at 808 nm as a function of the concentration of PEI-AgBiS₂ nanodots.

Calculation of the photothermal conversion efficiency.

The photothermal conversion efficiency (η) is calculated using equation (1):

$$\eta = \frac{hS(T_{\max} - T_{\text{surr}}) - Q_0}{I(1 - 10^{-A_\lambda})} \quad (1)$$

where h is the heat transfer coefficient, S is the surface area of the container, T_{\max} and T_{surr} were the equilibrium temperature and ambient temperature, respectively. Q_0 is the heat associated with the light absorbance of the solvent, A_λ is the absorbance of PEI-AgBiS₂ nanodots at 808 nm, and I is the laser power density.

The value of hS is obtained from the formula (2):

$$\tau_s = \frac{m_d C_d}{hS} \quad (2)$$

Where τ_s is the time constant of the sample system, m_d and C_d are the mass of water and the heat capacity, respectively.

According the formula (3):

$$t = -\tau_s \ln \theta \quad \text{and} \quad \theta = \frac{\Delta T}{\Delta T_{\max}} = \frac{T - T_{\text{surr}}}{T_{\max} - T_{\text{surr}}} \quad (3)$$

the τ_s was determined to be 184.2 s (Fig. 2g). Substituting hS value into equation (1), the η of PEI-AgBiS₂ nanodots can be calculated.

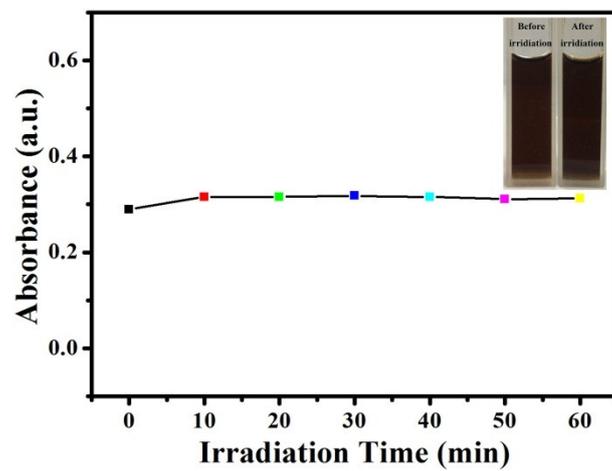


Fig. S11 The absorbance of PEI-AgBiS₂ at 808 nm as a function of 808 nm NIR laser irradiation time. The inset shows the digital photograph of PEI-AgBiS₂ nanodots solution before and after 60 min laser irradiation.

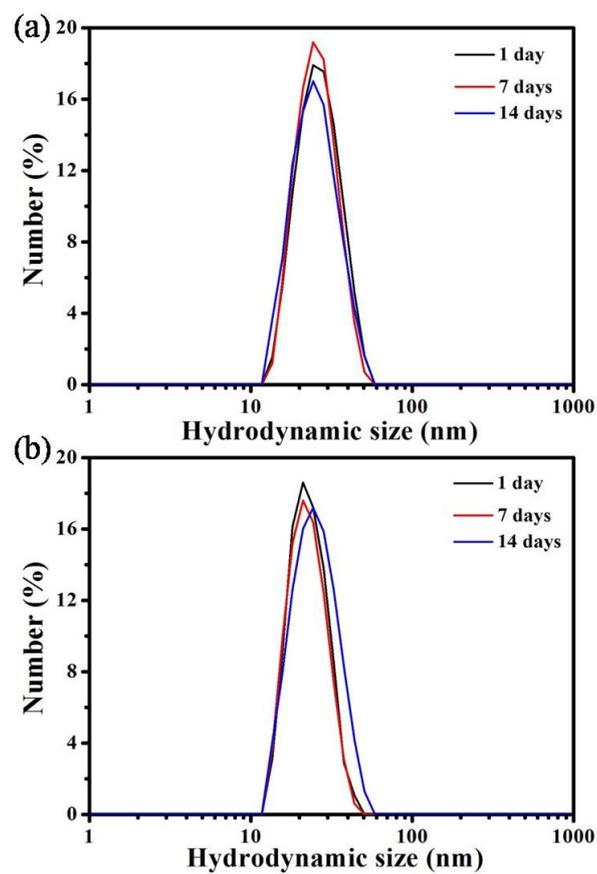


Fig. S12 The size distribution of PEI-AgBiS₂ nanodots in (a) aqueous and (b) PBS solutions at different time.

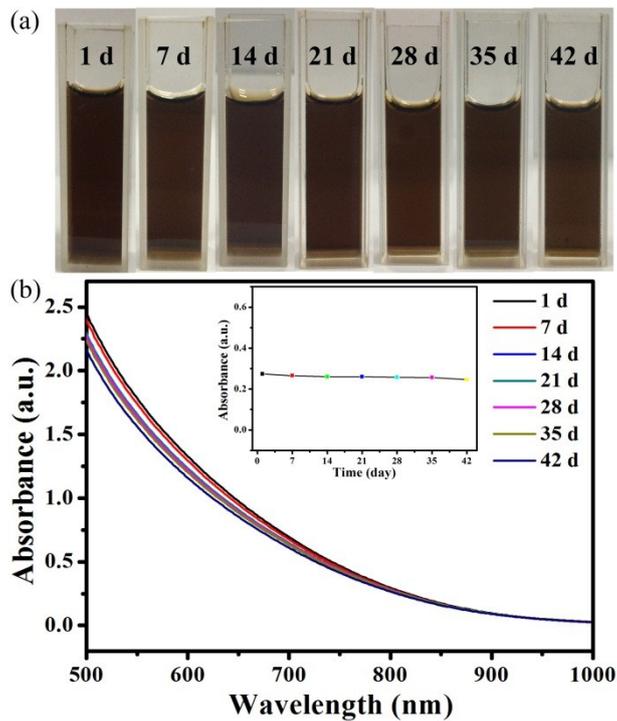


Fig. S13 (a) The digital photograph of PEI-AgBiS₂ nanodots aqueous solutions varies with time. (b) The absorption spectra of PEI-AgBiS₂ nanodots aqueous solutions at different time. The inset shows the the absorbance of PEI-AgBiS₂ at 808 nm as a function of time.

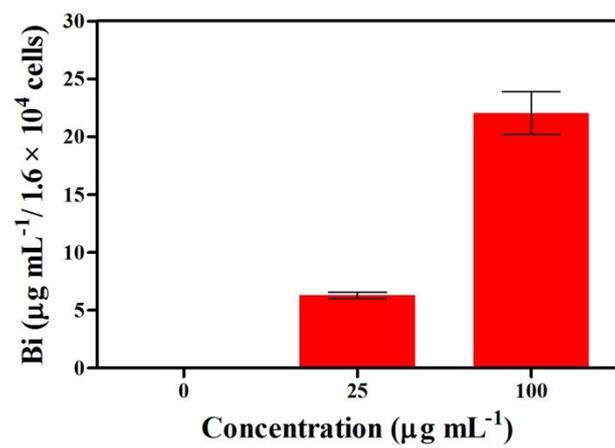


Fig. S14 Concentration-dependent cellular uptake measured by ICP-MS.

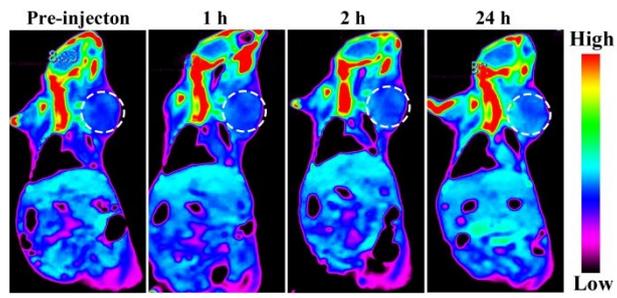


Fig. S15 *In vivo* CT images of the tumor-bearing mice after intravenous injection of PEI-AgBiS₂ nanodots at timed intervals (tumor, circles).

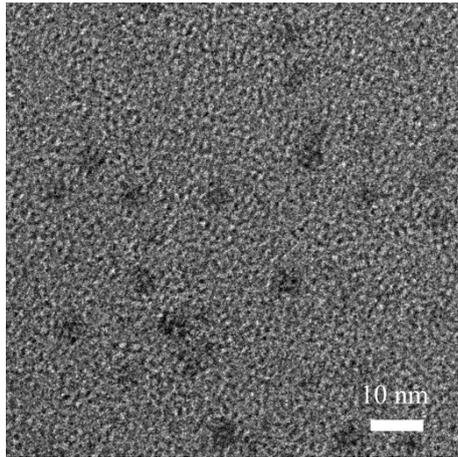


Fig. S16 TEM image of feces collected at 24 h after intravenous injection.

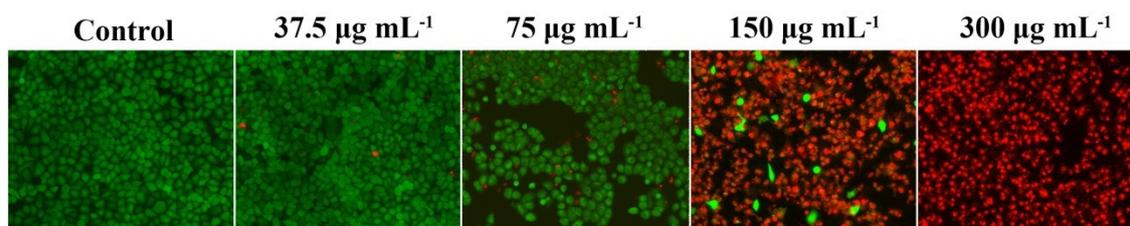


Fig. S17 Confocal fluorescence images of HepG2 cells after incubation with various concentrations of PEI-AgBiS₂ nanodots under 808 nm NIR laser irradiation (1.2 W cm⁻², 6 min). The cells were costained with calcein acetoxymethyl ester (green, living cells) and propidium iodide (red, dead cells).

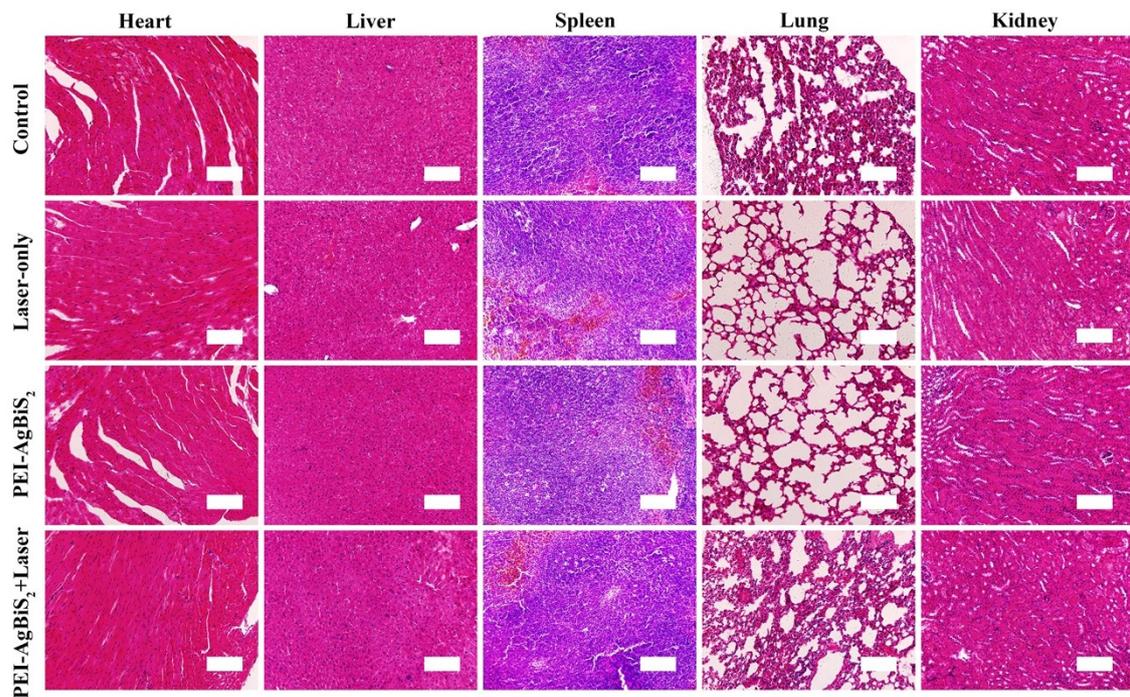


Fig. S18 Hematoxylin and eosin (H&E) stained images of major organs (heart, liver, spleen, lung, and kidney) of mice from different groups after various treatments. Scale bar stands for 100 µm.