Electronic Supplementary Information

Integration of IR-808 and thiol-capped Au-Bi bimetallic nanoparticles for NIR light mediated photothermal therapy and imaging

Peipei Jia,[a] Hongjiao Ji,[a] Shikai Liu,[a] Rui Zhang,[a] Fei He,[a,*] Lei Zhong,[b,*] and Piaoping Yang[a,*]

[a] Key Laboratory of Superlight Materials and Surface Technology, Ministry of Education, College of Material Sciences and Chemical Engineering, Harbin Engineering University, Harbin, 150001, P. R. China

[b] Department of Breast Surgery, Second Affiliated Hospital of Harbin Medical University, Harbin 150086, China

* E-mail: hefei@hrbeu.edu.cn; yangpiaoping@hrbeu.edu.cn; zhonglei@hrbmu.edu.cn

Experimental section

Reagents and materials. Bismuth nitrate pentahydrate ($Bi(NO_3)_3$ $5H_2O$, 99.0%), tetrachloroauric(III) acid ($AuCl_3$ HCl $4H_2O$, Au≥47.8%), sodium borohydride ($NaBH_4$), ethylene glycol (EG), methanol, ethanol, 4′,6-diamidino-2-phenylindole (DAPI), folic acid (FA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI) were purchased from Beijing Chemical Regent Co., Ltd. And Calcein AM were purchased from Sigma-Aldrich. Co. LLC. IR-783 (98.0%) were purchased from Tokyo Chemical Industry Co., Ltd. Tetraoctylammonium bromide (TOAB, 98%), glutathione (GSH, 98%), 4-mercaptobenzoic acid, N-hydroxysuccinimide (NHS, 98%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochlo-ride (EDC, 98%) were purchased from Sigma-Aldrich Co., LLC. All the chemical reagents and materials above-mentioned were used without purification.

ROS detection of samples. DCFH-DA was used to a chemical probe to confirm reactive oxygen species by measuring the fluorescence intensity of DCF by photoluminescence spectra. DCFH was obtained by mixing DCFH-DA (0.5 mL, 1 mmol/L) with a methanol solution of NaOH (0.01 mol/L, 2 mL) and stirring for 30 min shielded from light. Briefly, the mixed solution including 2 mL DCFH (25 mmol/L) and 2 mL Au-Bi-GSH@IR808 nanoparticles solution were irradiated with 808 nm NIR laser for various times (0, 1, 3, 6, 10 min) under dark conditions, respectively. Then, the photoluminescence spectra of the samples were tested to further analyse the amount of ROS generation. The intracellular ROS measurement of

Au-Bi-GSH@IR808 was by a typical procedure. HeLa cells were incubated with Au-Bi-GSH@IR808 for 3 h, using phosphate buffer saline (PBS) washed to remove the impurities and non-internalized nanoparticles, then DCFH-DA (20 μM, 1 mL) was added into fresh culture media and incubated for 20 min. After irradiated upon 808 nm laser (0.5 W/cm^2) for divers times, the cells were collected, samples were prepared, and the samples were further observed via CLSM.

***In vitro* cellular uptake.** The cellular uptake process of HeLa cells was investigated by using a CLSM. At first, HeLa cells were planted in six-well plates and inoculated at 37 ℃ with 5% CO_2 for 12 h. Then the Au-Bi-GSH@IR808 (800 μg/mL) was added to the culture medium incubated for 0.5, 1, and 3 h, respectively. After the cells were washed with PBS for three times, 1 mL of glutaraldehyde (2.5%) was used to incubate the cells for 10 min. Then further rinsed by PBS and the nucleus were stained by DAPI (20 μg/mL, 1 mL) and washed by PBS again. Finally, the images of cells were analyzed by using a Leica TCS SP8 instrument.

Hemolysis experiment of Au-Bi-GSH@IR808. In a typical approach, red blood cells were obtained and rinsed with 0.9% saline several times. Then, the red blood cells were diluted with PBS (10 mL). Au-Bi-GSH@IR808 with a series of concentrations (25, 50, 100, 200, 400 and 800 μg/mL) were mixed with the red blood cells and with deionized water and PBS as a positive and negative control, respectively. The samples were kept steady for 1 h, after that they were centrifuged and the absorbances of the upper supernatants were measured for further calculations.

Biodistribution. 25 Tumor-bearing mice were randomly divided into 5 groups

with same number, and the Au-Bi-GSH@IR808 NPs were injected intravenously. Major organs and tumors of mice were collected at different times (1, 3, 12, 24 h) after injection for treatment. The concentration of Au and Bi in solution was determined by ICP-AES.

Histological examination. The histological analysis was performed after two weeks treatment. Less than 1 cm × 1 cm of tissues of heart, lung, liver, kidney, spleen, and tumor of the representative mice in five groups were excised. Then the excised tissues were successively dehydrated using buffered formalin, ethanol of various concentrations, and xylene. Thereafter, the dehydrated tumors and organs were embedded in liquid paraffin, and sliced to 3mm × 5 mm for hematoxylin and eosin (H&E) staining. Finally, stained slices were observed using an optical microscope.

***In vitro* and *in vivo* X-ray CT imaging.** The *in vitro* CT imaging was conducted on a Philips 64-slice CT scanner at a voltage of 120 kV. For the *in vitro* experiments, the Au-Bi-GSH@IR808 was diluted into different concentrations and then placed in a line for CT imaging measurements. And for the *in vivo* experiments, the mice were first anesthetized by intraperitoneal injection of chloral hydrate (10%, 0.03 mL/g of mouse). Then, 100 μL of Au-Bi-GSH@IR808 solution was injected intratumorally into the mice for scanning.

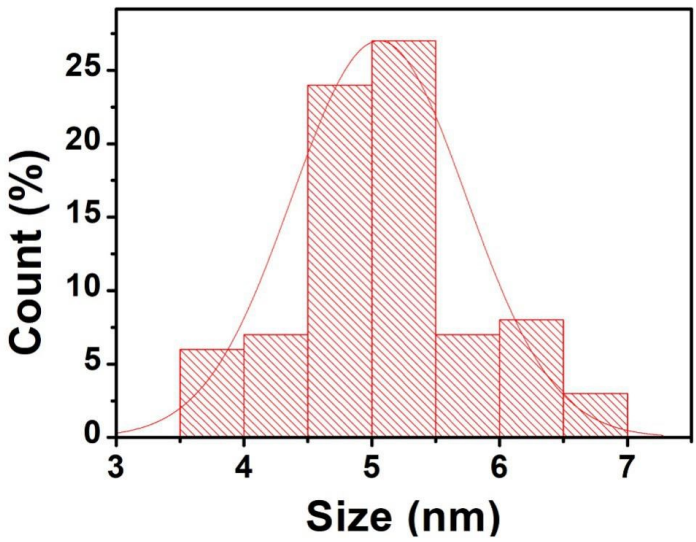

Fig. S1. Particle size distribution of Au-Bi-GSH@IR808.

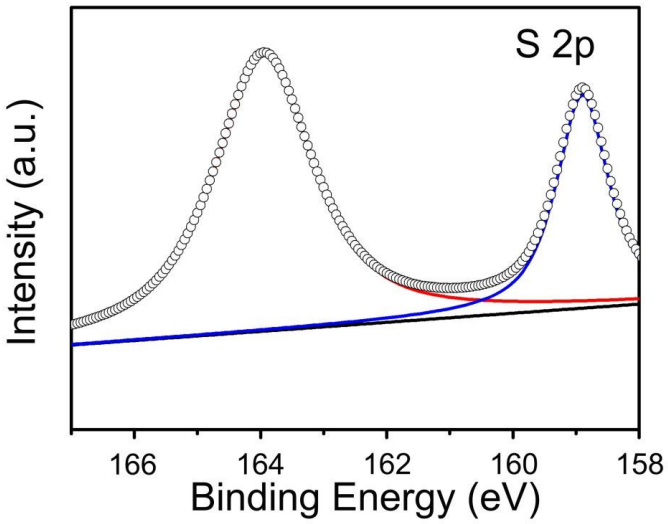

Fig. S2. High resolution S 2p spectra of Au-Bi-GSH@IR808 NPs, fitted to two energy components centered at around at 158.9 eV and 163.9eV.

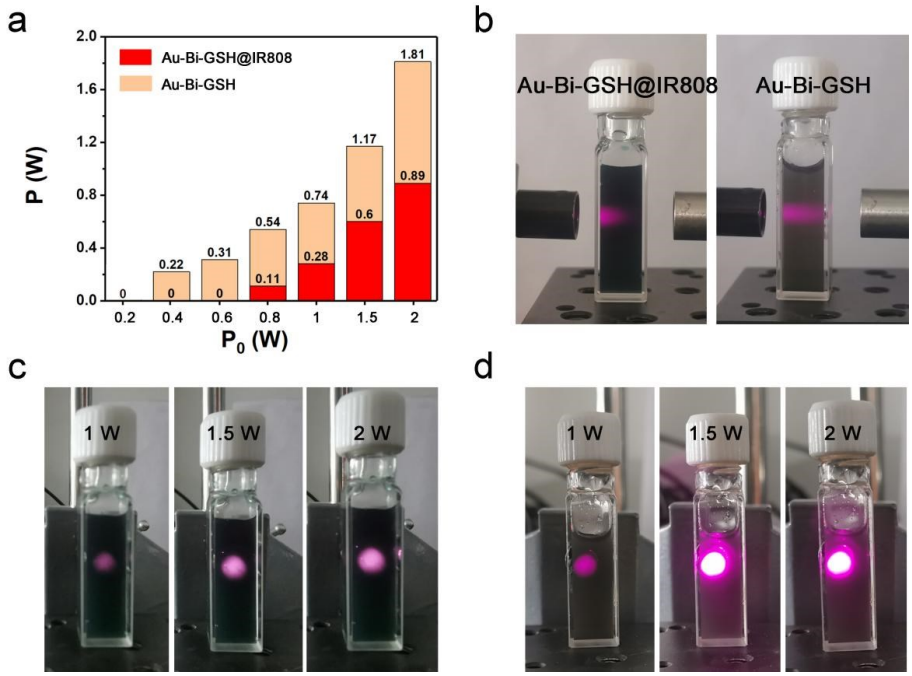

Fig. S3. (a) Laser (808 nm) power before (P_0) and after (P) passing through the samples of Au-Bi-GSH, and Au-Bi-GSH@IR808 with equal Bi concentration of 100 mg/mL. (b) Digital images of the 808 nm laser beam (P_0 = 1 w) through the Au-Bi-GSH, and Au-Bi-GSH@IR808 samples. (c) Digital images of the 808 nm laser (with different P_0) spots after passing through the Au-Bi-GSH@IR808. (d) Digital images of the 808 nm laser (with different P_0) spots after passing through the Au-Bi-GSH.

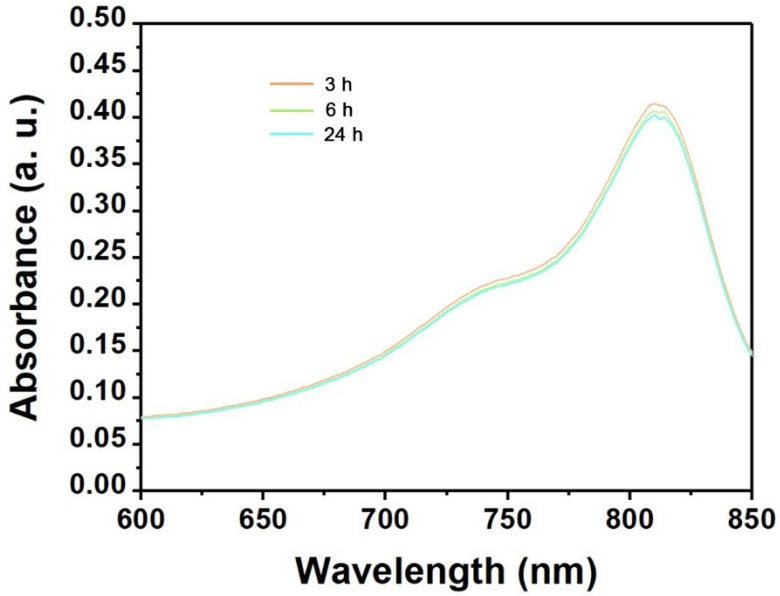

Fig. S4. UV-vis absorption spectra of Au-Bi-GSH@IR808 NPs solutions for 3 h, 6 h, 24 h storage at 20 ℃.

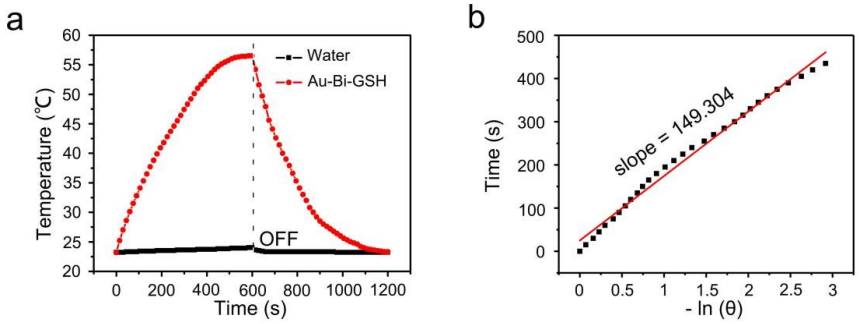

Fig. S5. (a) The temperature curve of the dispersion corresponds to the on/off of the laser. (b) The time versus -lnθ curve of Au-Bi-GSH, from the cooling period of panel.

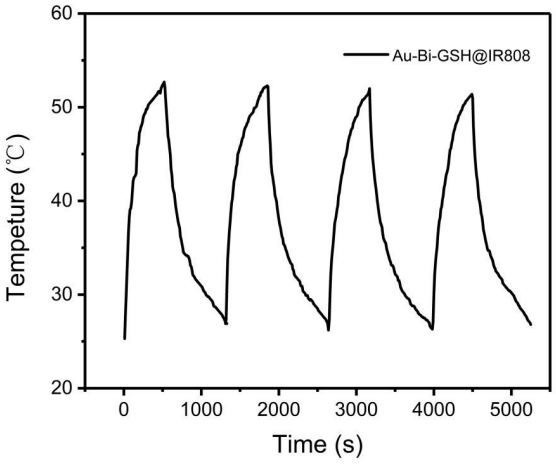

Fig. S6. Temperature curve of Au-Bi-GSH@IR808 solution during laser ON/OFF cycles.

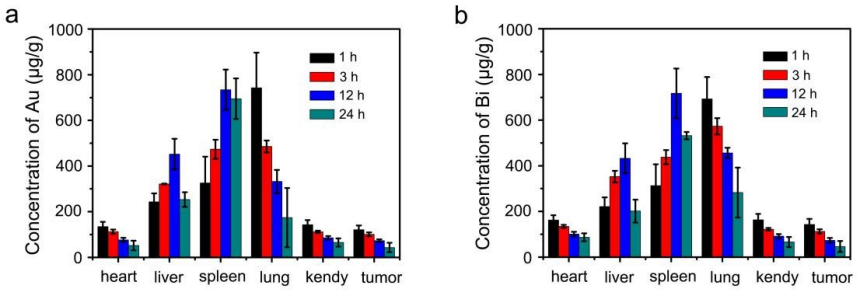

Fig. S7. The biological distribution of Au (a) and Bi (b) in major organs of mice after intravenous injection of Au-Bi-GSH NPs intravenous at different times intervals.

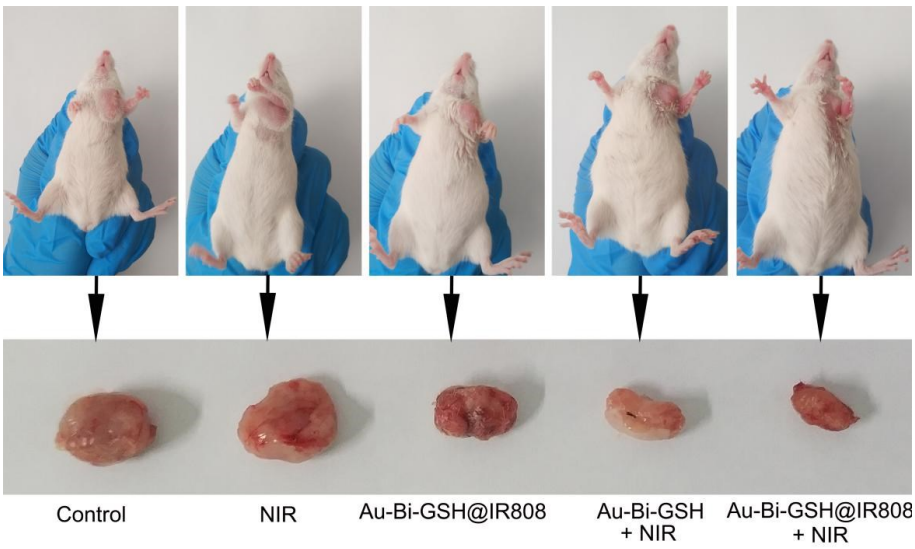

Fig. S8. The photographs of mice after treatments from representative Balb/c mice after 14 days treatment and the corresponding images of digital photos of excised tumors.

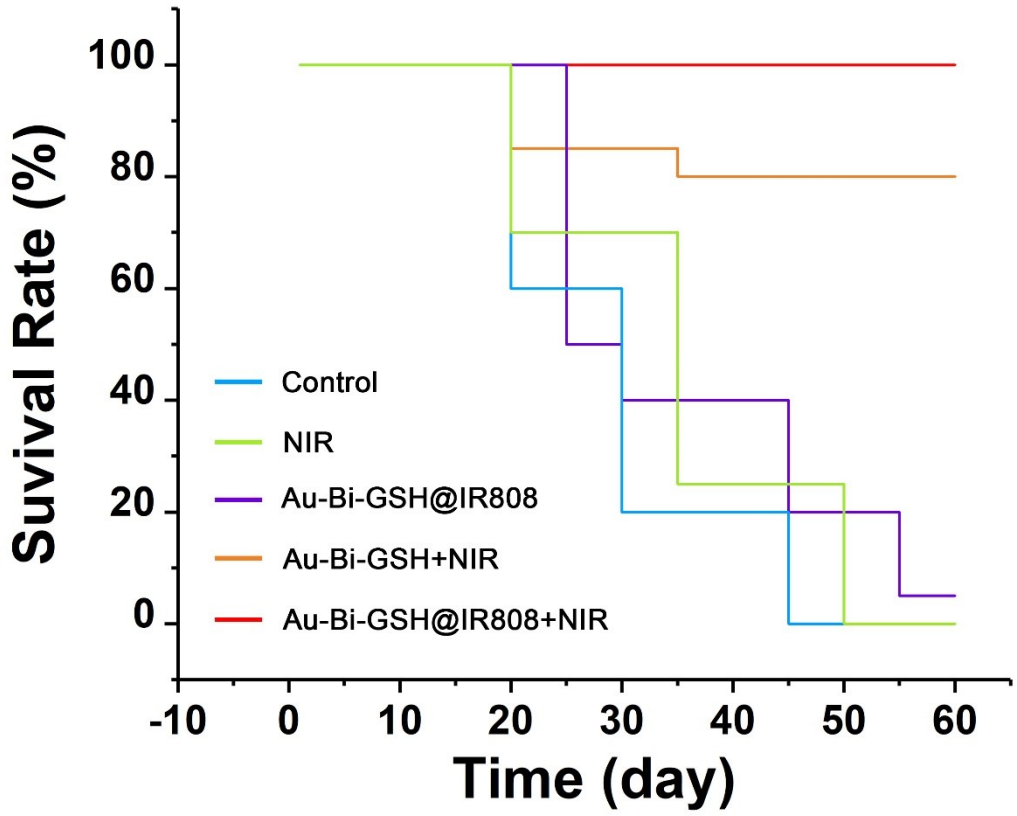

Fig. S9. Survival rate of the mice in different treatment groups.

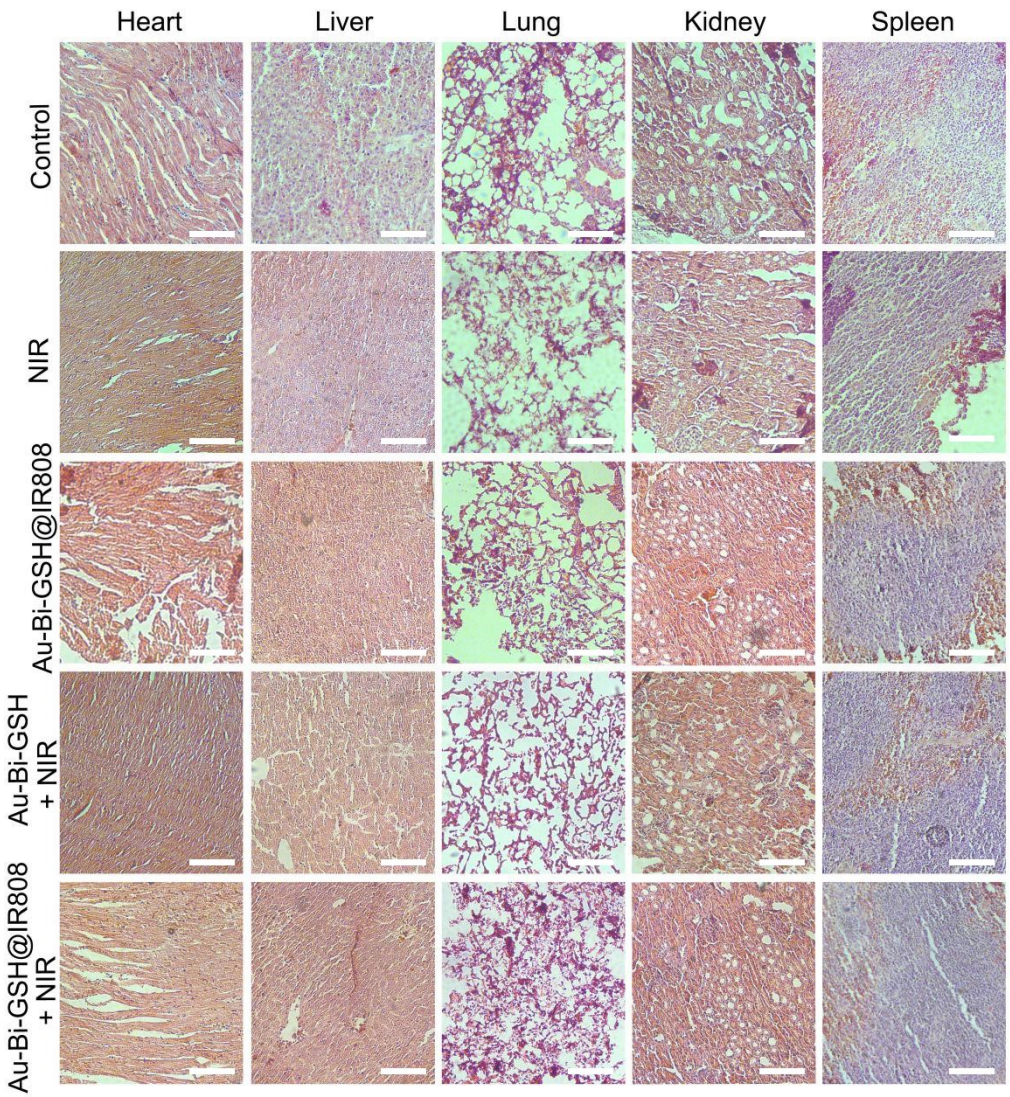

Fig. S10. Histological changes of heart, liver, lung, kidney and spleen in different groups (control, NIR, Au-Bi-GSH@IR808, Au-Bi-GSH + NIR, Au-Bi-GSH@IR808 + NIR) were by H&E-stained after 14 days of treatment. All images share the same 50 μm scale.