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Supporting Information

Acidic-driven aggregation of selenol-functionalized zwitterionic gold nanoparticles improves photothermal treatment efficacy of tumors

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Experimental section

Materials and instruments

Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and sodium dodecylsulfate (SDS) were obtained from China National Pharmaceutical (Shanghai, China); glutathione (GSH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma. All chemicals were analytical grade and used without further purification. Ultrapure water was purified to a resistivity of 18.2 MΩ·cm. Lys-Lys-Lys-Lys-{Se-Cys}, Lys-Lys-Lys-Lys-Cys, Glu-Glu-Glu-Glu-{Se-Cys} and Glu-Glu-Glu-Glu-Cys were synthesized and purified by Karebay Biochem (Ningbo, China). The human breast cancer cells MCF-7 were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cell culture products, unless mentioned otherwise, were purchased from Invitrogen (USA).

The transmission electron microscopy (TEM) was carried out on a Hitachi HT7700 transmission electron microscope (JEOL, Japan) and the samples were prepared *via* carbon-coated copper grids. Centrifugation was performed on an Eppendorf 5417R Centrifuge. Zeta potential and dynamic light scattering measurements were performed on a Malvern Zeta Sizer Nano (Malvern). Absorbance was measured in a microplate reader (Synergy 2, Biotek, USA). All pH values were measured by a pH-3c digital pH meter (LeiCi, China) with a combined glass-calomel electrode.

Preparation of the Au NPs

The 13 nm Au NPs were synthesized by the classical sodium citrate reduction method reported before. Typically, 70 mL $HAuCl_4$ (0.01% w.p.) aqueous solution was heated to boiling point with vigorous stirring, and 3.5 mL of trisodium citrate solution (1% w.p.) was rapidly added under stirring. The solution color changed from pale yellow to colorless and finally to burgundy. The solution was kept boiling for additional 20 min, and then cooled down to room temperature under stirring. Afterwards, the solution was filtered by using a 0.45 μm Millipore membrane filter and stored in refrigerator at 4 °C for further experiments.

Cell culture

Dulbecco's modified Eagle's medium (DMEM) was used for the MCF-7 cells culture, which supplemented with 10% fetal bovine serum and 100 U/mL 1% antibiotics (penicillin/streptomycin), and incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

Cell toxicity

The toxicity was measured *via* the following MTT assay in the MCF-7 cells. The cells were dispersed in replicated 96-well microtiter plates and incubated at 37 °C in 5% CO_2 and 95% air for 24 h. After the original medium was removed, different concentrations of the Au-Se-C_4-N_6 (0, 1, 2, 3, 4, 5 nM) were respectively added to each well, and then incubated for 4h with different concentration respectively. The original medium was removed and replaced with another medium containing MTT (0.5 mg/mL). The cells were incubated at 37 °C for another 4 h, and then the medium was removed. DMSO (100 μL) was added to the cells to dissolve the produced formazan. The absorbance at

490 nm was recorded for each well. The viability was calculated based on the recorded data. All the experiments were repeated at least three times.

Animals

Female BALB/c nude mice aged 6 weeks were subcutaneously injected with a suspension of MCF-7 cells (2×10^6) in PBS. The tumors were allowed to grow until they reached a suitable size (150-200 mm^3). The tumor sizes were measured with a caliper every two days and their volumes were calculated using the formula: volume = (tumor length) × (tumor width)^2/2. Relative tumor volume was calculated based on v/v_0(where v_0 is the initial tumor volume).

Supporting Figures

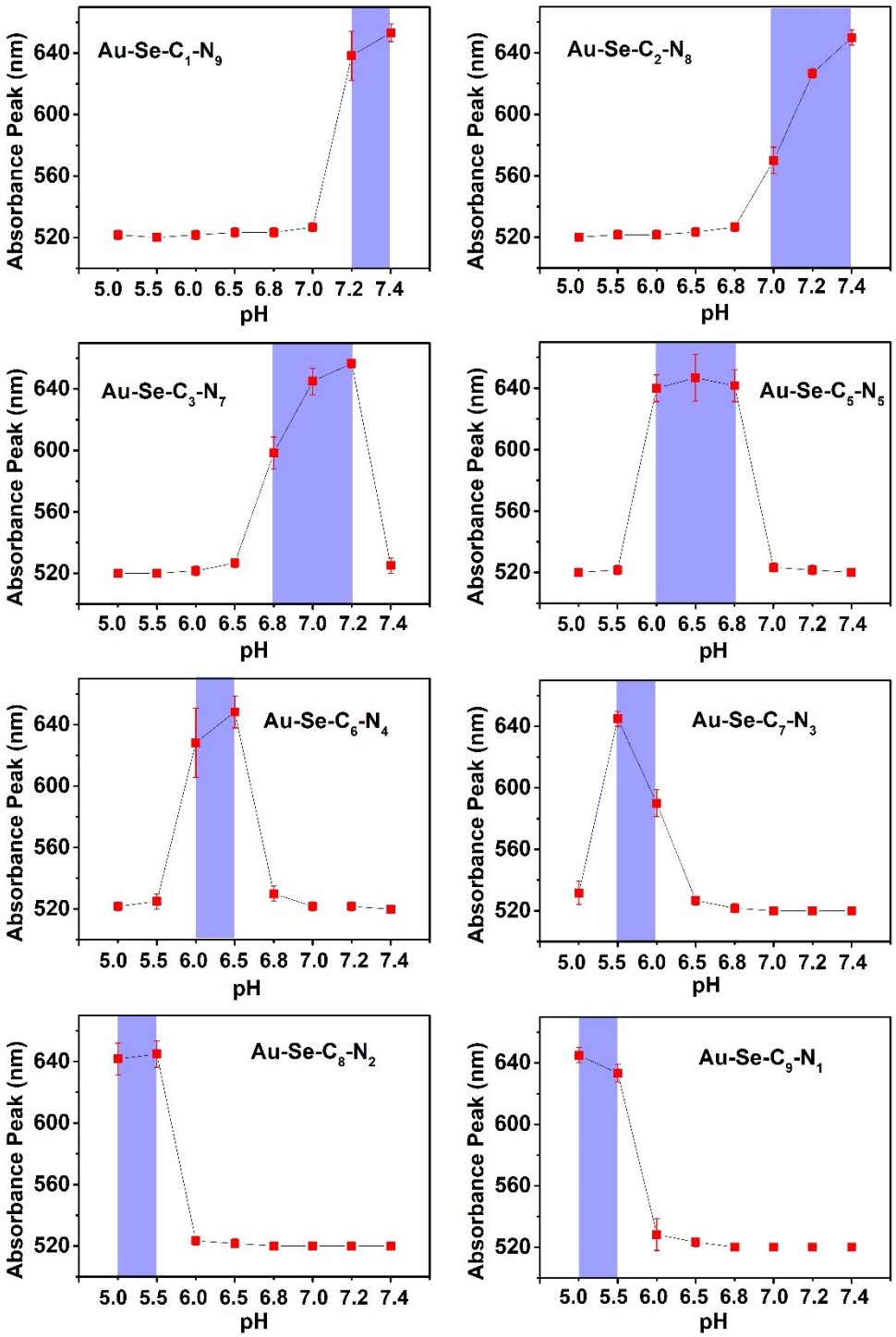

Fig. S1 Absorbance peak values of Au-Se-C_x-N_y with different X:Y molar ratios detected by UV−vis spectrum at different pH values.

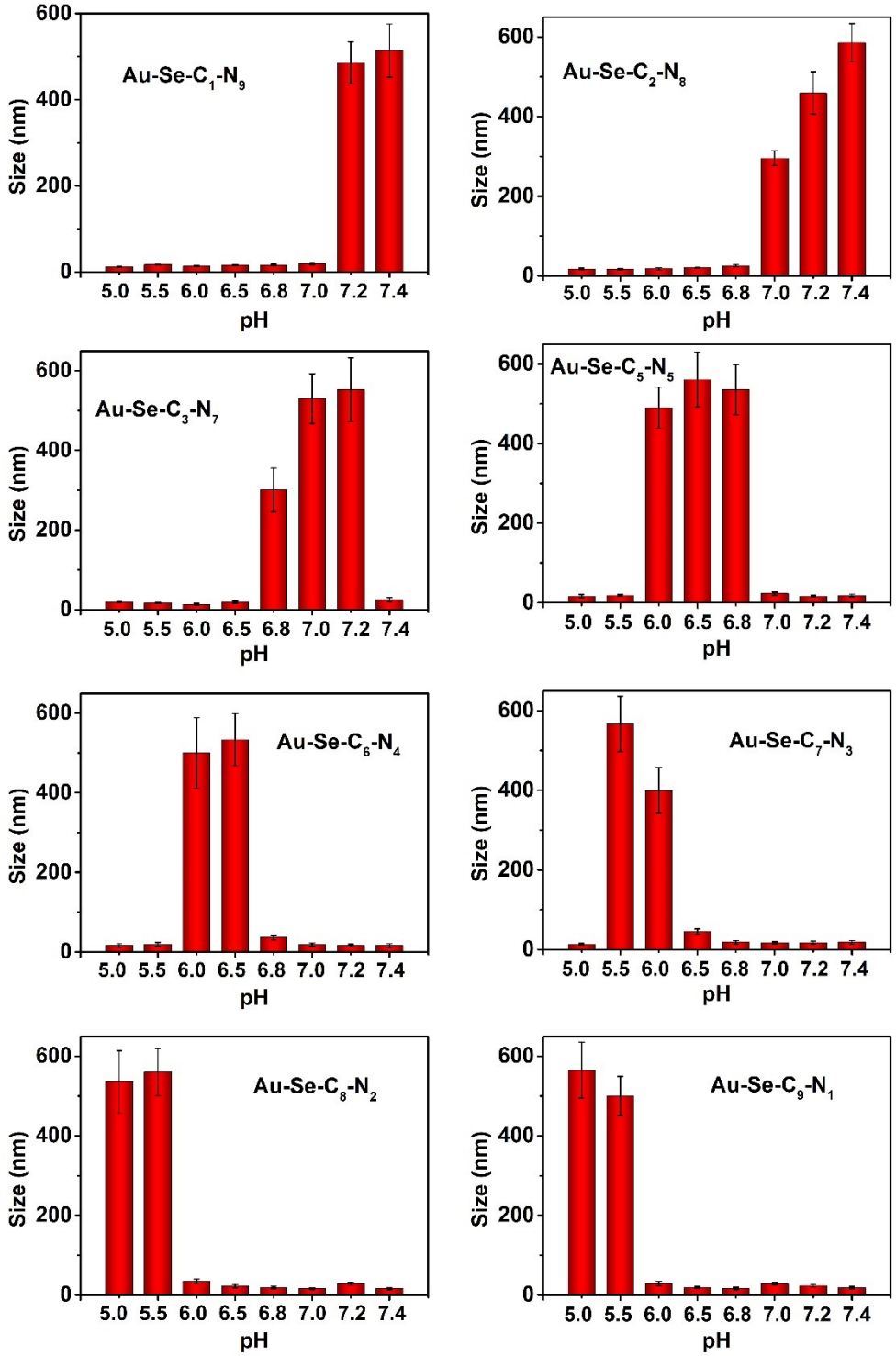

Fig. S2 Sizes of Au-Se-C_x-N_y with different X:Y molar ratios in different pH PBS solution.

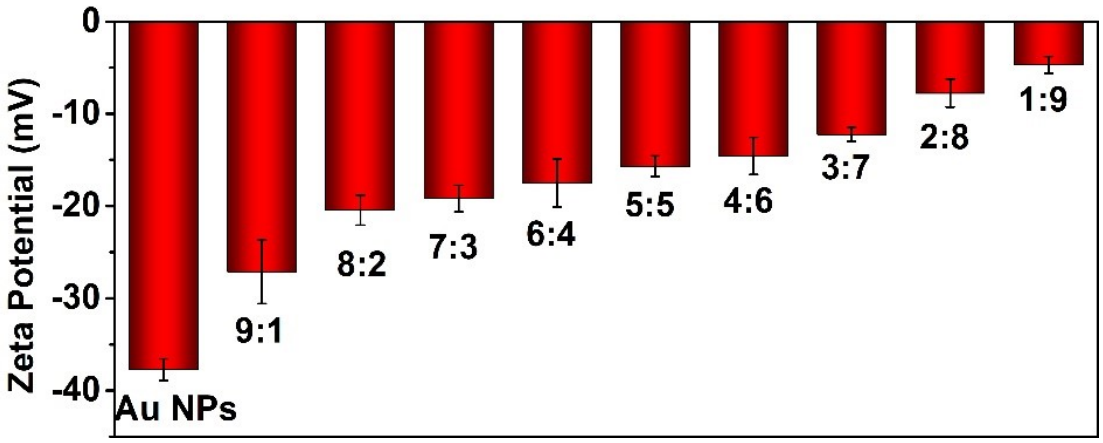

Fig. S3 Zeta potential of the Au NPs and Au-Se-C_x-N_y with different X:Y molar ratios at pH 7.4.

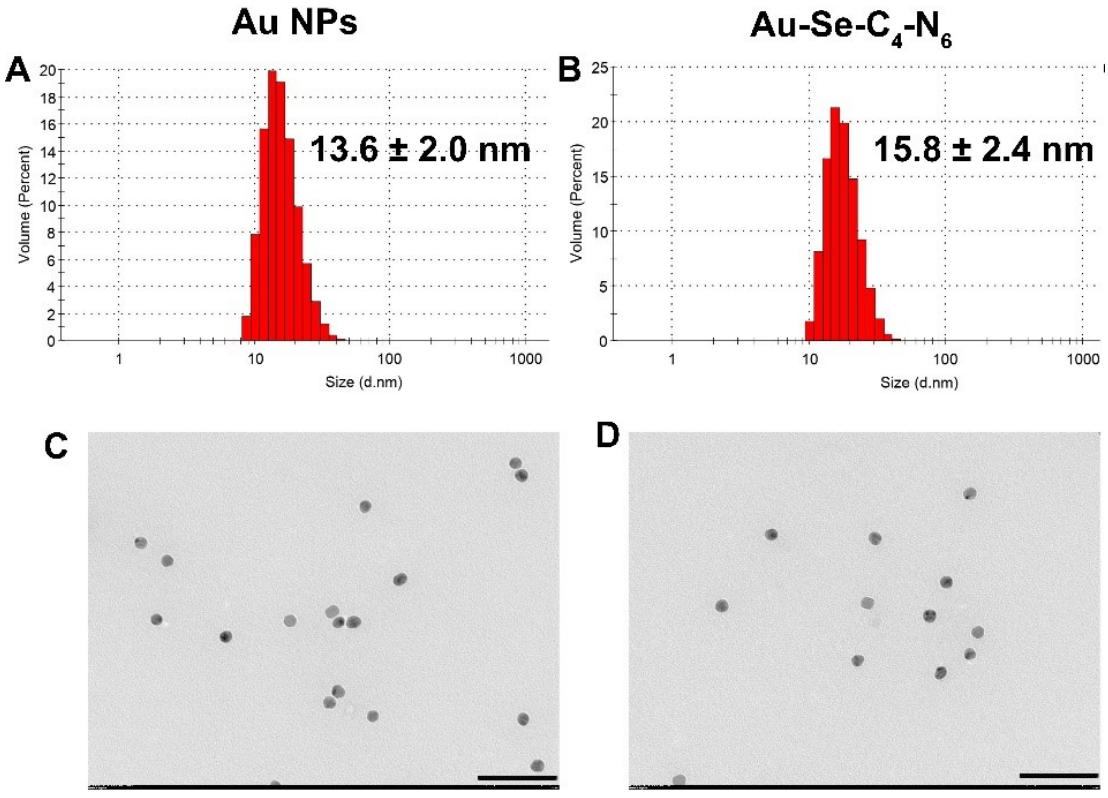

Fig. S4 The size distribution of (A) Au NPs and (B) Au-Se-C_4-N_6 determined by dynamic light scattering (DLS). The transmission electron microscopy (TEM) images of (C) Au NPs and (D) Au-Se-C_4-N_6 (scale bars in images are 100 nm).

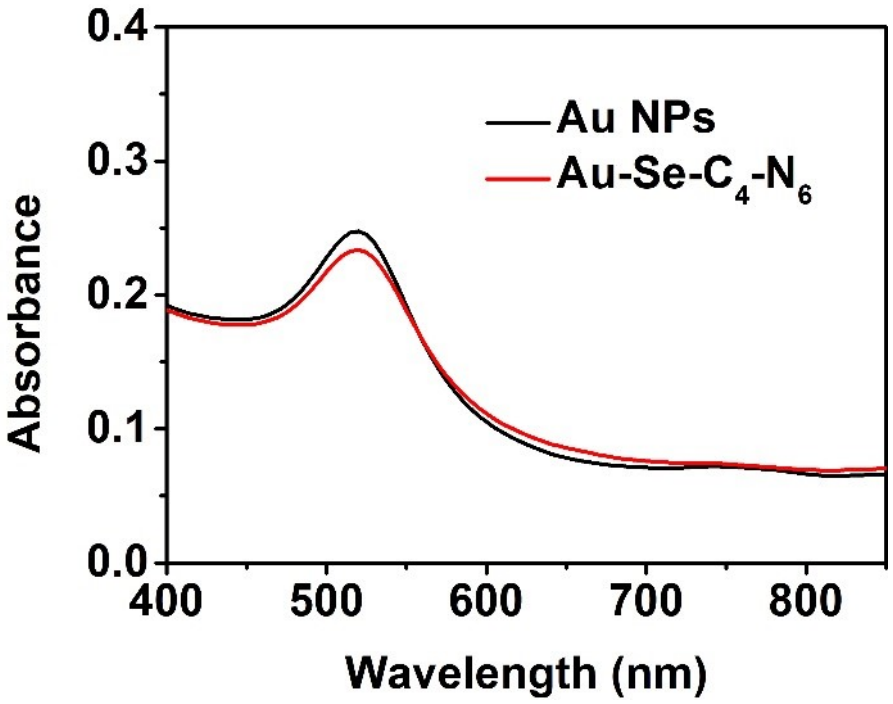

Fig. S5 UV−vis absorption spectra of the Au NPs and Au-Se-C_4-N_6.

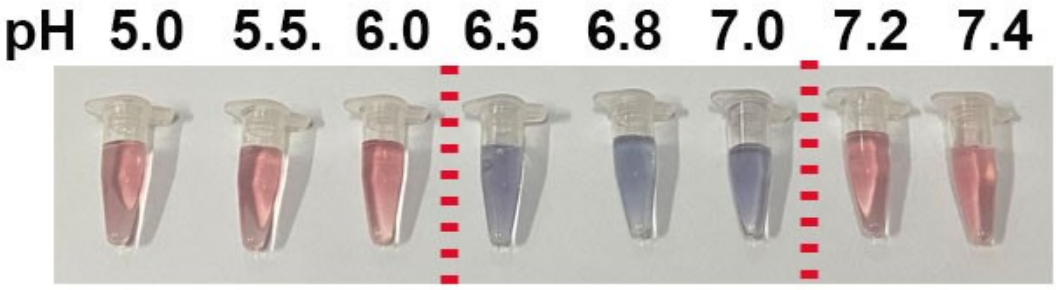

Fig. S6 Digital images of Au-Se-C_4-N_6 at different pH conditions. Clear red color means excellent dispersion of Au NPs. Samples between two dash lines are aggregated Au NPs (blue). The others are nonaggregated Au NPs.

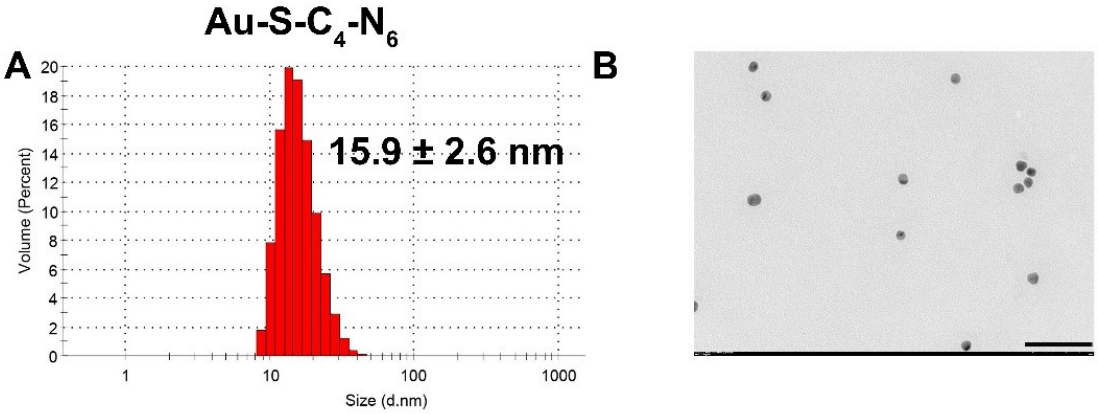

Fig. S7 (A) The size distribution of Au-S-C_4-N_6 determined by DLS. (B) The TEM images of Au-S-C_4-N_6 (the scale bar is 100 nm).

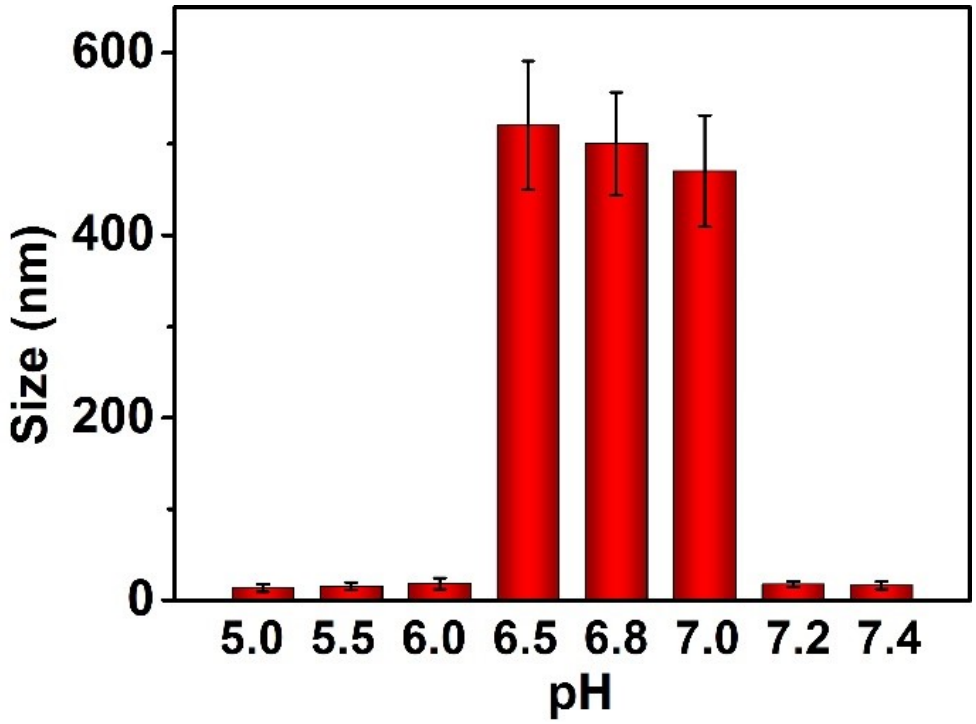

Fig. S8 Sizes of Au-S-C_4-N_6 in PBS buffer (10 mM) solution at different pH values.

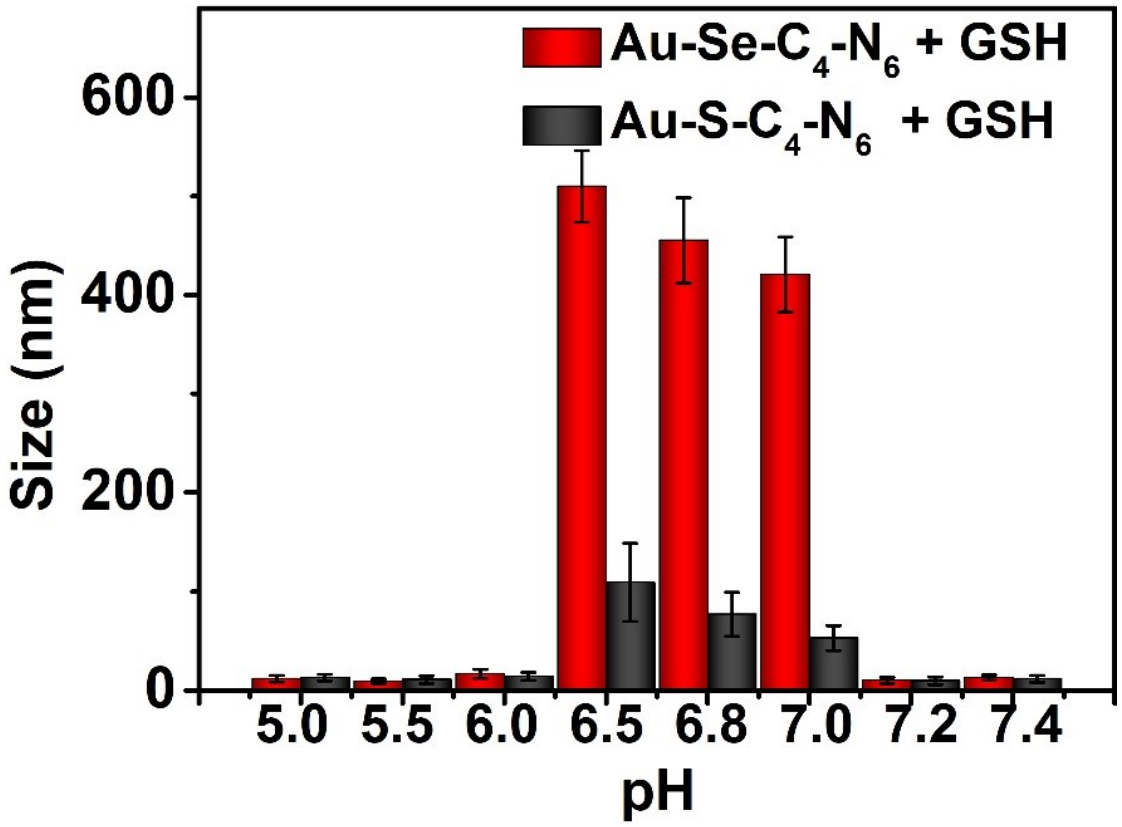

Fig. S9 Sizes of (A) Au-Se-C_4-N_6 and (B) Au-S-C_4-N_6 in PBS buffer (10 mM) solution in present of GSH at different pH values.

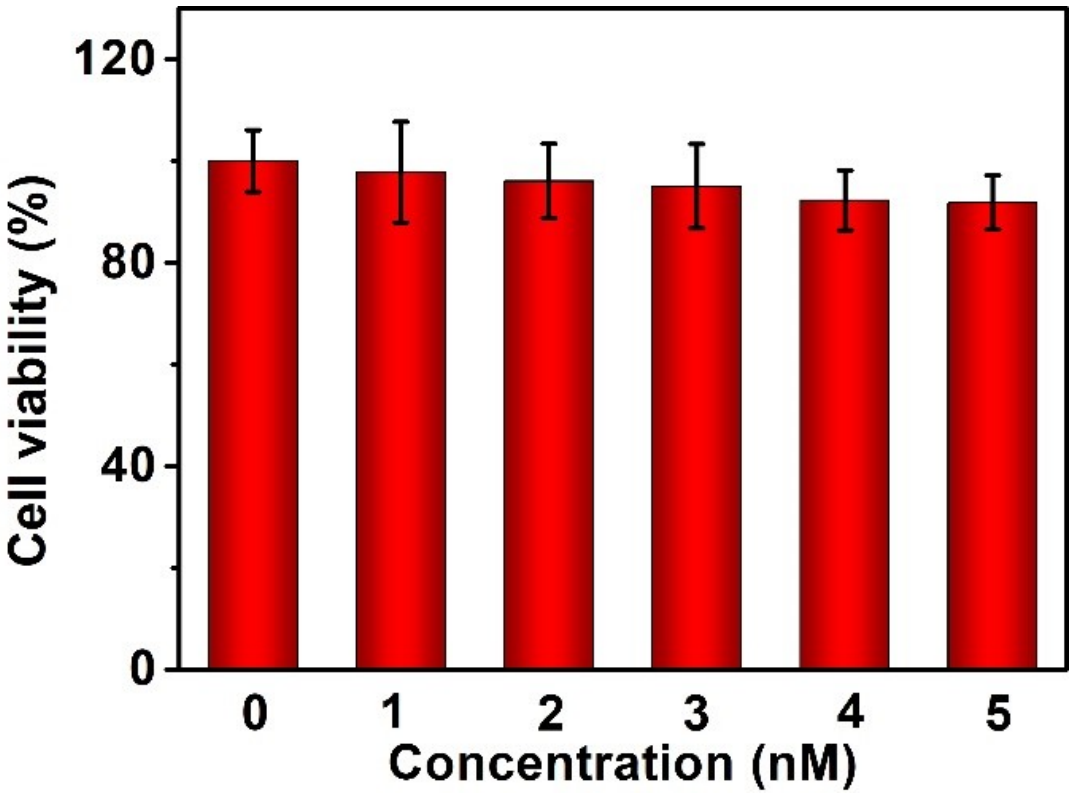

Fig. S10 Cell cytotoxicity after incubation with Au-Se-C_4-N_6 at different concentrations for 24 h.

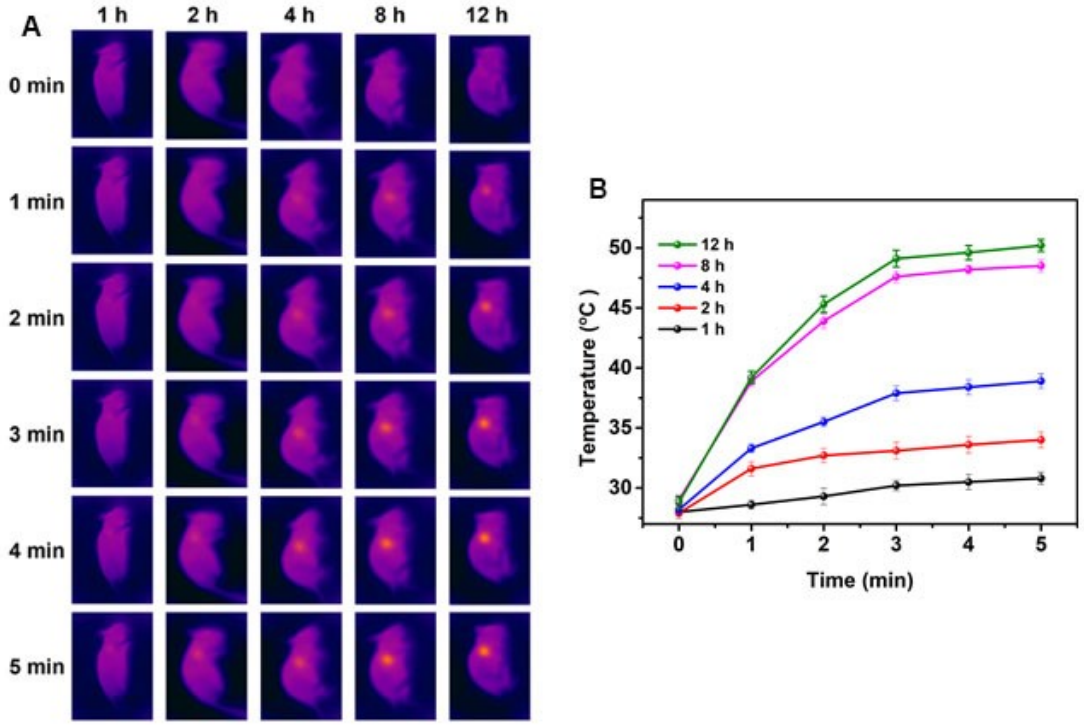

Fig. S11 (A) Time-dependent infrared thermal images and (B) temperature changes at the tumor sites of MCF-7-tumor-bearing mouse subjected to 5 min laser irradiation (808 nm, 2 W/cm^2) at 1 h, 2 h, 4 h, 8 h, or 12 h after being intravenous injection administered with Au-Se-C_4-N_6.

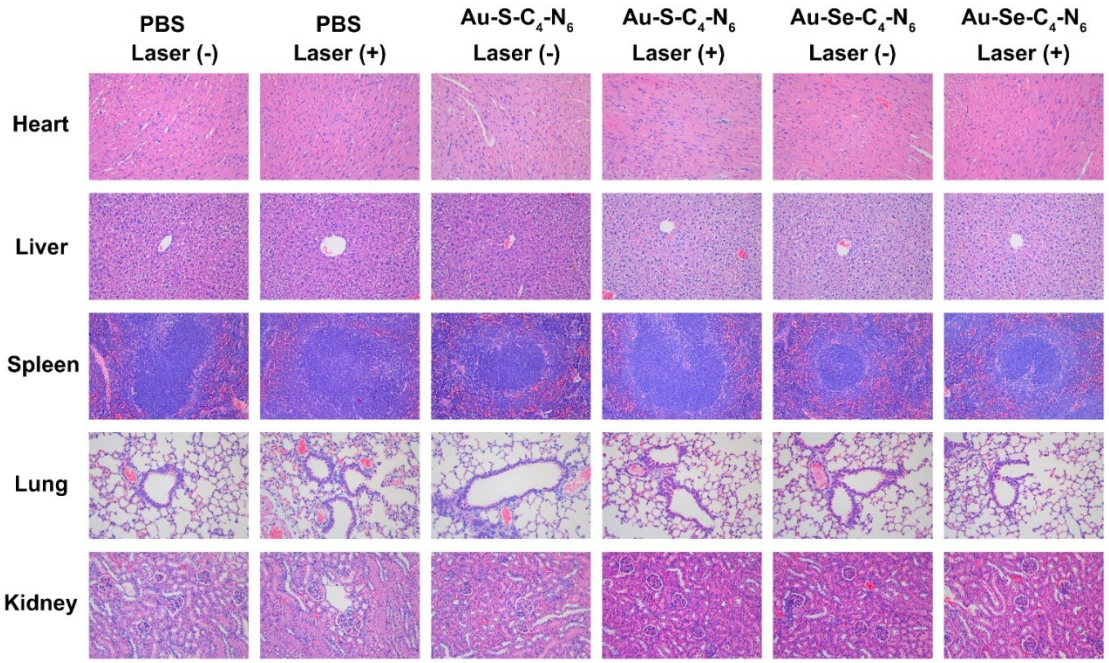

Fig. S12 Representative images of H&E staining for heart, liver, spleen, lung and kidney collected on day 14 after killing the mice.