Supporting Information

Amine-terminated PEG Functionalized Gold Nanostars for X-ray/CT Imaging and

Photothermal Therapy

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Supplementary Figures



Supplementary Fig 1. TEM images of gold seeds prepared for the growth of GNSs. Scale bar: 50 nm and 20 nm.



Supplementary Fig 2. Hydrophilic diameter of of GNS-mPEG and GNS-PEG-NH₂ determinated by DLS measurement.



Supplementary Fig 3. Relative cell viability of 293T cells incubated with GNS-mPEG and GNS-PEG-NH₂ at the gold concentration from 0.5 mg/L to 10 mg/L without (w/o) irradiation after 24 h.



Supplementary Fig 4. X-ray images of GNS-PEG-NH₂ and iodixanol with different concentrations (1: 0.5 mg/L, 2: 2.5 mg/L, 3: 12.5 mg/L, 4: 25 mg/L) in gray-scale and pseudo-color-scale.



Supplementary Fig 5. The gray-scale images of mice after injection of different concentrations of GNS-PEG-NH₂. (IR: intensity ratio)



Supplementary Fig 6. Tumor growth curves of tumor-bearing mice after treatment with PBS, GNS-mPEG and GNS-PEG-NH₂ without (w/o) and with (w/) irradiation, respectively. The tumor volumes were normalized to their initial sizes. Statistical difference has been indicated by asterisks. (p = 0.0166 for GNS-PEG-NH₂ with laser vs. PBS with laser, 6 mice per group).



Supplementary Fig 7. (a) The CT images of MCF-7 tumor-bearing mice with the injection of $GNS-PEG-NH_2$ before or after laser irradiation. The red circles indicated the location of the tumors. (b) The corresponding CT values of tumors challenged by $GNS-PEG-NH_2$ before or after laser irradiation.



Supplementary Fig 8. The reconstructed three-dimensional (3D) CT images of MCF-7 tumor-bearing mice treated with PBS or $GNS-PEG-NH_2$ before or after laser irradiation. The red circles indicated the location of the tumors.

Supplementary methods

1. Synthesis of gold seeds

The seed solution was prepared by adding 3 mL of 1 % sodium citrate solution to 100 mL of boiling 1.0 mM HAuCl₄ solution under vigorous stirring, leading to the solution color turned from no color to dark red. The sizes and morphologies of gold seeds were characterized by TEM.

2. Cell culture and viability assay of 293T

Human embryonic kidney cells (293T) were purchased from ATCC (Manassas, VA) and maintained in DMEM/10% FBS as previously described. MTT assay was used to test the cell viability of 293T treated with different concentrations of GNS-mPEG and GNS-

PEG-NH₂ (0 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 5 mg/L and 10 mg/L) for 24 h. The absorbance at 570 nm for cells was recorded by spectrophotometer described above.

3. X-ray imaging of GNS-PEG-NH₂ in vitro

The aqueous solution of GNS-PEG-NH₂ with four different concentration (0.5 mg/L, 2.5 mg/L, 12.5 mg/L and 25 mg/L) was recorded by an IVIS Lumina XR (Xenogen Corporation-Caliper, Alameda, CA, USA) and the commercial available contrast agent iodixanol with the same concentration was used as control.

4. X-ray imaging of GNS-PEG-NH₂ in vivo

Two-flank tumor-bearing mice (n=3) were chosen to scan using the above X-ray imaging system. GNS-PEG-NH₂ was intratumoral injected to each tumor with the concentration of 2.5 mg/L, 5 mg/L and 7.5 mg/L, respectively.

5. CT imaging of GNS-PEG-NH₂ in vivo

GNS-PEG-NH₂ with high concentration (12.5 mg/L) was injected to tumor to increase attenuation values for *in vivo* CT imaging. Images were recorded to compare with the change of CT values before and after laser irradiation (1 W/cm², 6 min, 808 nm). The reconstructed three-dimensional (3D) CT images were got though the workstation before and after laser irradiation as previously described.