## Supporting Information

## Tumor-targeted nanoprobes for enhanced multimodal imaging and synergistic photothermal therapy: core-shell and dumbbell Gd-tailored gold nanorods

Jinchang Yin,<sup>a</sup> Deqi Chen,<sup>a</sup> Shuangshuang Wu,<sup>a</sup> Chaorui Li,<sup>a</sup> Lizhi Liu,<sup>b</sup> and Yuanzhi Shao<sup>\*,a</sup>

<sup>a</sup> School of Physics, State Key Laboratory of Optoelectronic Materials and Technologies, Sun Yat-sen University, Guangzhou 510275, P. R. China.

<sup>b</sup> State Key Laboratory of Oncology in Southern China, Imaging Diagnosis and Interventional Center, Cancer Center, Sun Yat-sen University, Guangzhou 510060, P. R. China.

\* E-mail: <u>stssyz@mail.sysu.edu.cn</u>



**Figure S1.** Size distribution histograms and the corresponding TEM images of gold nanorods (a), CSNAs with thin (b) and thick (c) shells. The mean length and diameter were obtained from GaussAmp fitting with 250 - 300 recognizable particles randomly chosen from some TEM images.



**Figure S2.** Dynamic light scattering (DLS) measuring size distribution of PEGylated AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> CSNAs with thin (a) and thick (b) shells in aqueous solutions. PDI is the abbreviation of poly dispersion index. The relatively low PDI indicates a good dispersity of the as-prepared CSNAs.



**Figure S3.** Fourier transmission infrared (FTIR) spectra measured from AuNRs (a), AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> CSNAs in the absence (b) and presence (c) of PEG encapsulation, as well as the well-determined SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> (d). The dot cycles in the spectra represent the change of amounts of C-H bonds, indicating the successful PEG encapsulation and the previous CTAB elimination.



**Figure S4.** The powder X-ray diffraction (XRD) of PEGylated AuNR@SiO<sub>2</sub>@ Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> CSNAs. The characteristic diffraction peaks centered at  $2\theta = 18^{\circ}$  and  $23^{\circ}$  could be assigned to the distinct diffractions of gadolinium oxide carbonate hydrate (PDF # 43-0604). The well-defined peaks centered at  $2\theta = 38^{\circ}$ , 44°, 65° and 78° are well consistent with the typical diffractions of cubic structured Au crystals (PDF # 04-0784).



**Figure S5.** Typical TEM images of PEGylated AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> CSNAs with a visibly clear core-interlayer-shell structure. The light-colored area around to the dark core denotes the silica. (a) A visible thin silica interlayer; (b) an ultrathick silica interlayer.



**Figure S6.** EDX spectra of the NAs in small-area detection with 300 kV voltage (a) and the CSNAs with thin (b) and thick (c) shells in wide-view detection with 120 kV voltage. The insets in (a) show the corresponding TEM image and the detailed Gd, Si, Au composition analysis taken within four different regions from the TEM grids. The insets in (b) and (c) show all acquired elements and the relevant weight percentage.



**Figure S7.** High-resolution X-ray photoelectron spectroscopy patterns of CSNAs. (a) Au4f XPS pattern. (b) Gd3d XPS pattern. The peaks assigned to  $Gd3d_{3/2}$ , and  $Gd3d_{5/2}$  were detected at the binding energy of around 1221.7 eV and 1189.4 eV. The peak at 1199.7 eV is the satellite peak of  $Gd3d_{5/2}$ . (c) Si2p XPS pattern. The peak is deconvolved into two bands: one arises from the Si-O-Si siloxane bonds of silica structure (103.0 eV); the other is attributed to the formation of Si-O-Gd bonds (101.9 eV). (d) C1s XPS pattern. Peaks at 289.7 eV and 284.8 eV denote carbon atoms in carbonate (CO<sub>3</sub><sup>2-</sup>) and C-C bonds.



**Figure S8.** Wide-view TEM image of typical PEGylated AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> DBNAs in low magnifications.



**Figure S9.** Size distribution histograms and the corresponding TEM images of dumbbell structured AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> NAs (a) and the previous AuNR@SiO<sub>2</sub> NAs (b). The mean length and end diameter were measured from 250 - 300 recognizable particles randomly chosen from some TEM images and obtained via GaussAmp fits.



**Figure S10.** Dynamic light scattering (DLS) measuring size distribution of PEGylated AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> DBNAs in aqueous solutions. PDI is the abbreviation of poly dispersion index. The relatively low PDI indicates a good dispersity of the asprepared DBNAs.



**Figure S11.** The powder X-ray diffraction (XRD) of PEGylated AuNR@SiO<sub>2</sub>@  $Gd_2O(CO_3)_2$  DBNAs. The characteristic diffraction peaks centered at  $2\theta = 18^\circ$  and  $23^\circ$  could be assigned to the distinct diffractions of gadolinium oxide carbonate hydrate (PDF # 43-0604). The well-defined peaks centered at  $2\theta = 38^\circ$ , 44°, 65° and 78° are well consistent with the typical diffractions of cubic structured Au crystals (PDF # 04-0784).



**Figure S12.** EDX spectra of the DBNAs in small-area detection with 300 kV voltage (a), dumbbell AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> NAs (b) and the previous AuNR@SiO<sub>2</sub> NAs (c) in wide-area detection with 120 kV voltage. The insets in (a) show the corresponding

TEM image and the detailed Gd, Si, Au composition analysis taken within four different regions from the TEM grids. The insets in (b) and (c) show all acquired elements and the related weight or molar percentage.



**Figure S13.** (a) Unfiltered and (b) elastic EELS-EFTEM images of the well-established AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> DBNAs without PEG encapsulation and the related gadolinium N mapping and carbon K mapping. The measured particles are situated inside the hole of the micro grid film that has been used to replace the regular carbon film.



**Figure S14.** High-resolution X-ray photoelectron spectroscopy patterns of CSNAs. (a) Au4f XPS pattern. The peaks at 87.4 eV and 84.0 eV are ascribed to the surface Au<sup>3+</sup> and Au<sup>0</sup> species of the exposed gold nanorods. (b) Gd3d XPS pattern. The peaks assigned to Gd3d<sub>3/2</sub>, and Gd3d<sub>5/2</sub> were detected at the binding energy of around 1221.6 eV and 1189.4 eV. The peak at 1199.7 eV is the satellite peak of Gd3d<sub>5/2</sub>. (c) Si2p XPS pattern. The peak is deconvoluted in two bands: one arises from the Si-O-Si siloxane bonds of silica structure (102.9 eV); the other is attributed to the formation of Si-O-Gd bonds (101.7 eV). (d) C1s XPS pattern. Peaks at 289.8 eV and 284.8 eV denote carbon atoms in carbonate (CO<sub>3</sub><sup>2-</sup>) and C-C bonds.



**Figure S15.** UV-visible-NIR spectra of CSNAs with thin and thick shells in aqueous solutions. The lines have been normalized to keep a same transverse absorption peak value. The longitudinal SPR absorption peak value of the core-thin NAs (centered at 773 nm) is larger than that of core-thick NAs (centered at 861 nm), and the core-thin shell NAs possess a stronger absorption to 800 nm laser lights.



**Figure S16.** Extinction spectra and the corresponding TEM images of AuNRs, AuNR@SiO<sub>2</sub> NAs and AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> NAs with two kind gold nanocores of aspect ratio at 3.3 (a, c, d, e) and 4.2 (b, f, g, h).



**Figure S17.** (a) Room temperature magnetization curves of PEGylated AuNR@SiO<sub>2</sub>@Gd<sub>2</sub>O(CO<sub>3</sub>)<sub>2</sub> CSNAs and DBNAs. (b) X-band EPR spectra at room temperature. All spectra are centered on  $g \approx 2$ . Line width  $\Delta$ Hpp means the distance from peak to valley.



**Figure S18.** *In vivo* MR images of 4T1 xenografted tumor in Balb/c nude mice ( $\approx 23$  mg) before and after injected with 10 µmol kg<sup>-1</sup> core-shell or dumbbell NAs into the tail vein of mice at 0 h (a, f), 0.5 h (b, g), 1 h (c, h), 2 h (d, i) and 6 h (e, j).

## Calculation of the photothermal conversion efficiency

The commonly-used equation to calculate the photothermal conversion efficiency of Au nanoparticles is the following equation reported by Roper, firstly.<sup>1</sup>

 $\eta = \frac{hS(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{800}})} \quad \text{Equation S1},$ 

where  $\eta$  is the conversion efficiency from incident laser to thermal energy; *h* is heat transfer coefficient and *S* is the surface area of the container;  $Q_{dis}$  denote the baseline energy inputted by the quartz cuvette cell. Both *hS* and  $Q_{dis}$  can be determined independently referring to the Reference 2 and 3 in details. *I* is the laser power used (1.0 W cm<sup>-2</sup>).  $T_{max}$ , the equilibrium temperatures of CSNAs and DBNAs solutions were measured as 53.22 °C and 55.77 °C (Figure 41);  $T_{surr}$  is the ambient temperature of the surroundings, which was detected as 23.01 °C.  $A_{800}$  is the absorbance of the sample solution irradiated at 800 nm. According to Figure 4a, we determine the absorbances of CSNAs and DBNAs aqueous solutions as 0.61 and 0.84. Using all data from SPR absorption spectra and in vitro photothermal conversion assays, we have calculated the photothermal conversion efficiencies of CSNAs and DBNAs as 17.2% and 20.6%.

## Reference

- 1 D. K. Roper, W. Ahn and M. Hoepfner, J. Phys. Chem. C, 2007, 111, 3636-3641.
- 2 C. M. Hessel, V. P. Pattani, M. Rasch, M. G. Panthani, B. Koo, J. W. Tunnell and B. A. Korgel, *Nano Lett.*, 2011, **11**, 2560-2566.
- 3 Q. Tian, F. Jiang, R. Zou, Q. Liu, Z. Chen, M. Zhu, S. Yang, J. Wang and J. Hu, *ACS Nano*, 2011, **5**, 2560-2566.



**Figure S19.** TEM images of DBNAs (a, b) and CSNAs (c, d) before (a, c) and after (b, d) illuminated with an 800nm laser for 10 min.



**Figure S20.** Longitudinal absorption peak intensities of DBNAs and CSNAs illuminated with an 800nm laser at different exposure times. Note that the aqueous samples were cooled first and measured at room temperature. The SPR absorption patterns have been normalized to keep a same transverse absorption peak value at 0.4.



**Figure S21.** *In silico* tumor growth models simulated using CompuCell3D software. This software can help to describe intracellular activity, explore angiogenesis and find

ways to prevent tumor growth. The temperature in this software model can be characterized using the volatility amplitude parameter T exerted in cell membrane. "T = 0" can be regarded as the ambient temperature, and the increased value of T is approximately equivalent to the elevated temperature. The time is denoted with Monte Carlo Steps (MCS). Using these various input parameters, we simulated tumor and vascular growth, and qualitatively appraise their changes induced by the elevated temperature. (Coordinate axis:  $50 \times 50 \times 50$ )



**Figure S22.** *In silico* time-dependent tumor volume changes at ambient and evaluated temperature calculated using CompuCell3D software. The dynamic curves can qualitatively confirm tumor growth inhibited and tumor cells killing induced by the elevated temperature.



**Figure S23.** Cell cycle analysis determined with mean fluorescence intensity (MFI) at FL2-A::PI-A channel for 48 h (mean  $\pm$  SD, n = 3).



**Figure S24.** The origin apoptosis data of L-O2 cells measured by flowcytometry stained with Annexin-V/PI at FL2-A channel for 48 h (mean  $\pm$  SD, n = 3). Q1 is the area where cells should not turn up; Q2 represents the apoptosis or dead cells in later period; Q3 represents the apoptosis cells in early stage; Q4 displays the normal living cells.