Electronic Supplementary Information:

Coral-shaped Au nanostructures for selective apoptosis induction during photothermal therapy

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1. Experiments and methods

Photoacoustic (PA) imaging

For PA imaging, different concentrations (5, 10, 20, 30, and 40 μM) of ICG and Au NCs (0.02−0.06 nM) in water were introduced into a phantom prepared with agarose gel and scanned at 810 nm with PA equipment (Vision128, iThera Medical Inc., Germany). The system houses 128 unfocused ultrasound transducers (with 5 MHz center frequency and 3 mm diameter) arranged in a hemispherical bowl filled with water and a temperature monitor of the water bath (37 °C). The mean intensity was quantified for the scanned area.

MTT assay

MCF-7 cells (1×10^4 cells per well) were first incubated with different concentrations of PEG-Au NCs (0–200 μg/mL) at 37 °C for 12 h in 96-well plates, and then repeatedly rinsed with PBS to remove excess PEG-Au NCs. MTT solution (10 μL; 5 mg/mL, pH 7.4) was then added to each well and incubated for 4 h. Afterwards, 100 μL dimethylsulfoxide (DMSO) was added to each well. Absorbance was recorded at 550 nm on a Synergy 2 microplate reader (Biotek). The viabilities of cells incubated with PEG-Au NCs were obtained by comparing with cells before incubation.

Flow cytometry

Flow cytometry was performed on a FACSCalibur flow cytometer (BD Biosciences). MCF-7 cells (1×10^6) were incubated with PEG-Au NCs (100 μg/mL) at 37 °C for 12 h, irradiated under an 808 nm laser at 0.5 W/cm^2 for 0–15 min, washed three times with PBS, dyed with Anti-annexin V-FITC and propidium iodide (PI) for 15 min in the dark, and then detected using flow cytometry under excitation at 488 nm. The fluorescence signals of FITC and PI were recorded at emission wavelengths of 515 nm and 560 nm, respectively.

Trypan blue staining

MCF-7 cells were incubated with PEG-Au NCs (100 μg/mL) at 37 °C in 96-well plates (1×10^4 cells per well) for 12 h. Then, one well was irradiated under an 808 nm laser at a power density of 0.5 W/cm^2 for 15 min. As controls, one well was incubated with 0.5 mM H_2O_2 for 12 h and another was incubated with 0.1 mL ethanol (EtOH). Then, 20 μL of 0.4% (w/v) Trypan blue solution was added to the wells and incubated for 5 min at room temperature. Afterwards, bright-field images were captured with an optical microscope (AxioObserver A1, Carl Zeiss).

LDH assay

MCF-7 cells were incubated with PEG-Au NCs (100 μg/mL) at 37 °C in 96-well plates (1×10^4 cells per well) for 12 h. Then, the cells were either irradiated under an 808 nm laser at a power density of 0.5 W/cm^2 for 15 min, incubated with the apoptotic inducer H_2O_2, or with the necrotic inducer ethanol (EtOH). Plates were then equilibrated at room temperature for 30 min. CytoTox-ONE reagent (20 μL; Sigma) was added to each well, and the plates were incubated for 10 min at room temperature. The fluorescence signal at 590 nm was recorded with an excitation source of 560 nm on a Synergy 2 microplate reader. LDH release was expressed relative to the basal LDH release from untreated cells.

Caspase-3 activity assay

MCF-7 cells (1×10^4 cells per well) were incubated with PEG-Au NCs (100 μg/mL) at 37 °C in 96-well plates for 24 h. Then, the cells were irradiated under an 808 nm laser at a power density of 0.5 W/cm^2 for 15 min, incubated with the apoptotic inducer H_2O_2, or with the necrotic inducer ethanol (EtOH), respectively. Then, Caspase-Glo3/7 reagent (100 μL; Promega) was added to each well, followed by incubation at room temperature for 30 min. Caspase 3 activity was evaluated by recording the luminescence of each sample using a microplate reader.

Western blot analysis

Cells were lysed in 0.3 mL lysis buffer (0.1% SDS, 1% NP-40, 50 mM HEPES [pH 7.4], 2 mM EDTA, 100 mM NaCl, 5 mM sodium orthovanadate, 40 mM p-nitrophenyl phosphate, and 1% protease inhibitor (Calbiochem). Cell lysate was centrifuged at 12,800 ×*g* for 25 min, and the supernatant was collected and denatured. Equal amounts of protein were then separated by 10%–15% SDS-PAGE and blotted onto polyvinylidene difluoride membrane. The membrane was blocked in 0.2% block-1 (Tropix, Foster City, CA) for 3–5 h at room temperature, followed by incubation overnight at 4 °C with primary antibodies (1:500–1:1000). The membrane was rinsed, and then incubated for 1 h with peroxidase-conjugated secondary antibodies (1:10000, Bio-Rad, Hercules, CA). Chemiluminescent detection was performed with the ECL kit from Pierce (Rockford, IL). Densitometric analysis was conducted using Molecular Analysis software (Bio-Rad).

2. Experimental determination of C_{abs} and C_{sca}

PA signal is generated due to the optical absorption of a material, and thus the signal is expected to be proportional to the absorption coefficient (μ_{abs}) of the material within a certain concentration range.[1,2] On the basis of this principle, we could convert the PA signal from a sample into the μ_{abs} by benchmarking against a linear calibration curve based on an organic dye that describes the relationship between the PA signal and the μ_{abs}. Indocyanine green (ICG) is used as the reference dye for calibration in this work because its molar absorption coefficient is well documented[3] and its absorption peak is at ~800 nm, which is close to the absorption peak of our synthesized Au NCs.

As depicted in Fig. S2A and 2C, the PA signal of the ICG and Au NCs linearly increase with their concentrations. The plot of the PA signal of ICG against its μ_{abs} show linear relationships (Fig. S2B); the linear dependence can be described using the following equation:

$$\mu_{abs} = 2.72 \times \text{PA signal} + 0.53 \quad \text{(S1)}$$

Using this linear equation, we can calculate the μ_{abs} of the Au NCs at different concentrations based the determined PA signal of the Au NCs. The extinction coefficient (μ_{ext}), which is the sum of the absorption coefficient (μ_{abs}) and the scattering coefficient (μ_{sca}), of the Au NCs can be derived from the Beer-Lambert law. Therefore, by combining with the extinction spectra, we could experimentally obtain the linear relationship between the extinction coefficient (μ_e) of Au NCs (as shown in Fig. S2D), and further determine the individual value of the μ_{sca} of the Au NCs based on the following equation:

$$\mu_{ext} = \mu_{abs} + \mu_{sca} \quad \text{(S2)}$$

Then, we would converted to these μ_{abs} and μ_{sca} to C_{abs} and C_{sca}, respectively, by taking into consideration the relationship:

$$\mu = N_{\text{Au NCs}} \times C \quad \text{(S3)}$$

where μ refers to the μ_{abs} or μ_{sca} (in m^{-1}) of the Au NCs; $N_{\text{Au NCs}}$ is the concentration of the Au NCs, expressed as the number of particles per m^3; and C refers to the C_{abs} or C_{sca}, in m^2).

3. Calculations of the photothermal conversion efficiency

According to the method proposed by Roper,[4] the temperature data were fit to a linearized energy balance derived from a description of the microscale thermal dynamics in the suspension to determine the photothermal conversion efficiency (η) (Fig. 2D), which was calculated with the following equation (S4):

$$\eta = \frac{h \cdot A(T_{max} - T_{surr}) - Q_0}{I \cdot (1 - 10^{-A_{808}})} \quad \text{(S4)}$$

where h is the heat transfer coefficient of the nanocomposites, A is the area cross section of irradiation, Q_0 is the heat dissipated from light absorbed by the quartz sample cell and water (this was measured independently as 25.7 mW using borosilicate glass cells containing water), I is the incident laser power, A_{808} is the absorbance of the Au NCs at 808 nm, T_{max} is the highest temperature that can be reached under irradiation, and T_{surr} is the surrounding temperature. The value of $h \cdot A$ can be derived from the following equation (S5):

$$\tau_s = \frac{\sum m_i C_{p,i}}{h \cdot A} \quad \text{(S5)}$$

where m_i and $C_{p,i}$ are the mass of and heat capacity of the irradiated system, respectively, including water, the quartz cell, and the Au NCs. The values for m_i are 0.5 and 5.7 g, while the values for $C_{p,i}$ are 4.2 J g^{-1} K^{-1} and 0.89 J g^{-1} K^{-1} for water and the quartz cell, [5,6] respectively. The time constant, τ_s, is defined as the slope of cooling time against –Ln(θ) (Fig. 2E), where θ is the temperature driving force, which is defined by equation (S6):

$$\theta = \frac{T_{surr} - T}{T_{surr} - T_{max}} \quad \text{(S6)}$$

where T is the temperature of the system. The value of η for the Au NCs was calculated to be 32%.

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4. Figures

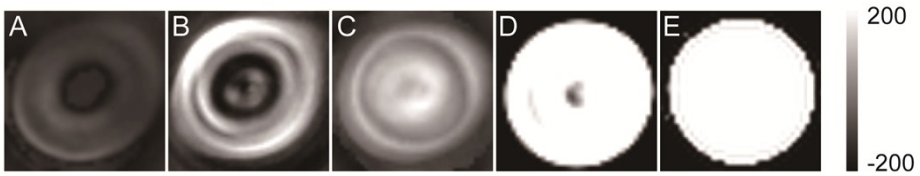

Fig. S1. Photoacoustic imaging of Au NCs with concentration of 0.02 nM (A), 0.03 nM (B), 0.04 nM (C), 0.05 nM (D), 0.06 nM (E).

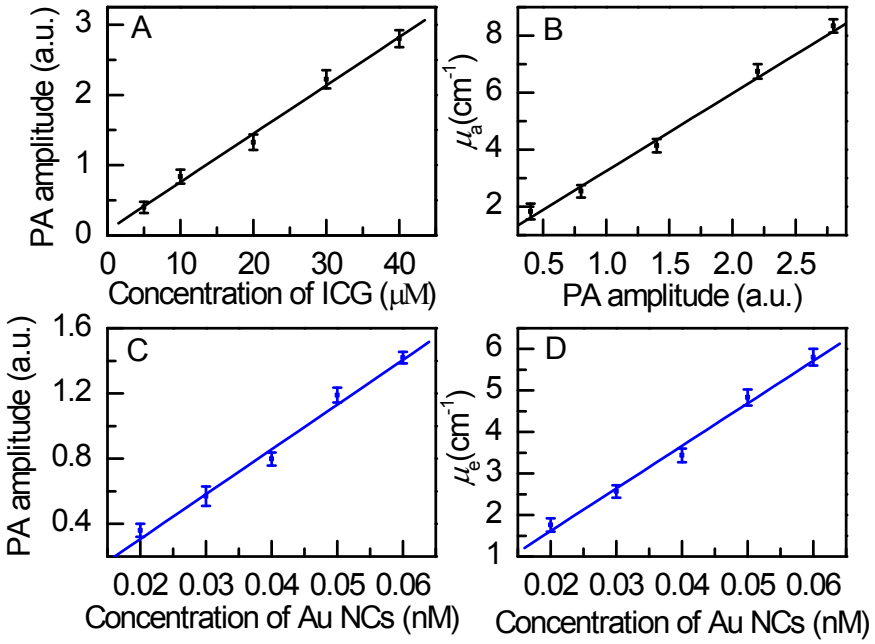

Fig. S2. (A) Plot of ICG PA signal amplitude against its concentrations. (B) The linear relationship between the absorption coefficient (μ_a) of ICG and PA signal derived from plot in (A). (C) Plot of Au NCs PA signal amplitude against their concentrations. (D) The linear relationship between the extinction coefficient (μ_e) and concentrations of Au NCs.

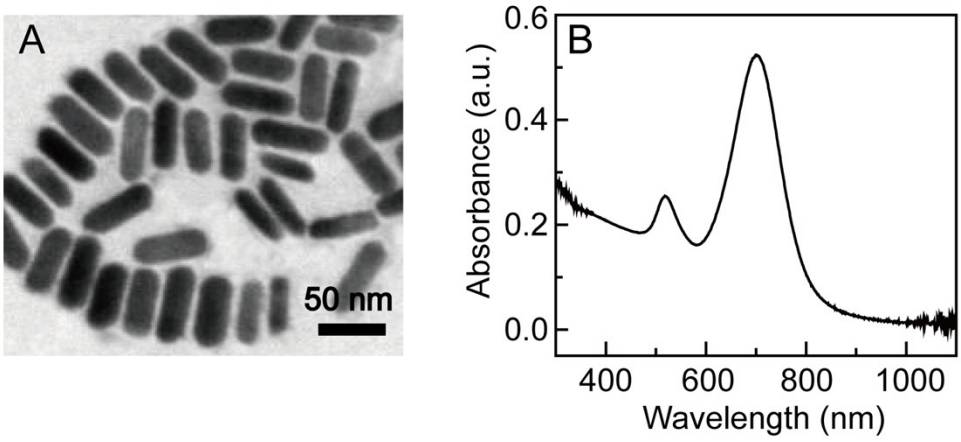

Fig. S3. TEM image (A) and UV-vis spectrum (B) of Au NRs.

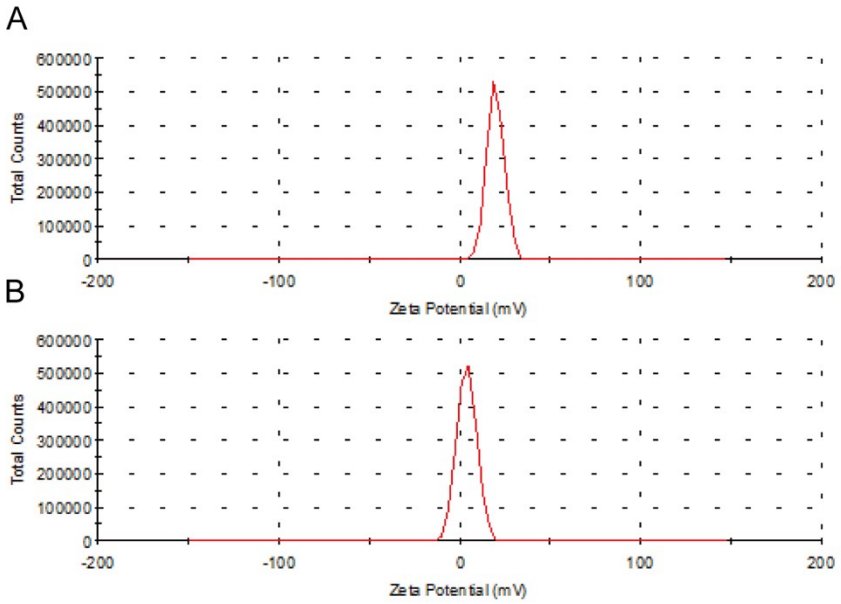

Fig. S4. The graph of zeta potential measurement of Au NCs (A) and PEG-Au NCs (B).

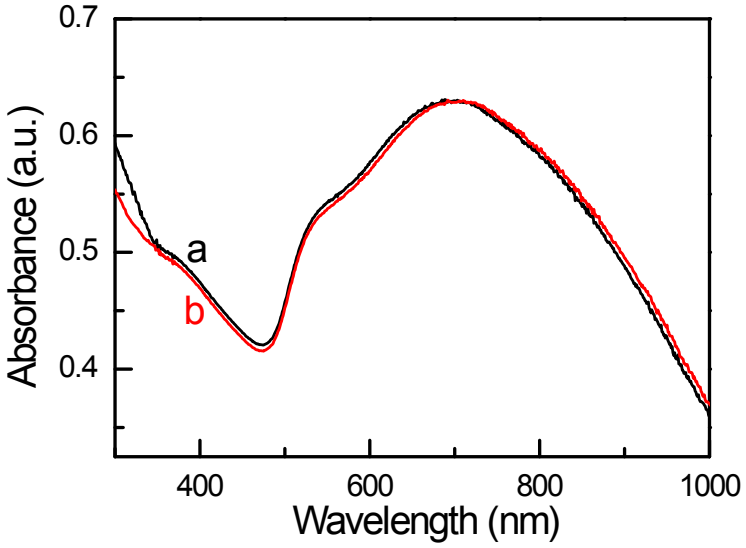

Fig. S5. UV-vis-NIR spectra of Au NCs (a) and PEG-Au NCs (b)

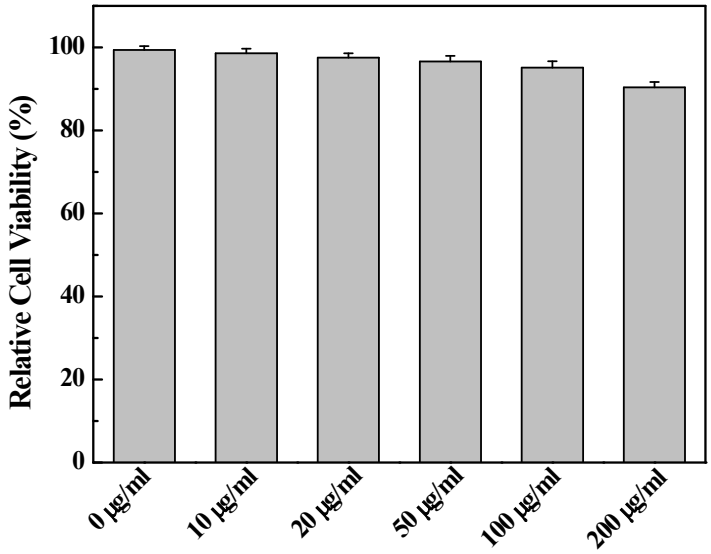

Fig. S6. Cell viability of MCF-7 cells upon incubation of Au NCs at concentration ranging from 0 to 200 μg/mL.

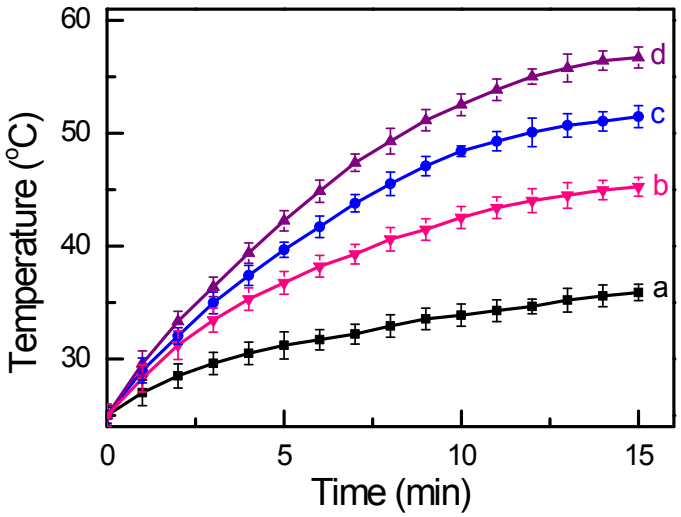

Fig. S7. Dependence of the temperature-irradiation time profiles on the power density of MCF-7 cells loaded with Au NCs (100 μg/mL). The power density of 808 nm laser 0.25 (a), 0.5 (b), 0.75 (c), and 1.0 W cm^{-2} (d).

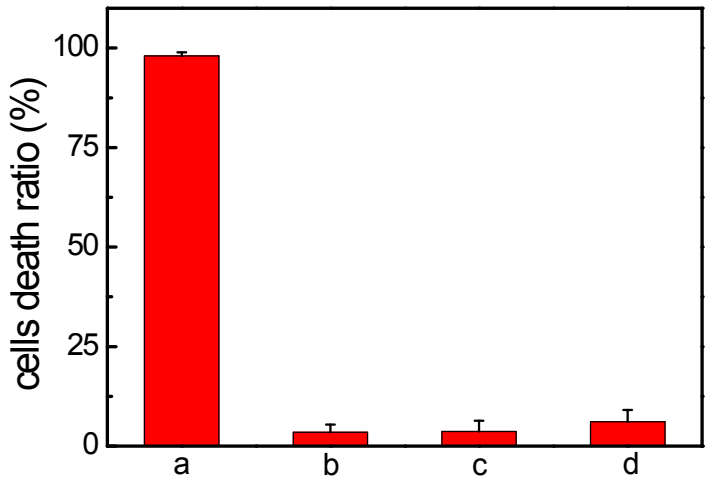

Fig. S8. Cells death ratio of MCF-7 cells treated by (a) injection with the Au NCs and irradiation for 15 min; (b) grow naturally (control); (c) injection with the Au NCs but without irradiation and (d) only laser irradiation for 15 min. The irradiation condition was 808 nm laser with power density of 0.5 W/cm^2.