Supporting Information

A New Theranostic System Based on Gold Nanocages and Phase-Change Materials with Unique Features for Photoacoustic Imaging and Controlled Release

Geon Dae Moon,[†,‡] Sung-Wook Choi,[†,§] Xin Cai,[†] Weiyang Li,[†]
Eun Chul Cho,[†] Unyong Jeong,[‡] Lihong V. Wang,[†] Younan Xia[†,*]

[†]*Department of Biomedical Engineering, Washington University, St. Louis, MO 63130,* [‡]*Department of*

Materials Science and Engineering, Yonsei University, 134 Shinchon-dong, [§]Current address: *Division of*

Biotechnology, The Catholic University of Korea, Gyeonggi-do, Korea

Corresponding author. E-mail: xia@biomed.wustl.edu

Experimental Section

Materials. Chloroauric acid (HAuCl₄, 99.9%), 1-tetradecanol (97%), Rhodamine 6G (R6G, 99%), methylene blue (MB, ≥ 82%), and methanol (≥99.9%) were obtained from Sigma-Aldrich and used as received. In all experiments, we used deionized (DI) water with a resistivity of 18 MΩ, which was prepared using an ultrapure water system (MILLIPORE).

Synthesis of Au Nanocages. Gold nanocages (AuNCs) with an edge length of 60 nm were prepared using the galvanic replacement reaction between Ag nanocubes with an edge length of 44 nm and HAuCl₄ according to a protocol described in our previous report (Skrabakak, S. E.; Au, L.; Li. X.; Xia, Y. *Nature Protocols* **2007**, *2*, 2182).

Loading Au Nanocages with 1-tetradecanol and Dye. The AuNCs dispersed in methanol were

added to a dye solution (R6G or MB) in 1-tetradecanol at 50 °C, followed by increasing the temperature to 90 °C to evaporate methanol. The above mixture was stirred for 5 h and then centrifuged with hot DI water at 14,000 rpm for 5 min to obtain the AuNCs loaded with 1-tetradecanol and dye. The retrieved AuNCs were washed with cold DI water at least 8 times before the release test.

Dye Release from the Au Nanocages by Heating and by High Intensity Focused Ultrasound (HIFU). The AuNCs loaded with 1-tetradecanol and dye were added into a vial and placed in an oil bath set to the designated temperature (25, 37, or 40 °C) and heated for different periods of time. At intervals, the solution was cooled with an ice bath, followed by centrifugation at 14,000 rpm for 5 min. The supernatant was then taken out for UV-vis spectral measurement. Likewise, HIFU was used to heat the sample and trigger the dye release at different powers (4, 8, and 12 W) for different periods of time.

Instrumentation. The transmission electron microscopy (TEM) images were captured using a microscope (FEI G2 Spirit Twin) operated at 120 kV. Samples were prepared by dropping an aqueous suspension of the particles on carbon-coated Cu grids, followed by drying at ambient temperature. The concentration of AuNCs was determined using an inductively-coupled plasma mass spectrometer (ICP-MS, Perkin Elmer): the concentration of Au ions was converted to the concentration of nanocages once the geometric dimensions of the nanocage had been determined from TEM images. Thermal gravimetric analysis (TGA, TA Instruments Q500, New Castle, DE) was conducted to characterize the AuNCs loaded with 1-tetradecanol. Water was evaporated by increasing the temperature at a rate of 10 °C/min to 120 °C and then kept at 120 °C for 20 min. After the sample had cooled down to 50 °C, TGA measurement began by increasing the temperature at a rate of 10 °C/min to 500 °C under nitrogen. UV-vis extinction spectra were recorded using a Cary 50 spectrometer (Varian, Palo Alto, CA). The HIFU transducer (TX 009, Philips) was operated at a central frequency of approximately 1.6 MHz, with a focal length of 40 mm and a focal spot 0.9 mm in diameter. It was driven by a continuous sinusoidal voltage produced by a function generator (33250A, Agilent) and passed through a radiofrequency amplifier (240L, ENI). The HIFU transducer and the targeted sample were both immersed in a water bath to provide ultrasound coupling between them.

Calculation of weight and volume fraction of 1-tetradecanol (the phase-change material, PCM) in Au Nanocages

The weight fraction of PCM in the AuNC is calculated as follows:

$$f_{PCM} = m_{PCM}/(m_{cage} + m_{PCM})$$

(f_{PCM}: weight fraction of PCM, m_{PCM}: weight of PCM, m_{cage}: weight of AuNC)

The density of the AuNC ($\rho_{Au\ Cage}$) is calculated as the mean value of Au and Ag because the AuNC is actually Au-Ag alloy (approximately 1:1 atomic ratio, based on ICP-MS):

$$\rho_{Au\ Cage} = \rho_{Au\text{-}Ag\ alloy\ cage} = (\rho_{Au} + \rho_{Ag})/2 = 14.85\ \text{g/cm}^3$$
$$(\rho_{Au} = 19.3\ \text{g/cm}^3, \rho_{Ag} = 10.5\ \text{g/cm}^3)$$

This leads to the calculation of the weight fraction of PCM (f_{PCM}) in the AuNC (here, we assume that the inner space of the AuNC is all occupied by PCM):

$$f_{PCM} = V_{PCM} \times \rho_{PCM} / (V_{Au\ cage} \times \rho_{Au\ cage} + V_{PCM} \times \rho_{PCM})$$
$$= b^3 \times \rho_{PCM} / [(c^3 - b^3) \times \rho_{Au\ cage} + b^3 \times \rho_{PCM}]$$

$\rho_{PCM} = 0.824\ \text{g/cm}^3$, for 1-tetradecanol

a: wall thickness of AuNC

c: edge length of AuNC

$b = c - 2 \times a$

Figure S1 shows the relationship between the weight fraction of PCM and the dimension of the AuNC. The volume fraction occupied by PCM in the AuNC can be calculated by the weight loss measured in the TGA measurement. When the edge length and wall thickness of the AuNC is 60 nm and 7.5 nm, respectively, the volume fraction ($V_{f,\ PCM}$) of PCM in the AuNC is calculated as follows:

$$V_{f,\text{PCM}} = V_{\text{PCM}}/V_{\text{inner cage}}$$

$$= (m_{\text{real, PCM}} / \rho_{\text{PCM}}) \times (\rho_{\text{PCM}} / m_{\text{ideal, PCM}})$$

$$= f_{\text{real}} / f_{\text{ideal}} = 3.4/4.05 = 0.84$$

Thus, PCM occupies 84% of the interior volume of the AuNC.

$V_{f,\text{PCM}}$: volume fraction of PCM in AuNC

V_{PCM}: volume occupied by PCM in AuNC

$V_{\text{inner cage}}$: interior volume of AuNC

$m_{\text{real, PCM}}$: weight of PCM measured from TGA analysis

$m_{\text{ideal, PCM}}$: weight of PCM calculated from the value of edge length and wall thickness of AuNC

f_{real}: weight fraction of PCM in AuNC from TGA analysis

f_{ideal}: weight fraction of PCM calculated from the value of edge length and wall thickness of AuNC

Estimate of loading capacity of Rhodamine 6G and methylene blue in Au nanocages

To obtain the mole of dye in each AuNC, a sample of AuNCs loaded with PCM and dye was added into methanol to fully dissolve the PCM and release all the dye molecules from the AuNCs. The concentration of AuNCs we used was about 1.2 nM and there were 7.2×10^{12} nanocages in each sample. The total mole of dye in the sample ($N_{dye, total}$) was obtained by comparing its peak absorbance with a calibration curve:

$$N_{R6G, total} = 5.81 \times 10^{-7} \text{ mol}$$
$$N_{MB, total} = 3.35 \times 10^{-7} \text{ mol}$$

Therefore, the mole of dye molecules encapsulated in each AuNC can be calculated as:

$$N_{Au \ cage} = 7.2 \times 10^{12} \text{ per sample}$$
$$N_{R6G} = N_{R6G, total}/N_{Au \ cage} = 8.06 \times 10^{-20} \text{ mol/cage}$$
$$N_{MB} = N_{MB, total}/N_{Au \ cage} = 4.65 \times 10^{-20} \text{ mol/cage}$$

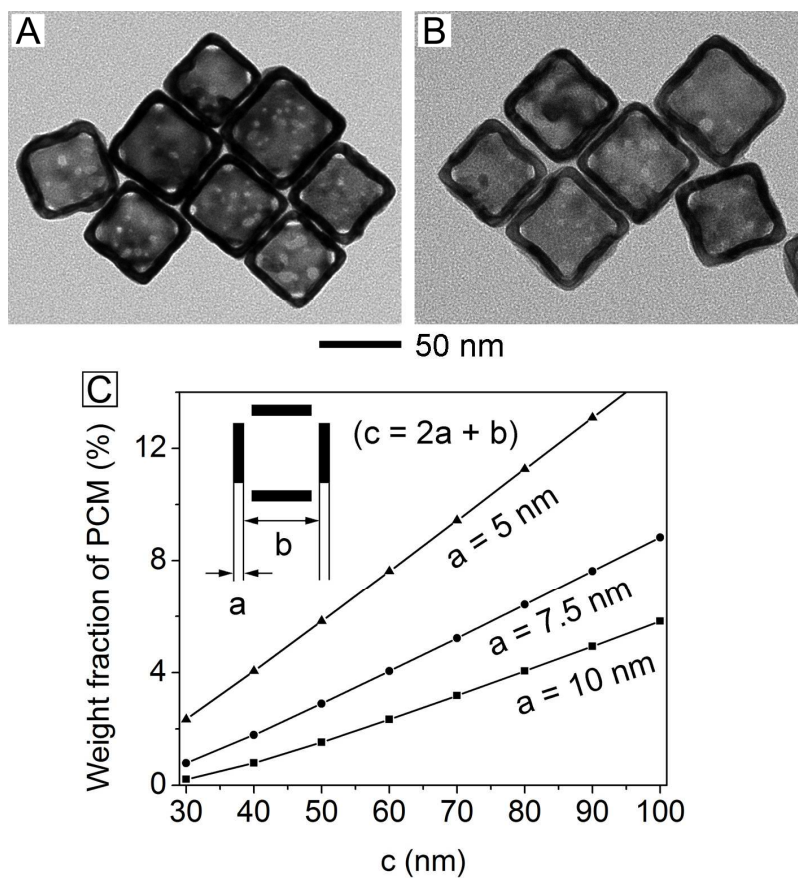These data indicate that the loading capacity of MB was about 57% of that of R6G.

Figure S1. TEM images of (A) the as-prepared AuNCs and (B) AuNCs after loading with 1-tetradecanol and R6G. (C) Calculated weight fraction of 1-tetradecanol in a AuNC as a function of the edge length (*c*) and wall thickness (*a*) of the nanocage.
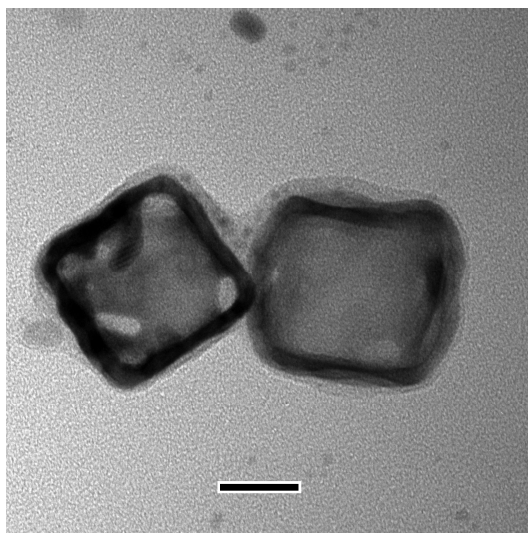
Figure S2. TEM image of some of the AuNCs loaded with PCM/dye after re-dispersion in water under brief sonication (for 10 sec), showing that a very small portion of 1-tetradecanol could leak out from the interiors of the AuNCs due to sonication (scale bar: 20 nm).
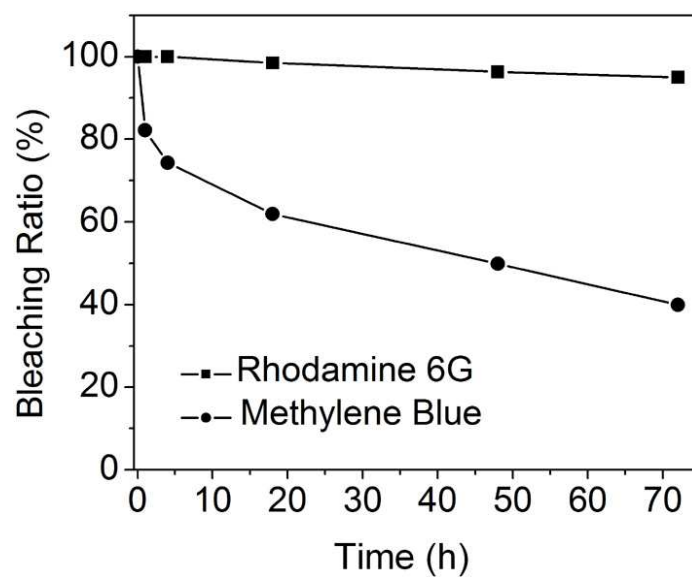
Figure S3. Photobleaching ratio of R6G and MB in aqueous solutions as a function of exposure time at room temperature.
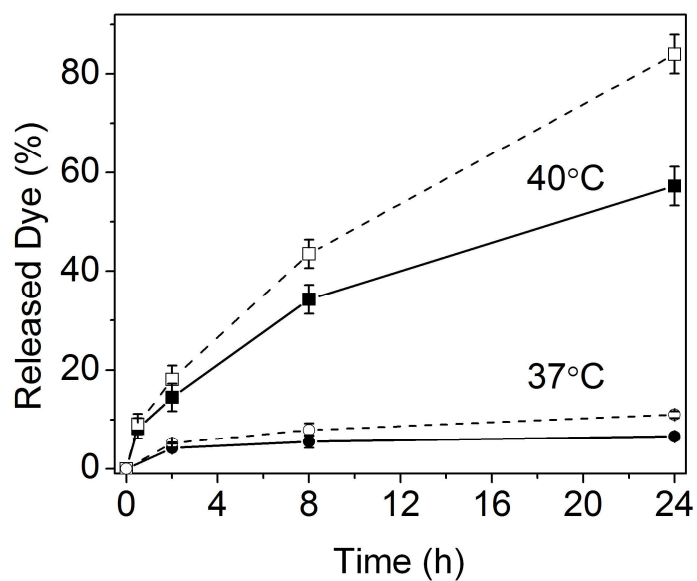
Figure S4. Release profiles of MB at 37 °C and 40 °C. The solid lines represent experimental data and the dashed lines are the release profiles corrected by considering photobleaching of MB.
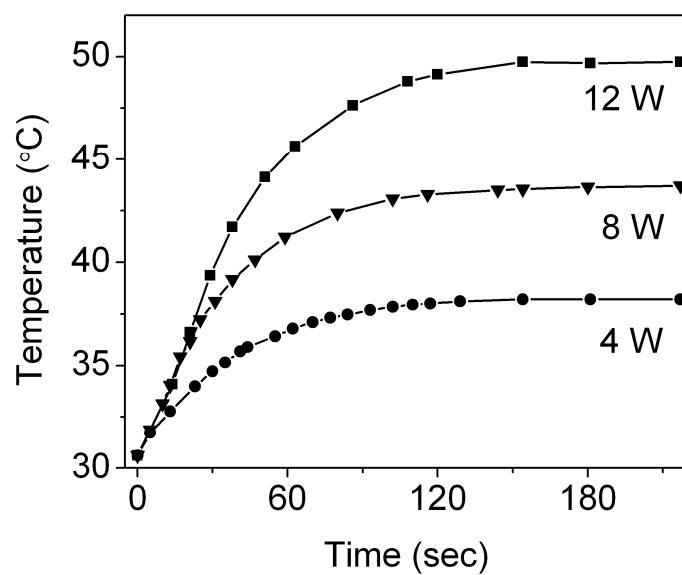
Figure S5. The changes in temperature for aqueous suspensions (0.2 nM) of AuNCs loaded with PCM and dye after irradiated by HIFU at different powers for different periods of time.