Supporting Information

1. Materials and characterizations

1.1 Materials

Silver trifluoroacetate (CF_3COOAg), poly(vinyl pyrrolidone) (PVP, MW≈55,000), sodium hydrosulfide (NaHS), gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2-thiobarbituric acid (TBA), cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), trypan blue solution (0.4%), rhodamine 6G (RG), and protoporphyrin IX (PPIX) were obtained from Sigma-Aldrich. Lauric acid (97%) was purchased from Spectrum Chemical Mfg. Corp. 2,2'-azobis[2-(2-imidazolin-2-yl) propane] dihydrochloride (AIPH) was purchased from Oxchem Corp. Phalloidin-Alexa Fluor 555, 2',7'-dichlorofluorescin diacetate (DCFHDA), 4',6-diamidino-2-phenylindole (DAPI, 1 $mg \cdot mL^{-1}$), Lysotracker Deep Red, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Thermal Fisher Scientific. All chemicals were used without further purification. Red blood cells were supplied by Innovative Research, Inc. Deionized (DI) water (18.2 MΩ.cm) used in all experiments was prepared by Milli-Q system (Millipore, USA).

1.2 Characterizations

Transmission electron microscopy (TEM) images were taken on a Hitachi HT7700 microscope operated at 120 kV. The thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) measurements were conducted using TGA Q50 (TA Instruments) and DSC Q200 (TA Instruments) under nitrogen atmosphere. Fluorescence images were captured using a Leica optical microscope (DMI 6000B).

2. Synthesis of Au nanocages

The Au nanocages (AuNCs) were synthesized by sacrificial galvanic replacement of Ag nanocubes in the presence of $HAuCl_4$ according to the method reported in our previous publication.[1]

3. Loading and releasing of AIPH

For the loading of AIPH into AuNCs, 3 mg of AuNCs was dispersed in a 0.5 mL of methanol solution containing 0.15 g of PCM and 0.2 g of AIPH. After stirring for 5 h, the solution was centrifuged (14,000 rpm, 5 min) and the supernatant was discarded, followed by two times of methanol washes to remove PCM adsorbed on the outer surface of AuNCs. The as-obtained AuNCs were denoted as Au-PCM-AIPH. The loading capacity of AIPH was determined by dissolving the Au-PCM-AIPH in methanol, and the characteristic absorption at 366 nm was measured on a UV-Vis spectrometer (Cary 60, Varian, Monrovia, USA).

The NIR-triggered release of AIPH was performed in an aqueous system. Briefly, 6 mg of Au-PCM-AIPH was dispersed in 2 mL of water. Then, the mixture was irradiated by an 808-nm diode laser (Power Technology Inc.) for a duration of 20 min at a power density of 0.2 or 0.4 $W \cdot cm^{-2}$. The release profiles of AIPH were analyzed by UV-Vis spectrometer post purification with centrifugation (14,000 rpm, 5 min) at different intervals (3, 5, 10, 20 min) after laser irradiation.

4. Generation of ABTS+· free radical

The generation of $ABTS^{+\cdot}$ was performed by taking advantage of the reaction between ABTS aqueous solution (2 $mg \cdot mL^{-1}$, 0.2 mL) and Au-PCM-AIPH aqueous solution (2 $mg \cdot mL^{-1}$, 0.2 mL). The mixture was protected from light irradiation and allowed to proceed for 2, 4, and 6 h at 37 °C or 44 °C. Then, the absorbance of diluted $ABTS^{+\cdot}$ solution (with DI water) in the range from 400 nm to 950 nm was recorded using a UV-Vis spectrometer.

5. Determination of lipid peroxidation

We choose mouse RBCs as a model to investigate the oxidative stress induced by Au-PCM-AIPH nanoparticles. The RBCs were separated from the plasma by centrifugation at 1,500 rpm for 10 min, followed by washing with PBS (pH = 7.4) for three times, and re-suspended in PBS to reach a hematocrit level of 20%.

Lipid peroxidation was evaluated by measuring the production of methane dicarboxylic aldehyde (MDA) based on thiobarbituric acid (TBA) reactivity. In a typical experiment, 0.2 mL of RBCs were mixed with 0.2 mL of Au-PCM-AIPH aqueous solution with varing concentrations. Then the mixture was irradiated with NIR laser for 30 min (0.2 $W \cdot cm^{-2}$), followed by incubation at 37 °C for 2 h. Sample without laser irradiation was used as a control. After adding 1% Triton X-100 (Sigma-Aldrich) for 15 min (4 °C), 200 μL of lysis solution was withdrawn and mixed with 200 μL of TBA solution. The mixture was incubated at 90 °C for 40 min and measured by UV-Vis spectrometer at 532 nm.

6. RBCs oxidative hemolysis

The Au-PCM-AIPH nanoparticles induced erythrocyte hemolysis was also investigated. 0.2 mL of 20% RBCs suspension was incubated with 0.2 mL PBS containing Au-PCM-AIPH or AuNCs (1.5 $mg \cdot mL^{-1}$) for 3 h at 37 and 44 °C respectively. Afterwards, the mixture was diluted with PBS, and the content of hemolysis was determined by measuring the absorbance ranging from 250 to 800 nm using a UV-Vis spectrometer.

7. Cellular uptake

7.1 Cell culture

Human non-small cell lung cancer cell line A549 cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI-1640 culture medium (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (Gibco) and penicillin/streptomycin (100 U/mL, Thermo Fisher Scientific).

7.2 Cellular uptake

To prepare rhodamine 6G-labeled Au-PCM (Au-PCM-RG), 3 mg of AuNCs were dispersed in methanol, followed by the dissolution of lauric acid (0.15 g) and rhodamine 6G (2 mg) under ultrasonication. After centrifugation and gently washing for 3 times, Au-PCM-RG was obtained.

For the cellular uptake, A549 cells (*ca.* 5×10^4 cells) were seeded into Petri-dish (μ-Dishes, ibidi). 24 h later, the cells were incubated with Au-PCM-RG (50 $\mu g\cdot mL^{-1}$) for 1 and 4 h, respectively. Then, the cells were washed and stained with Lysotracker Deep Red. After fixing with 4% formaldehyde, the cells were stained with DAPI and observed using a fluorescence microscope.

8. ROS generation in cells

The intracellular generation of ROS was monitored using 2',7'-dichlorofluorescein diacetate (DCFHDA) probe. Briefly, A549 cells were co-cultured with free AIPH or Au-PCM-AIPH nanoparticles at the AIPH concentration of 20 $\mu g\cdot mL^{-1}$ for 2 h at 37 °C. Afterwards, non-specific absorbed particles were removed by PBS washing for three times, followed by irradiation with an 808-nm laser for 30 min. During the irradiation, the temperature was monitored by an IR camera (FLIR E60) and controlled between 42 °C and 45 °C by adjusting the power density of the laser. Then, the cells were incubated with 10 μM DCFHDA for 30 min at 37 °C, and the distribution of ROS was investigated by fluorescent imaging. For the hypoxic group, cells were incubated in the culture medium containing $CoCl_2$ at the concentration of 100 μM.

9. Membrane blebbing of A549 cells

A549 cells were co-cultured with Au-PCM-AIPH nanoparticles at the AIPH concentration of 20 $\mu g\cdot mL^{-1}$ for 2 h and irradiated with an 808-nm laser for 30 min. During the process, the temperature was controlled between 42 °C and 45 °C by adjusting the power density of the laser. Then the cells were incubated for another 4 and 8 h in normoxic and hypoxic media, respectively. After staining with a lipophilic membrane dye Dil, the cells were imaged with a fluorescence microscope.

10. Cytoskeleton morphology

Phalloidin-Alexa Fluor 555, a marker for actin filaments (red), was used in combination with DAPI (nuclei, blue) for characterization of cytoskeleton morphology. A549 cells (*ca.* 5×10^4 cells) were seeded into petri-dish and then incubated overnight to reach a confluence of 80%. After incubation with free AIPH (200 $\mu g\cdot mL^{-1}$) or Au-PCM-AIPH (AIPH: 20 $\mu g\cdot mL^{-1}$) for 2 h at 37 °C, the

experimental group were then irradiated with an 808-nm laser for a duration of 10 min or 30 min, followed by culturing for another 12 h. During the NIR irradiation, the temperature was controlled between 42 °C and 45 °C. For hypoxic cells, they were treated under identical conditions except the presence of hypoxia-mimic culture medium (containing 100 μM $CoCl_2$). For the staining, cells were first fixed with 4% paraformaldehyde for 20 min, and permeabilized with 0.1% Triton X-100 for 5 min. Thereafter, the F-actin filament and nucleus of the cells were labeled with Phalloidin-Alexa Fluor 555 and DAPI, respectively. The morphology of the cells was imaged using a fluorescence microscope.

11. Cytotoxicity evaluation of Au-PCM-AIPH nanoparticles

To determine the cytotoxicity, A549 cells (1×10^4 cells per well) were seeded into a 96-well plate and incubated for 24 h at 37 °C. After being rinsed with PBS, these cells were incubated with free AIPH and Au-PCM-AIPH at various concentrations for 2 h at 37 °C. Afterwards, the cells were irradiated with an 808-nm laser for 20 min. During the irradiation process, the temperature was monitored by an IR camera and controlled between 42 °C and 45 °C by adjusting the power density of the laser. Then the cells were cultured for another 12 h. Cells without being irradiated by NIR laser were taken as control group. Cell viability was determined using a standard MTT assay. For the hypoxic group, cells were incubated with the culture medium containing $CoCl_2$ at a concentration of 100 μM.

12. Cytotoxicity evaluation of photodynamic therapy (PDT)

To better understand the therapeutic potential of Au-PCM-AIPH against hypoxic cells, photodynamic therapy based on PPIX was carried out under identical conditions for comparison. The cytotoxicity of PDT exerted on A549 cells in normoxia or hypoxia conditions were evaluated. A549 cells were incubated with PPIX at a concentration of 20 $\mu g\cdot mL^{-1}$ for 2 h in the presence or absence of $CoCl_2$ (100 μM). After washing with PBS, the cells were irradiated with a 405-nm diode laser (Existotem Laser) at a power density of 40 $mW\cdot cm^{-2}$ for a duration of 3 min or 5 min.

Then the cytotoxicity was evaluated by standard MTT assay. Furthermore, a trypan blue exclusion assay was carried out as a straightforward way to determine cell death. Briefly, the treated cells were stained with 0.4% trypan blue for 10 min, followed by PBS washing to remove excess trypan blue, and imaged using a bright-field microscopy.

Reference

[1] S. E. Skrabalak, L. Au, X. Li, Y. Xia, *Nat. Protoc.* **2007**, *2*, 2182-2190.

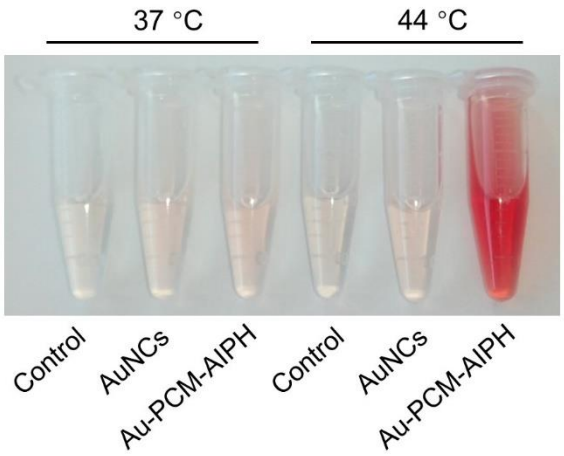

Figure S1. Au-PCM-AIPH induced RBC hemolysis. Photographs of RBCs treated with AuNCs (1.5 mg·mL^{-1}) and Au-PCM-AIPH (1.5 mg·mL^{-1}) at 37 and 44 °C, respectively, for 3 h.

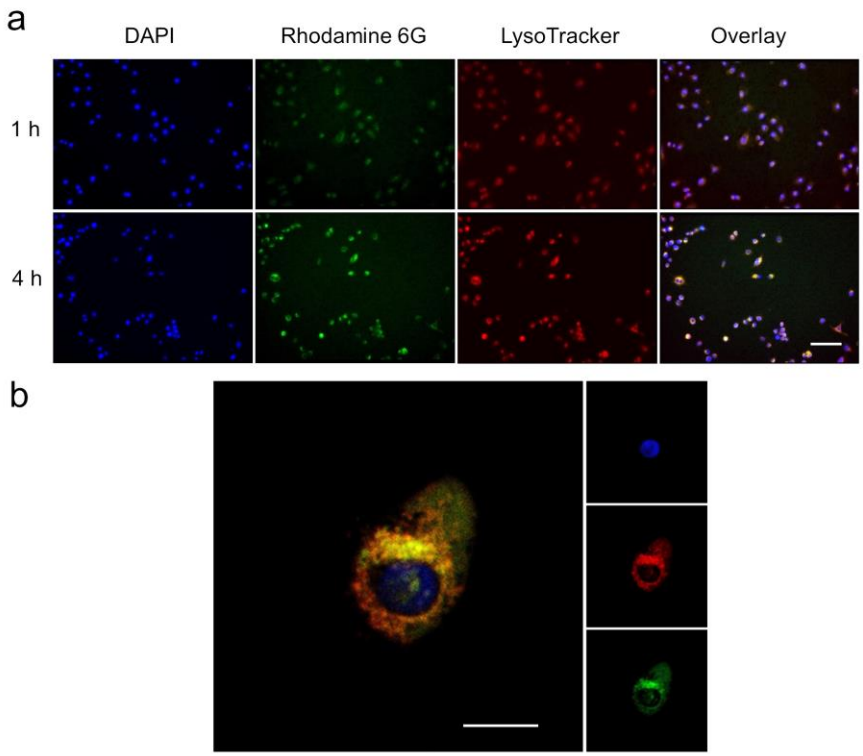

Figure S2. a) Fluorescence images of A549 cells incubated with Au-PCM-RG nanoparticles for 1 and 4 h, respectively. Scale bar =100 μm. b) Fluorescence images with high magnification of A549 cells incubated with Au-PCM-RG nanoparticles for 4 h. Scale bar = 25 μm.

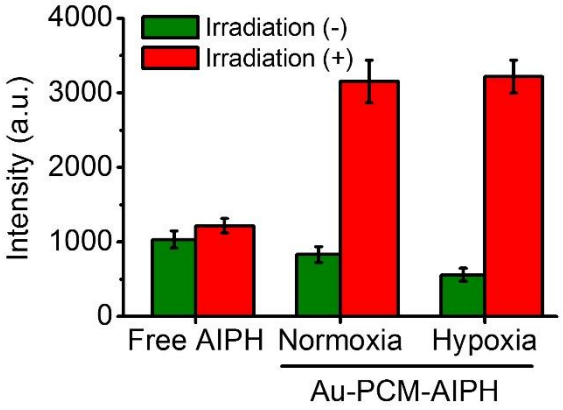

Figure S3. Fluorescence intensity of DCFHDA in A549 cells. Cells were treated with AIPH (20 μg·mL^{-1}) or Au-PCM-AIPH (AIPH: 20 μg·mL^{-1}) in normoxic or hypoxic culture media for 2 h and then irradiated with the NIR laser for 30 min. The values were represented as mean ± standard deviation (n = 3).

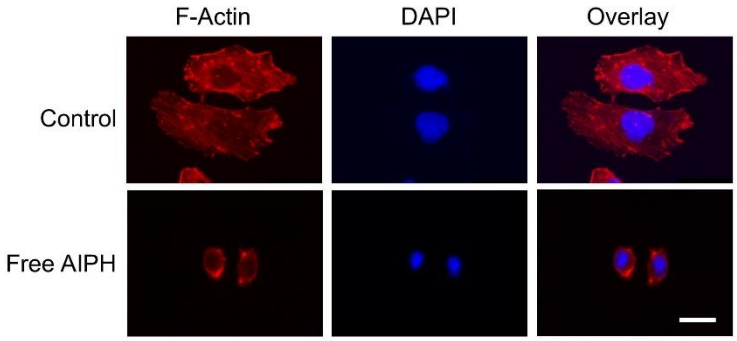

Figure S4. F-actin morphology of A549 cells treated with free AIPH (200 μg·mL^{-1}) for 12 h and then stained with Phalloidin-Alexa Fluor 555 (a F-actin staining dye, red) and DAPI (a nuclear staining dye, blue). Scale bar = 25 μm.

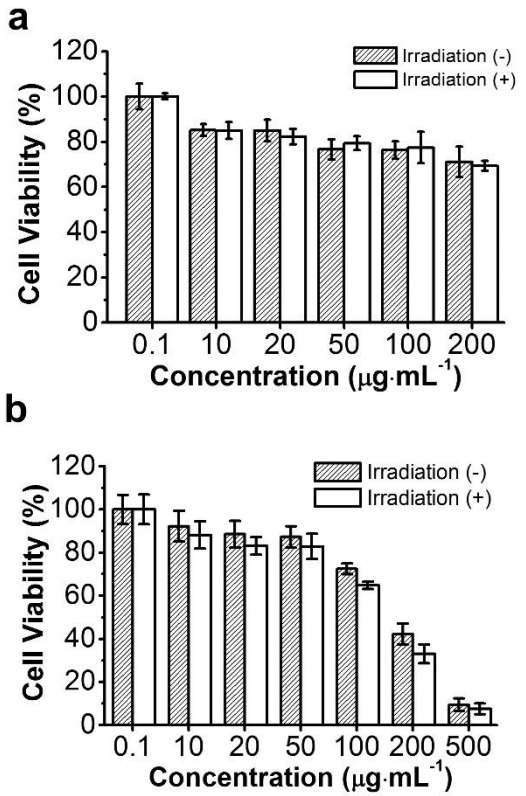

Figure S5. Cell viability of A549 cells after incubation with (a) pristine AuNCs and (b) free AIPH with and without NIR laser irradiation. The temperature was monitored by an IR camera and controlled between 42 °C and 45 °C by adjusting the laser power. The values were represented as mean ± standard deviation (n = 6).

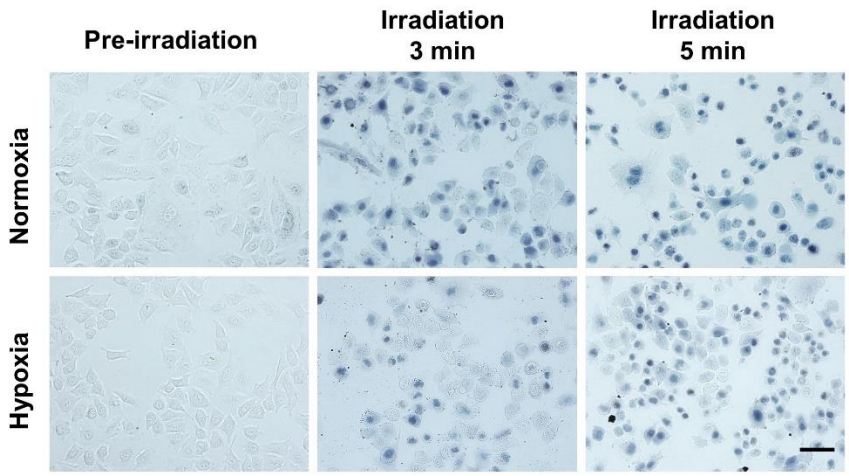

Figure S6. Optical micrographs of A549 cells incubated with 20 μg·mL^{-1} PPIX for 2 h in hypoxic or normoxic media and then irradiated with a 405-nm laser at a power density of 40 mW·cm^{-2} for 3 and 5 min, respectively. Blue color indicates dead cells (Trypan blue staining). Scale bar = 60 μm.