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SUPPLEMENTARY INFORMATION

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Supplementary Information for "Gold Nanocages Covered by Smart Polymers for Controlled Release with Near-Infrared Light"

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Chemicals & Materials

The monomer, *N*-isopropylacrylamide (NIPAAm, 99%) was obtained from Sigma-Aldrich and re-crystallized in hexane prior to use. Acrylamide (AAm, 99%) was purchased from Sigma-Aldrich and recrystallized in methanol before use. Copper(I) bromide (CuBr), *N,N,N',N',N'*-pentamethyldiethylenetriamine (PMDETA), poly(ethylene glycol) methyl ether (MPEG, M_W≈5,000), *N,N'*-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), doxorubicin hydrochloride (Dox), 5-(3-nitrophenylazo) salicylic acid sodium salt (alizarin yellow), and diethyl ether were obtained from Sigma-Aldrich and used as received. Tetrahydrofuran (THF), methanol, acetic acid, and cellulose dialysis tubes were obtained from Fisher Scientific and used as received. The disulfide-containing initiator, bis(2-hydroxyethyl) disulfidebis(2-bromo propionate) (BHEDS$(BP)_2$) was synthesized according to the literature (see Ref. 13 of the main text). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) and Dulbecco's Phosphate Buffered Saline (DPBS) were purchased from Invitrogen and used as received. Micro BCA Protein Assay kit was obtained from Thermo Scientific and used as received. Lysozyme (from chicken egg white) and *Micrococcus Lysodeikticus* (lyophilized, washed with 0.01 M EDTA at pH=7.0 and then 0.15 M NaCl) were obtained from Sigma-Aldrich and used as received.

Instrumentation

A tunable Ti:sapphire laser (LT-2211A, LOTIS TII) pumped by a Q-switched Nd:YAG laser

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(LS-2137/2, LOTIS TII) was used as the light source for the drug-release experiments. The laser beam had a ~6.5 ns pulse duration and a 10 Hz pulse-repetition rate. The output wavelengths range from 730 to 820 nm, with a peak at 790 nm. The average power of the laser could be tuned in the range of 0.2-0.4 W. The energy was attenuated by several optical components for the laser-triggered release. In addition, a ground glass with 660 grit number (from Thorlabs) was used to expand the laser beam homogeneously. In all experiments, the average power was kept constant, and the power density was adjusted by controlling the beam diameter. The power density was controlled in the range of 10-40 mW/cm^2, corresponding to an energy density of 1-4 mJ/cm^2, which are well below the safety limit (20 mJ/cm^2) of pulse energy for skin at 800 nm in the absence of any contrast agent (see American National Standard for Safe Use of Lasers: ANSI Z136.1-2000). A UV-Vis-NIR spectrometer (Cary 50 Bio, Varian Inc.) was used to obtain the absorbance spectra.

For gel permeation chromatography (GPC) measurements, the polymer sample (10 mg) was dissolved in 5 mL of tetrahydrofuran (THF), followed by filtering over a nonsterile PTFE filter with a pore size of 0.45 μm. The measurements were performed at 34 ^oC with a Waters 150-C Plus GPC equipped with a Waters 410 differential refractometer, a Waters 2487 dual wavelength absorbance UV-vis detector set to 254 nm, a Polymer Laboratories PL-ELS 1000 evaporative light scattering detector, and a Jordi Gel DVB 105 Å, a PL Gel 104 Å, a Jordi Gel DVB 100 Å, and a Waters Ultrastyragel 500 Å column setup. THF was used as an eluent at a flow rate of 3 mL/min. Number-average (M_n) and weight-average (M_w) molecular weights were determined from calibration plots constructed with polystyrene standards.

We characterized the polymer-covered nanocages by thermal gravimetric analysis (TGA) using Hi-Res TGA 2950. The analysis measures weight loss as a function of temperature or time. The sample was added to a platinum pan and then the pan was placed on the sample holder of the TGA instrument. Water in the sample was removed by ramping at 10 ^oC/min to 120 ^oC and holding isothermally for 20 min under argon. After the sample had been cooled down to 50 ^oC, the TGA measurement was recorded by ramping at 10 ^oC/min to 525 ^oC under argon. The decomposition temperature of the copolymer was measured to be around 415 ^oC. The

hydrodynamic diameters of the polymer-covered nanocages were measured using dynamic light scattering (DSL) with a Zetasizer Nano-ZS from Malvern Instruments.

Cell Culture Study

The cultured SK-BR-3 breast cancer cells were transferred to 12 wells of a 24-well plate and kept at 37 ^{o}C before use. Each well contained 4×10^{4} cells in 1.5 mL of DPBS. Half an hour before the addition of Dox-loaded Au nanocages to the wells and after the releasing experiments, the plate was kept at 37 ^{o}C, below the LCST (39 ^{o}C) of the copolymer. Each experiment was repeated three times. A total of 6 wells were used for the controls (C-1 and C-2). **C-1**: with laser irradiation in the absence of Au nanocages at a power density of 20 mW/cm^2 for 2 min; **C-2**: with laser irradiation in the presence of empty Au nanocages at a power density of 20 mW/cm^2 for 2 min. Dox-loaded Au nanocages were added to the next 6 wells. The cells were exposed to laser for 2 min and 5 min at a fixed power density of 20 mW/cm^2. After exposure to the laser, the samples were incubated for 24 h. Subsequently, 30 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and 270 μL of the medium were added to each well. After incubation for 3 h, the supernatant in each well was aspirated. 300 μL of isopropanol was added to each well before the plate was examined using a microplate reader (Infinite F200 series, Tecan Group Ltd.). Cell viability was determined from the absorbance at 560 nm relative to the controls. In this case, the absorbance represents the amount of formazan formed due to the reaction between the mitochondria of viable cells and the MTT assay.

Bioactivity Test for the Released Lysozyme

The bioactivity of lysozyme was determined from the rate of lysis for *Micrococcus lysodeikticus*. 0.1 mL of DPBS buffer containing 0.05 mg *Micrococcus Lysodeikticus* was added to a 96-well plate. 0.1 mL of native and released lysozymes (at the same concentration) was added to the wells containing *Micrococcus Lysodeikticus*, and the samples were immediately examined using a microplate reader. The absorbance at 450 nm was recorded every minute. We then plotted the absorbance at 450 nm against the concentration of lysozyme for both native and released lyszoymes. The ratio of the slopes for these two linear fits provides the percentage of enzyme bioactivity after going through the loading and releasing processes.

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Potential Rupture of the Au-Thiolate Bond and thus Desorption of the Polymer from the Surface of Gold Nanocages during Exposure to a Pulsed Laser

It has been reported by a number of studies (*J. Am. Chem. Soc.*, **2006**, *128*, 2426; *J. Am. Chem. Soc.*, **2006**, *128*, 3709; and *ACS Nano*, **2009**, *3*, 80) that the Au-thiolate bond could be ruptured to cause desorption for the thiolate molecules during exposure to a pulsed laser. In the present work, the polymer on Au nanocages did not come off during laser irradiation due to the use of a much lower power density. In our study, we typically used an energy density of 1 mJ/cm^2 in the nanosecond regime and the peak power density of each pulse was on the order of 10^5 W/cm^2. For all other studies mentioned above, the energy density in the sub-picosecond regime that caused the Au-thiolate bond to dissociate was 38, 0.8, and 1.68 mJ/cm^2, respectively. In addition, their peak power density of each pulse was in the range of 10^9 to 10^{11} W/cm^2. Essentially, their peak power densities were 4-6 orders of magnitude higher than the nanosecond pulse we used in the present work. We suspect that the high peak power density generated by the sub-picosecond pulsed laser is responsible for the observed dissociation of the Au-thiolate bond on an electron-phonon coupling time scale (typically within ~1 ps for Au, as reported in another study, *J. Am. Chem. Soc.*, **2006**, *128*, 2426). To our knowledge, that is no report on the dissociation of the Au-thiolate by exposure to a nanosecond pulsed laser. The melting of our Au nanocages at an energy density of 4 mJ/cm^2 may cause some of the thiolate molecules to desorb from the surface of the Au nanocages but this energy density is much higher than the range of 1-2 mJ/cm^2 we typically used for the laser-triggered release.

A Plausible Mechanism for the Laser Trigged Release

For our system, we assume that each Au nanocage has essentially a "monolayer" of pNIPAAm on its surface. Since each polymer chain is tethered to the nanocage via a Au-thiolate bond, the time scales associated with the phase transition should be consistent with the phase transition of a polymer chain rather than a network of polymer chains like in a smart "gel" system (where a phase separation is involved). That said we can estimate the time constant (τ) for the polymer folding, or collapse, according to the diffusion-collision model [1, 2]:

$$\tau^{-1} \sim 3DR_{MIN} / (R_{MAX})^3$$

where R_{MAX} and R_{MIN} are the hydrodynamic radii of the coiled and globular states of the pNIPAAm chain on the nanocage (and these we determined from DLS measurements to be 24 and 17 nm, respectively), and D is the diffusion constant associated with the polymer chain. From the parameters of the laser system we used, we estimate that the nanocages will be above the LSCT for about 6.5 ns during the irradiation of each pulse. Solving for D we find that the pNIPAAm chain would need a diffusion constant of 3×10^{-7} m^2 s^{-1}. We note that typical values of D for pNIPAAm in solution are 10^{-8}-10^{-10} m^2 s^{-1} [3]. This difference between D is likely due to the fact our polymers are tethered to a solid surface. This has two important effects: (i) we can assume that each polymer chain is more or less isolated from each other as we have formed a "monolayer" on the nanocage, and (ii) the surface of the nanocage will stabilize the hydrophobic globular conformation of the polymer as it limits its contact with the surrounding water. These could reduce the folding time, or increase the polymer's diffusion, as the polymers are not overlapped and the energy barrier to the globular state may be lowered. Recent experiments show that the coil-globular transition of pNIPAAm occurs in solution on timescales near 30 μs [3]. We suspect that surface confinement of the pNIPAAm chains will result in faster phase transition, although we admit that transitions on the nanosecond timescale are not well-known. However, nanoscale heat transport is a very new concept and a recent report suggests that the heat propagated through the monolayer coating on a nanoparticle via molecular vibrations on the picosecond timescale, forcing the molecules to change conformation [4]. Once the polymer chain is in the globular state, it might take a longer timescale to go back to the coil state so the pores on the Au nanocage can remain open for a certain period of time. There might be other mechanisms responsible for the observed release under irradiation by a pulsed laser. More fundamental studies are needed in order to completely resolve this issue.

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[4] J. A. Carter, Z. Wang, D. D. Dlott, *J. Phys. Chem. A*, **2008**, 112, 3523.

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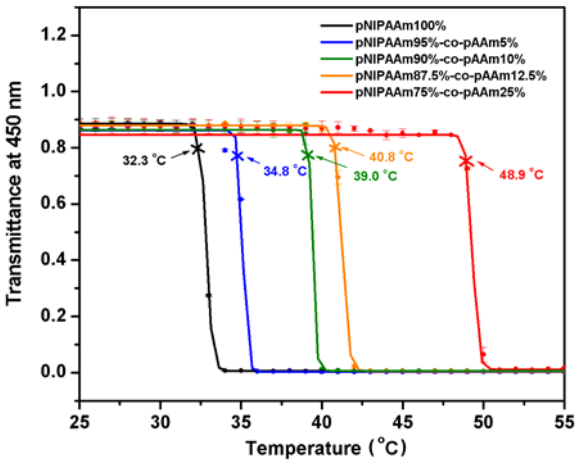

Figure S1 The LCST (or cloud point) measured spectrosphotometrically with the solution being heated at a rate of 1 ^{o}C/min. The temperature at 90% light transmittance (at 450 nm) of the original polymer solution was defined as the LCST.

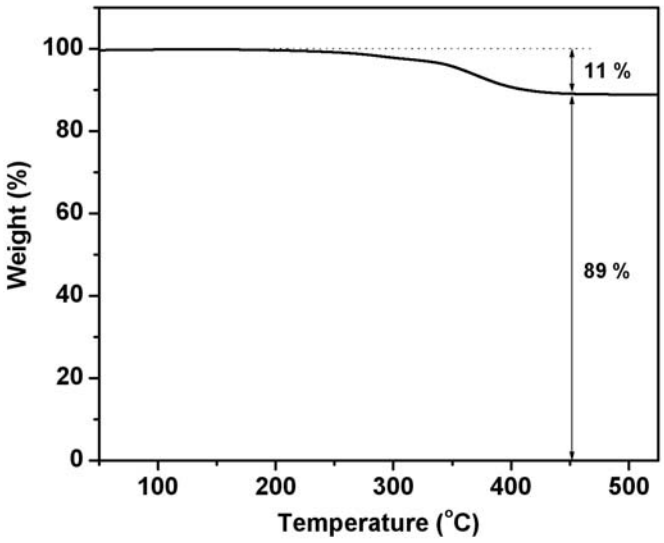

Figure S2 TGA analysis of the copolymer-covered Au nanocages. The 11% weight loss up till 450 ^{o}C corresponds to the desorption and decomposition of the copolymer.

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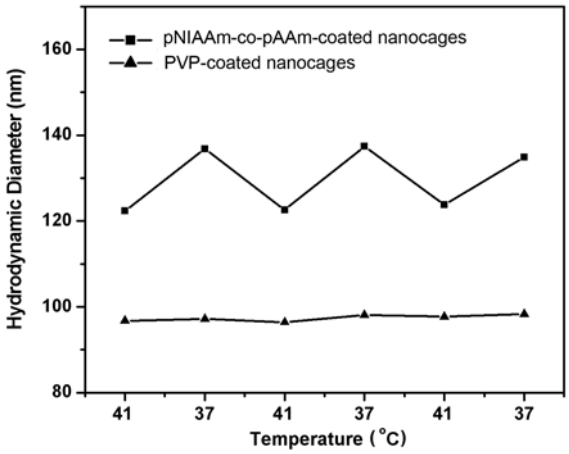

Figure S3 Plots of dynamic light scattering data showing the mean hydrodynamic diameters of Au nanocages as a funciton of solution temperature. We tested both Au nanocages covered by PVP and the copolymer. Note that only the copolymer-covered Au nanocages showed reversible size changes in response to temperature variations. The lines were added for aiding visulization purpose only.

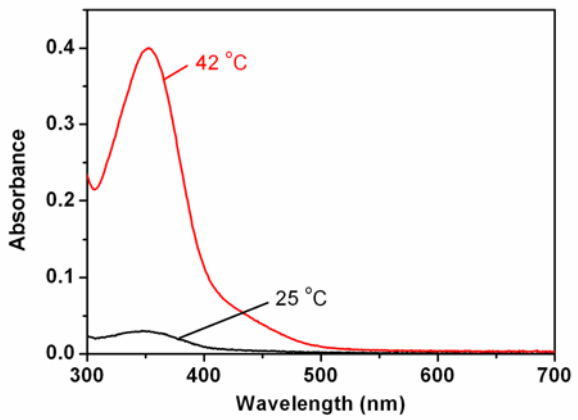

Figure S4 UV-vis absorbance spectra of alizarin-PEG released from the copolymer-covered Au nanocages at two different temperatures: 42 ^{o}C (red) and 25 ^{o}C (black). These results indicate that the release of alizarin-PEG was very negligible under ambient conditions and was increased drastically as the sample was heated to a temperature above the LCST (39 ^{o}C) of the copolymer.

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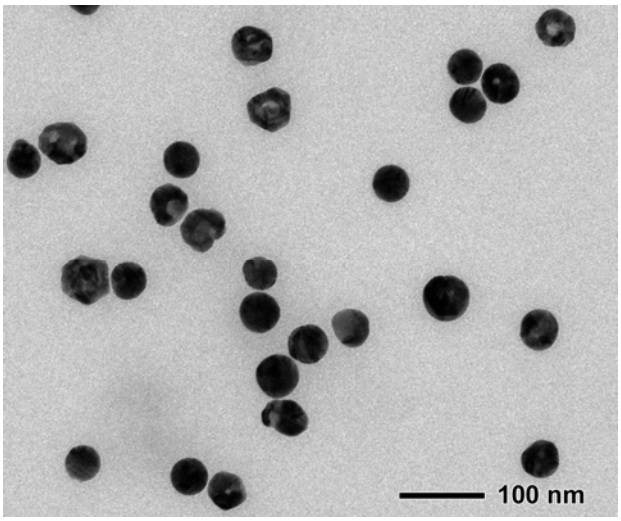

Figure S5 TEM image of melted Au nanocages upon exposure to the laser at a power density of 40 mW/cm^2. In this case, the nanocages have been transformed into more or less solid particles.

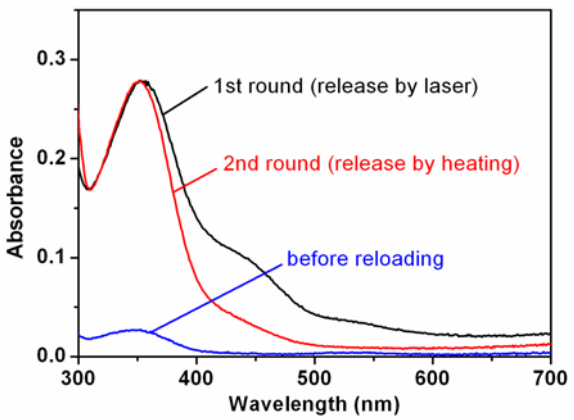

Figure S6 Absorbance spectra of alizarin-PEG released from copolymer-covered Au nanocages. The Au nanocages were used first for dye loading and laser-triggered release (Figure 2b), loaded again and then released by heating.

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Table S1 Characterization of the pNIPAAm and pNIPAAm-*co*-pAAm.

NIPAAm/AAm (molar ratio)	LCST[a] (^{o}C)	M_n ($\times 10^4$)	M_w ($\times 10^4$)	Polydispersity index (PDI=M_w/M_n)
100 : 0	32.3	6.87	12.4	1.80
95 : 5	34.8	6.77	11.9	1.76
90 : 10	39.0	6.93	12.1	1.75
87.5 : 12.5	40.8	7.24	12.8	1.77
75 : 25	48.9	7.89	14.1	1.79

[a]The LCST was determined from Figure S1.