Supporting Information

Plasmonic nanopowders for photothermal therapy of tumors

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S1. Extinction spectra of CTAB-coated GNRs before and after freeze-drying

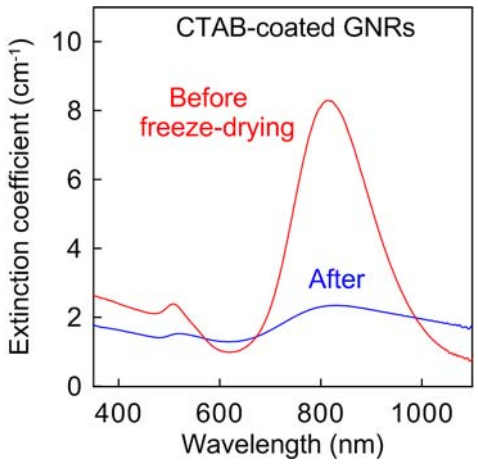

Figure S1. Extinction spectra of as-prepared CTAB-coated GNRs and those after freeze-drying, redispersion in water, and sonication.

S2. TEM images of CTAB- and PEG-coated GNRs before and after freeze-drying

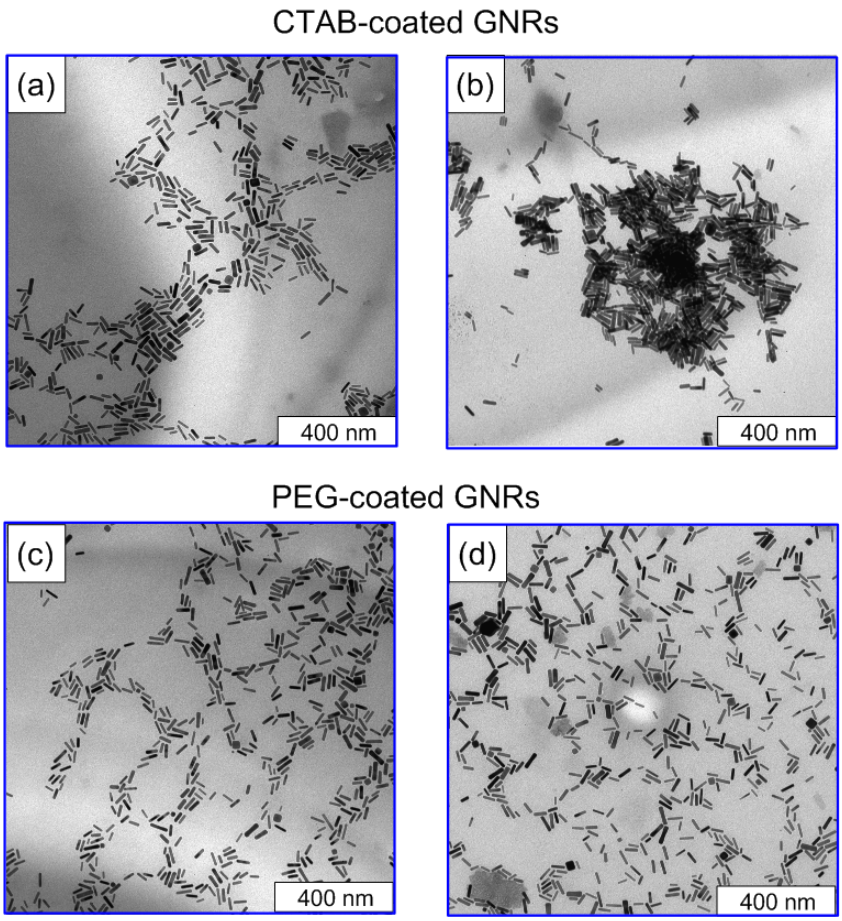

Figure S2. TEM images of CTAB-coated (a, b) and PEG-coated (c, d) GNRs before (a, c) and after (b, d) freeze-drying and redispersion in water.

S3. Fabrication of silver nanocubes. Au–Ag nanocages were prepared by a two-step process previously reported by Skrabalak *et al.*,[1] with a minor modification.[2] In the first step, Ag nanocube templates were prepared. Briefly, 30 mL of ethylene glycol was added to a 250-mL round-bottomed flask and was heated in an oil bath at 150 °C under magnetic stirring. After 50-min preheating, a flow of argon was introduced at a rate of 1200 mL/min. Ten min after, a sodium sulfide solution in EG (0.35 mL, 3 mM) was quickly injected into the preheated EG solution, followed by injection of a PVP solution in EG (7.5 mL, 20 g/L) and 8 min later by injection of a silver nitrate solution in EG (2.5 mL, 48 g/L). Shortly after the addition of $AgNO_3$, the reaction solution went through four distinct stages of color change, from golden yellow to deep red, reddish gray, and then green ocher within about 30 min. The reaction solution was then quenched by placing the reaction flask in an ice-water bath. The resultant product was washed by

centrifugation (12000 g, 30 min) and was redispersed in acetone, followed by redispersion in ethanol to remove excess EG and PVP. Finally, the Ag nanocubes were redispersed in 40 mL of ethanol. In a typical synthesis, the product contained 0.05 g of solid Ag, corresponding to cubic nanoparticles with an average edge length of 43 ± 5 nm, a number concentration of 1.4×10^{15} L^{-1}, and an extinction coefficient of about 370 cm^{-1} at 454 nm (the resonance optical density of a 1:50 diluted suspension in a 2-mm cuvette equals 0.64).

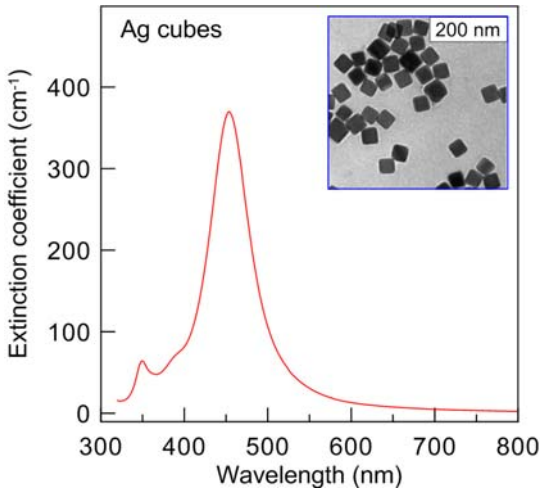

Figure S3. Extinction spectrum and TEM image of as-prepared silver nanocubes.

S4. Fabrication of Au–Ag nanocages. The isolated Ag nanocubes were converted into Au–Ag nanocages *via* the galvanic replacement reaction.[1] Briefly, a PVP water solution (100 mL, 1 g/L) was heated at 100 ^{0}C under magnetic stirring, followed by injection of 2 mL of the as-prepared nanocubes suspension in ethanol. Three min after, 10 mL of a 1mM $HAuCl_4$ solution was added drop by drop during 10 min. The addition of $HAuCl_4$ to the reaction mixture was accompanied by four distinct changes in color, from orange yellow to deep red, purple, and finally light blue. The appearance of a light blue color indicates the successful formation of Au nanocages owing to the galvanic replacement reaction between solid Ag and Au^{3+} ions. The reaction solution was then quenched by placing the reaction flask in an ice-water bath. This was followed by the addition of NH_4OH (0.7 mL, 30% wt) to dissolve solid AgCl formed as a byproduct. The nanocages were washed by centrifugation (12000 g, 20 min) and were redispersed in water three times. Finally, the Au–Ag nanocages were redispersed in 100 mL of

water. In a typical synthesis, the product contained nanocages with an average edge length of about (45–50) ± 5 nm and an extinction coefficient of about 3.5 cm^{-1} at resonance wavelength near 745 nm, depending on the Ag/Au conversion ratio. The predicted concentrations of Ag and Au are 8 mg/L and 15 mg/L, respectively.

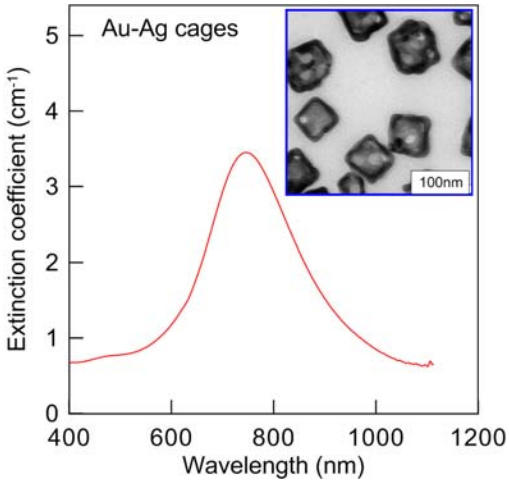

Figure S4. Extinction spectrum and TEM image of as-prepared Au–Ag nanocages.

S5. Synthesis of 56-nm colloidal gold particles

Fifty-nm colloidal gold nanoparticles were prepared by citrate reduction of $HAuCl_4$[3]. Briefly, 100 mL of water was boiled in an Erlenmeyer flask fitted with a reflux condenser, on a magnetic stirrer for 20 min. Then, 1 mL of 1% aqueous $HAuCl_4$ and 0.88 mL of sodium citrate (10 mg/mL) were added sequentially. This resulted in the formation of colloidal gold particles of quasispherical shape at a total mass-volume concentration of 57 mg/L. The extinction coefficient of this colloid was about 2.9 cm^{-1} at 530 nm (Fig. 5S).

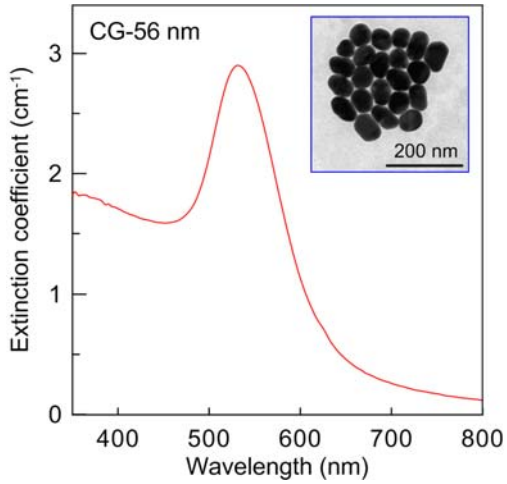

Figure 5S. Extinction spectrum and TEM image of 56-nm colloidal gold nanoparticles.

S6. Implanted Ehrlich carcinoma tumor image

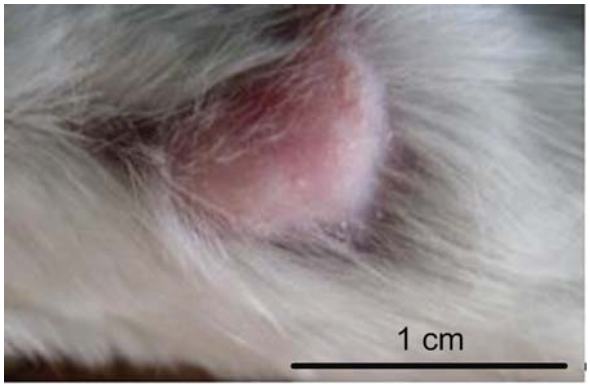

Figure 6S. Ehrlich carcinoma tumor at 14 days after implantation.

S7. Toxicity of PEG-coated GNRs after freeze-drying

To evaluate the toxicity of PEG-coated GNRs after freeze-drying, we examined the viability of HeLa cells (MTT assay) at GNR concentrations ranging from 1 to 100 mg/L. HeLa cells (Saratov Science Research Veterinary Station, Saratov, Russia) were maintained at 37 ^{o}C and 5% CO_2 in complete DMEM medium (Biolot Co., Russia) supplemented with 10% bovine serum, 300 mg/L L-glutamine, 100 mg/L ampicillin, 50 mg/L gentamicin, and 2.5 mg/L amphotercin B (all from Biolot). The cell suspension was centrifuged at 1000 g for 5 min and then was redispersed in Hanks' solution (Biolot). One mL of the cell suspension (10^8 cells/L) was incubated with 0.2 mL of free GNR solutions for 24 h. Cell viability was measured by the MTT assay.[4] In brief, after incubation with GNRs, the cells were centrifuged at 1000 g for 5 min, redispersed in 0.5 mL of MTT solution (Sigma, USA), and incubated for 1 h in the dark at 37º C. Then, the cells were centrifuged at 1000 g for 5 min and were redispersed in 0.5 mL of DMSO (ASC Grade DMSO, Amresco, USA) to dissolve formazan crystals. The samples were centrifuged at 12,000 g for 5 min in 1.5 mL Eppendorf tubes (Eppendorf Minispin), and 0.2 mL of the supernatant liquids was transferred to 96-well plates. Absorbance values at 492 nm were collected on a Multiskan Ascent microplate reader (Thermo Fisher Scientific Inc).

Figure 7S shows that no toxicity of freeze-dried GNRs was observed for all concentrations in the range 1–100 mg/L. The somewhat enhanced MTT activity of the treated cells can be

attributed to experimental errors, and the differences between control and experimental data are within statistical tolerance.

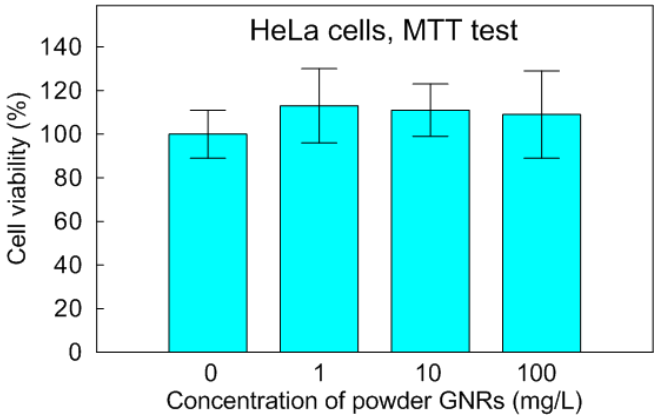

Figure 7S. MTT assay for redispersed freeze-dried GNRs by using HeLa cells.

S8. Video file

The video file "GNR-PEG-SH-Powder.wmv" illustrates convenient work with the fabricated powder including, its transfer from the storage vessel for weighing, redispersion in water, and spectral measurements.

References

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2. Khlebtsov, B.; Panfilova, E.; Khanadeev, V.; Bibikova, O.; Terentyuk, G.; Ivanov, A.; Rumyantseva, V.; Shilov, I.; Ryabova, A.; Loshchenov, V.; Khlebtsov, N. Nanocomposites Containing Silica-Coated Gold–Silver Nanocages and Yb–2,4-Dimethoxyhematoporphyrin: Multifunctional Capability of IR-Luminescence Detection, Photosensitization, and Photothermolysis. *ACS Nano* **2011**, *5*, 7077–7089.

3. Frens, G. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nature Phys. Sci.* **1973**, *241*, 20–22.

4. Oez, S; Platzer, E.; Welte, K. A. A Quantitative Colorimetric Method to Evaluate the Functional State of Human Polymorphonuclear Leukocytes. *Blut* **1990**, *60*, 97–102.