Supporting Information for

Enhanced Photothermal Effect of Plasmonic Nanoparticles Coated with Reduced Graphene Oxide

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I. General

All chemical reagents (graphite flakes, cysteamine, N_2H_4-H_2O, NH_4OH, H_2SO_4, H_3PO_4, $KMnO_4$, $HAuCl_4$, tissue culture grade water) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received without further purification. C-flat™ carbon grids (Electron Microscopy Sciences, Hatfield, PA, USA) and HR-TEM (JEOL-2010, Japan, 200 kV) were used for TEM analysis. A Delsa Nano C particle analyzer (Beckmann Coulter, Danver, MA, USA) was used for particle size and zeta potential measurements. Extinction spectra were obtained with an Agilent 1100 series UV spectrometer. FT-IR spectra were collected using a Bruker Alpha FT-IR system (Billerica, USA). Fluorescence microscopy images were captured using a Olympus FSX 100 Microscope (Pennsylvania, USA). Fiber-coupled laser diodes (LDX 3415-808, RPMC laser Inc., MO, USA) were controlled by a 4320 laser source (Arroyo instruments, San Luis Obispo, USA) and cooled by a 212 DIL LaserMount (Arroyo instruments). The laser beam spot was controlled using collimators (12.7 mm) manufactured by Ceramoptec® (Longmeadow, USA). The solution temperature was monitored by use of a digital precision clinical thermometer (Alpha techniques (3000i), Irvine, USA).

II. Method

II-1. Preparation of nanosized graphene oxide (GO)

GO was prepared by a modified Hummer's method.[1] A 9:1 mixture of concentrated H_2SO_4/H_3PO_4 (360:40 mL) was added to a mixture of graphite flakes (3.0 g, 1 wt equiv). With stirring and cooling in an ice bath, $KMnO_4$ (18.0 g, 6 wt equiv) was slowly added into the reaction mixture, producing a slight exotherm to 35 ~ 40 °C. The reaction was then heated to 50 °C and stirred for 12 h. The reaction was cooled to room temperature and poured onto ice (400 mL) containing 30% H_2O_2 (3 mL). The solution was then filtered through a metal U.S. Standard testing sieve (W.S. Tyler, 300 μm). The filtrate was centrifuged (8,000 rpm for 2 h), and the supernatant was decanted off. The remaining material was then washed in succession with 200 mL of water, 200 mL of 30% HCl, 200 mL of ethanol, and water again until solution pH reached 5.0 ~ 6.0. The resulting suspension was filtered with a PTFE membrane with a 0.45 μm pore size and then lyophilized to produce a fluffy GO powder. GO powder (5.0 mg) was dissolved in triple distilled water (> 18 M**Ω,** 10 mL), and then exfoliated by prolonged sonication

(35% amplitude, 500 W, 2 hr) until the entire size distribution was below 150 nm, then centrifuged (18,000 rpm, 20 min) to remove precipitates (un-exfoliated large GO sheets). The supernatant was analyzed by dynamic light scattering (DLS). DLS analysis (0.05 mg/mL) showed a narrow size distribution (average 60 nm) of GO sheets after centrifugation (**Fig. S2**). Importantly, the nanosized GO sheets were freely dispersible in water without aggregation. The freshly prepared nanosized GO sheets were characterized using high resolution transmission electron microscopy (HR-TEM), UV-Vis spectroscopy, FT-IR spectroscopy (**Figs. S3, S4-A, B)**. The HR-TEM image showed multilayer GO sheets, and the selected-area electron diffraction (SEAD) pattern also revealed a multilayer single crystalline state of prepared GO sheets (**Fig. S3**). Solution based reduction with hydrazine in the presence of trace amount of NH_4OH (28%, w/w) successfully converted GO to the reduced state of graphene oxide (r-GO) as shown by UV-Vis and FT-IR spectra (**Fig. S4**).[2]

II-2. Preparation of GO-AuNS, rGO-AuNS, and GO-AuNR, rGO-AuNR

10 mL of AuNS solution synthesized as previously described[3] (OD = 1.0 at 800 nm) was centrifuged to produce AuNS pellets, then 5 mL of 20 μM of cysteamine solution in DMF was added and incubated for 30 min with brief sonication every 10 min. The solution was centrifuged again, and dispersed in 10 mL of water. The resulting 10 mL of positively charged cysteamine-modified AuNS solution (1.0×10^9 particles/mL, 1.6 pM) was dropped into a large excess of negatively charged nanosized GO solution (0.5 mg/mL, 20 mL), and gently vortexed for 1 hr, centrifuged (4,000 rpm, 15 min, 2 times), and finally redispersed in 10 mL of water. Separately, 10 mL of AuNR solution (as prepared in DW[4]) (3.5×10^{10} particles/mL, 0.056 nM) was dropped into the GO solution (0.5 mg/mL, 20 mL), and incubated for 1 hr, then centrifuged (12,000 rpm, 15 min, 2 times), and redispersed in 10 mL of water. Finally, to reduce the GO layer on the particles, the pH of the GO coated AuNS and AuNR solutions were adjusted between 10.0 – 11.0 using trace amounts of ammonium hydroxide solution (28 %, w/w), then hydrazine hydrate (50 μL, Aldrich) was quickly added to the solution[2]. The reaction mixtures were allowed to stir for 1 hr at 95 °C then cooled, centrifuged, and redispersed in distilled water. The solution color became slightly darker after GO reduction. The reduced GO-coated particle solutions were stable

for a longtime at room temperature without requiring any additional surfactant.

II-3. Preparation of GO-SiO_2 NPs and r-GO SiO_2 NPs.

1.0 mL of amine-modified silica nanoparticles (2.5% (w/w), average size; ~ 118.6 nm, cat #; 140-324-10, Microsphere-Nanosphere, NY, USA), was dispersed in DW, incubated with 2.0 mL of GO solution (0.5 mg/mL, dark brown solution in DW) for 30 min, and then centrifuged (14,000 rpm/15 min) to remove the supernatant (pale yellow solution). The pale yellow color of the supernatant indicated that almost all the GO sheets were bound on amine–modified silica nanoparticles. The resulting yellow pellets were redispersed in DW (2 mL) and centrifuged again to fully remove unbound GO sheets in the supernatant. The resulting pellets were redispersed in DW (10 mL, particle number concentration = 1.425×10^{12} particles/mL). The particle solution changed from white to light yellow, indicative of successful GO layer formation. Next, 5 mL of GO-silica solution was reacted with hydrazine hydrate (20 μL) in the presence of ammonium hydroxide (28%, (w/w)) for 1 hr at 95 °C. The solution was then centrifuged (14,000 rpm/15 min), redispersed in DW, and diluted with DW to adjust particle concentration to 1.0×10^9 or 3.5×10^{10} particles/mL (**Fig. S6**).

III. Analysis

III-1. Raman analysis

Raman spectra of samples were measured using a Kaiser Optical Hololab 5000R Raman Microscope using an excitation wavelength of 785 nm (50 × objective, 50 mW). Sample solutions were deposited on quartz slides, then dried at room temperature. The fluorescence background for the GO spectra was subtracted using the equipment's software.

III-2. MTS assay

The cytotoxicity and time-dependent cell viability were measured using a MTS assay (CellTiter 96® AQueous One Solution Reagent (Promega, Medison, USA)). Human umbilical vein endothelial cells (HUVECs) cells, purchased from Lonza (NJ, USA), were seeded into 96-well plate (1×10^4 cells per well) in EG2 media (Cat. No. CC-3165, Lonza) and grown for 24 h. The media was then removed and EG2 media (200 μL per well) containing NPs at two different

concentrations (1.6 pM for AuNS, 0.056 nM for AuNR) was added to each well. 10% DMSO in EG2 media was used as a positive control (complete cell death) and fresh media was used as a negative control. After 24 h incubation, the media were removed, and replaced with EG2 media (200 μL per well) containing 10% MTS reagent followed by incubation for another 1 h at 37°C, 5% CO_2. The absorbance intensity at 490 nm was recorded by a plate reader (Gen5, Biotek, Winooski, USA).

III-3. Live/dead assay

A live/dead assay (Abcam, ab115347, Cambridge, USA) was used to stain cells in culture media (2X solution of dye in EG2 media), then imaged with a fluorescence microscope (Olympus FSX 100 Microscope, Pennsylvania, USA).

III-4. Statistics.

Statistical analysis was performed using Graphpad prism V5.00 (Graphpad software, Inc., CA, USA). One way analysis of variance (ANOVA) with Bonferroni post-test was used to determine statistical significance. A p-value < 0.05 was considered to be significant.

Figures S1-4 demonstrate the schema for the production process (S1), the formation of nanoscale GO < 150 nm (nano GO, S2), their morphology and crystalline structure (S3), and changes in UV-Vis and FT-IR spectra showing reduction of GO (S4).

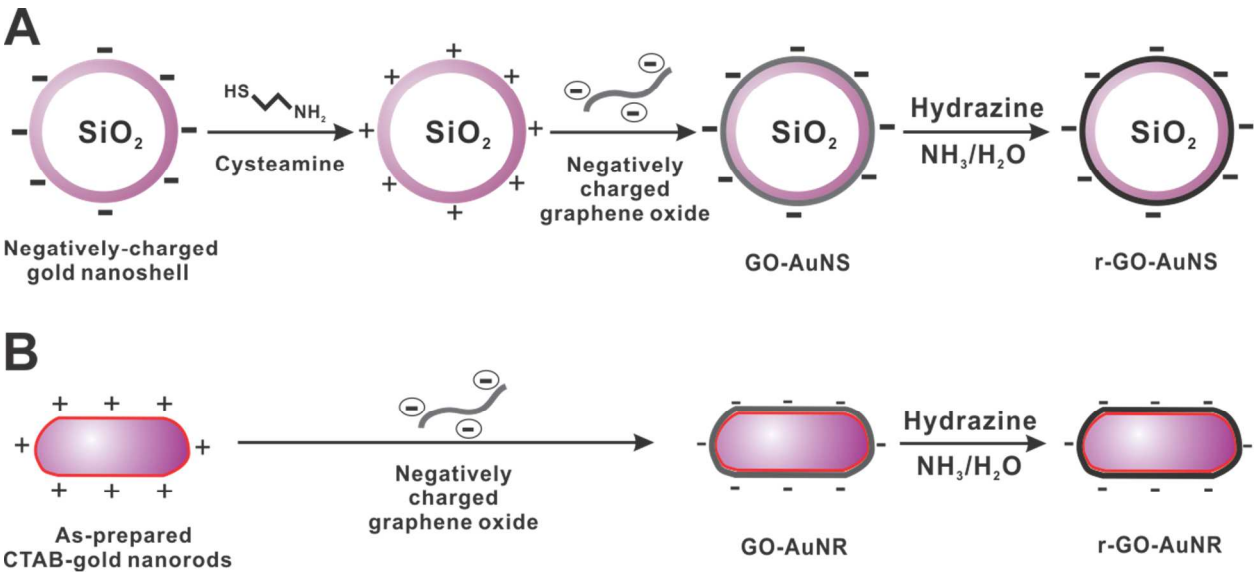

Figure S1. Schema of layer-by-layer procedure to produce (**A**) GO-AuNS and r-GO-AuNS, and (**B**) GO-AuNR and r-GO-AuNR.

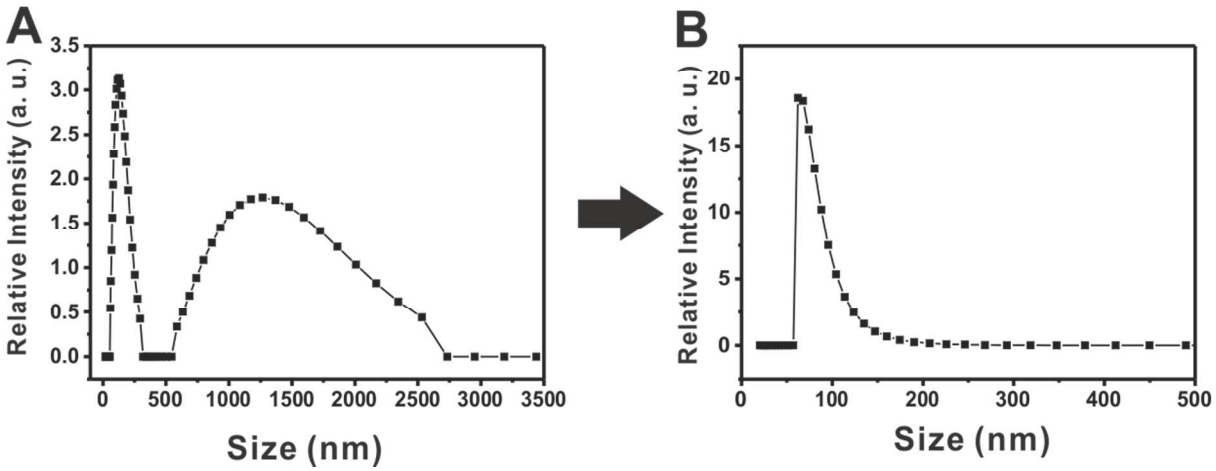

Figure S2. Size distributions of graphene oxide (GO) sheets (0.05 mg/mL in DW) (**A**) before exfoliation, and (**B**) after exfoliation (by sonication, 35% amplitude, 500 W, 2 hr) and purification (by centrifugation at 18,000 rpm for 15 min). Exfoliation produced GO sheets < 150 nm.

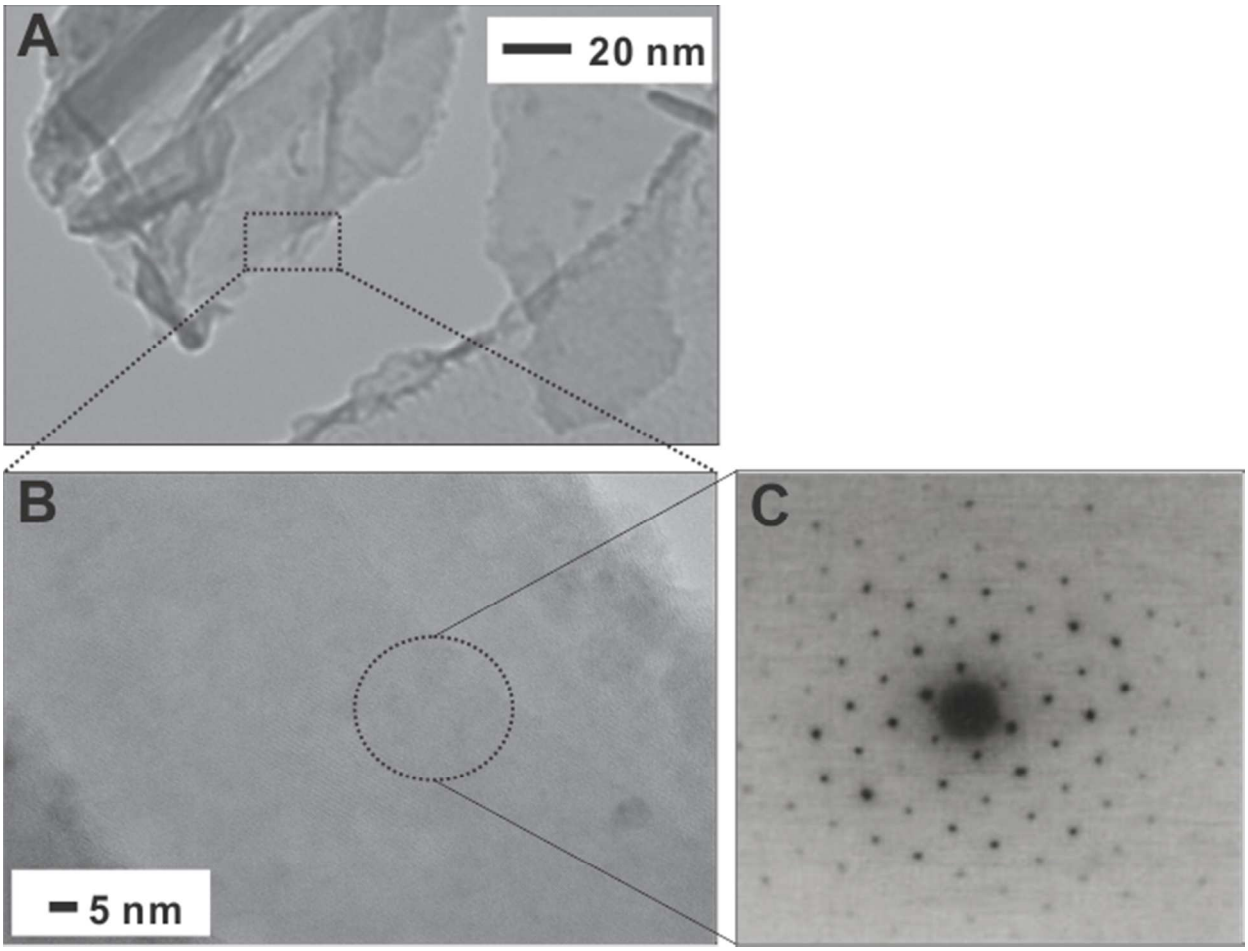

Figure S3. (**A**) HR-TEM imaging of nanosized GO. (**B**) Magnified view of the area in panel A denoted by a dotted square. (**C**) Selected-area electron diffraction (SEAD) pattern of the region in panel B denoted by a dotted circle, showing well-preserved crystalline structure even after extensive exfoliation.

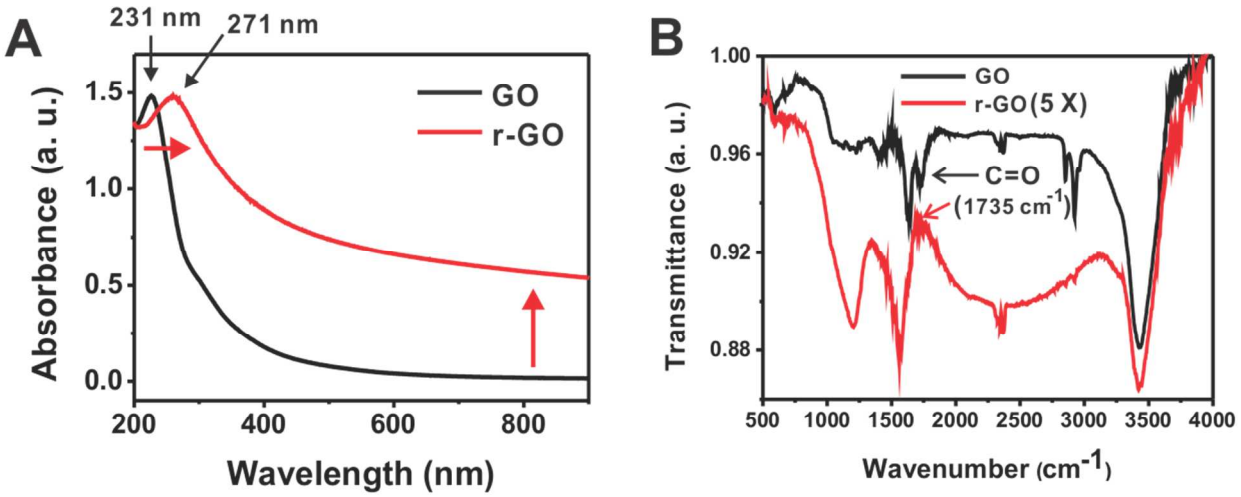

Figure S4. Changes in UV-Vis and FT-IR spectra with reduction of GO. (**A**) UV-Vis and (**B**) FT-IR spectra of nanosized graphene oxide (GO) and chemically reduced graphene oxide (r-GO). **Fig. S3-A** shows a red-shift and increased absorption due to the recovered electronic states of r-GO.[2,5] **Fig. S3-B** shows the disappearance of the C=O peak at 1,735cm which indicates successful formation of r-GO.[2,5] Note that no C=O peak (arrows) can be observed in the r-GO FT-IR spectrum (red line) even though the concentration of r-GO was 5 times higher than that of GO).

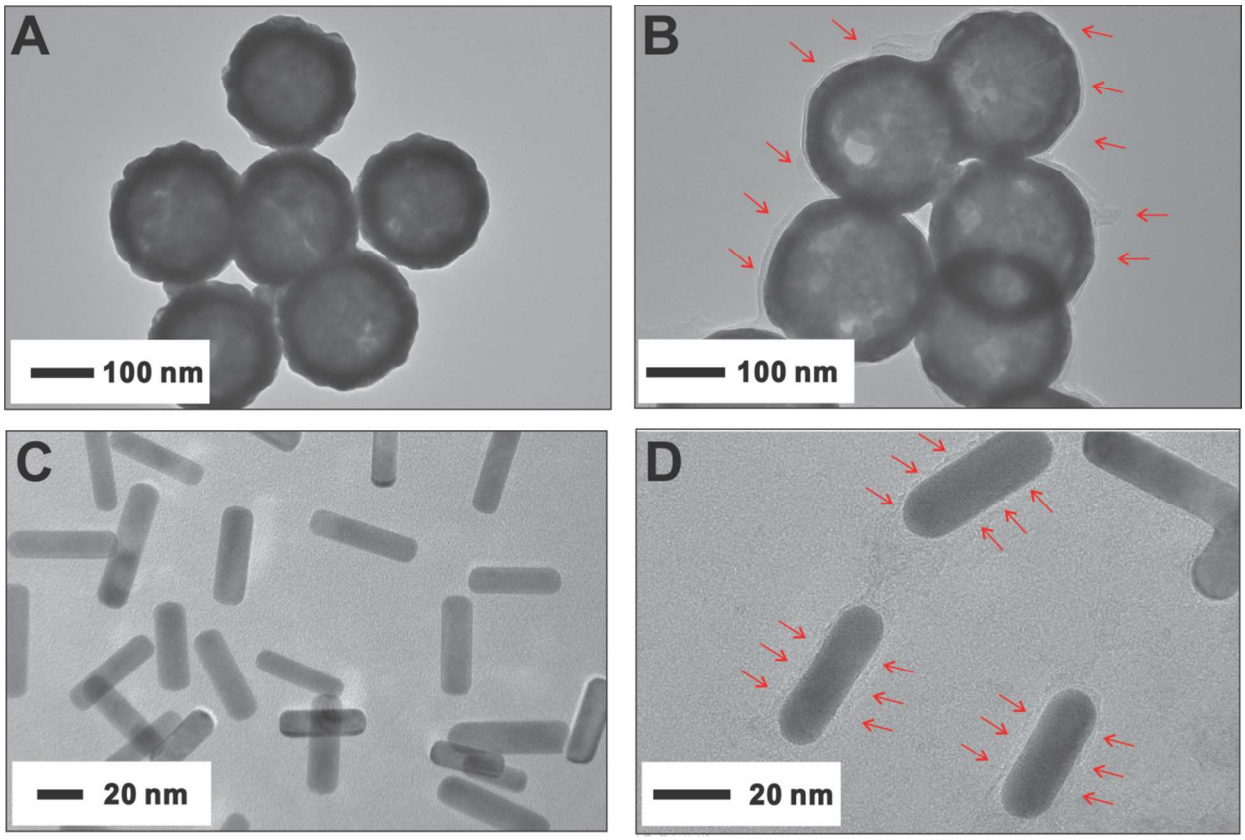

Figure S5. Representative HR-TEM images of (**A**) AuNS, (**B**) r-GO-AuNS, (**C**) AuNR, and (**D**) r-GO-AuNR. Red arrows indicate layers of r-GO on the particles.

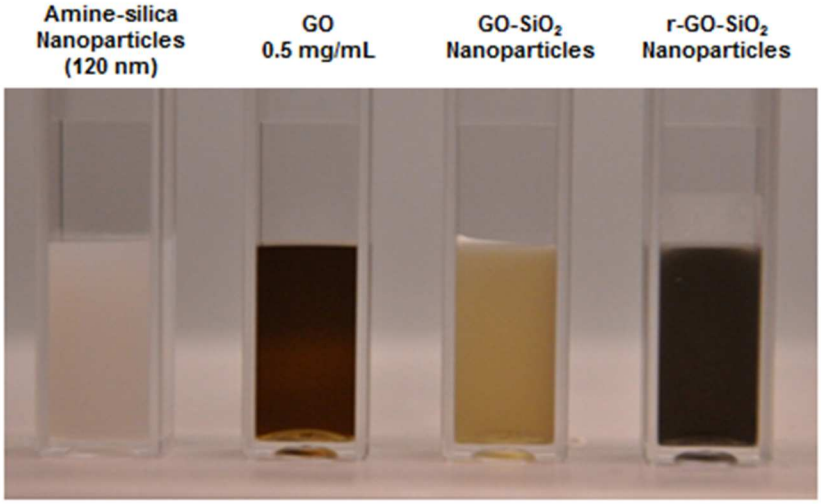

Figure S6. Images of amine-silica nanoparticle (25 mg/mL, 1.4×10^{13} particles/mL), GO solution (0.5 mg/mL), GO-modified silica nanoparticle (1.4×10^{13} particles/mL), and r-GO silica nanoparticle (1.4×10^{13} particles/mL).

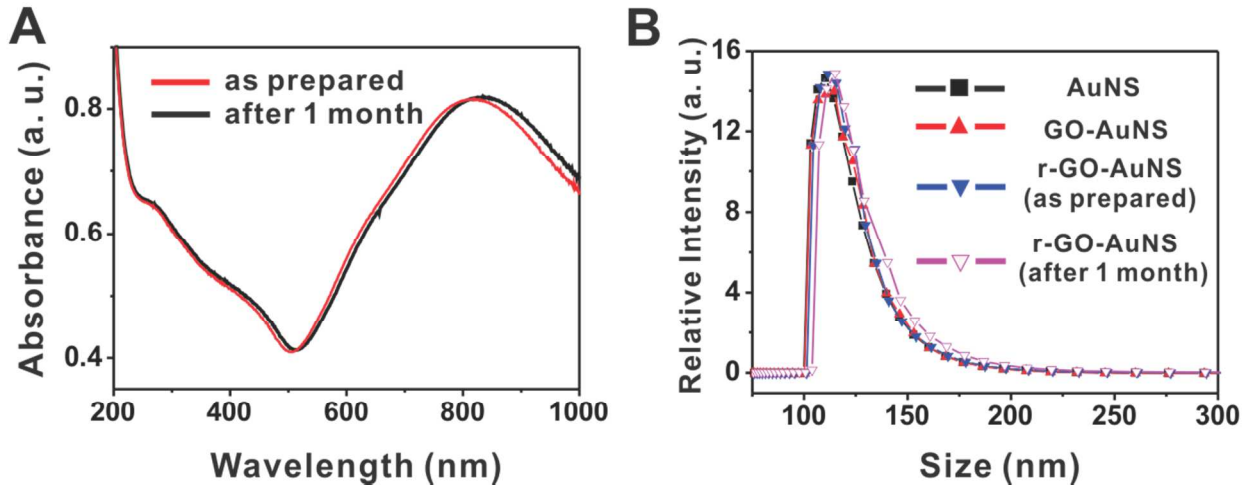

Figure S7. Colloidal stability of r-GO AuNS, (A) UV-Vis spectra of r-GO AuNS in distilled water. Red line: as prepared; back line: after 1 month at room temperature. (B) DLS data of AuNS, GO-AuNS, r-GO AuNS (as prepared), and r-GO AuNS (after 1 month at room temperature).

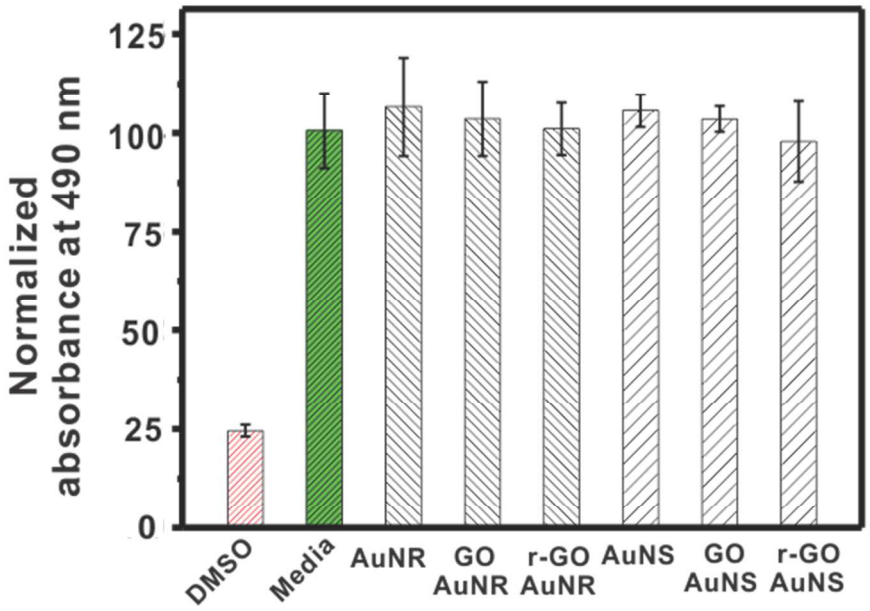

Figure S8. Viability of human vascular endothelial cells exposed to AuNS (OD 1.0, 1.6 pM) and AuNS (OD 1.0, 0.056 nM) with or without modification with GO or r-GO. DMSO: 10 % DMSO, which kills all cells; 25% absorbance reflects no living cells. Media: media without additives. Data are means ± SD; N = 6.

[References]

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