Supplementary Information

Graphene Oxide Wrapped Gold Nanoparticles for Intracellular Raman Imaging and Drug Delivery

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Experimental Section

Materials

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), citrate, 4',6-diamidino-2-phenylindole (DAPI), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin (Dox), dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), formaldehyde, graphite,hydrochloric acid (HCl, 37%), hydrogen peroxide (H_2O_2, 30%), nitric acid (HNO_3, 68%), phosphate buffered saline (PBS) buffer, potassium permanganate($KMnO_4$), sodium hydrochloride (NaOH), sodium tetrahydridoborate ($NaHB_4$), sulfuric acid (H_2SO_4, 98%), and trypsinwere were purchased commercially.

Instruments

Transmission electron microscopy (TEM) images were taken by JEOL 2010 TEM at 200kV. X-Ray diffraction (XRD) pattern was collected by X'Pert powder XRD. Zeta potential value was measured by MavernNanosizer. FT-IR spectra were recordedby Fourier transformed infrared spectrometer. UV-vis spectra were recorded by UV-vis 2501 Spectrometer. Raman spectra and mapping were performed by WITec 488nm Confocal Raman system. A microplate reader (infinite 200 PRO, Tecan) was employed for the MTT assay. Confocal microscopy images were taken by a confocal microscope (Leica TCS SP5, 63× oil objective).

Preparation of nano-size graphene oxide (NGO)

GO was prepared by a modified Hammer's method.[1] Typically, graphite (0.5 g) was suspended in concentrated H_2SO_4 (15 mL) under an ice bath. $KMnO_4$ (1.5g) was added gradually with mild stirring. The mixture was kept stirring at 50 °C for 3h, followed by the addition of distilled water (35mL). After stirring for 15min, distilled water (150 mL) was added to terminate the reaction. Then, H_2O_2 (10mL, 30%) was added and a bright yellow solution was obtained. The mixture was then washed by HCl solution (250mL, 10%) and distilled H_2O. In order to exfoliate the oxidized graphite, the product was treated with an ultrasonic probe at 250W for 1h and centrifuged at 8000rpm for 30min. The supernatant was collected as the GO stock solution. Then, the as-prepared GO solution was refluxed with HNO_3 (5.2M) at 80°C for 24h to re-oxidize the GO. After the mixture solution was cooled down to room temperature, NaOH pallets were slowly added under an ice batch to neutralize the GO solution. Then, the mixture solution was dialyzed (1.4kDa) against de-ionized (DI) H_2O for three days to remove excessive salts. Afterwards, the re-oxidized GO solution was treated with probe sonication at 500W for 2h to cut GO into ultrasmall sheets. Finally, the NGO solution was collected by further filtration through 400nm syringe filter.

Synthesis of Au@NGO nanoparticles

The $HAuCl_4$ solution (1mL, 2.5mM) was mixed with NGO solution (1mL, 0.5mg mL^{-1}) under stirring. The $NaBH_4$ solution (3×100μL, 20mM) was added slowly for three times with 2h interval. Then, the mixture was stirred at room temperature for 24h. The formed Au@NGO nanoparticles were collected by centrifugation and washed by DI H_2O for three times. After a mild sonication for 15min, the Au@NGO solution was filtered through 200nm syringe filter to remove irregular by-products if any.

Confocal Raman spectra and cell image mapping

The Raman study was conducted by using 488nm laser (20mW) for excitation. The Raman spectra were recorded by using a 20×objective with 2s acquisition time. For Raman spectra collected from HeLa cells, cover slides were placed in a 6-well plate. HeLa cells were seeded onto the 6-well plate at a density of 1×10^4 cells/well in DMEM medium and grown on the cover slide for 24h. Then, the cells were treated with various concentrations of Au@NGO nanoparticles for 24h. After that, the cells were washed with PBS for three times, and then transferred onto clean silicon wafer for Raman measurements. For the cell imaging, spatial

Raman mapping was carried out by using a 20×objective with a resolution of 250×250 pixels in an area of 80×80μm. The acquisition time for each pixel is 25ms.

Cell TEM

After HeLa cells were treated with Au@NGO (40 μg mL^{-1}) for 24h, the culture medium was removed and the cells were washed by PBS for three times. Then, the cells were fixed with 2.5% glutaraldehyde (SPI, USA) at 4°C overnight. Hereafter, cells were washed with 0.1% phosphate buffer and post-fixed with 1% osmium tetraoxide (SPI, USA) at room temperaturefor 1 h. Fixed cell pellets were further washed and performed dehydration in increasing grades of ethanol (25%-100%) and pure acetone. Infiltration in SPI-Pon™-Araldite® (SPI, USA) resin was started at room temperature overnight followed by pure resin embedding at 60°C for 72 h. Blocks were sectioned by an ultramicrotome (Leica Ultracut UCT) and ultrathin sections were stained with lead citrate. Grids were viewed under a JEOL 2010 100 kV transmission electron microscope.

Confocal laser scanning microscopy (CLSM) images

Intracellular uptake of Au@NGO nanoparticles and sustained Dox release were investigated using HeLa cells *in vitro*. For the Dox loading, Au@NGO nanoparticles were suspended in concentrated Dox solution (1mg mL^{-1}) and the mixture was stirred for 24h. Then, the Dox loaded Au@NGO nanoparticles were collected by centrifugation and washed by PBS. HeLa cells were first seeded in 6-well plateat a density of 2×10^5 cells/well and grown on a cover slide in the DMEM medium for 24 h. Then, the cells were exposed to Dox loaded Au@NGO nanoparticles (20 μg mL^{-1}) for a certain time. After that, the medium was removed and cells were washed with PBS for three times. The cells were fixed with 4.0% formaldehyde and then the cell nucleus was stained with DAPI (10nM). The cells were further washed with PBS for three times before observation with confocal microscopy.

MTT cytotoxicity assay

HeLa cells were seeded into 96-well plate at a density of 1×10^4 cells/well in DMEM medium and grown for 24h. Then, the cells were treated with Au@NGO, Dox loaded Au@NGO and free Dox for 24h, respectively. After that, the medium was replaced with new medium, and the cells were further cultured for different times (0h, 24h and 48h) before performing the MTT assay. For the MTT assay, the old medium was replaced with new medium (100 μL) containing MTT (0.5mg mL^{-1}). After 4h incubation, the medium was again

replaced with DMSO (100μL). The plate was shaked for 15min before measuring the absorbance intensity at 565 nm by a microplate reader (infinite 200 PRO, Tecan). The cell viability related to the control wells that only contain cell culture medium was calculated by $[A]_{test}/[A]_{control}$, where $[A]_{test}$ and $[A]_{control}$ are the average absorption intensities of the test and control samples (n=8), respectively.

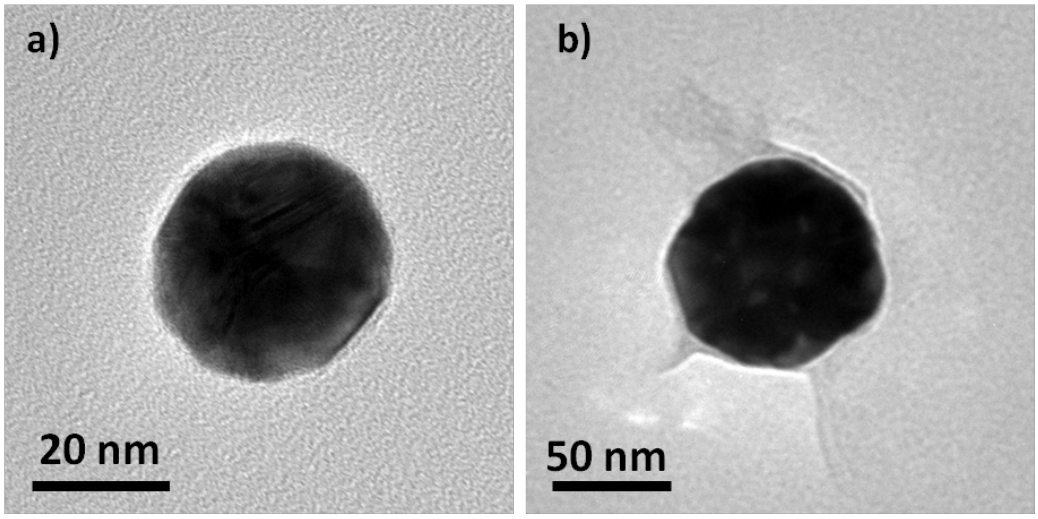

Figure S1. TEM images of a) citrate reduced bare gold nanoparticles and b) Au@NGO nanoparticle. By comparison, the graphene oxide sheets wrapped on the Au@NGO nanoparticles were clearly observed.

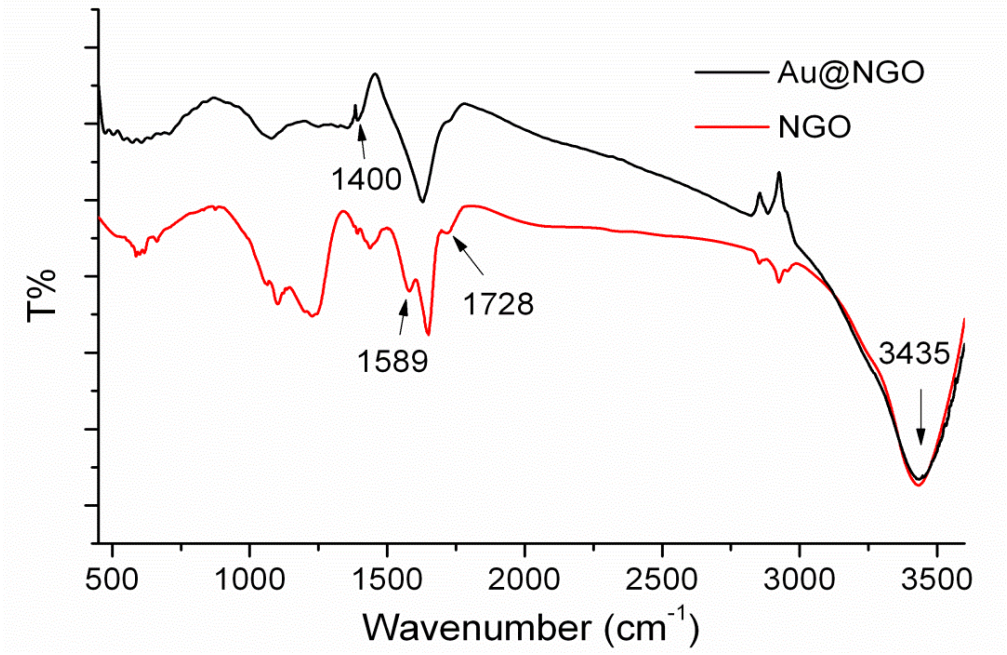

Figure S2. FT-IR spectra of Au@NGO (black curve) and NGO (red curve).

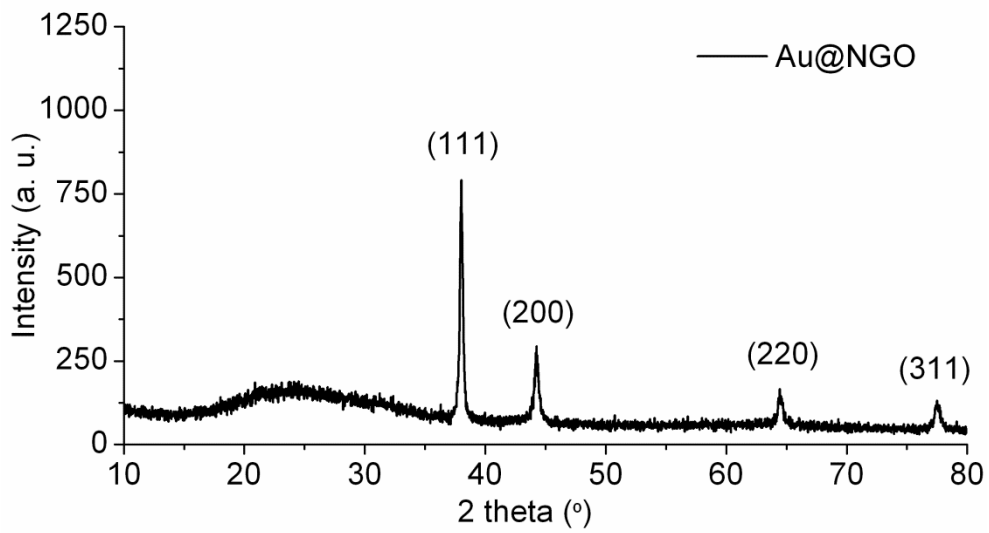

Figure S3. X-ray diffraction pattern of Au@NGO nanoparticles.

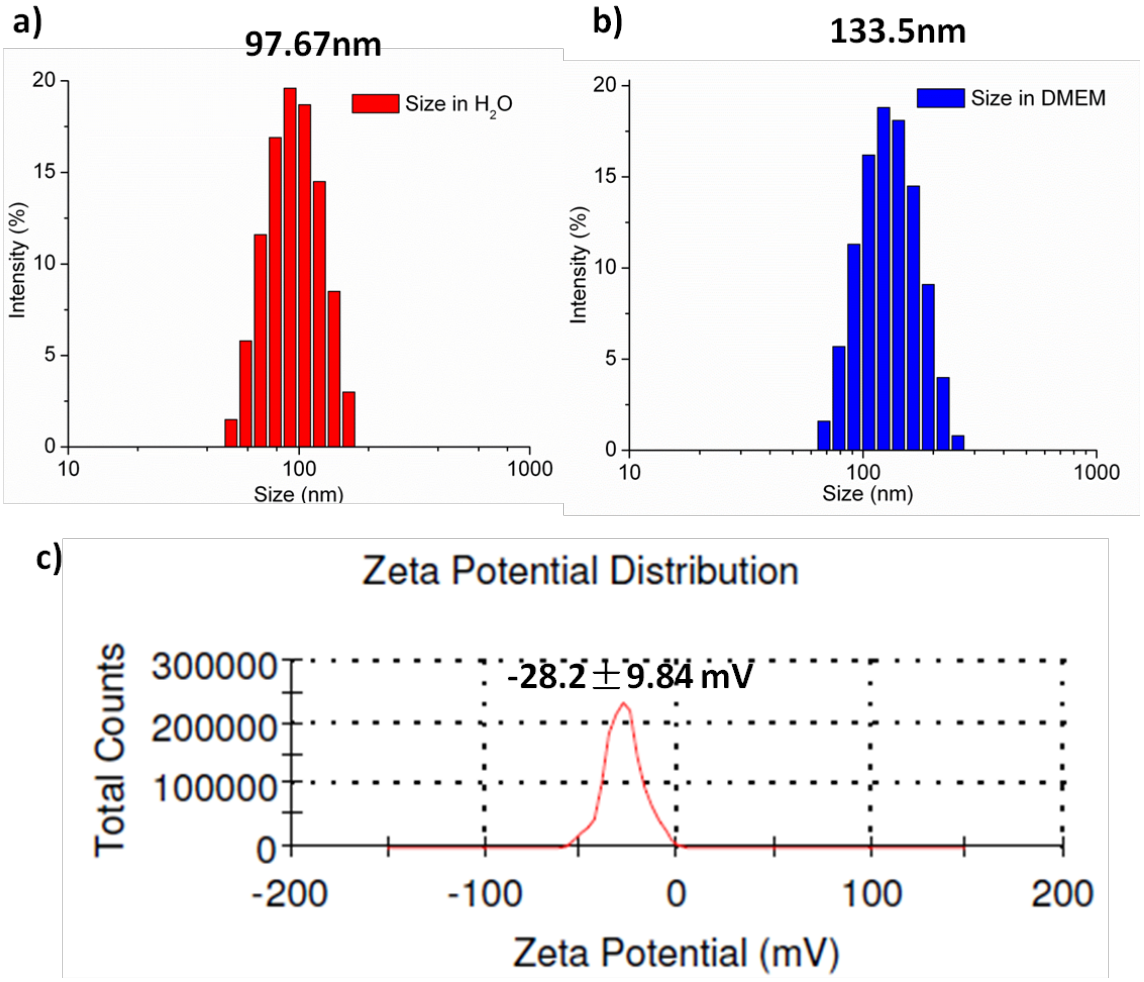

Figure S4. Dynamic light scattering (DLS) size distribution of the Au@NGO nanoparticles in a) DI H_2O and b) Dulbecco's Modified Eagle's Medium (DMEM). c) Zeta-potential measurements of the Au@NGO nanoparticles in DI H_2O.

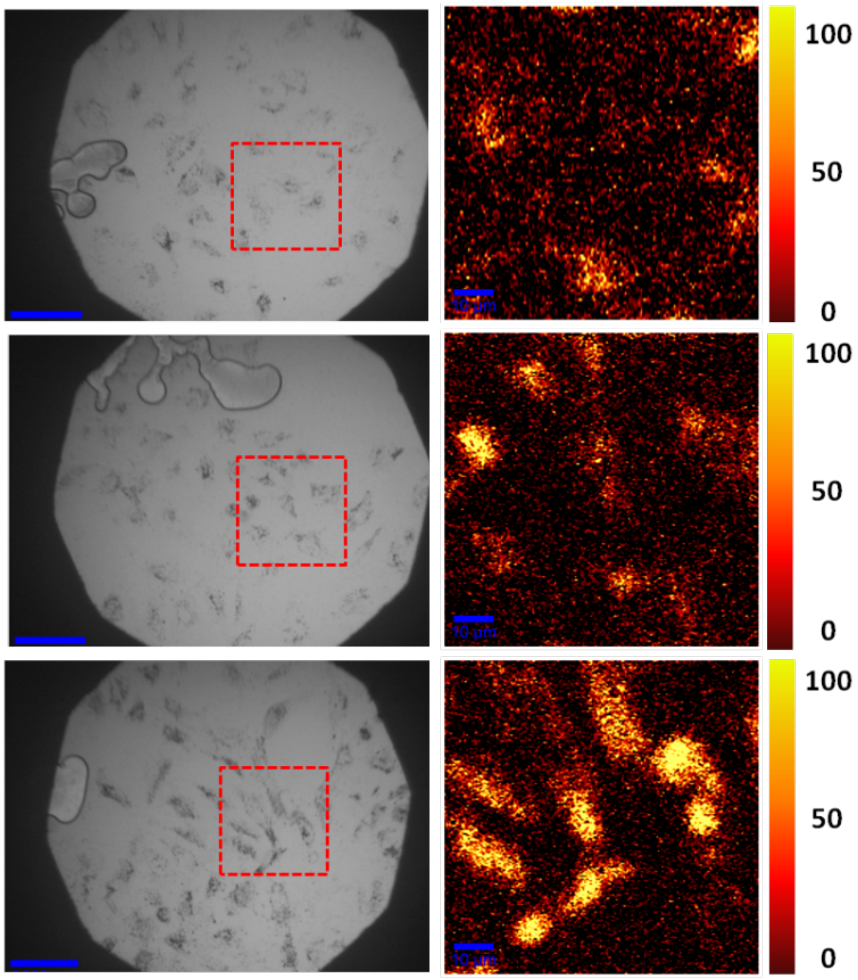

Figure S5. Confocal Raman mapping of HeLa cells treated with Au@NGO at various concentrations (from top to bottom: 20, 40, 80 μg mL^{-1}). The scale bars in bright field and mapping images are 50μm and 10μm, respectively.

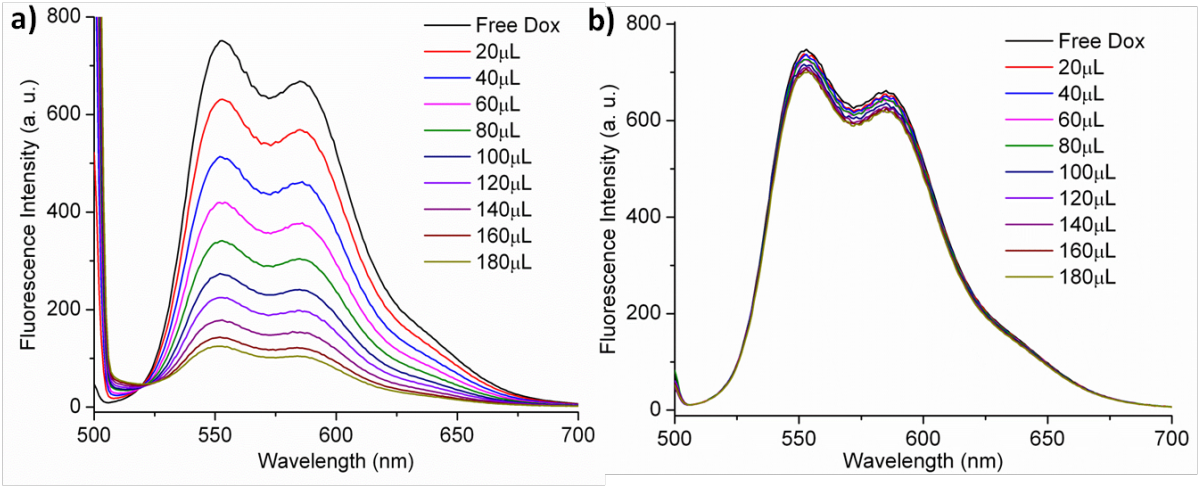

Figure S6. a) Quenching of the Dox fluorescence by the addition of various amounts of Au@NGO solution (1μg mL^{-1}), and b) equivalent volume of DI H_2O.

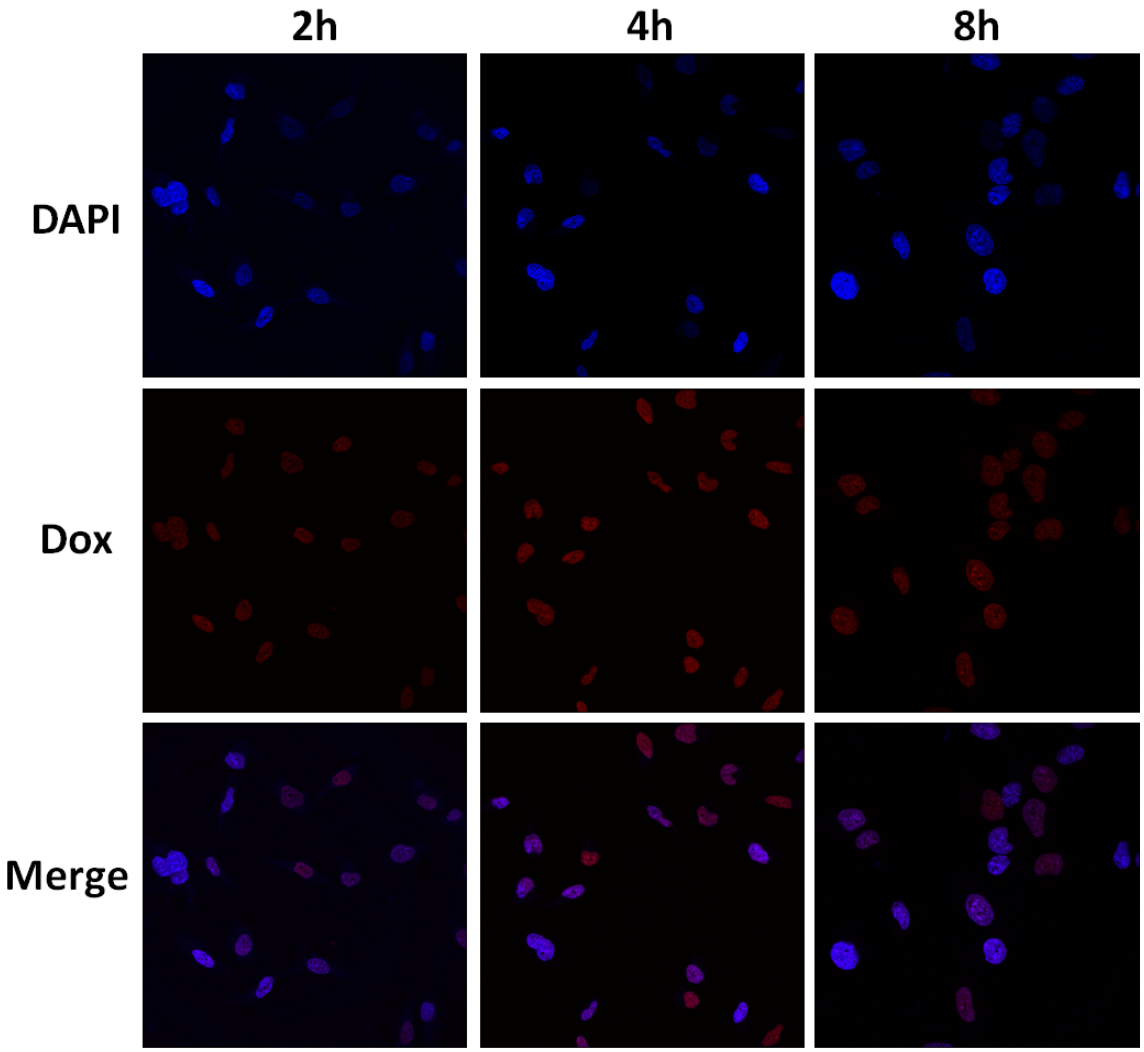

Figure S7. CLSM images of HeLa cells treated with free Dox (0.5 μg mL^{-1}) for various times.

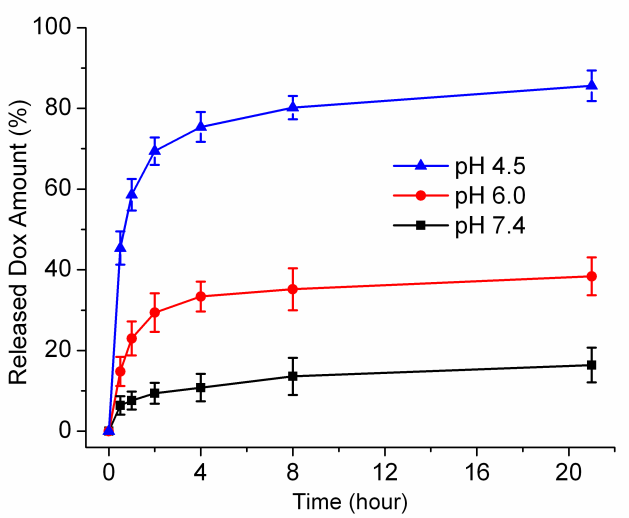

Figure S8. Dox release profiles of Dox-loaded Au@NGO at various pH conditions.

Reference:

[1] W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339.