

Supporting Information

Materials and Instrumentation

Glutathione and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (USA). Sodium borohydride (NaBH_4 , 98%) and silver nitrate (AgNO_3 , 99.99%) were obtained from Alfa Aesar. Dulbecco's modified Eagle's medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Invitrogen. A549 cells were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai). All other reagents were all of analytical reagent grade and used as received. Nanopure water (18.2 MH; Millpore Co., USA) was used throughout the experiment.

UV-vis absorption spectra were recorded on a JASCO V-550 UV/Visible spectrophotometer. Transmission electron microscopy (TEM) images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. Fluorescence images were captured using an Olympus BX-51 optical equipped with a CCD camera. Dynamic light scattering (DLS) was measured by Malvern Instruments, equipped with the Dispersion Technology Software 5.03.

Plasmid DNA Preparation

The plasmid pEGFP–N2 (4.7 kb) containing enhanced green fluorescent protein (EGFP) encoding DNA under control of the cytomegalovirus (CMV) promoter was propagated in *E. coli* and purified using the Plasmid Giga Kit (Qiagen, Valencia, CA). Purified pEGFP–N2, with the A260/A280 purity between 1.8 and 1.9, was dissolved in TE buffer at 1 mg/mL and stored at $-20\text{ }^\circ\text{C}$.

Synthesis of silver deposition on pDNA scaffolds

The pDNA-templated silver deposition was synthesized by reduction of AgNO_3 with NaBH_4 in the presence of pDNA. Briefly, pDNA (1 μg , 100 μL) was mixed with excess of AgNO_3 (10^{-3} M , 1 μL). After 15 min incubation, a freshly prepared NaBH_4 ($5 \times 10^{-4}\text{ M}$, 10 μL ; AgNO_3 and NaBH_4 were mixed in a 1:5 molar ratio) was dropped into the above aqueous solution under vigorous stirring. After mixture, the resulting colloidal silver solution was stirred for another 30 min. After the synthesis of the

pDNA-AgNPs composites, the AgNPs were further stored at 4 °C for 24 h before use to remove the excess sodium borohydride.

Cell cultures

The A549 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Gibco). The cells were kept at 37 °C in a humidified 45 atmosphere containing 5% CO₂.

Cytotoxicity assays

MTT assays were used to probe cellular viability. A549 cells were seeded at a density of 5000 cells/well (100 μL total volume/well) in 96-well assay plates. After 24 h incubation, the as-prepared pDNA-AgNPs, at the indicated concentrations, were added for further incubation of 48 h. To determine toxicity, 10 μL of MTT solution (BBI) was added to each well of the microtiter plate and the plate was incubated in the CO₂ incubator for an additional 4 h. Then the cells were lysed by the addition of 100 μL of DMSO. Absorbance values of formazan were determined with Bio-Radmodel-680 microplate reader at 490 nm (corrected for background absorbance at 630 nm). Three replicates were done for each treatment group.

Expression of the enhanced green fluorescent protein (EGFP) gene

A549 cells were seeded at a density of 5×10^4 cell/well in 24-well plates and cultivated under 37 °C and 5% CO₂ overnight. Prior to transfection, medium was exchanged with 500 μL fresh basic medium. Then the naked pDNA, pDNA-AgNPs, and pDNA-Lipo containing 1 μg of pDNA were incubated with the cells for 6 h, thereafter, the culture medium was again replaced with fresh medium and further incubated for another 48 h. After that, the imagings were captured using an Olympus BX-51 optical equipped with a CCD camera.

Statistical analysis

All data were expressed in this article as mean result ± standard deviation (SD). The statistical analysis was performed by using Origin 8.0 software.

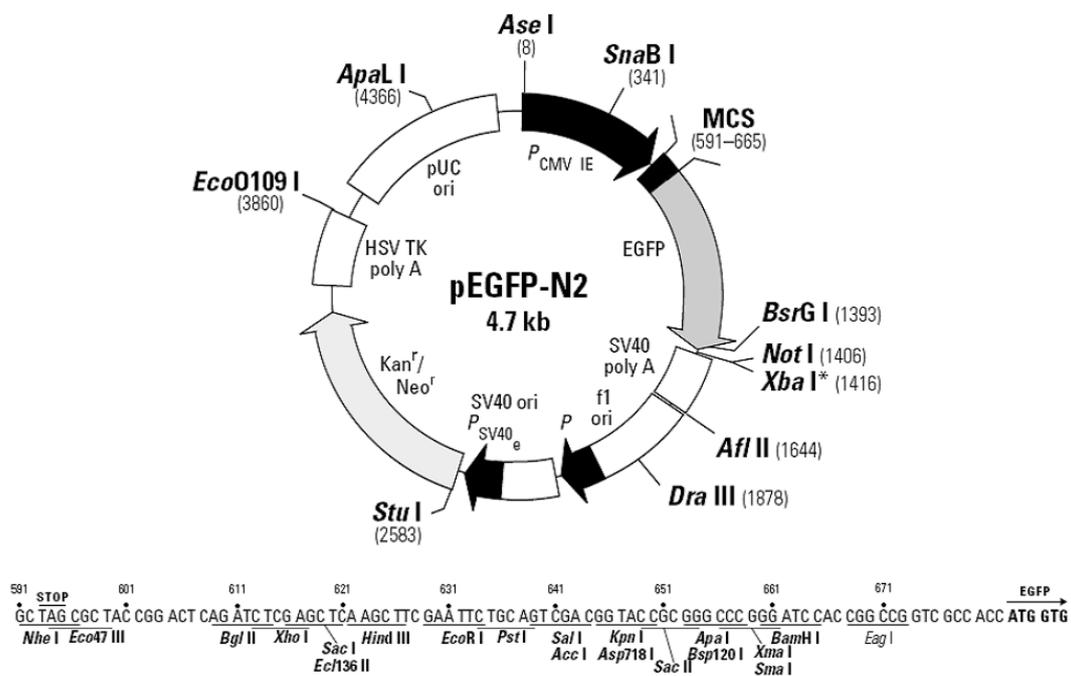


Fig. S1 Restriction map and multiple cloning site (MCS) of pEGFP-N2.

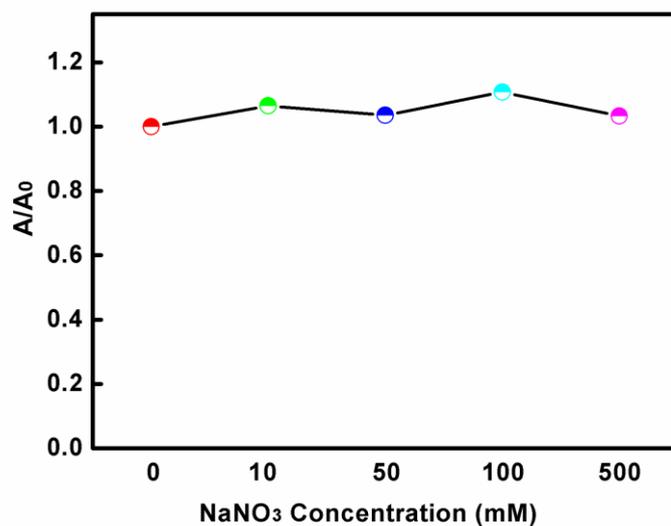


Fig. S2 Absorbance ratio of pDNA-AgNPs as a function of ionic strength, where A_0 and A are the absorbance of pDNA-AgNPs at 410 nm in the absence and presence of NaNO_3 , respectively.

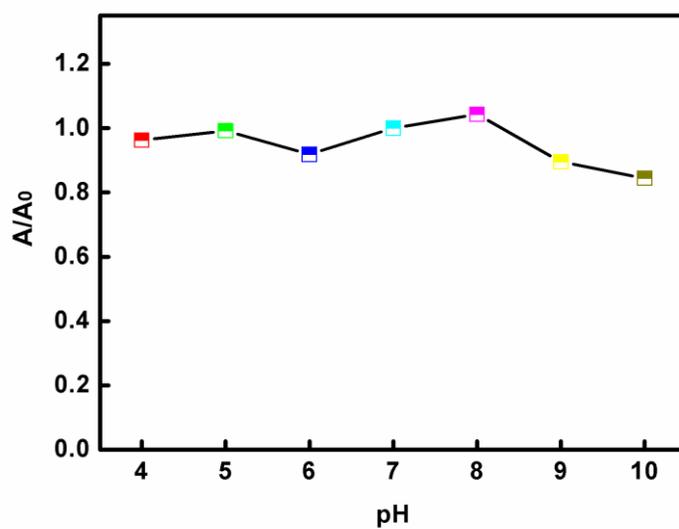


Fig. S3 Absorbance ratio of pDNA-AgNPs as a function of pH, where A_0 and A are the absorbance of pDNA-AgNPs at 410 nm in pH 7 and other pH values, respectively.

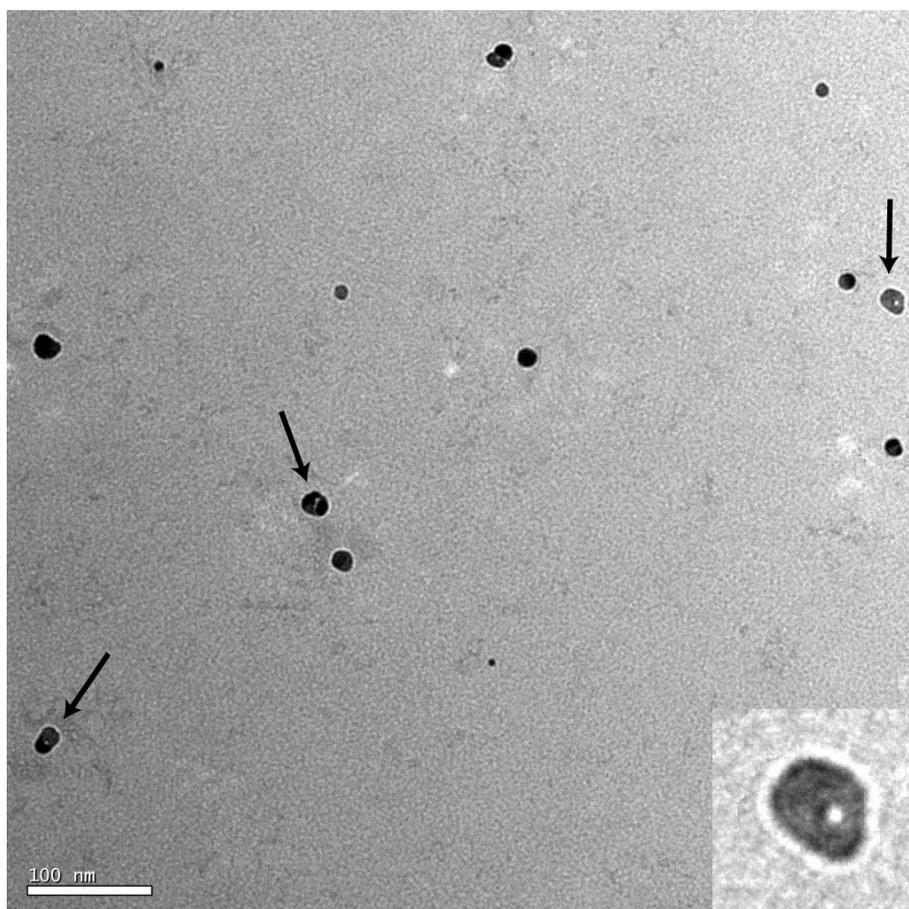


Fig. S4 TEM image of the AgNPs synthesized with AgNO₃ and NaBH₄ mixed in a 2:1 molar ratio. The inset is the magnified image of a single ring-shaped nanoparticle selected in the TEM image.

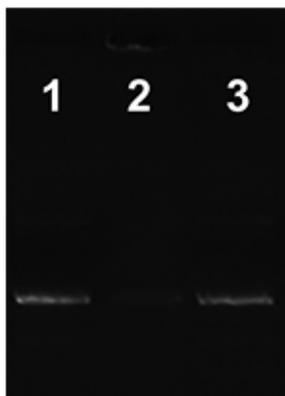


Fig. S5 Agarose gel electrophoresis of pDNA-AgNPs before and after GSH treatment. Lane 1: original DNA plasmid; Lane 2: pDNA-AgNPs without GSH treatment; Lane 3: pDNA-AgNPs with GSH treatment.

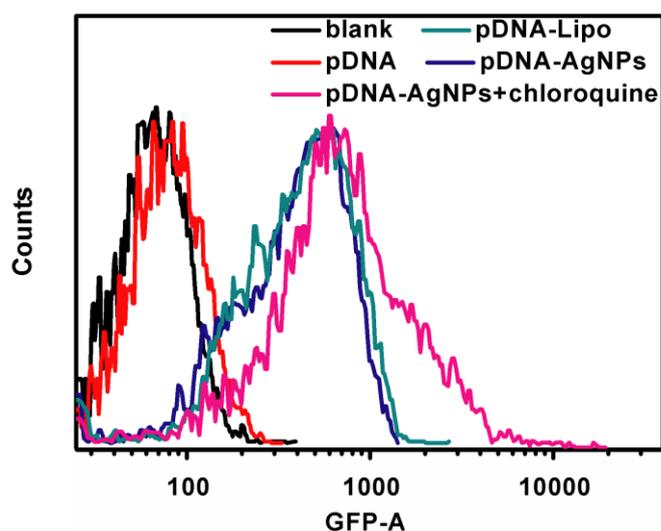


Fig. S6 Flow cytometry measurement of EGFP gene expression in A549 cells. Transfection was performed at a dose of 1 µg/well of pDNA. Cells without treatment (black), treated with free pDNA (red), pDNA-AgNPs (blue), pDNA-Lipo (green) and pDNA-AgNPs with chloroquine (pink), respectively.