# Supplementary Information

# Self-Assembly of Gold Nanoparticles Shows Microenvironment-Mediated Dynamic Switching and Enhanced Brain Tumor Targeting

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## Methods

### Synthesis of HS-pH-DOX

Synthesis of compound 1: Briefly, 30 mmol (3.18g, 2.679mL) of methyl thioglycolate (MTG) was dissolved in 10 mL ethanol and added dropwise into 36mmol of hydrazine hydrate (1.15 g, 1.114 mL). The mixture was heated to reflux for 12 h. Solvent and excess hydrazine hydrate were evaporated under vacuum and dried overnight to obtain compound 1 as light yellow oil (2.98 g, 93.7% yield). Synthesis of DOX-thiol: with respect to Todd's method [30], several steps were optimized. Doxorubicin hydrochloride (58 mg, 0.10 mmol) and anhydrous sodium sulfate (100 mg, 0.70 mmol) were dissolved in 10 mL methanol. Next, compound 1 (0.6 mmol, 63.7 mg) was dissolved in 5 mL methanol by dropwise addition over a period of 5 min. The reaction mixture was stirred at room temperature for 12 h while being shielded from light. Salt was removed by suction filtration, and the filtrate was concentrated by evaporation under vacuum. The solution was precipitated into acetonitrile to produce HS-pH-DOX as a dark red solid (35 mg, 55.5% yield).

### Photothermal Treatment with NIR Light *In Vitro*

U87 cells were incubated with 50 nm Gold nanoassemblies and monodispersed Au nanoparticles in a 96-well cell-culture plate at 37 °C and 5% $CO_2$. After the internalization of the gold NMs for

24 h and washing with PBS 3 times, the treated cells were irradiated by an 808 nm NIR laser at a power density of 2 W $cm^{-2}$ for 10 min. A standard cell viability assay MTT was conducted to determine the cell killing efficiency after photothermal ablation. Cell viability was normalized to a control group without any treatment.

**ROS Assay**

The U87 cells were plated in 24-well plates at a density of $1 \times 10^5$ cells/well and then exposed to EGF-SA-AuNPs and EGF-AuNPs of 12.5 nm concentration for 24 h. The cells were harvested and incubated with 500 μL of 10 μM DCFH-DA for 30 min at 37 °C. After incubation, the dye was washed twice with serum free medium, and intracellular fluorescence was detected under 488 nm excitation using confocal laser scanning microscope.

**Cell Cytotoxicity Assay**

U87 cells were seeded into 96-well cell culture plates at a density of $4\times10^4$ cells per well. After incubating overnight, the cells were treated with EGF-AuNPs, EGF-SA-AuNPs, DOX-SA-AuNPs, and DOX-EGF-SA-AuNPs at a series of Au concentrations (i.e., 50, 25, 12.5, 6.3 and 3.1 nM) and incubated for 72 h. The cell killing efficiencies were determined by MTT assays, according to the instructions provided in the Cell Proliferation Kit I (MTT; Roche Applied Sciences, Indianapolis). Briefly, a mixed solution (0.5 mg/mL) of MTT and fresh culture medium were added to each well and incubated for 4 h at 37°C and 5% $CO_2$. Absorbances were measured at a test wavelength of 570 nm and a reference wavelength of 630 nm using a microplate reader (ELx808, BioTek).

***In Vivo* Therapeutic Study**

U87 tumor-bearing mice were established as described above. Seven days after implantation, the mice were randomly divided into 5 groups (6 mice per group), namely a saline group, a free DOX group, a DOX-EGF-AuNPs group, a DOX-SA-AuNPs group and a DOX-EGF-SA-AuNPs group. The mice were intravenously injected with each formulation at 1.5 mg/kg (DOX concentration) on days 8 and 15. The survival rates of the mice were monitored and recorded every day, and mouse body weight was measured.

**Results**

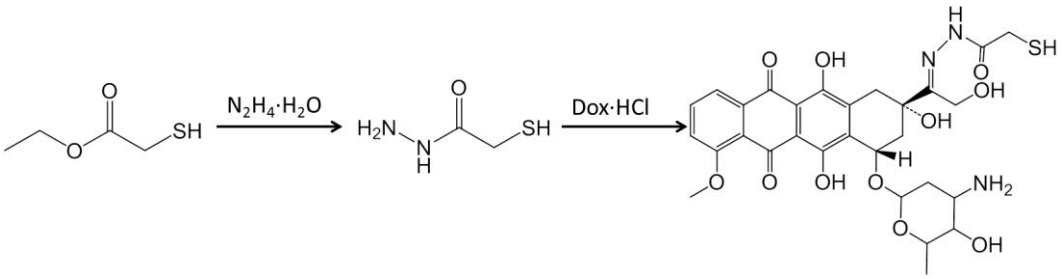


**Figure S1 Synthesis route of HS-pH-DOX.**

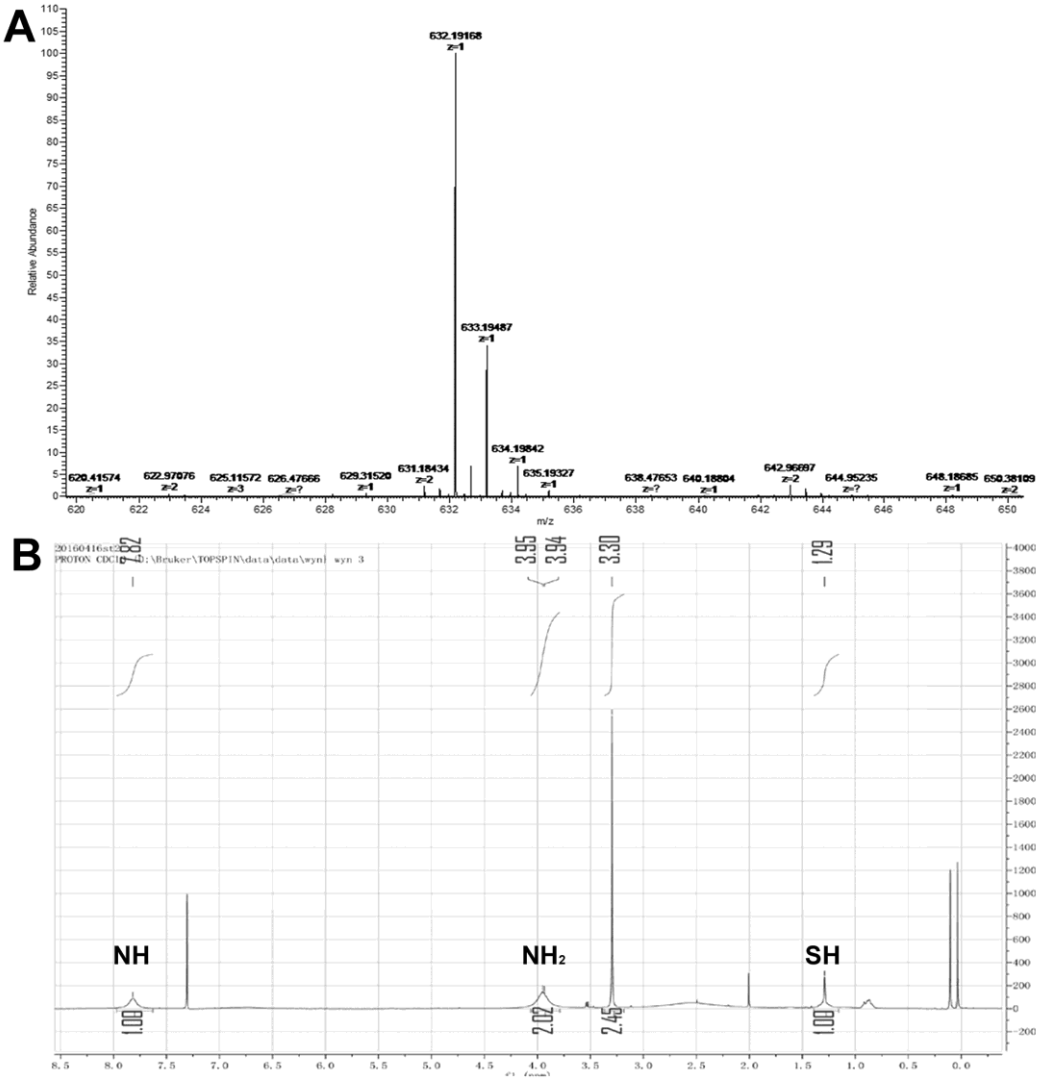


**Figure S2** (A) Mass spectrometry spectra for the molecular weight analysis of HS-pH-DOX. (B) $^{1}H$ NMR spectra in $CDCl_3$ show the representative functional group magnetic displacement of intermediate compound 1.

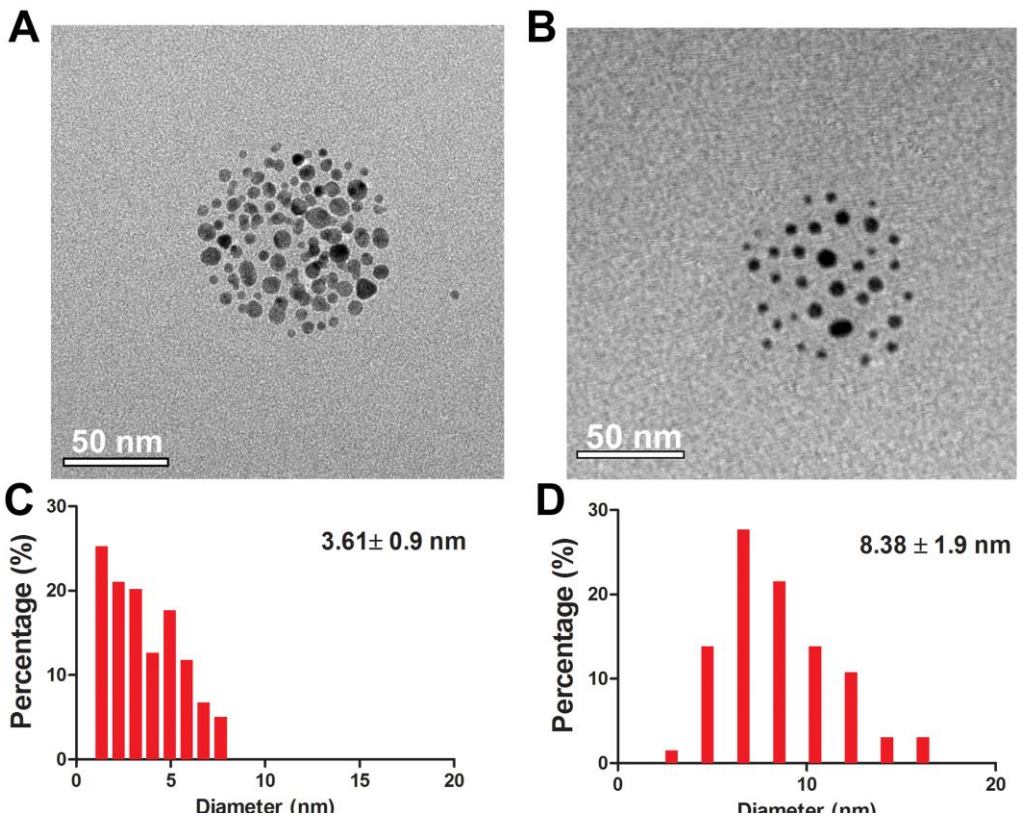


**Figure S3 Characterization of self-assembled AuNPs.(A and B)** Representative TEM images of SA-AuNPs and DOX-EGF-SA-AuNPs, respectively. **(C and D)** Histograms of the interparticles distance from TEM images by measuring the diameter between gold nanoparticles.

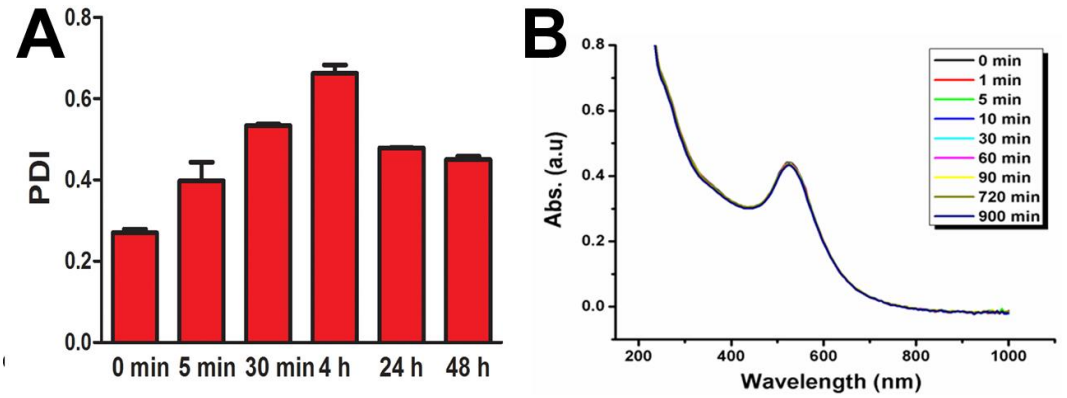


**Figure S4** (A) Polydispersity index (PDI) of DOX-EGF-SA-AuNPs with 10 mM GSH at different time-points (5 min, 30 min, 4 h, 24 h and 48 h) via DLS. (B) UV-vis spectra of DOX-EGF-SA-AuNPs with 10 mM GSH at different time intervals.

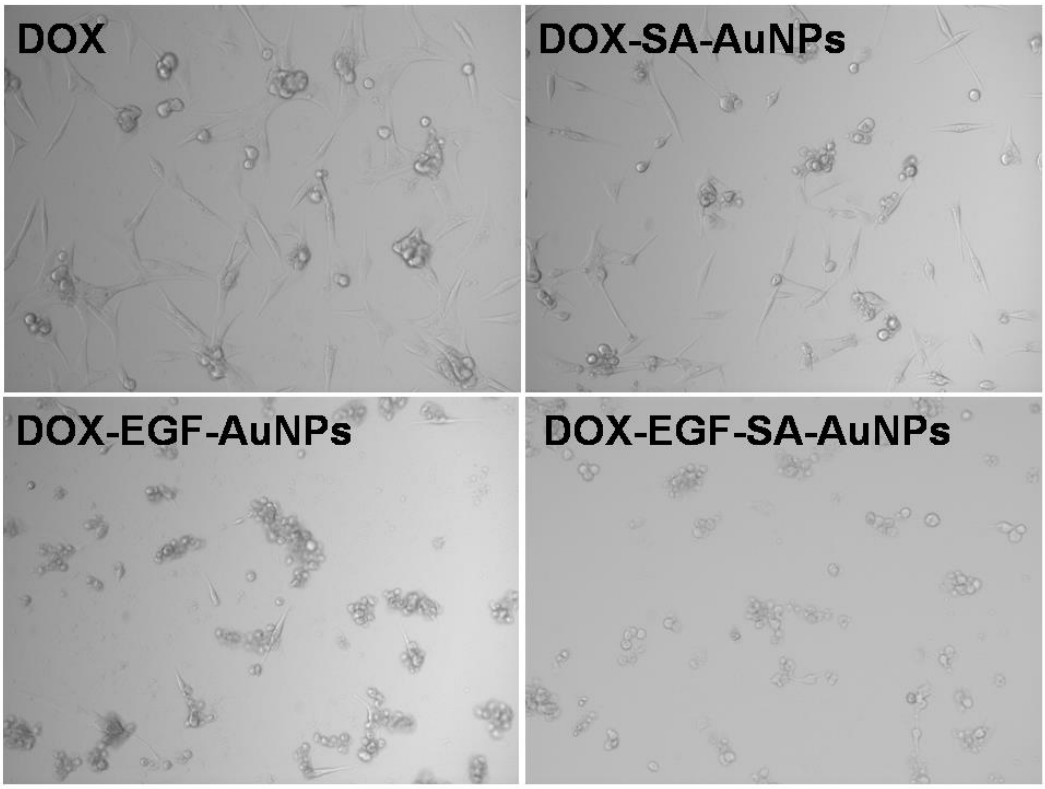


**Figure S5** Microscope images of U87 cells treated with 1 μM DOX, DOX-SA-AuNPs, DOX-EGF-AuNPs or DOX-EGF-SA-AuNPs at 72 h.

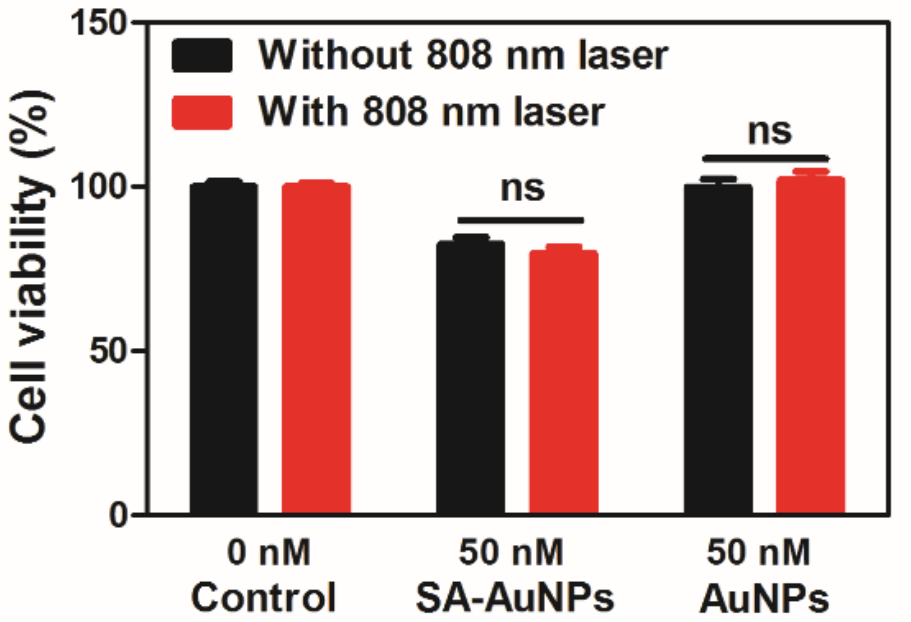


**Figure S6** Relative viabilities of U87 cells incubated with SA-AuNPs and AuNPs (with or without the pretreatment of NIR laser irradiation) of different concentrations (50 and100 nM) for 24 h.

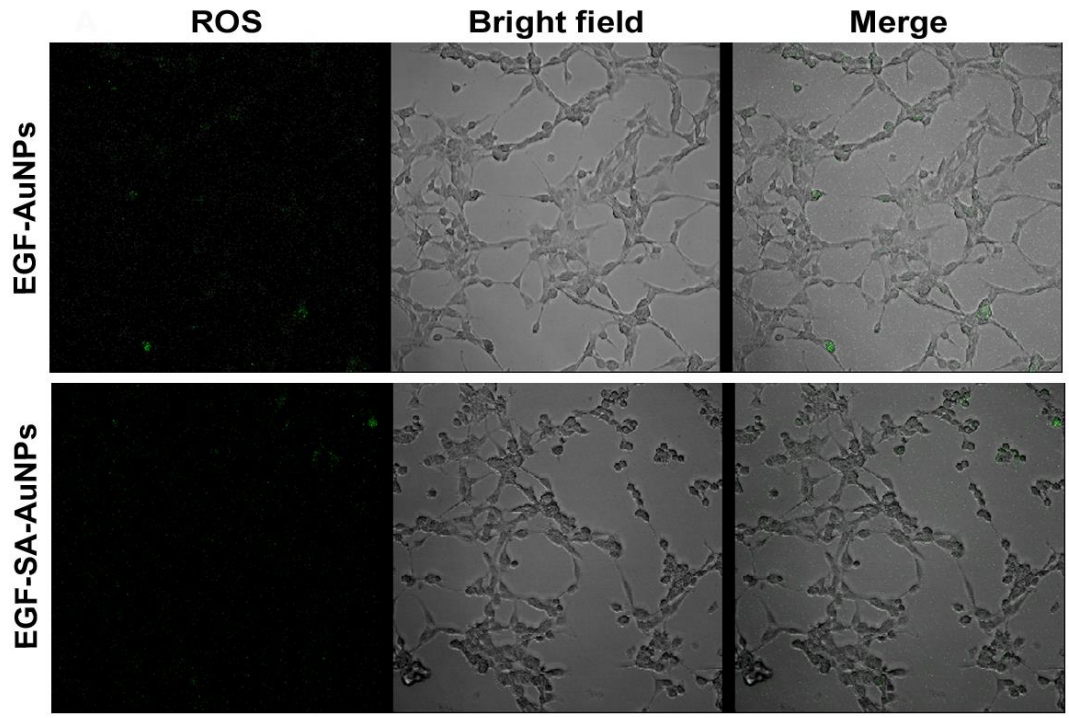


**Figure S7** Intracellular ROS generation of U87 cells incubated with EGF-AuNPs, and EGF-SA-AuNPs as determined by a DCFH-DA assay.

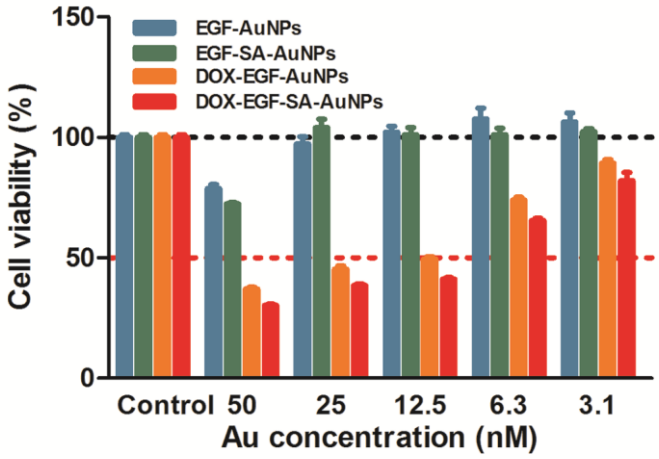


**Figure S8** Cell viability of U87 cells were assessed by MTT assay for cells exposed to the different Au concentrations of EGF-AuNPs, EGF-SA-AuNPs, DOX-EGF-AuNPs and DOX-EGF-SA-AuNPs for 72 h.