Supporting Information

Aggregation-Induced Emission-Active Au Nanoclusters for Ratiometric Sensing and Bioimaging of Highly Reactive Oxygen Species

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Experiment Section

Chemicals and Instruments

Hyaluronic acid (HA, MWz40 KDa), ethylenediamine (EDA) and L-glutathione (GSH) were purchased from Aladdin (Shanghai, China). Fluorescein isothiocyanate (FITC), 1-Ethyl-3(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium (sulfo-NHS) were purchased from Alfa Aesar. Hydrogen tetrachloroauratetrihydrate ($HAuCl_4 \cdot 3H_2O$), lipopolysaccharide (LPS), and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma-Aldrich. Dialysis bags (molecular weight cut off = 1000Da) were obtained from Sangon (Shanghai, China). Other chemicals were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., LTd. (Shanghai, China). All solutions were prepared using Nanopure water (18.2 MΩ; Millpore Co., USA). TEM images were recorded by a JEOL 1011 transmission electron microscope operating at 200 kV. Fluorescence spectra were measured by JASCO FP6500 spectrophotometer (JASCO International Co. LTD., Tokyo, Japan). UV-Vis absorption spectra were measured by JASCO V-550 UV/Visible spectrophotometer (JASCO International Co. LTD., Tokyo, Japan).

Synthesis of GSH-AuNCs

Freshly prepared $HAuCl_4$ solution (20 mM, 1 mL) and GSH solution (100 mM, 0.3

mL) were mixed with 8.7 mL of ultrapure water at 25°C. The mixture was heated to 70°C under gentle stirring (500 rpm) for 24 h. The solution was re-dissolved in nanopure water followed by being dialyzed in a dialysis bag (retained molecular weight: 1000 Da) and used accordingly. The obtained AuNCs solution was stored at 4°C.

Preparing of FITC-EDA-HA

The covalent conjugation of hyaluronic acid (HA) and ethylenediamine (EDA) was achieved by using the cross-linking reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS). Firstly, HA (50 mg) was hydrated in water overnight. Then HA was activated by EDC (2 mg/mL) and NHS (2 mg/mL) in 2-(N-morpholino)ethanesulfonic acid (MES) buffer (20 mM, pH 6.0) for 30 min. 20 mL of phosphate buffered saline (PBS, 100 mM, pH 7.4) was added to the mixture, followed by the addition of 3 mL of EDA at room temperature with continuous stirring for 24 h. The solution was dialyzed in nanopure water for 3 days. Secondly, in order to conjugate FITC onto to HA, the synthesized EDA-HA and 5 ml of FITC (1 mg/ml) were mixed in 20 ml ultrapure water. The mixture was stirred at room temperature in dark for 48 h. Finally, the resulting solution was further dialyzed in a dialysis bag (retained molecular weight: 1000 Da) and FITC-EDA-HA was obtained.

Preparing of FHAuNCs

Firstly, 5 mL of AuNCs solution (1.6 mg/mL) was mixed with 20 mL of PBS (10 mM, pH 7.4). 20 mL of FITC-EDA-HA (10 mg/mL) solution was added to the above solution and the mixture was incubated under constant stirring at room temperature for 12 h. The obtained nanocomplexes were purified by washing with water 3 times to remove the unreacted FITC-EDA-HA and AuNCs. Finally, the resulting FHAuNCs were dialyzed in water.

Generation of different hROS.

Commercially available H_2O_2 and NaClO were directly diluted to prepare stock solutions of H_2O_2 and ClO^-, respectively. The concentrations of H_2O_2 and ClO^- were determined by their UV/vis absorbance at 240 nm and 292 nm, respectively. ·OH was obtained through Fenton reaction by mixing $FeCl_2$ with H_2O_2 at a molar ratio of 1:10 at 37°C for 30 min. The concentration of ·OH was equal to that of ferrous ion. $ONOO^-$ was obtained by the reaction between $NaNO_2$ and H_2O_2 at a molar ratio of 1:5. It was used from stock solution in NaOH. The concentration of $ONOO^-$ was determined by the absorbance at 302 nm.

hROS detection

The fluorescence spectra of the FHAuNCs (50 μg/mL) in PBS (10 mM, pH=7.4) were measured 5 min after the addition of hROS. PBS used for the above experiments was purged with N_2 for 1 h before the measurement.

Cell culture

HeLa, HEK-293T and RAW cells were cultured with regular growth medium consisting of high glucose DMEM. The cell growth media were supplemented with 10% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin and cultured at 37℃ in a 5% CO_2 humidified environment.

In vitro cytotoxicity assay

The cellular viability was tested by Methyl thiazolyl tetrazolium (MTT) assay. HeLa, HEK-293T and RAW cells were seeded in 96-well plates at a density of 5000 cells/well, respectively. FHAuNCs at the indicated concentrations were added to the cells and further incubated for 24 h. 10 μL of MTT solution (BBI) was added to each well of the plate. The cells were incubated in the CO_2 incubator for an additional 4 h. Then 100 μL of DMSO was added to lyse the cells. Absorbance values of formazan at 490 nm were measured using a Bio-Rad model-680 microplate reader.

In vitro hROS assay

200 mL of 0.02 mg/mL FHAuNCs in PBS were incubated with different concentrations of hROS at 37℃ for 2 h. Then the fluorescence intensities of the samples were measured upon the excitation at 430 nm. Quantification of different hROS was achieved by the plot of the ratio values of I_{570}/I_{520} versus hROS concentrations.

Detection of Endogenous hROS in living cells

RAW 264.7 and HeLa cells were seeded into 24-well plate and incubated for 24 h in a humidified atmosphere containing 5% CO_2 at 37℃. LPS (1 μg/mL) was added and incubated for 8 h, followed by PMA (5 μg/mL) treatment for 1 h. Then, FHAuNCs (10 μg/mL in culture medium) were added to the cells and incubated for 3 h. The cells were washed three times before fluorescence imaging.

In another experiment, cells were treated with LPS (1 μg/mL) for 8 h, and then incubated with PMA (5 μg/mL) for 1 h. Subsequently, the medium was changed and the cells were treated with a medium containing uric acid (250 μM) and DMSO (0.5%) for 15 min, followed by incubation with the FHAuNCs (10 μg/mL in culture medium) for 4 h.

Ratiometric images were obtained using Image J software.

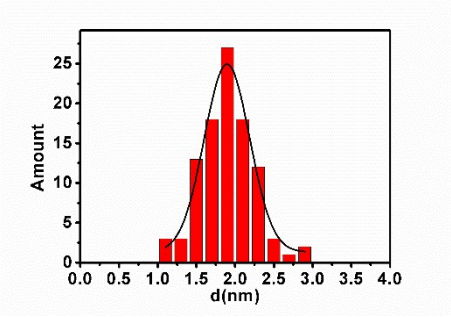

Figure S1 Histogram of the diameter distribution of GSH-AuNCs. The total number of nanoparticles counted for the histogram was 100.

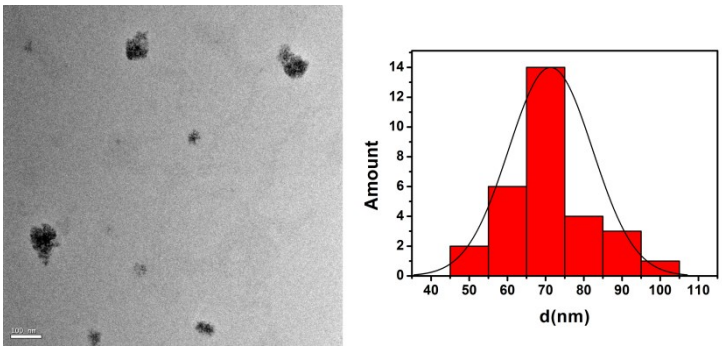

Figure S2 TEM image and particle statistics of FHAuNCs at low magnification.

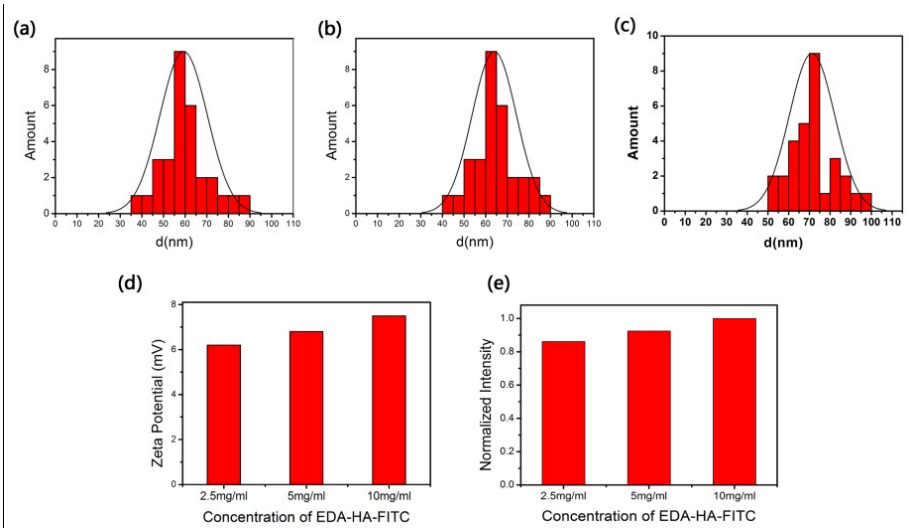

Figure S3 Particle size statistics of the self-assembled FHAuNCs with (a) 2.5 mg/mL, (b) 5 mg/mL and (c) 10 mg/mL of FITC-EDA-HA, respectively. (d) Zeta potentials of the self-assembled FHAuNCs with 2.5 mg/mL, 5 mg/mL and 10 mg/mL of FITC-EDA-HA. (e) Fluorescence intensities of FHAuNCs with 2.5 mg/mL, 5 mg/mL and 10 mg/mL of FITC-EDA-HA at 570 nm.

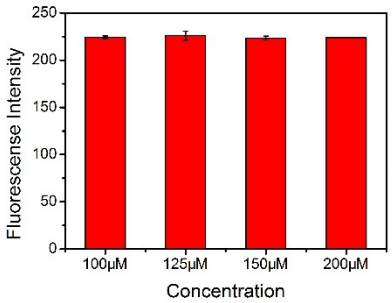

Figure S4 Fluorescence intensities of AuNCs-EDA-HA treated with hROS over 100 μM.

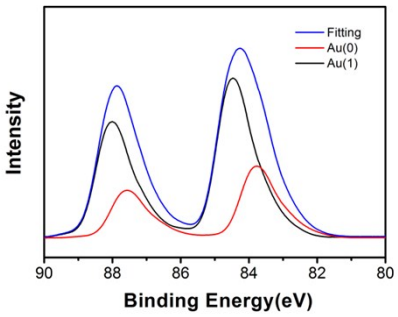

Figure S5 XPS characterization of FHAuNCs after being treated with •OH.

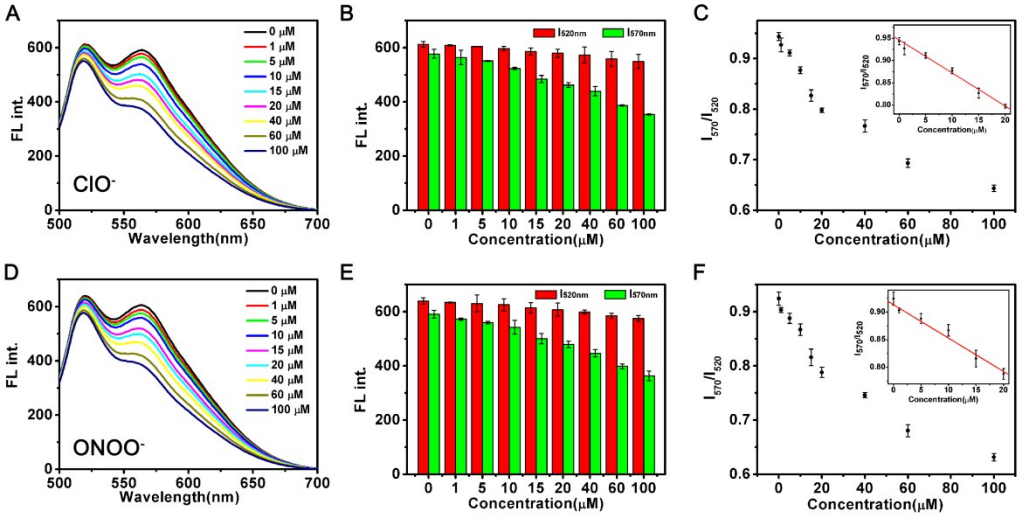

Figure S6 (A, D) Fluorescence spectra of FHAuNCs treated with different hROS with different concentrations upon excitation at 430 nm. (B, E) The change of fluorescence intensities at 520 nm and 570 nm with the increase of hROS concentrations. (C, F) The ratio I_{570}/I_{520} versus hROS concentrations. Inset is the linear region of the plot.

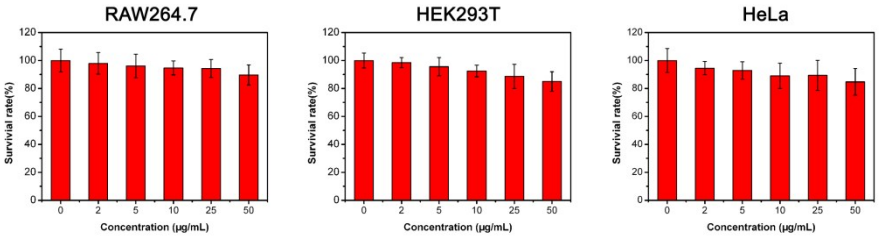

Figure S7 The survival rates of RAW264.7, HEK239T, and HeLa cells after being treated with FHAuNCs for 24 h.

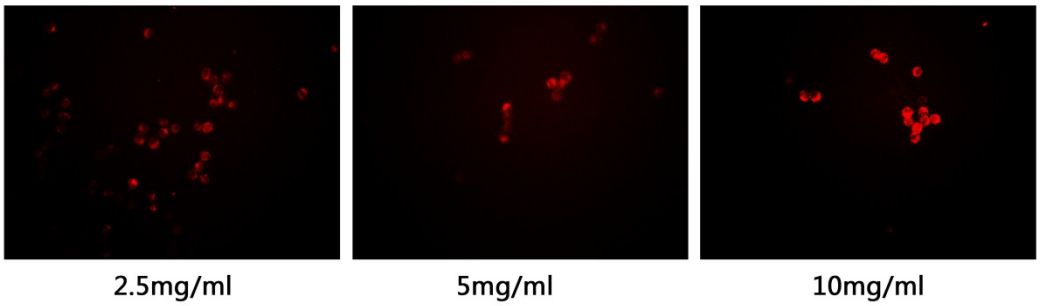

Figure S8 The cell uptake of FHAuNCs with different EDA-HA contents.

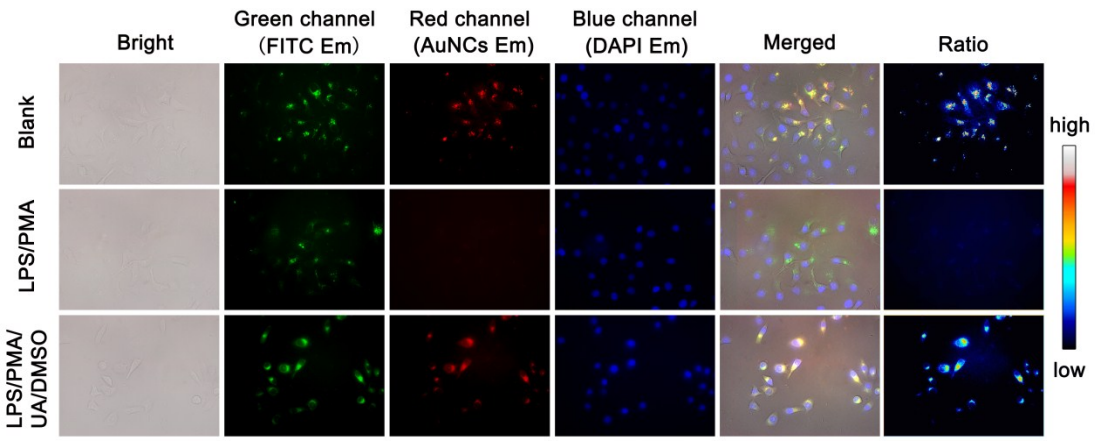

Figure S9 Fluorescence images of HeLa cells treated with FHAuNCs.