

Supporting Information

Materials. Silver nitrate (AgNO_3 , 99.9995%) and sodium borohydride (NaBH_4 , 98%) were purchased from Alfa Aesar and used without further purification. Intercalating dye Acridine Orange (AO) and Thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (USA). Dulbecco's modified Eagle's medium (DMEM) and Fetal Bovine Serum (FBS) were purchased from Invitrogen. The HEK-293T cell line and HeLa cell line were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai). All other reagents were of analytical reagent grade and used as received. Nanopure water (18.2 M Ω ; Millipore Co., USA) was used in all experiments and to prepare all buffers. DNA oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). The sequences were as follows: DNA1: 5'-AAT GTG CTA ACT CCT CCC ACC CAC CCG CCC A-3'; DNA2: 5'-GGG AGG GAG GGA GGG AAG GAG TTA GCA CAT T -3'. The sequences 5'-CCT CCC ACC CAC CCG CCC-3' was used as a template for AgNCs synthesis and the sequences 5'-GGG AGG GAG GGA GGG AAG GAG-3' was designed for signal enhancement of AgNCs.

Instrument. Fluorescence measurements were carried out by using a JASCO FP-6500 spectrofluorometer (Jasco International Co., Japan). Electronic absorption spectra were acquired using a CARY 300 UV/Visible spectrophotometer (Varian Inc., Palo Alto, CA). TEM images were recorded using a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. Fluorescence images were captured using confocal laser scanning microscopy (CARL ZEISS LSM 700, Germany). The excitation wavelength was 488 nm. The fluorescence signals were simultaneously acquired using multitrack mode with green channel of 500-550 nm and red channel of 560-600 nm.

Synthesis of single-strand ssDNA1-AgNCs. The silver nanoclusters were synthesized by cooling the solution of DNA1 and AgNO_3 to 0 °C and then adding NaBH_4 , followed by vigorous shaking for 2 min. The final concentrations were 15 μM for the DNA1, 90 μM for AgNO_3 , and 180 μM for NaBH_4 . The reaction mixture was kept in the dark at 4 °C for 24 hours before use. Experiments were carried out in 25 mM phosphate buffer at pH 7.0. For pH-effect experiments, buffers with the desired pH values containing a total concentration of 25 mM phosphate were used.

Synthesis of double-strand dsDNA-AgNCs. The as-prepared ssDNA1-AgNCs solution was mixed and vortexed with the enhancer DNA2 solution at molar ratio of 1:1, then diluted to 10 μM by 25 mM phosphate buffer. After hybridization for 12 h at room temperature in the dark, the hybridized double-strand dsDNA-AgNCs was obtained.

Synthesis of probe dsDNA-AO-AgNCs. AO (3.79 μM , 15 μL) was added to dsDNA-AgNCs nanohybrids (10 μM , 900 μL). After 5 min of incubation, the probe dsDNA-AO-AgNCs was obtained. The probe was then diluted to desired concentration used for thiols detection. Fluorescence experiments were excited at 500 nm, and emission spectra were collected from 510 to 650 nm.

Cell culture. The HEK-293T cell line and HeLa cell line were grown at 37 °C in an atmosphere of 5% (v/v) CO_2 in air, in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated FBS, 1.5 g/L NaHCO_3 , 100 units/ml penicillin, 100 mg/mL streptomycin. The

media was changed every three days, and the cells were digested by trypsin and resuspended in fresh complete medium before plating.

Cytotoxicity assays. HEK-293T cells and HeLa cells were seeded at a density of 5000 cells/well (100 μ L total volume/well) in 96-well assay plates for 24 h. Then, the as-prepared probe, at the indicated concentrations (0, 10, 20, 40, 80, 160, 320, 640, 960 nM) were added to the cell culture medium. Cells were incubated with probe for 24 h. To determine toxicity, 10 μ L of MTT solution was added to each well of the microtiter plate and the plate was incubated in the CO₂ incubator for additional 4 h. The media was removed and DMSO (100 μ L) was added into each well. The plate was then gently swirled for 2 min at room temperature at dark to dissolve all formed precipitate. Absorbance values were determined with Bio-Rad model-680 microplate reader at 490 nm (corrected for background absorbance at 630 nm). The cell viability was estimated according to the following equation: Cell Viability (%) = (OD_{Treated}/OD_{Control}) \times 100%. Where OD_{Control} was obtained in the absence of probe, and OD_{Treated} obtained in the presence of probe.

Detection of thiols. In a typical test, the diluted probe solution (1 μ M, 400 μ L) was mixed with certain amounts of analytes. The mixture was incubated at room temperature for 1 min and then fluorescence spectra were recorded under excitation at 500 nm. (In the experiment of selectivity, we added our probe (10 μ M, 40 μ L) to Nanopure water (355 μ L) and then mixed with various amino acids (200 μ M, 10 μ L). The mixture was incubated at room temperature for 1 min and then fluorescence spectra were recorded under excitation at 500 nm. Therefore, the final concentration of amino acids was 5000 nM.)

Detection of thiols in vitro. HEK-293T cells and HeLa cells were seeded into 24-well plate and allowed to adhere for 24 h in a humidified atmosphere containing 5% CO₂ at 37 $^{\circ}$ C. Then, the cells were incubated with probe (200 nM) in culture medium for 4 h. The cells were washed three times before fluorescence imaging.

Flow Cytometry Analyses. HEK-293T cells and HeLa cells were seeded at a density of 1,000,000 cells/well (1 mL total volume/well) in 6-well assay plates for 12 h. Then, the as-prepared probe with a concentration of 200 nM were added to the cell culture medium. Cells were incubated with probe for 4 h, and then digested with trypsin. Cells were centrifuged at 3000 rpm for 5 min, washed three times with buffer and then redispersed in the buffer. The mean fluorescence was determined by counting 10,000 events by BD LSRFortessa cytometer.

Quantitative Detection of Biothiols in Cell Samples. Since GSH is the most abundant low molecular weight biothiols in mammal cells,¹ to evaluate the accuracy of the detection, the cell extraction solutions with different added amounts of standard GSH were subjected to the quantitative detection of biothiols by our method.² HeLa cells and HEK-293T cells were seeded at a density of 5000 cells/well (100 μ L total volume/well) in 96-well assay plates for 24 h. The cells were washed with PBS (3 \times) and harvested by treatment with trypsin. Cell density and viability, defined as the ratio of the number of viable cells over the total number of cells, of the cultures were determined by trypan blue staining and a Neubauer hemacytometer (Qiujiing, Shanghai, China). The numbered cells (1.0 \times 10⁶ cells/ mL for HeLa, 5.0 \times 10⁵ for HEK-293T) were washed with an isotonic saline solution three times, and lysed with sonication (30% amplitude, 3 pulse) for 1 min. Then the diluted probe solution (1 μ M, 400 μ L) was mixed with certain amounts of cell

extraction solutions. The mixture was incubated at room temperature for 1 min and then fluorescence spectra were recorded under excitation at 500 nm. For the ratio increase studies known of GSH were added to cell extraction solutions and the total thiol concentrations were then determined.

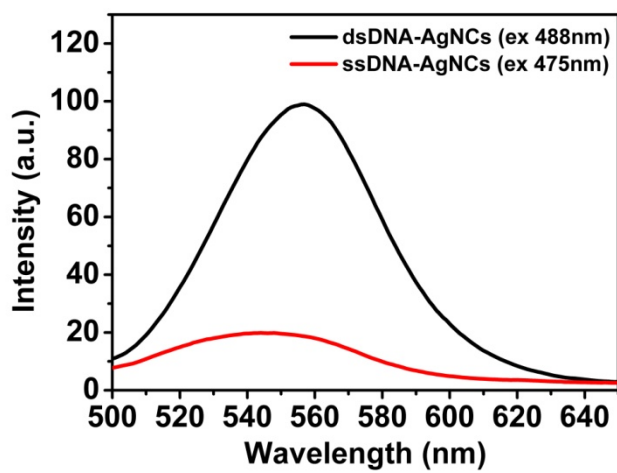


Fig. S1 Fluorescence emission spectra of dsDNA-AgNCs and ssDNA-AgNCs.

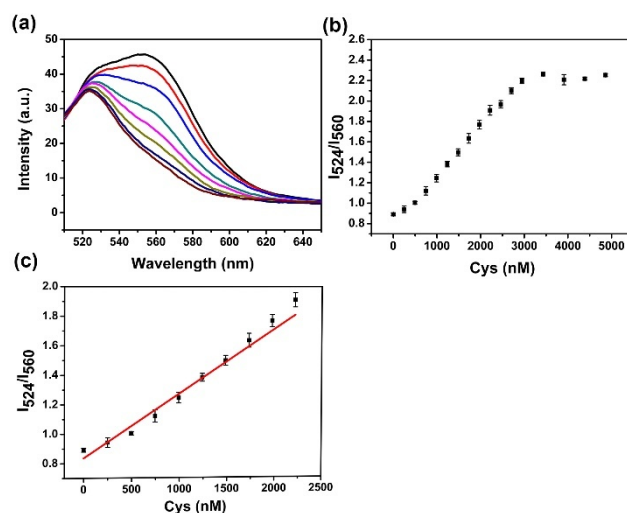


Fig. S2 Detection of Cys. (a) Fluorescence emission spectra of dsDNA-AO-AgNCs when increasing Cys concentrations (b) Plots of the ratio (I_{525}/I_{560}) as a function of the Cys concentration. (c) The linear plot. Error bars were estimated from at least three independent measurements. (The concentration of our probe is 1000 nM)

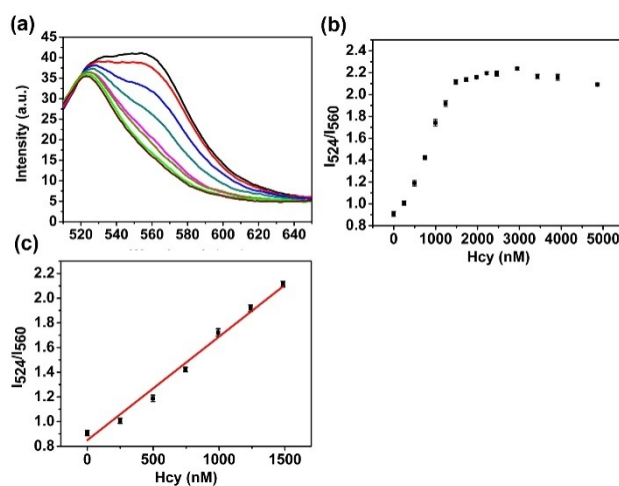


Fig. S3 Detection of Hcy. (a) Fluorescence emission spectra of dsDNA-AO-AgNCs when increasing Hcy concentrations. (b) Plots of the ratio (I_{525}/I_{560}) as a function of the Hcy concentration. (c) The linear plot. Error bars were estimated from at least three independent measurements.

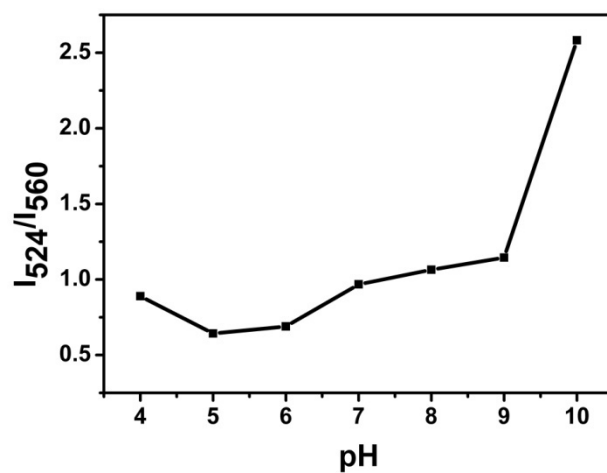


Fig. S4 Effect of pH on the probe.

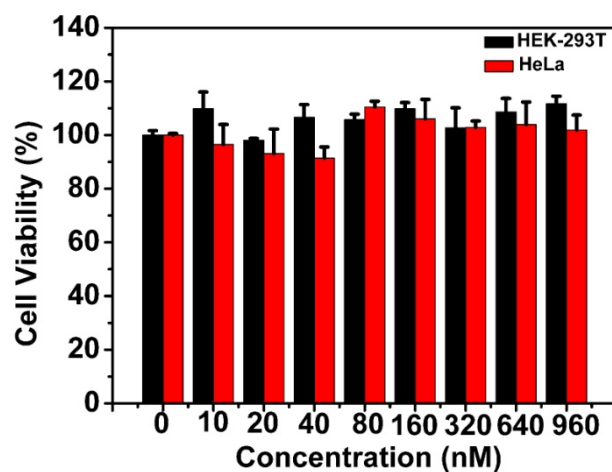


Fig. S5 Cytotoxicity of HeLa and HEK-293T cells evaluated by MTT assays after incubation with our probe in different concentrations for 24 h. Error bars were estimated from at least three independent measurements. The concentration of our probe was 200 nM.

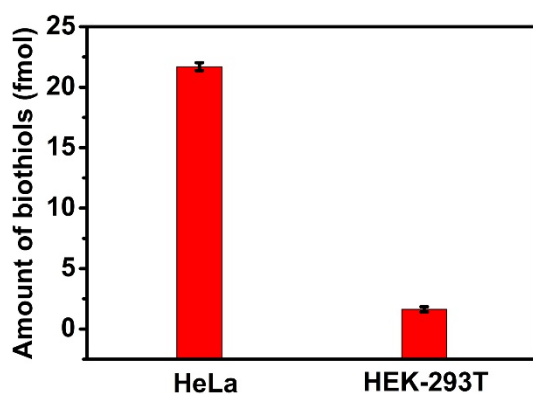


Fig. S6 Average amounts of total biothiols in a single HeLa and HEK-293T cell.

Table S1. Determination of biothiols in HeLa cell.

| Sample | Spiked (μM) | I_{524}/I_{560} | Found (μM) | Recovery (%) |
|--------|--------------------------|-------------------|-------------------------|--------------|
| 1 | 0 | 0.894 | 0.060 | |
| 2 | 0.5 | 1.104 | 0.566 | 101.2 |
| 3 | 1 | 1.323 | 1.093 | 103.3 |

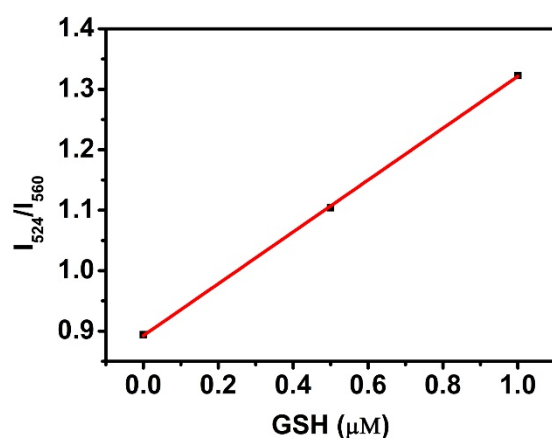


Fig. S7 Standard addition method used for the determination of total biothiols in HeLa cell.

Table S2. Determination of biothiols in HEK-293T cell.

| Sample | Spiked (μM) | I_{524}/I_{560} | Found (μM) | Recovery (%) |
|--------|--------------------------|-------------------|-------------------------|--------------|
| 1 | 0 | 0.877 | 0.02 | |
| 2 | 0.1 | 0.921 | 0.12 | 100 |
| 3 | 0.2 | 0.984 | 0.25 | 115 |

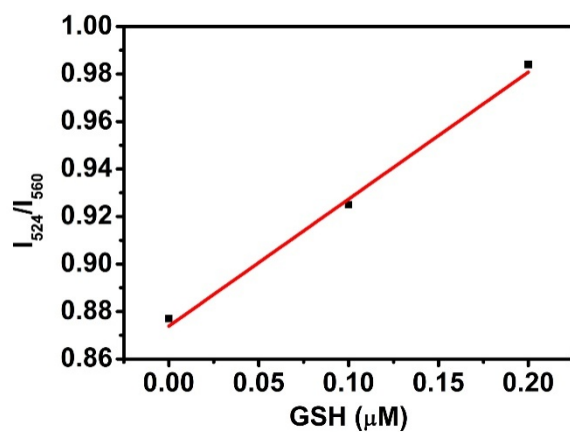


Fig. S8 Standard addition method used for the determination of total biothiols in HEK-293T cell.

1. (a) Ayers, F. C.; Warner, G. L.; Smith, K. L.; Lawrence, D. A. *Anal. Biochem.* 1986, 154, 186; (b) Lee, M. H.; Han, J. H.; Lee, J. H.; Choi, H. G.; Kang, C.; Kim, J. S. *J. Am. Chem. Soc.* 2012, 134, 17314; (c) Meister, A.; Tate, S. S. *Annu. Rev. Biochem.* 1976, 45, 559.
2. (a) Z. Dai, L. Tian, Z. Ye, B. Song, R. Zhang and J. Yuan, *Anal. Chem.*, 2013, 85, 11658; (b) W. Wang, H. Xin, H. Shao and W. Jin, *J. Chromatogr. B*, 2003, **789**, 425; (c) W. Jin, Q. Dong, X. Ye and D. Yu, *Anal Biochem*, 2000, **285**, 255.