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Supporting Information

Sensitive and Rapid Detection of Glutathione Based on Fluorescence-Enhanced "Turn-on" strategy

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1. Experiments

1.1 Synthesis of disulfide linker (AEDP)

Synthesis of AEDP was conducted according to previous work[1]. In general, cysteamine (10 mmol) and 3-mercaptopropionic acid (10 mmol) were dissolved in 30 mL ammonium hydroxide, following adding ferrous sulfate in a round-bottomed flask under stirring in ice bath. Then, 16% (w/w) hydrogen peroxide was dripped slowly into the flask until the color of the mixture turned from deep green to light yellow. The

mixture was steamed at room temperature for 20 min to remove excess ammonia. pH value of the mixture was adjusted to 2 before it was stirred for 30 min in ice bath. Next, the mixture was filtered and aqueous phase was washed with ethyl acetate. Lower aqueous phase was collected and pH was slowly adjusted to 7 with ammonia. The resulting product was separated by column chromatography (ethyl acetate: acetic acid: water= 8 : 2 : 1). Finally, crude product was recrystallized by 90% (v/v) n-propanol and 70 mg of 3-[(2-Aminoethyl) dithio]propionic acid (AEDP) was acquired. ^{13}C NMR (150.9 MHz, D_2O): δ 180.19, 37.76, 36.42, 34.16, 33.95. ^{1}H NMR (600 MHz, D_2O): δ 3.28 (t, *J* = 6.68 Hz, 2H), 2.88 (dt, *J* = 14.10, 6.40 Hz, 4H), 2.52 (t, *J* = 6.64 Hz, 2H) (Fig. S1 in Supporting information).

1.2 Preparation of AEDP-FITC

1 mL triethylamine/methanol (v : v=1 : 100) was poured into a 1.5 mL tube with FITC (48 mM) and AEDP (40 mM) in it. Reaction mixture was put into 35 ℃ water bath for 6 h in dark. After that, solvent was removed by speed vacuum centrifugation and dried AEDP-FITC could be stored at 4 ℃ for several weeks in dark.

1.3 Optimization of reaction temperature and time

20 μL, 20 mM GSH solution was added into 1.98 mL Au@PLL-AEDP-FITC. The reaction solutions were put into 25 ℃, 30 ℃, 37 ℃, 40 ℃, 45 ℃ water bath respectively and reacted for 30 min in dark. 100 μL solution was added into 96 black microtiter plates and fluorescence spectra were measured on Molecular Devices SpectraMax iD5 Microplate Reader with excitation wavelength at 470 nm. Each experiment was repeated three times. Then, under the optimized temperature, 20 μL, 20 mM GSH solution was added into 3.96 mL Au@PLL-AEDP-FITC. The solution was reacted for 0.5 h, 1.0 h, 1.5 h, 2.0 h, 2.5 h, 3.0 h in water bath respectively in dark. Fluorescence spectra were measured on Molecular Devices SpectraMax iD5 Microplate Reader with excitation wavelength at 470 nm. Parameters were set as: Method: Mono/Mono, Excitation wavelength (Ex): 470 nm, Emission wavelength (Em) Start: 510 nm, Em Stop: 600 nm, Em Step: 2 nm, Photomultiplier tube (PMT): Auto, Read Height: 1 mm.

1.4 Cell culture

Hela cells were grown in regular commercially available DMEM supplemented with 10% FBS and grown in a 100 mm×20 mm cell culture dish at humidified atmosphere with 5% CO_2 at 37℃ in a cell culture incubator. The culture medium was replaced once every three days with fresh medium.

The Hela cells at about 70% confluences were replaced by fresh medium with no FA for several hours and harvested using trypsinization with 1.5 mL of trypsin ethylene diamine tetra acetic acid (EDTA) solution at 37℃ for 1-2 min to detach the cells from the dish. The trypsin was neutralized by adding 4.5 mL of fresh supplemented 10% FBS medium, and the harvested cells in the medium suspension were transferred into a 15 mL centrifuge tube and centrifuged at 1200 rpm for 2 min. After the supernatant was removed, the Hela cells were resuspended in DMEM with 10% FBS and move to a new dish with a specific proportion. New DMEM with 10% FBS was added into the dishes and cells were cultured.

1.5 Determination of the Detection Limit

The detection limit was calculated based on the fluorescence titration. In the absence of GSH, the fluorescence emission spectrum of Au@PLL-AEDP-FITC was measured by eleven times and the standard deviation of blank measurement was achieved. To gain the slop, the fluorescence intensity at 522 nm was plotted to the concentration of GSH (in standard GSH Kit OD_{412} value was plotted to the concentration of GSH). So, the detection limit was calculated with the following equation:

Detection limit = $3\boldsymbol{\sigma}/\mathbf{k}$

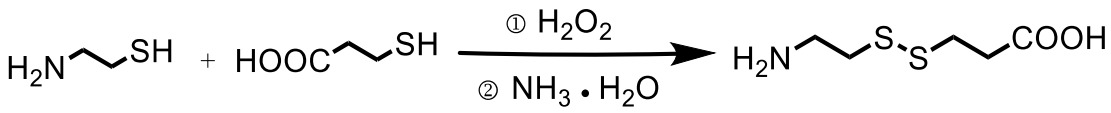
Where $\boldsymbol{\sigma}$ is the standard deviation of blank measurement, $\mathbf{k}$ is the slop between the fluorescence intensity or OD_{412} value versus GSH concentration.

1.6 MTT Assay

To evaluate the biocompatibility and cell cytotoxicity of Au@PLL-AEDP-FITC, Hela cells

were chosen as the model of MTT Assay. HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), washed twice with ice-cold phosphate buffer solution (PBS) before detaching at the density of 70%-80%, detached from dish using trypsin, and 5×10^3 cells in 100 μL DMEM (10%FBS) were added into 96-well plate each plate. After 6-24 hours Hela cells were attached on the bottom of wells. Old medium was removed and 150 μL DMEM (10%FBS) with 7.5 μL, 15 μL, 30 μL, and 75 μL Au@PLL-AEDP-FITC were added into each well respectively. Each concentration of Au@PLL-AEDP-FITC was added into 5 wells. Hela cells in 96-well plate were cultured for 6 h and 24 h respectively. After culturing 20 μL MTT solution (dissolved in PBS) were added into each well with a final concentration of 0.5 mg mL^{-1}. Medium with MTT were removed and 150 μL DMSO were added into each well after Hela cells were cultured for 4 h. The 96-well plate was shaken for 10 min and OD_{490} value was taken on Molecular Devices SpectraMax iD5 Microplate Reader.

2. Results



FigureS1 Synthesis pathway of 3-[(2-Aminoethyl)dithio]propionic acid (AEDP).

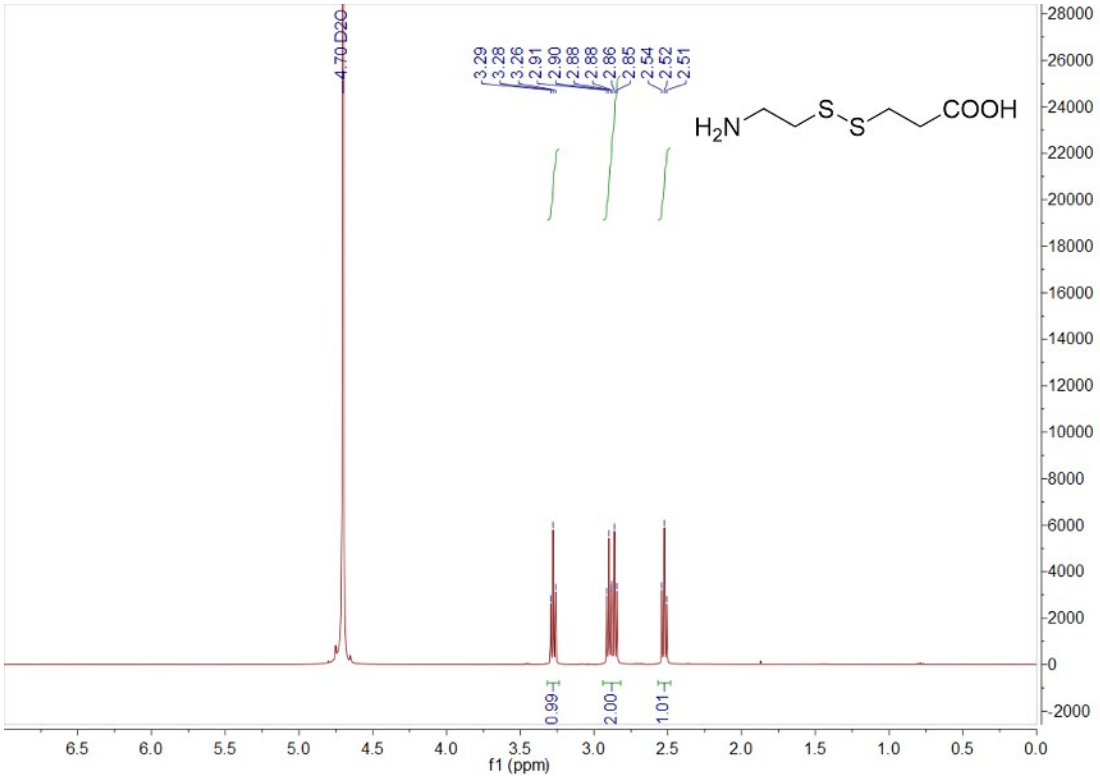


Figure S2 ^{1}H NMR spectrum of AEDP (deuterium reagent: D_2O).

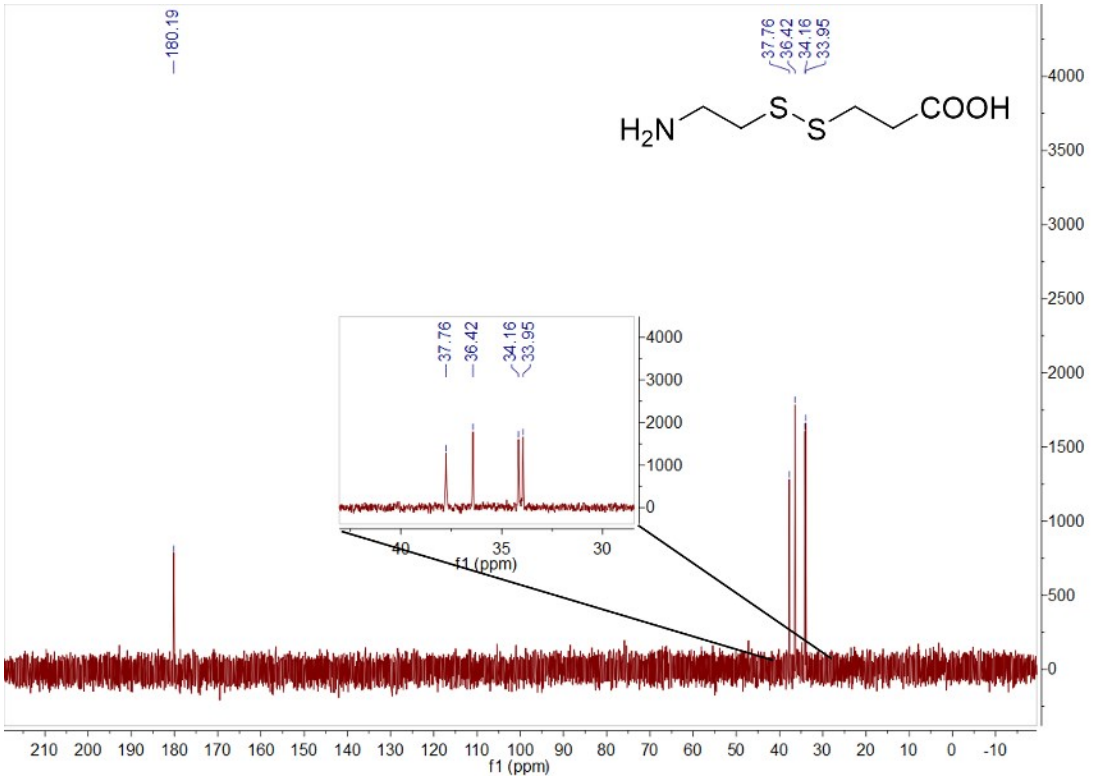


Figure S3 ^{13}C NMR spectrum of AEDP (deuterium reagent: $CDCl_3$).

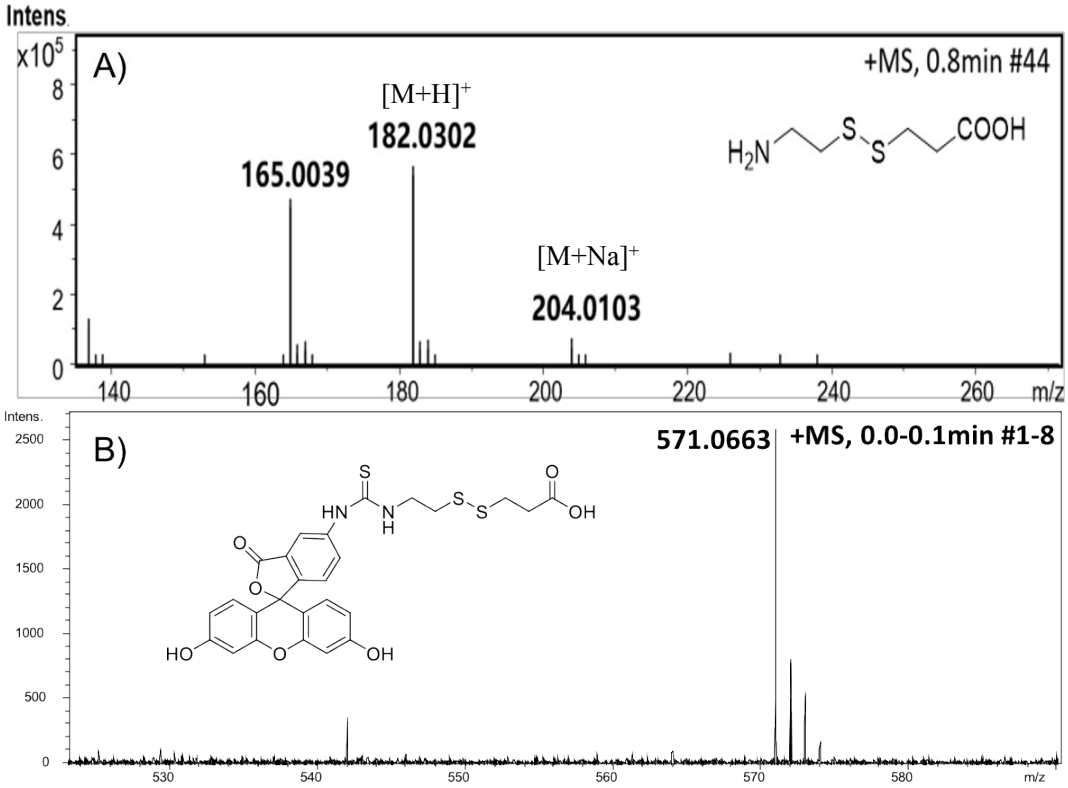


Figure S4 High resolution mass spectrum of fluorescent linkers. All the results were detected in $[M+H]^+$ style. A) High resolution mass spectrum of AEDP. Calculated $[AEDP+H]^+$ *m/z* 182.0309, measured $[AEDP+H]^+$ *m/z* 182.0302, Δ<5 ppm; B) High resolution mass spectrum of AEDP-FITC. Calculated $[AEDP\text{-}FITC+H]^+$ *m/z* 571.0667, measured $[AEDP+H]^+$ *m/z* 571.0663, Δ<5 ppm.

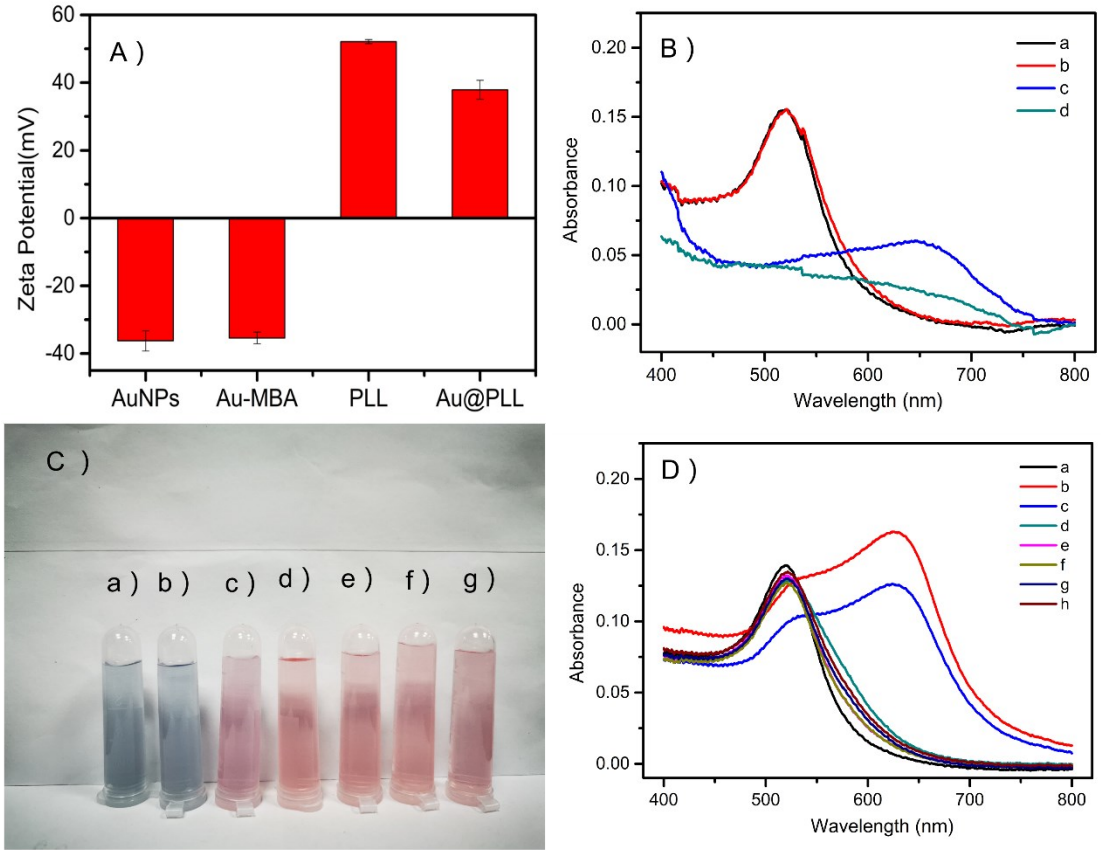


Figure S5 Characterization of AuNPs, Au@PLL, and Au@PLL-AEDP-FITC. A) Zeta Potential of Au NPs, Au-MBA, PLL and Au@PLL. B) UV-Vis absorption spectra of AuNPs modified with different molecular weights of PLL. From a to d, AuNPs, Au@PLL(Mw<5000), Au@PLL(Mw=30000~70000), Au@PLL(Mw=150000~300000), respectively. C) Color change of AuNPs modified with different concentrations of PLL(Mw<5000). From a to g are Au/PLL=4/1, Au/PLL=2/1, Au/PLL=1/1, Au/PLL=1/2, Au/PLL=1/3, Au/PLL=1/4 Au/PLL=1/5 respectively. D) UV-Vis absorption spectra of AuNPs modified with different concentrations of PLL(Mw<5000). From a to h, AuNPs, Au/PLL=4/1, Au/PLL=2/1, Au/PLL=1/1, Au/PLL=1/2, Au/PLL=1/3, Au/PLL=1/4, Au/PLL=1/5 respectively.

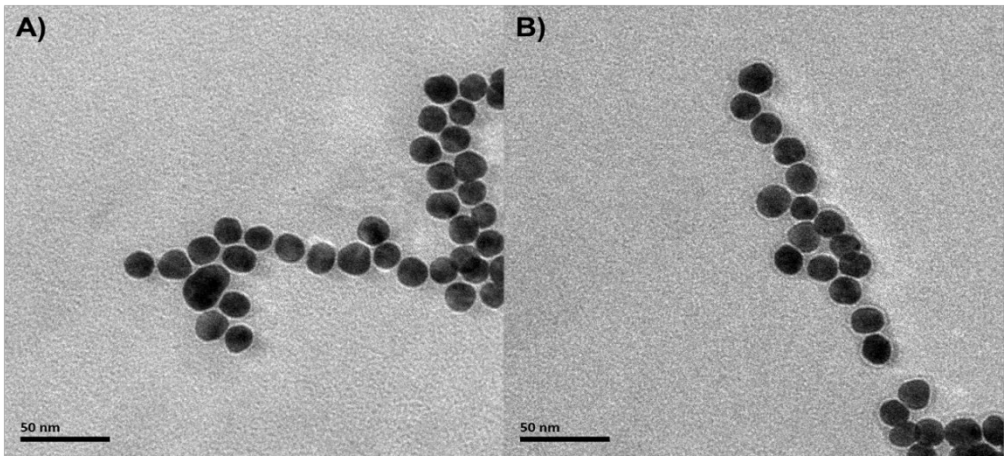


Figure S6 TEM images of Au@PLL and Au@PLL-AEDP-FITC. A) Au@PLL. B) Au@PLL-AEDP-FITC.

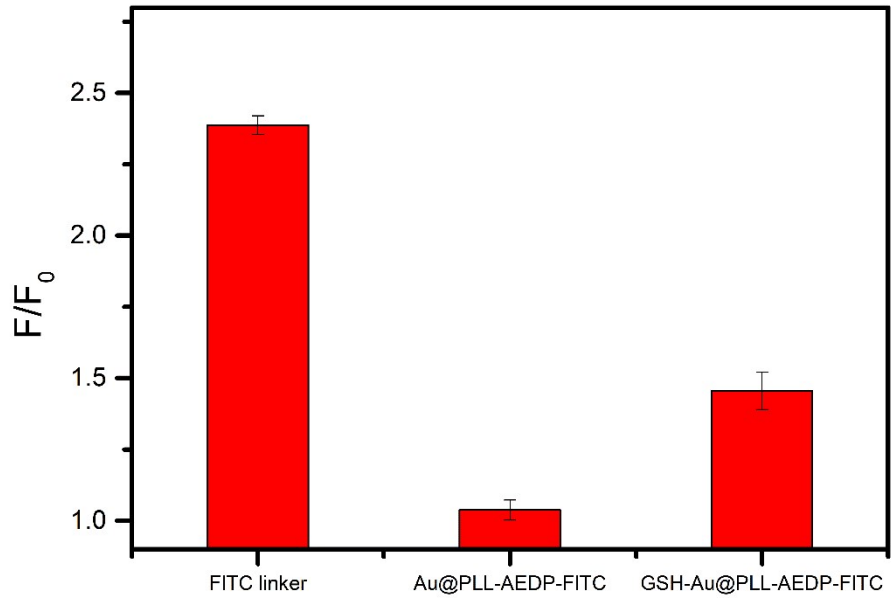


Figure S7 FRET process of Au@PLL-AEDP-FITC.

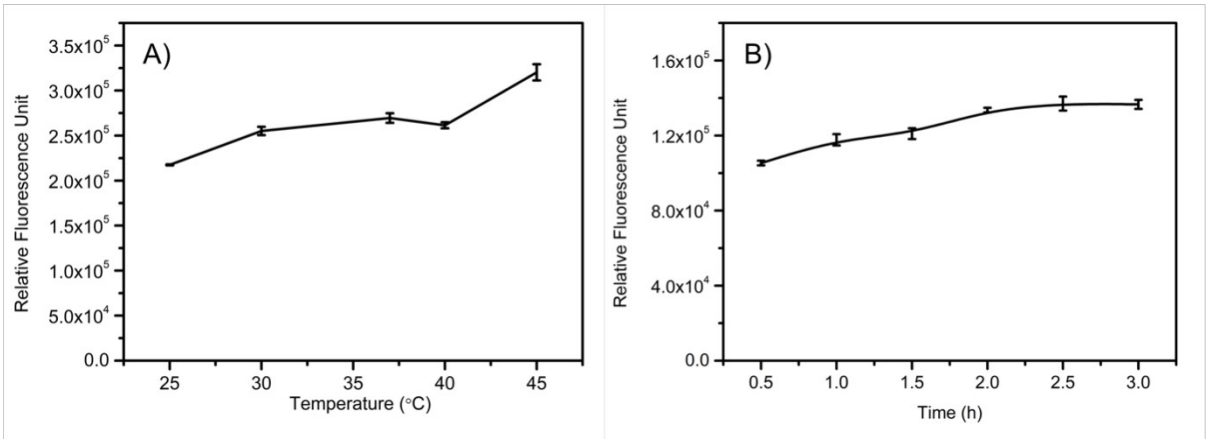


Figure S8 Optimization of experiment conditions. A) Reaction temperature; B) Reaction time.

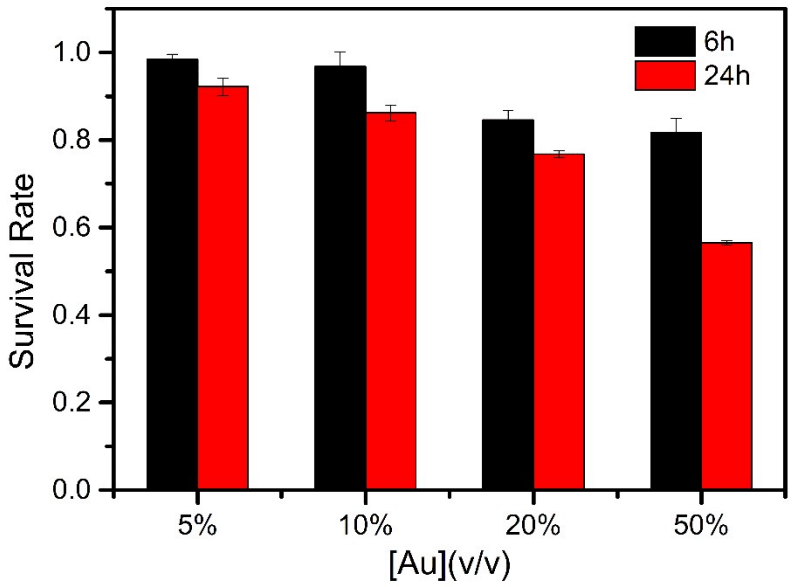


Figure S9 Biocompatibility and cell cytotoxicity of Au@PLL-AEDP-FITC in Hela Cells. Concentration of Au stands for the volume ratio between Au@PLL-AEDP-FITC and DMEM (10%FBS).

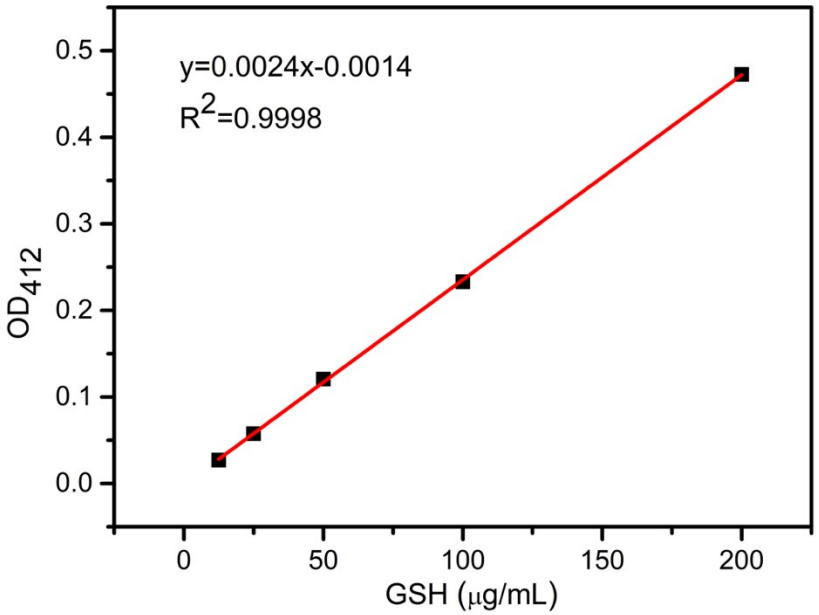


Figure S10 Calibration curve of commercial GSH Kit. LOD of GSH Kit was calculated according to the 3σ rules as 615 nM.

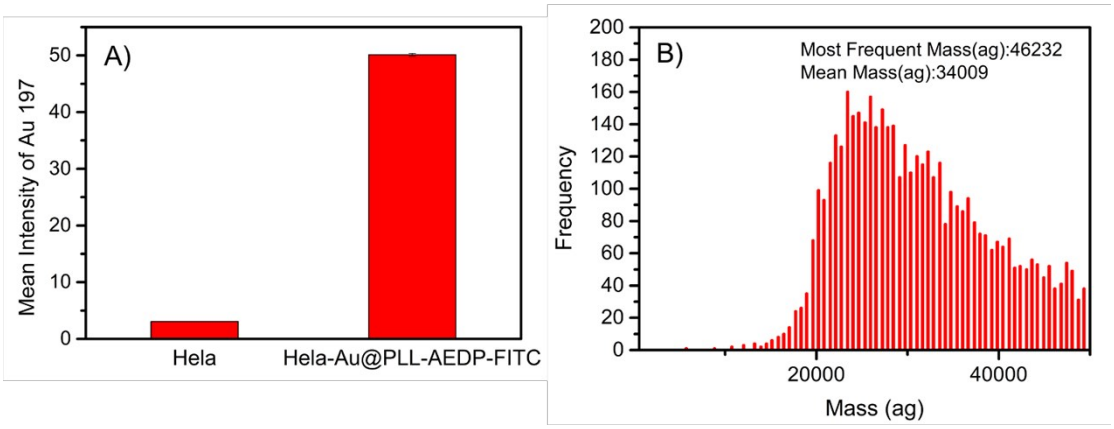


Figure S11 Single cell ICP-MS experiments of Au@PLL-AEDP-FITC in Hela cells. A): mean intensity of Au 197 in Hela cells and Au@PLL-AEDP-FITC treated Hela cells. B): frequency of Au mass in single Hela cell. Cells were incubated for 24 h with Au@PLL-AEDP-FITC, washed with PBS, and diluted at a concentration of 10^5/mL in ddH_2O before ICP-MS experiment.

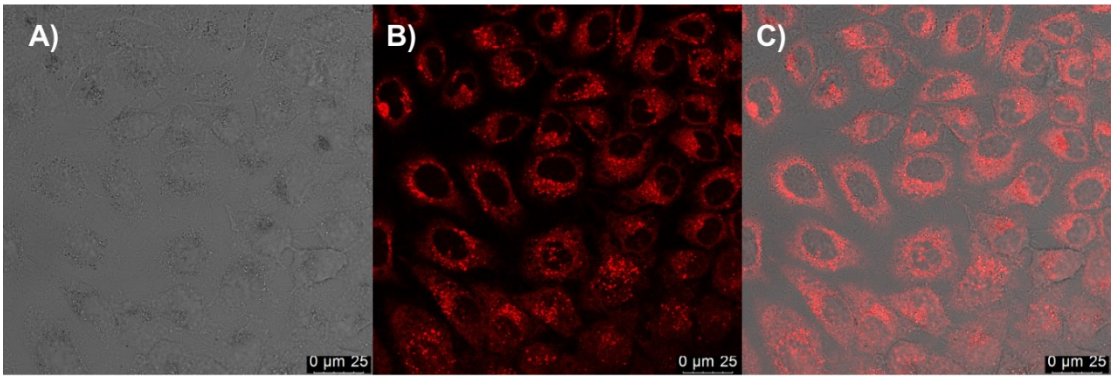


Figure S12 Confocal fluorescence images of HeLa cells using lyso-tracker. (A) Bright field, (B)fluorescence and (C) merge images of HeLa cells. Cells were incubated with 200 µL Au@PLL-AEDP-FITC for 24 h, treated with lyso-tracker for 20 min, rinsed with 10 mM PBS, then used for confocal experiment. Black dark dots in bright field indicates the Au@PLL-AEDP-FITC probe. Lysosome was treated with lyso-tracker and showed in red in fluorescence and merge images.

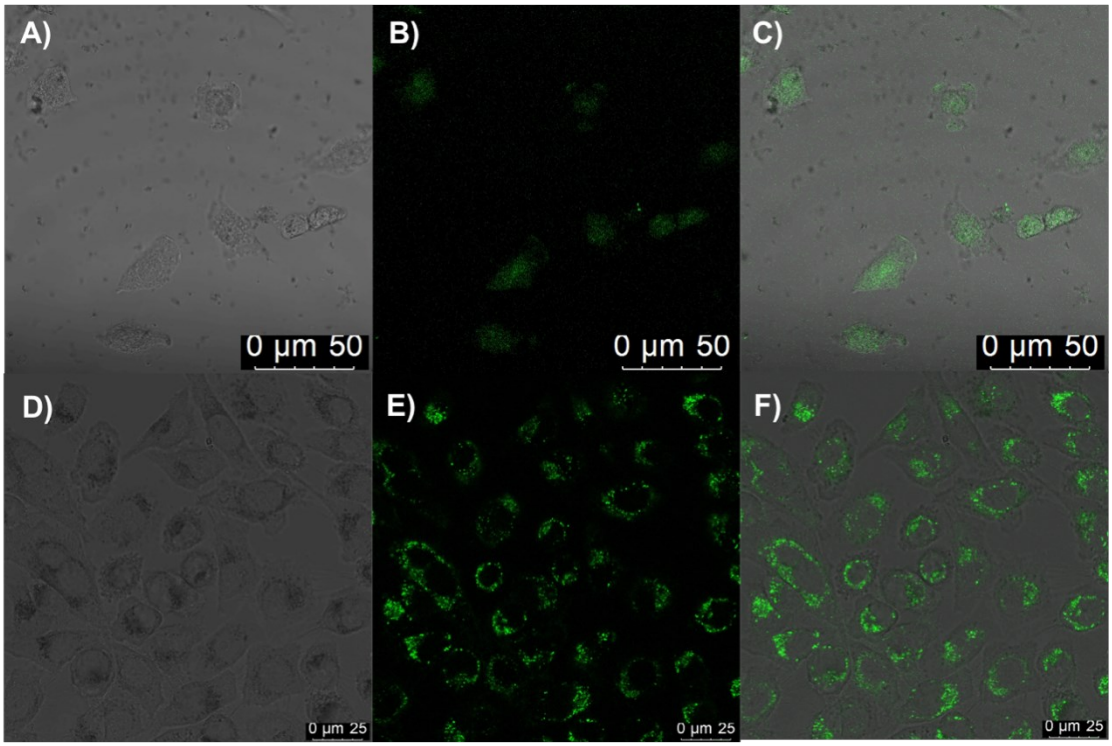


Figure 13 Time-dependent confocal fluorescence images of HeLa cells. (A) Bright field, (B)fluorescence and (C) merge images of HeLa cells incubated with 200 μL Au@PLL-AEDP-FITC for 4 h; (D) Bright field, (E) fluorescence and (F) merge images of HeLa cells with 200 μL Au@PLL-AEDP-FITC for 24 h.

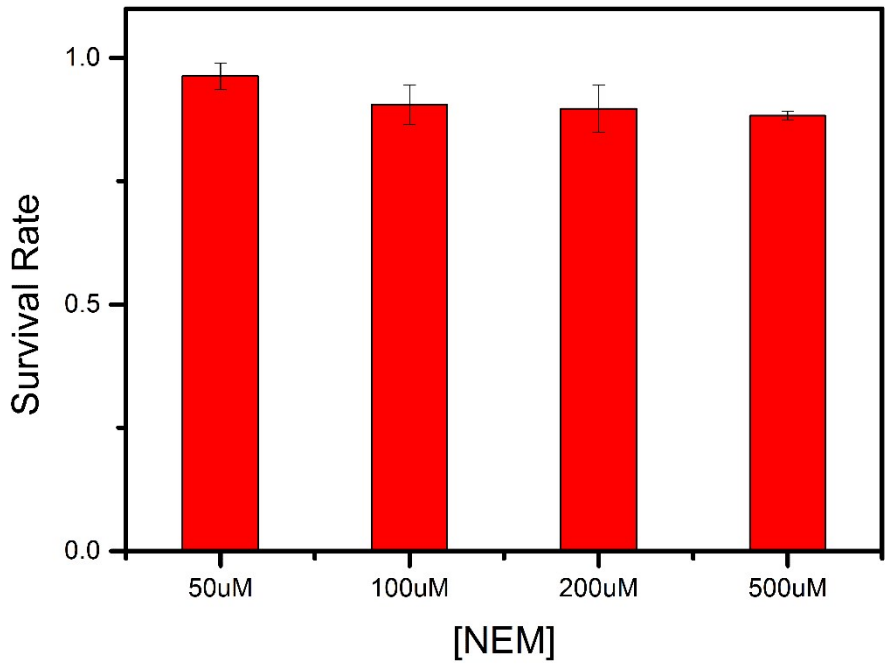


Figure S14 MTT result of different concentration *N*-ethylmaleimide in Hela cells.

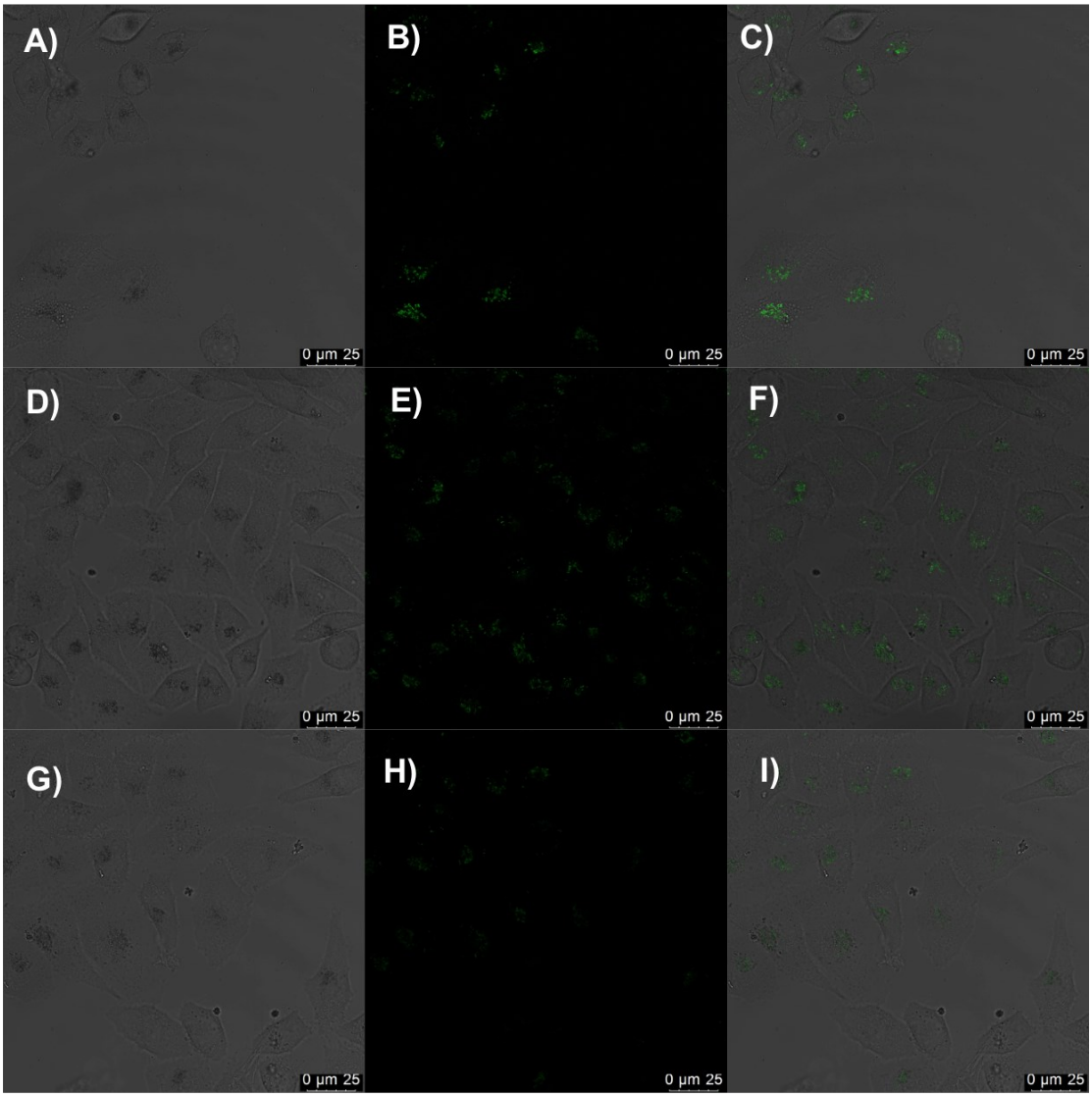


Figure S15 Confocal fluorescence images of HeLa cells pre-incubated with different concentration of *N*-ethylmaleimide for 20 min. (A) Bright field, (B)fluorescence and (C) merge images of HeLa cells pre-incubated with 100μM *N*-ethylmaleimide. (D) Bright field, (E) fluorescence and (F) merge images of HeLa cells pre-incubated with 200μM *N*-ethylmaleimide. (G) Bright field, (H)fluorescence and (I) merge images of HeLa cells 500μM *N*-ethylmaleimide. All the Hela cells were incubated with 200 μL Au@PLL-AEDP-FITC for 24 h after the pre-incubation of *N*-ethylmaleimide.

Table S1 Determination of GSH in Hela cell lysate by two different methods

Cell lysate	GSH Concentration(μM)		
	Dilute 2-folds	Dilute 6-folds	Average
Au@PLL-AEDP-FITC	152.11±2.89	46.62±1.06	291.96±1.98
GSH Kit	143.35±0.78	43.48±3.41	273.78±2.10

Reference:

1. J. E. Eager and W. E. Savige, *Photochem. Photobiol.*, 1963, **2**, 25-37.