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Supplementary Information

Mercaptopyrimidine-directed gold nanoclusters: a suitable fluorescent probe for intracellular glutathione imaging and selective cancer cells identification

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Experimental section

Reagents and instruments

DAMP, GSH, thiazolyl blue (MTT), and buthionine sulfoximine (BSO) were purchased from Sigma-Aldrich (St. Louis, USA). Chloroauric acid ($HAuCl_4$), glutamic acid (Glu), glycine (Gly), lysine (Lys), Cys, NAC, methionine (Met), glutamine (Gln), phenylalanine (Phe), arginine (Arg), serine (Ser), glucose and other reagents are of analytical grade from Sinoreagent (China). The commercial GSH assay kit was purchased from Beyotime Biotechnology Co. Ltd. (China). Phosphate buffer saline (PBS) and all other solutions were prepared throughout by ultrapure water (18.2 MΩ cm^{-1}, Millipore, USA).

The fluorescence and UV−vis spectra were recorded using a Shimadzu RF-5301PC fluoremeter (Japan) and a Biomate 3S spectrophotometer (Thermo Fisher, USA), respectively. The X-ray photoelectron spectra (XPS) and mass spectroscopy (MS) were measured on a PHI 5000 VersaProbe X-ray photoelectron spectrometer (ULVAC-PHI, Japan) and a matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) 4800 plus mass spectrometer (Applied Biosystems, USA), respectively. The zeta potential was obtained by Nano ZS Zetasizer 90 (Malvern, UK). The transmission electron microscopy (TEM) were carried out on a JEM-2100 microscope operating at 200 kV (JEOL, Japan). The MTT assays were executed using a MK3 microplate reader (Thermo Fisher, USA). The confocal microscopic images were shot on a Nikon Ti-E confocal microscopy (Nikon, Japan).

Synthesis of AuDAMP

For typical synthesis of AuDAMP, 2 mL of DAMP (10 mM, dissolved in 50% ethanol solution) and 1 mL of $HAuCl_4$ (10 mM) were added to 7 mL of ultrapure water. The mixed solution was heated to 70 °C and continually reacted under gentle stirring (300 rpm) for 10 h. The red-emitting AuDAMP was formed. To obtain the optimal synthetic conditions, parameters including thiol-to-Au molar ratios, solution pH, reaction temperatures and times were varied. The synthesized Au NCs were purified using an ultrafiltration tube (molecular weight cut-off 3 KDa) and centrifugation to remove bulk gold and free DAMP.[1] The purified AuDAMP solution was stored in refrigerator for further characterization.

Determination of the Quantum Yield

Fluorescence quantum yield (QY) of AuDAMP was determined by using Rhodamine B (QY = 95% in ethanol) as the fluorescence standard.[2] The QY was calculated using the following equation.

$$QY_{Au}\ (\%) = QY_R \times (A_R F_{Au}/A_{Au} F_R) \times (n_{Au}/n_R)^2$$

where A is the value of absorbance at the excitation wavelength; F is the value of area in the corrected emission spectrum; and n is the refractive index of the solvents used. Subscripts R and Au represent the Rhodamine B and the AuDAMP, respectively.

Cytotoxicity assays

The *in vitro* cytotoxicity of Au NCs was evaluated by classical MTT assays.[3] Four types of cell lines, i.e., normal liver L02 cells, cancerous liver HepG2 cells, normal lung AT II cells, and cancerous lung A549 cells were purchased from KeyGen Biotech. Co. Ltd. (Nanjing, China) and were respectively inoculated in a sterile microtiter plates (96-well) for 8 h and then incubated with different concentrations of Au NCs in culture media (DMEM) at 37 °C (5% CO_2) for 24 h. All cell experiments were performed according to the guidelines for Biological Experimentation with the approval of the National Institute of Biological Science and Animal Care Research Advisory Committee of Southeast University. The MTT solution (20 μL, 5 mg/mL) was injected in every well and further cultivated for 4 h. The cell supernatant was discarded and then 150 μL of dimethylsulfoxide was added. The absorbance (A) was measured at 490 nm using a Thermo MK3 microplate reader. The cell viability was estimated based on the following equation:

$$\text{Cell viability (\%)} = A_{treated} / A_{control} \times 100\%$$

where $A_{control}$ was obtained without Au NCs and $A_{treated}$ was obtained with Au NCs.

Assay of GSH in buffer solution (PBS)

Aliquots of a stock solution of GSH (200 mM) were mixed into Au NCs solutions (1 mL), reaching a final concentration of 0.1−100 mM. The fluorescence intensity of the mixture was immediately read by using fluoremeter. The selectivity of this fluorescence probe for GSH was evaluated by examining the fluorescence response of this probe to other main biomolecule including Glu, Gly, Lys, Cys, NAC, Met, Gln, Phe, Arg, Ser, and glucose in PBS. To further evaluate the selectivity of the Au NCs nanosensor toward GSH, the competitive experiments were performed by adding same concentration of GSH to the solution containing the potential interfering substances mentioned above.

Intracellular GSH imaging

L02, HepG2, AT II, and A549 cells were cultured in confocal dishes, respectively. Au NCs were added to each dish with a final concentration of 100 μg/mL and were further incubated for 3 h. In the parallel experiments with decreasing intracellular GSH levels, the cells were pretreated with BSO as a GSH synthase inhibitor before reaction with Au NCs. Then, the cells were fully washed with sterile PBS and immediately observed under a confocal laser scanning microscope (CLSM, Nikon Ti-E, Japan). In addition, GSH was added to the Au NCs-treated cells, and incubated for 2 hours before observation on a CLSM.

Quantitative evaluation of GSH in cells

L02, HepG2, AT II, and A549 cells before and after treatment with BSO were collected, and the concentrations of intracellular GSH were measured by using a commercial kit (Beyotime Co.), respectively. The detailed procedures were operated according to the instructions. Measurements of the absorbance of 5-thio-2-nitrobenzoic acid at 412 nm can be employed to evaluate the GSH level of the sample.

Supplementary Figures and Tables

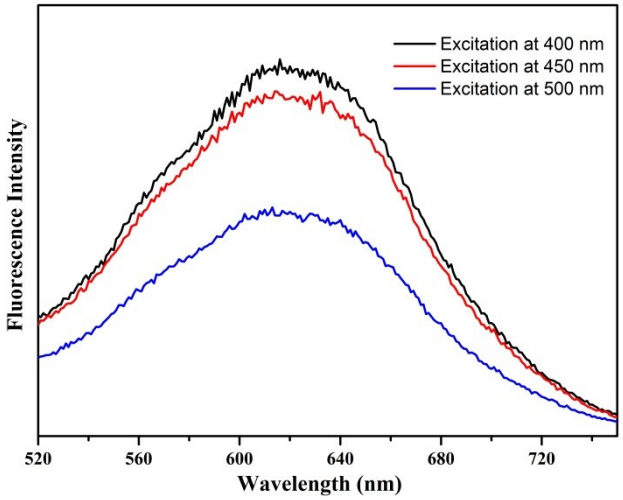

Fig. S1. Fluorescence emission spectra of AuDAMP upon excitation at 400 (black), 450 (red), and 500 (blue) nm, respectively.

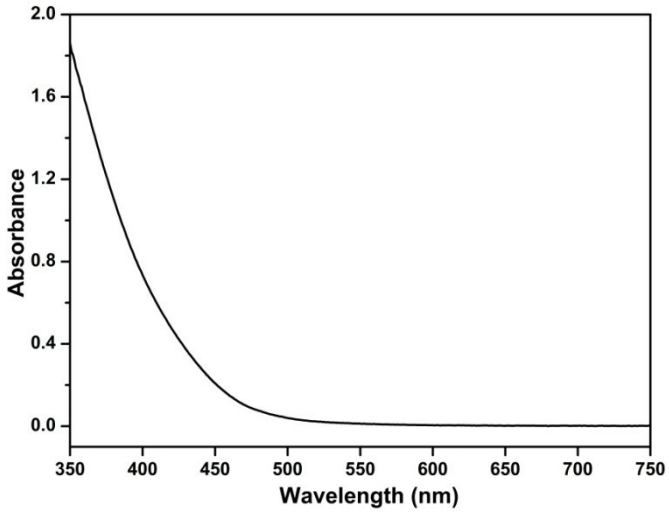

Fig. S2. UV-vis absorption spectrum of AuDAMP aqueous solution.

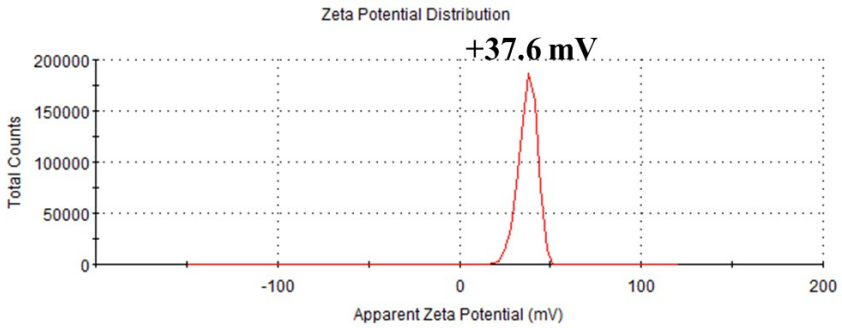

Fig. S3 The zeta potential of AuDAMP.

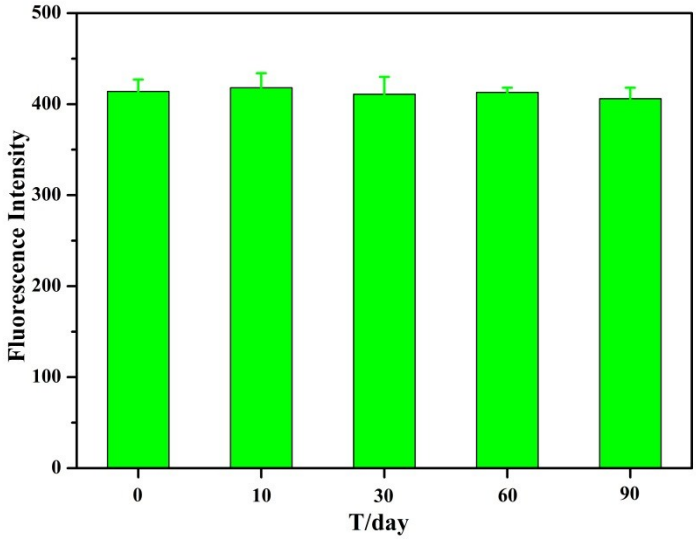

Fig. S4. The fluorescent stability of AuDAMP aqueous solution at room temperature.

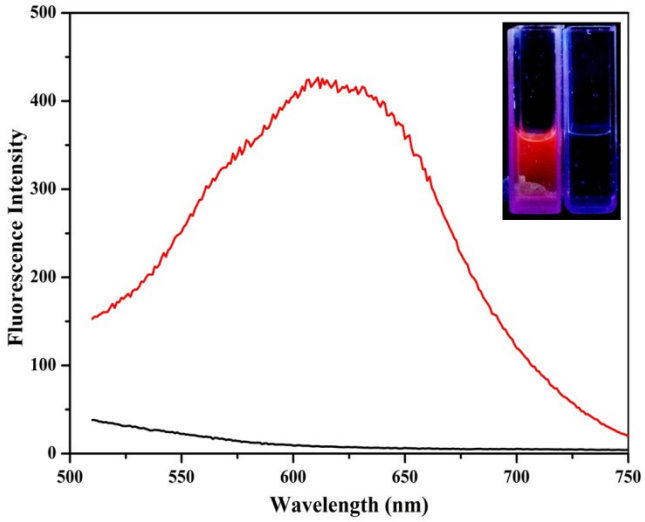

Fig. S5. Fluorescence emission spectra of AuDAMP before (red) and after (black) addition of $NaBH_4$ (excitation wavelength 420 nm). Inset shows the photographs of AuDAMP before (left) and after (right) addition of $NaBH_4$ under UV illumination.

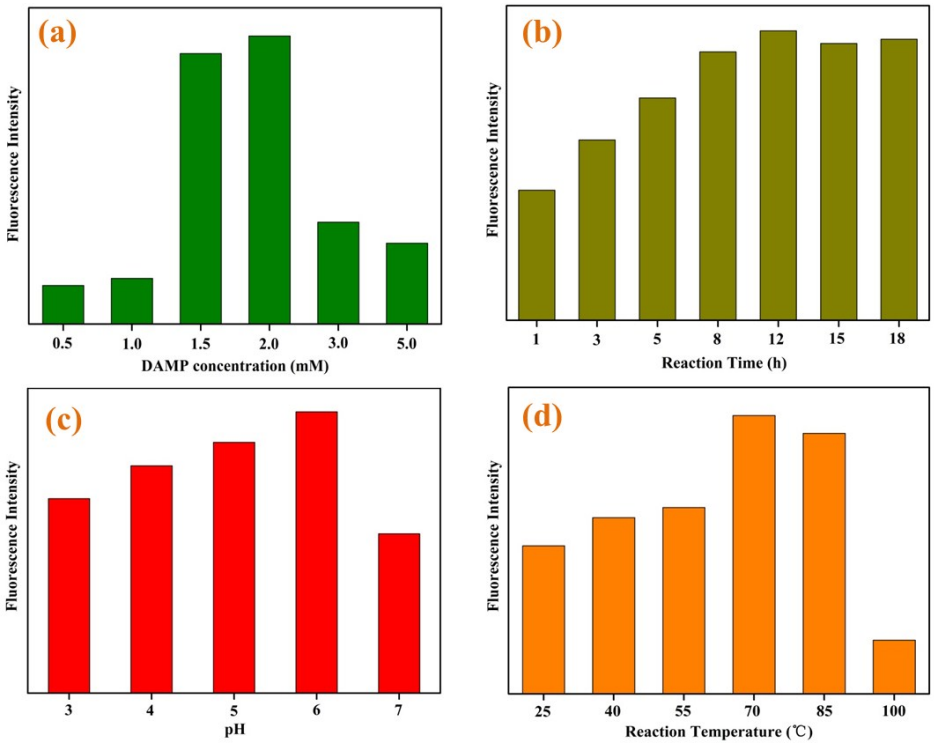

Fig. S6. Effects of the thiol-to-Au molar ratios (a), reaction time (b), pH values (c), and temperature (d) on the fluorescent intensities of AuDAMP.

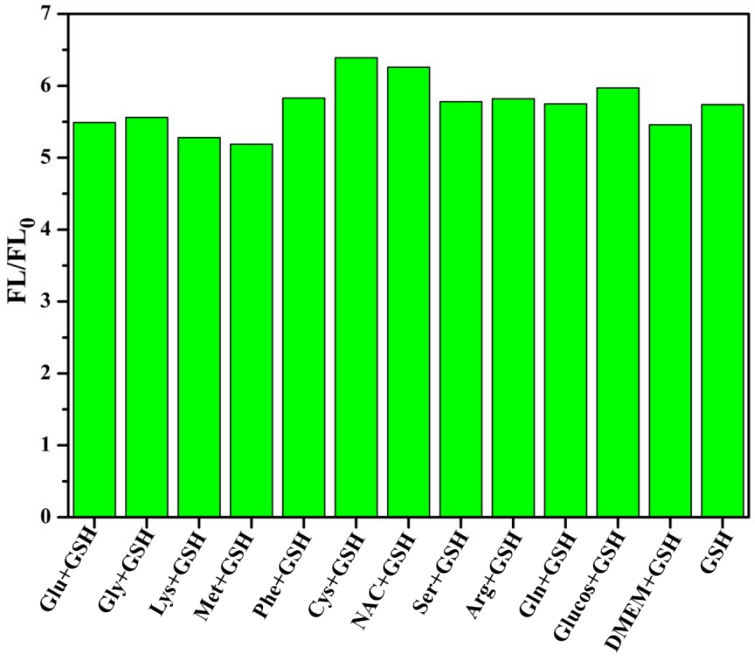

Fig. S7. Competitive assay of the AuDAMP toward GSH in the presence of common potential interfering substances.

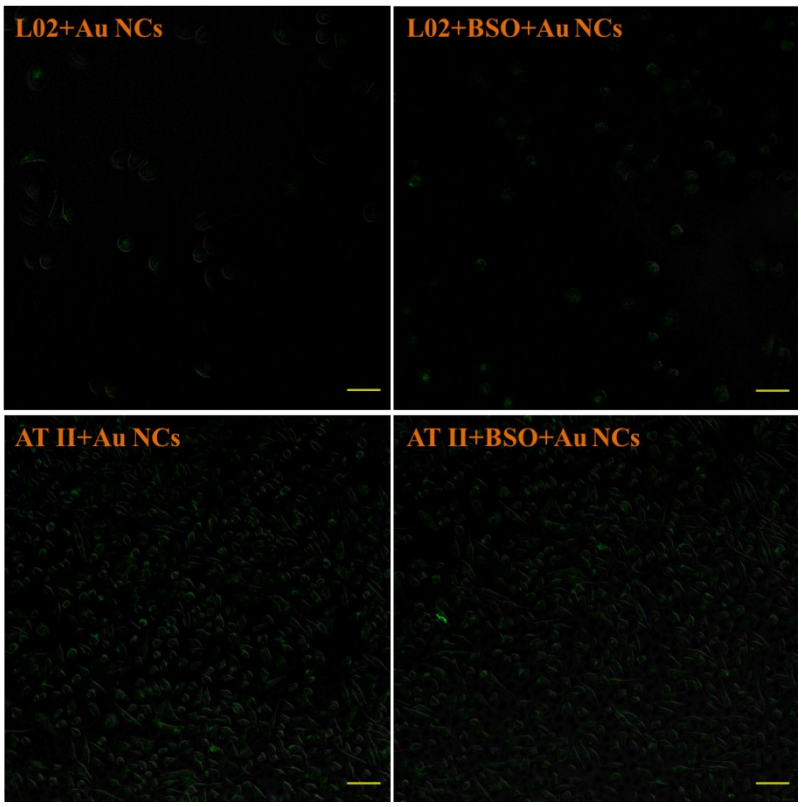

Fig. S8. CLSM images of L02 and AT II cells by incubation of AuDAMP with pretreatment with BSO. Scale bar: 100 μm.

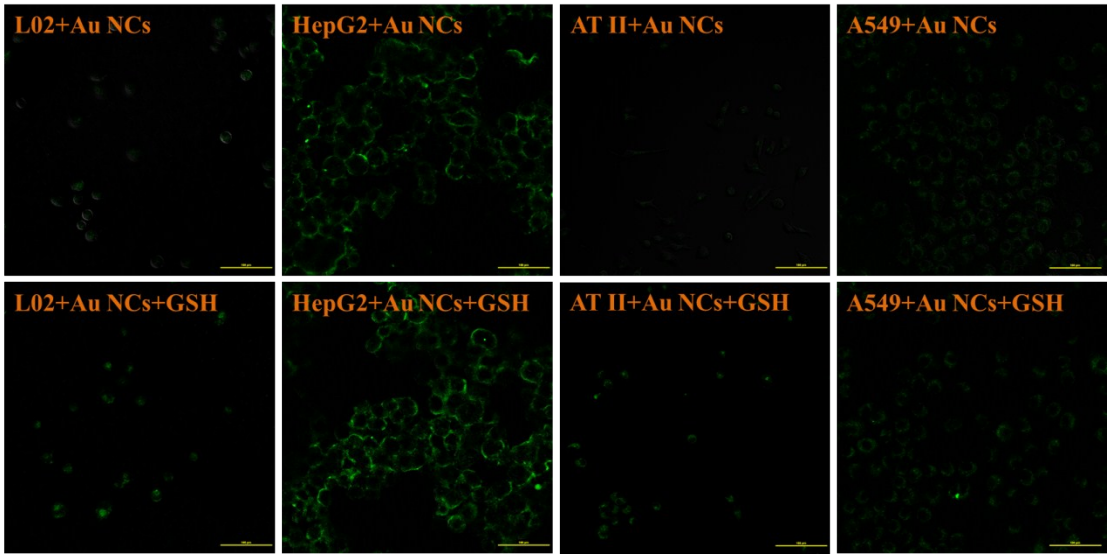

Fig. S9. CLSM images of L02, HepG2, AT II, and A549 cells incubated with AuDAMP and further incubated with added GSH (10 mM). Scale bar: 100 μm. The results show that the fluorescence intensity of cells, particularly in normal cells (i.e. L02 and AT II), is further increased after 2 hours incubation.

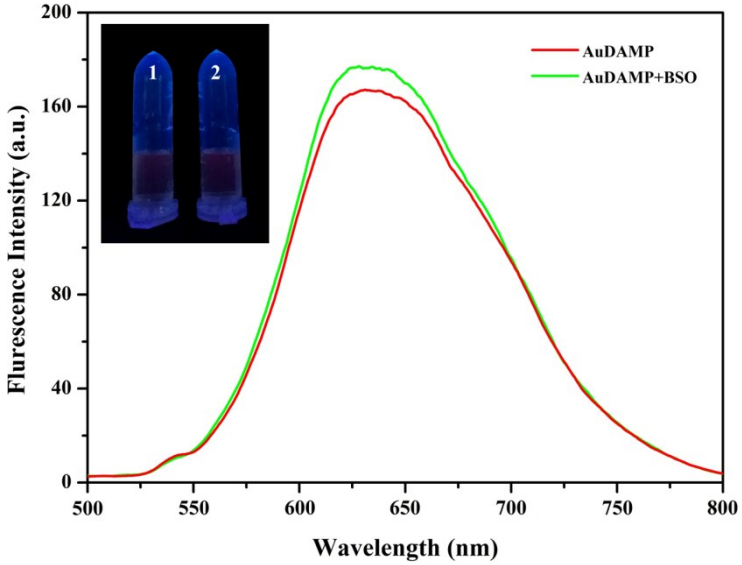

Fig. S10. Fluorescence emission spectra of AuDAMP before (red) and after (green) addition of BSO (excitation wavelength 420 nm). Inset shows the photographs of AuDAMP before (1) and after (2) addition of BSO under UV illumination.

Table S1. Identification of MS m/z peaks.

Number	Peak m/z	Calculated m/z	Formula
1	818.95	817.98	$[Au_{13}(DAMP)_5]^{4-}$
2	852.93	853.52	$[Au_{13}(DAMP)_6]^{4-}$
3	1014.89	1017.54	$[Au_9(DAMP)_9]^{3-}$
4	1048.84	1050.52	$[Au_{17}(DAMP)_6]^{4-}$
5	1210.82	1214.54	$[Au_{12}(DAMP)_9]^{3-}$
6	761.07	760.93	$[Au_8(DAMP)_6(GSH)_2]^{4-}$
7	930.70	930.35	$[Au_{13}(DAMP)_6(GSH)]^{4-}$
8	971.09	971.64	$[Au_{13}(DAMP)_5(GSH)_2]^{4-}$
9	1135.18	1135.29	$[Au_9(DAMP)_5(GSH)_3]^{3-}$

Table S2. The intracellular GSH concentration before and after BSO treatment

Cell lines	GSH concentration (mM)	
	untreated	treated
HepG2	7.2±0.4	2.6±0.2
L02	1.4±0.1	0.9±0.2
A549	5.8±0.3	1.5±0.1
AT II	1.7±0.4	1.2±0.1

Note: Data expressed as mean ± standard deviation.

Reference

1 H. Jiang, Y. Zhang, X. Wang, *Nanoscale*, 2014, **6**, 10355.

2 Y. Zhang, H. Jiang, W. Ge, Q. Li, X. Wang, *Langmuir*, 2014, **30**, 10910.

3 Y. Zheng, W. Liu, Y. Chen, H. Jiang, H. Yan, I. Kosenko, L. Chekulaeva, I. Sivaev, V. Bregadze, X. Wang, *Organometallics*, 2017, **36**, 3484.