Supporting Information

A Gold Nanocarrier and DNA-Metal Ligation-Based Sensing Ensemble for Fluorescent Assay of Thiol–Containing Amino Acids and Peptides

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Chemicals and Apparatus. $HAuCl_4 \cdot 4H_2O$ (AR) was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The DNA oligonucleotide sequences (P1: 5'-FAM-CTT CTT TG AAA AAA AAA AAA AAA A CTT TGT TG-SH-3'; P2: 5'-FAM-CTT CTT TG AAA AAA AAA AAA AAA A CAA AGA AG-SH-3') was synthesized and purified by HPLC from TaKaRa Biotechnology Co., Ltd., (Dalian, China). The probe was labeled at the 5'-end with a carboxyfluorescein (6-FAM) dye and at the 3'-end with a thiol group (-SH). The DNA concentration was identified according to UV absorption at 260 nm. Reduced L-glutathione (GSH), L-cysteine (Cys), homocysteine (Hcy) and other amino acids were purchased from Sigma-Aldrich Chemical Co. and used as received. HeLa cells were obtained from the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China). All other chemicals (AR) were commercially available and used without further purification. Solutions were prepared using deionized water (18.3 MΩ·cm) produced from a Millpore water purification system. They were deoxygenated by bubbling with purified Nitrogen.

The fluorescence spectra were measured using a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ). Both the excitation and emission slit widths were set to 5.0 nm. FAM was excited at λ_{ex} = 480 nm, and its fluorescence emission was followed at λ_{em} = 520 nm. The UV-vis spectra were recorded using a

Hitachi U-4100 UV-vis spectrophotometer (Kyoto, Japan). pH was measured by model 868 pH meter (Orion). Temperature was controlled by PolyScience 9112 refrigerating/heating circulators. For cell imaging, the scanning confocal microscopy (Olympus FluoView 500) was used.

Preparation of the Nanoprobe System. The 13 nm-AuNPs were first produced from gold precursors by citrate reduction[1] and the synthesized nanoparticles were characterized and quantified by absorptivity of a UV-vis absorption spectrum at 520 nm ($\varepsilon = 2.7 \times 10^8$ M^{-1} cm^{-1}).[2] Then, 4 nmol of (alkanethiol)oligonucleotide (P1 or P2) was added to 5 ml of 13 nm Au nanoparticle solution. After ~16 h, the colloid solution was brought to 10 mM in phosphate (NaH_2PO_4/Na_2HPO_4) by adding 0.1 M pH 7 concentrated buffer, and was followed brought to 500 nM in 3-Mercaptopionic acid to block the uncovered surface of gold nanoparticles. In the subsequent salt aging process, colloids were first brought to 0.05 M in NaCl by dropwise addition of 2 M NaCl solution and allowed to stand for 8 h, salted to 0.1 M and allowed to age for another 8 h, then salted to 0.2 M for standing 8 h, and finally salted to 0.3 M in NaCl. To remove excess thiol-DNA, the solution was centrifuged for 25 min at 12000×g. Following removal of the supernatant, the red oily precipitate was then washed with a 10 mM HEPES (2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer solution (pH 7.4, 0.1 M $NaNO_3$, 5 mM KNO_3). After being washed two times, the colloid was redispersed in 5 ml of 10 mM HEPES buffer solution, and stored in a refrigerator (4 ^{0}C).

In Vitro Detection of Thiol–Containing Amino Acids and Peptides Using the Sensing Ensemble. 50 μL of the prepared P1-AuNPs was incubated with 0.5 μM Hg^{2+} solution in 500 μL of 10 mM HEPES buffer for 2 h so that the fluorescence-quenched hairpin-structured nanoprobes could be formed. Then, freshly prepared GSH/Cys stock solution or other samples was added and incubated at room temperature (25 ^{0}C) for 1 h. After reaction, the resulting solution was subjected to fluorescence measurements. The fluorescence spectra were recorded at room

temperature in a quartz cuvette on an PTI QM4 Fluorescence System. The excitation wavelength was 480 nm and the emission wavelengths were in the range from 500 to 600 nm with both excitation and emission slits of 5 nm. The time-dependent fluorescence responses were recoded immediately after the addition of analytes at an excitation wavelength of 480 nm (slit 5 nm) and an emission wavelength of 520 nm (slit 5 nm). (Note: due to the high toxicity of Hg^{2+}, the solutions after assays should be discarded following the waste disposal procedure.)

Live Cell Imaging of Thiol–Containing Amino Acids and Peptides Using the Proposed Nanoprobes. HeLa cells were grown in RPMI 1640 medium (Thermo Scientific HyClone) supplemented with 15% heat-inactivated fetal bovine serum (Invitrogen), 100 U/mL penicillin and 100 U/mL gentamicin at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 24-well culture plate, and grown overnight on glass coverslips at the bottom of the plate. When the cells were ~80% confluent, the coverslips were washed three times with 10 mM HEPES buffer solution (pH 7.4, 0.1 M $NaNO_3$, 5 mM KNO_3). Then, 20 μL of the P1-AuNPs/Hg^{2+} probes was mixed with 480 μL of HEPES buffer containing 0.5 μM Hg^{2+}, and the mixture was added in each well. After incubation for 1 h, the coverslips were then washed twice with cold HEPES buffer and mounted on a glass slide. After another 1 h, fluorescence images were acquired using a water dipping objective (60×, NA 1.2) on a confocal laser scanning fluorescence microscope setup consisting of an Olympus IX-70 inverted microscope with an Olympus FluoView 500 confocal scanning system. Ar+ laser (488 nm) was used as excitation source, and a 505-525 nm bandpass filter was used for fluorescence detection. Three-dimensional images were taken per micrometer by scanning the samples across a defined section along the z-axis.

References

[1] K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735–743.

[2] W. Zhao, W. Chiuman, M. A. Brook and Y. Li, *ChemBioChem,* 2007, ***8***, 727–731.

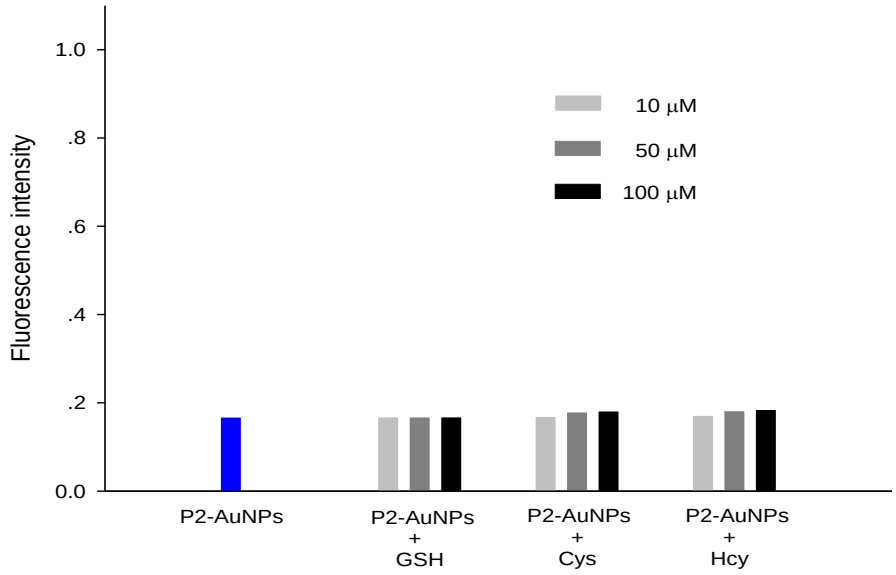

Figure S1 Stability of the adsorption of thiol-DNA on gold surface under the presence of Cys/Hcy/GSH with different concentrations. 50 μL of the prepared P2-AuNPs was incubated with/without Cys/Hcy/GSH in 500 μL of 10 mM HEPES buffer (pH 7.4, 0.1 M $NaNO_3$, 5 mM KNO_3) for 1 h, and then the fluorescence intensity of the reaction solution was recorded at 520 nm with an excitation wavelength of 480 nm.

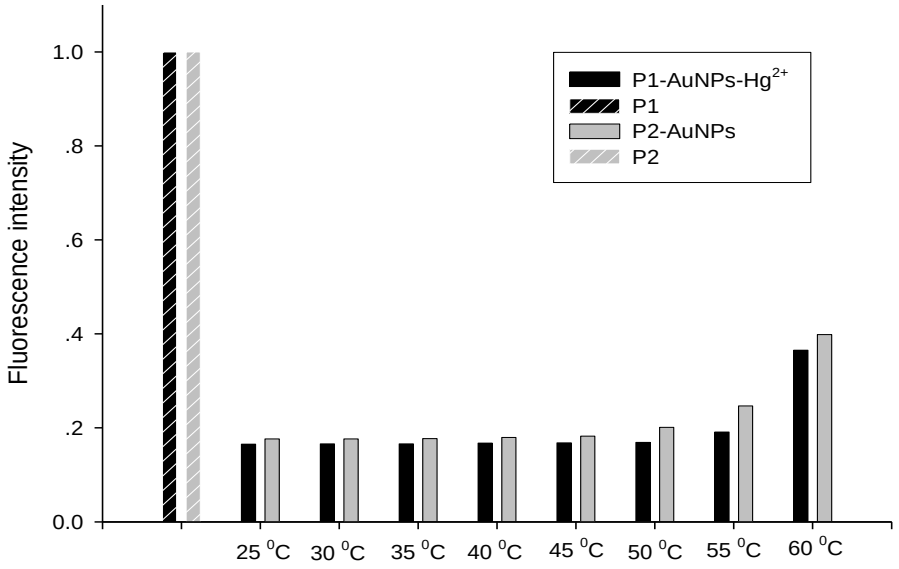

Figure S2 Stability of the probe ensemble under the different temperatures. The concentration of Hg^{2+} is 0.5 μM in 10 mM HEPES buffer (pH 7.4, 0.1 M $NaNO_3$, 5 mM KNO_3). The fluorescence intensity was recorded at 520 nm with an excitation wavelength of 480 nm.

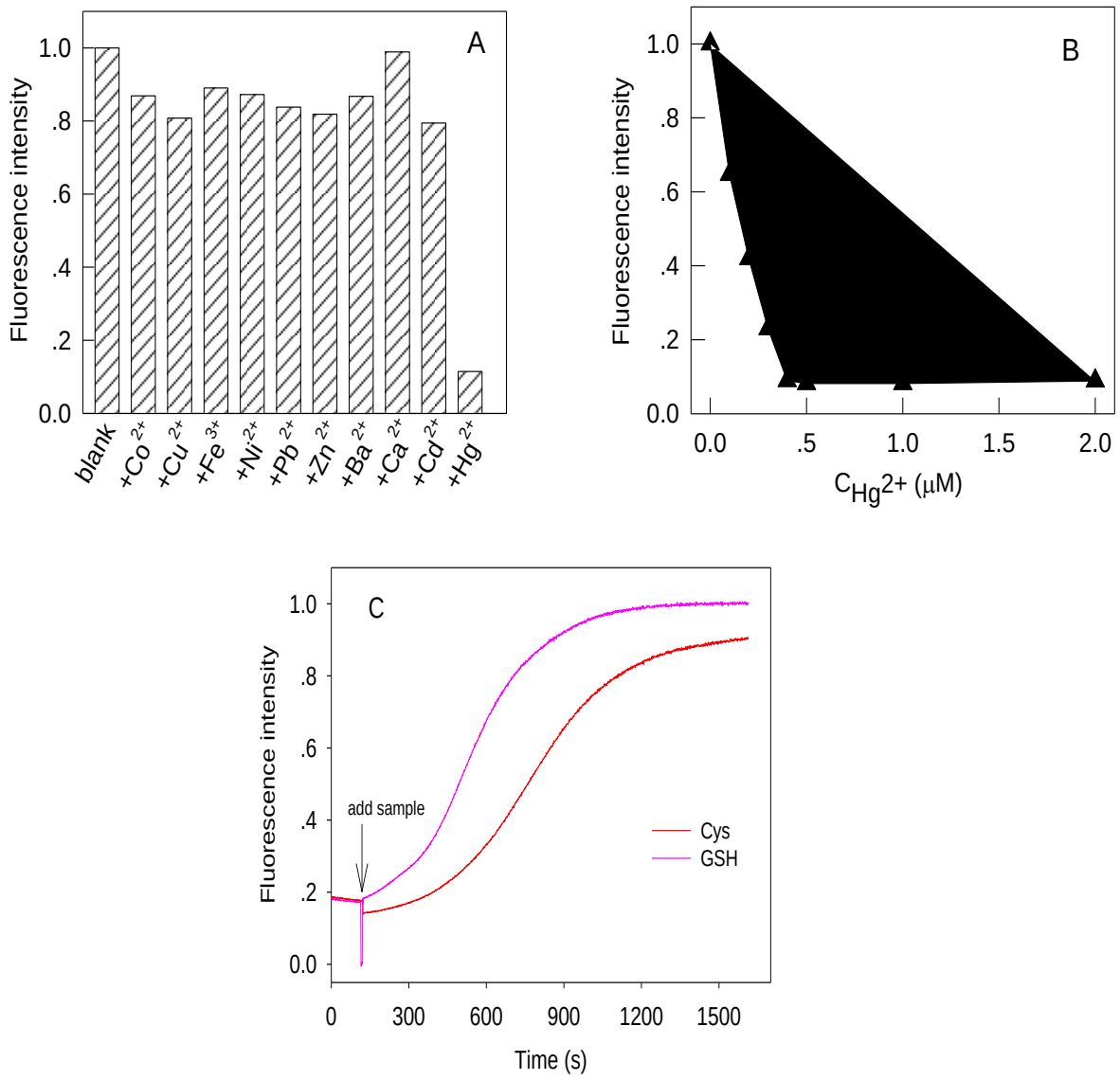

Figure S3 (A) Fluorescence emission of P1-AuNPs with different metal ions. The concentrations of the metal ions are all 10 μM; (B) Fluorescence emission of P1-AuNPs with different concentration of Hg^{2+}; (C) Real-time fluorescence records of P1-AuNPs/Hg^{2+} upon additions of 10 μM Cys (red) or GSH (green); The concentration of Hg^{2+} is 0.5 μM. Buffer solution: 10 mM HEPES buffer (pH 7.4, 0.1 M $NaNO_3$, 5 mM KNO_3). The fluorescence intensity was recorded at 520 nm with an excitation wavelength of 480 nm.

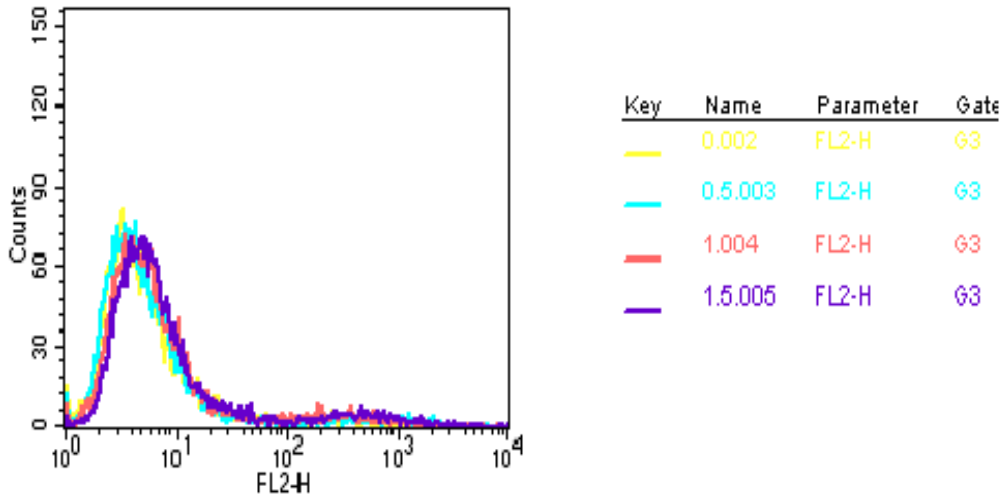

Figure S4 The effect of the toxicity of Hg^{2+} for HeLa cells by flow cytometric analysis. HeLa cells were first incubated with different concentrations of Hg^{2+} (0 (yellow line), 0.5 μM (cyan line), 1.0 μM (red line), 1.5 μM (purple line)) for 3 h at 37 ^{0}C**,** then these cells were treated by using propidium iodide (PI) dye and the flow cytometric analysis was carried out.

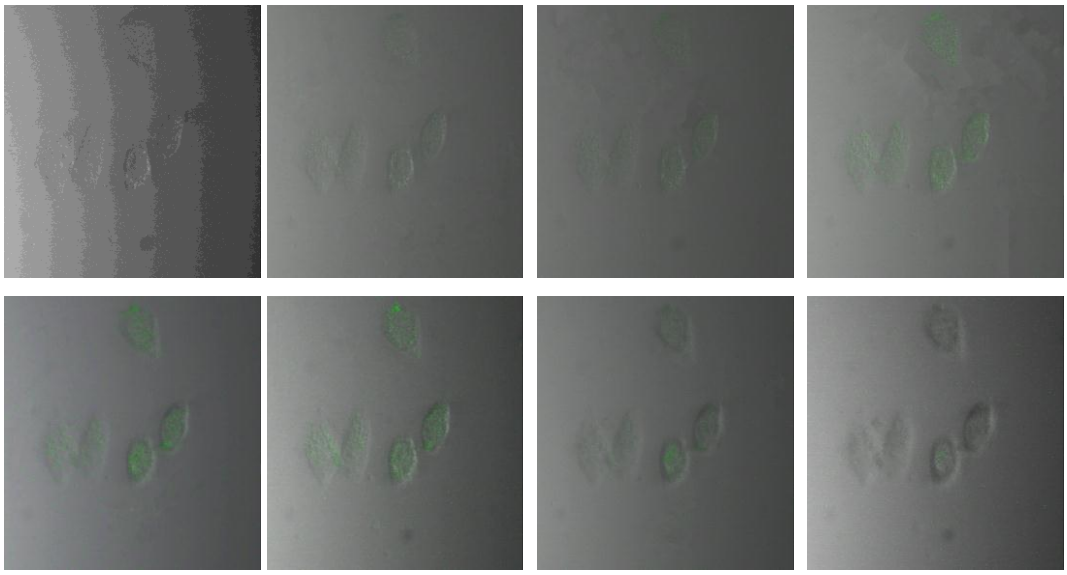

Figure S5 Z-scanning confocal fluorescence microscopy images (Overlap of fluorescence and bright-field images) of HeLa cells incubated with P1-AuNPs/Hg^{2+}. Confocal optical z sections in 1 μm intervals were taken through the whole cells.