

**Supporting Information to accompany:**

**Modulating the optical color of Au nanoclusters and its application of  
ratiometric photoluminescence detection**

Yan Xiao, Jie Zhou, Miaomiao Chen, Wei Wen, Xiuhua Zhang,\* and Shengfu Wang

Hubei Collaborative Innovation Center for Advanced Organic Chemical Materials,  
Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic  
Functional Molecules & College of Chemistry and Chemical Engineering, Hubei  
University, Wuhan, Hubei 430062, PR China.

CORRESPONDING AUTHOR. Dr. Xiuhua Zhang, E-mail: zhangxh@hubu.edu.cn,  
Tel: 86-27-8721-7886, Fax: 86-27-6875-4067. Address: College of Chemistry and  
Chemical Engineering, Hubei University, Wuhan, Hubei 430062, PR China.

## **Experimental details.**

**Reagents:** Glutathione (GSH), Gold(III)chloride trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ), silver nitrate ( $\text{AgNO}_3$ ), bovine serum albumin (BSA) and piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyethyleneimine (PEI, Mw  $\sim$ 70 000), cysteine, homocysteine, urea and sodium citrate was obtained from Aladdin Reagent, Ltd. (Shanghai, China). The rest of the chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and at least analytical grade. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore,  $18.2 \text{ M}\Omega \text{ cm}^{-1}$  resistivity).

**Instrumentation:** The size and morphology of Au nanoclusters and graphitic carbon nitride dots (g-CNDs) were characterized by a JEM-2010 transmission electron microscope with an acceleration voltage of 200 kV. X-ray photoelectron spectroscopy (XPS) was performed on a Kratos Axis Ultra LD spectrometer using a monochromatic Al K $\alpha$  source. The zeta potentials measurements were performed with Malvern Zetasizer Nano ZS ZEN3600 (Malvern Instruments, UK). FI-IR spectra were measured on a NICOLET 5700 FTIR Spectrometer (Thermoelectron, USA) with the KBr pellet technique. The UV-vis absorption spectra were recorded using a UV-2550 UV-vis spectrometer (Shimadzu, Japan). The photoluminescence (PL) measurements were performed with a RF-5301 PC fluorometry (Shimadzu, Japan). The time-resolved photoluminescence was measured by using a FLS980 Lifetime Spectrometer (Edinburgh, England). The photoluminescence images were obtained with a confocal laser scanning microscope (Leica TCS SP5, Germany).

**Synthesis of Au NCs.** In a typical synthesis, a freshly prepared aqueous solution of  $\text{HAuCl}_4$  (20 mM, 4 mL) was added into a GSH aqueous solution (40 mM, 3 mL) under vigorous stirring at 25 °C for 10 minutes. After the mixture turned clear, 43 mL ultrapure water was added to the reaction solution. Thereafter, the mixture was heated to 70 °C under gentle stirring for 12 h. A light-yellow aqueous solution of Au NCs of was thus formed. The supernatants were then dialyzed against ultrapure water with a dialysis

membrane (3500 molecular weight cutoff) for one day to purify the Au NCs. The Au NCs solution was stored at 4 °C until use.

**Sensing of Silver ions.** To detect Ag<sup>+</sup>, A 25 μL aqueous solutions of Au NCs (0.5 mg/mL) and 5 μL of 2.5 mg/mL PEI was first mixed with 270 μL of HEPES buffer (10 mM, pH 7.2) solution and then was incubated with 3 μL of a solution with various concentrations of silver ions on water bath at 70 °C for 40 minutes. The mixtures were then subjected to photoluminescence measurements. The excitation wavelength was 420 nm. To examine the specificity of this method towards silver ion, some metal ions were added in place of silver ions with the same experimental conditions and procedures.

**Experimental Calculation of Limit of Detection (LOD) Value.** The detection limit was calculated based on the method reported in the previous literature.<sup>1</sup> The photoluminescence emission spectrum of blank sample was measured by eleven times and the standard deviation of blank measurement was achieved. The ratio of the photoluminescence intensity at 650 and 565 nm ( $F_{650}/F_{565}$ ) was plotted as a concentration of Ag<sup>+</sup>. The detection limit was calculated by using detection limit  $3\sigma/k$ : where  $\sigma$  is the standard deviation of blank measurement, k is the slope between the photoluminescence intensity ratios ( $F_{460}/F_{650}$ ) versus Ag<sup>+</sup> concentration.

**Synthesis of graphitic Carbon Nitride Dots (g-CNDs).** The water-soluble g-CNDs were synthesized based on a solid-phase method. In brief, a mixture of 0.101 g urea and 0.080 g sodium citrate was ground in the solid state until a uniform powder formed. Then, the mixture was placed in an autoclave and heated to 180 °C for 1 h. The resultant yellowish mixture was purified by washing with ethanol for three times and collected by centrifugation at 12000 rpm. The as-obtained g-CNDs were then dialysis against pure water through a dialysis membrane (3500 molecular weight cutoff) for 24 h.

**Synthesis of PEI-Ag(I)/Au NCs.** A 2 mL aqueous solution of Au NCs (0.5 mg/mL) was mixed with 40 μL of 25 mg/mL PEI and 30 μL of 20 mM AgNO<sub>3</sub> and quickly vortexed for one minute. Subsequently, the obtained solution was incubated on water

bath at 70 °C for 40 minutes to generate PEI-Ag(I)/Au NCs. The PEI-Ag(I)/Au NCs solution was stored at 4 °C until use.

**Preparation of g-CNDs-NCs Nanoprobe.** A 150  $\mu$ L of 0.3 mg/mL PEI-Ag(I)/Au NCs in HEPES buffer (10 mM, pH 7.2) was added to 150  $\mu$ L of 0.3 mg/mL g-CNDs solution, followed by gentle shaking for 30 min. This leads to the electrostatic adsorption of PEI-Ag(I)/Au NCs with g-CNDs to form of g-CNDs-NCs nanoprobe.

**Sensing of Glutathione.** For the detection of GSH, 300  $\mu$ L of 0.15 mg/mL as-prepared g-CNDs-NCs nanoprobes in HEPES buffer (10 mM, pH 7.2) was incubated with 3  $\mu$ L of a solution with various concentrations of GSH at 37 °C for 1 h. Thereafter, the mixtures subjected to photoluminescence measurements. The excitation wavelength was 380 nm. In order to evaluate the selectivity of the nanoprobe for GSH, some other species, including metal ions, hydrogen peroxide and biomolecules were added in place of GSH with the same experimental conditions and procedures.

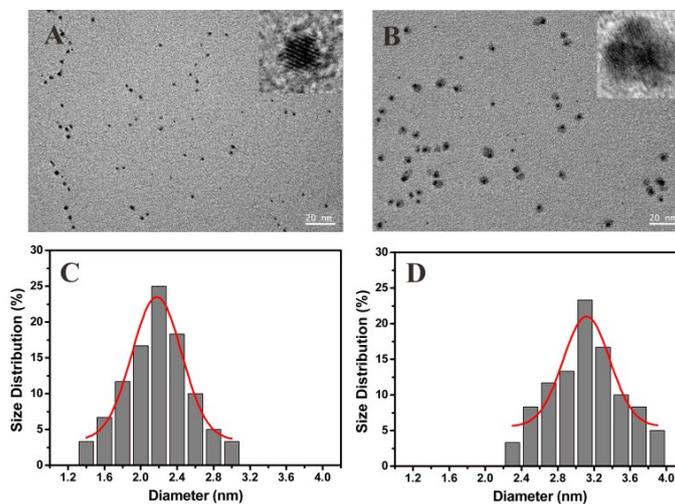
**Cell Culture.** HeLa cells were cultured in DMEM supplemented with 1% penicillin/streptomycin and 10 % fetal bovine serum (FBS), and incubated in an atmosphere of 5/95 (v/v) of CO<sub>2</sub>/air at 37 °C.

**Evaluation of Cytotoxicity.** The cellular cytotoxicity of the nanoprobe was evaluated using the standard MTT assay. HeLa cells were cultured in 96-well plates at 37 °C under an atmosphere of 5/95 (v/v) of CO<sub>2</sub>/air for 12 h. The medium was next replaced by fresh medium containing various concentrations of g-CNDs-NCs nanoprobes and incubated for 24 h. Then, cells were rinsed twice with phosphate buffer saline and 20  $\mu$ L 5.0 mg/mL MTT solution was added into each well, followed by incubation for 4h under the same condition. The medium was then replaced with 200  $\mu$ L DMSO. After shaking for 10 min, the absorbance at 570 nm was measured by microplate reader (Bio-Rad, Model 550, USA). Cell survival rate was calculated by  $A/A_0 \times 100\%$  (A and A<sub>0</sub> are the absorbance of experimental group and control group, respectively).

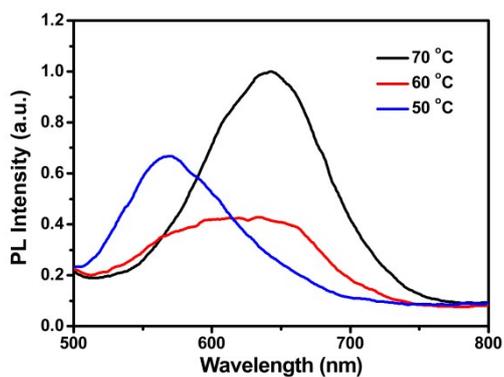
**Nanoprobe for bioimaging.** One day before imaging, the cells were passed and plated into confocal dishes to grow with adherence. Prior to imaging experiments, the cells

were washed with phosphate buffered saline for three times, and then incubated with 0.4 mg/mL g-CNDs-NCs nanoprobe for 4 h at 37 °C. For negative and positive control groups, before the incubation with g-CNDs-NCs nanoprobe, the HeLa cells were initially pretreated with 0.5 mM N-methylmaleimide (NMM) and GSH for 1 h at 37 °C. Cell imaging was then carried out after washing the cells with PBS.

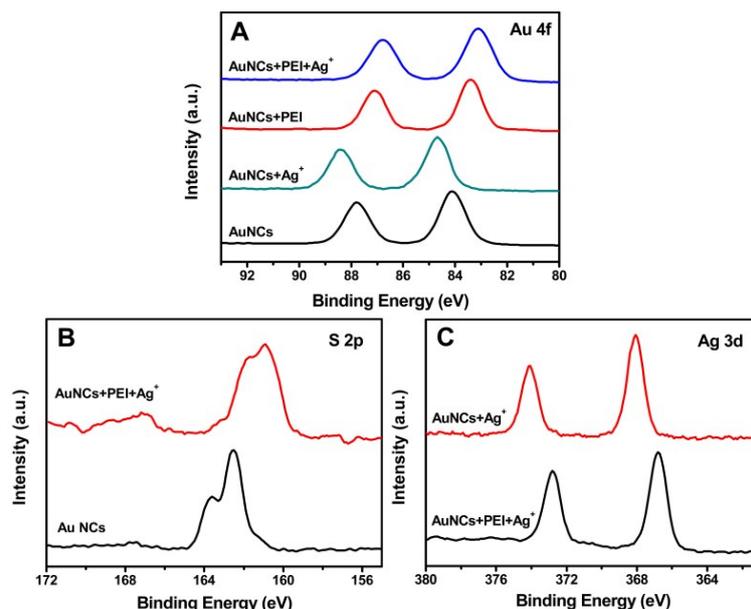
## Supplementary Figures



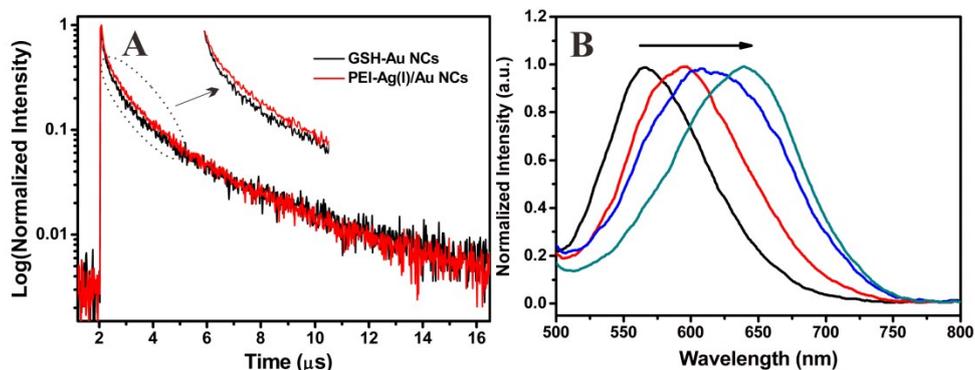
**Figure S1.** (A-B) TEM and HR-TEM (inset) images of the as-synthesized GSH-Au NCs (A) and PEI-Ag(I)/Au NCs (B). (C-D) Size distribution histogram of GSH-Au NCs (C) and PEI-Ag(I)/Au NCs (D).



**Figure S2.** Photoluminescence spectra of GSH-Au NCs (0.04 mg/mL) after incubating with PEI (0.04 mg/mL) and 30  $\mu$ M  $\text{Ag}^+$  under different temperature for 40 min. Excitation wavelength: 420 nm.



**Figure S3.** (A) X-ray photoelectron spectroscopy (XPS) spectrum of Au 4f for the GSH-Au NCs (black), GSH-Au NCs after heating treatment with  $\text{Ag}^+$  (green), with PEI (red), and with PEI and  $\text{Ag}^+$  (blue), respectively. (B) XPS spectrum of S 2p for GSH-Au NCs (black) and the as-formed PEI-Ag(I)/Au NCs (red). (C) XPS spectrum of Ag 3d for the PEI-Ag(I)/Au NCs (black) and the GSH-Au NCs after heating treatment with  $\text{Ag}^+$  (red).

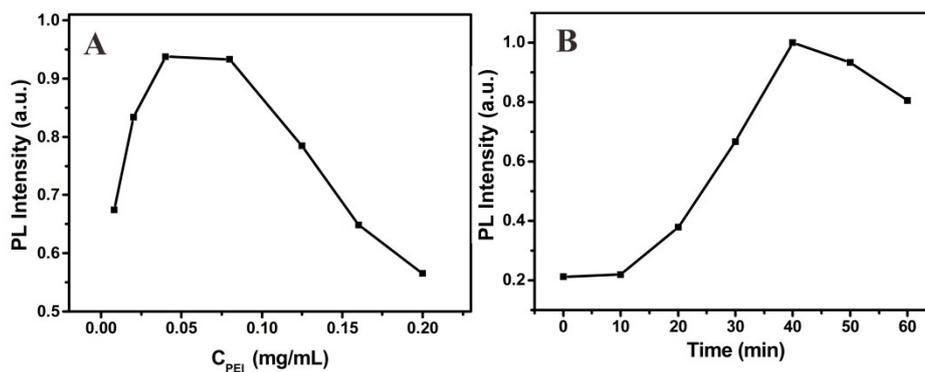


**Figure S4.** (A) Time-resolved photoluminescence decay curves of GSH-Au NCs (black) and PEI-Ag(I)/Au NCs (red). Excitation wavelength: 375 nm. (B) Tunable photoluminescence spectra of PEI-Ag(I)/Au NCs prepared under different  $\text{Ag}^+$  concentration (0, 5, 15, 30  $\mu\text{M}$ ). Excitation wavelength: 420 nm.

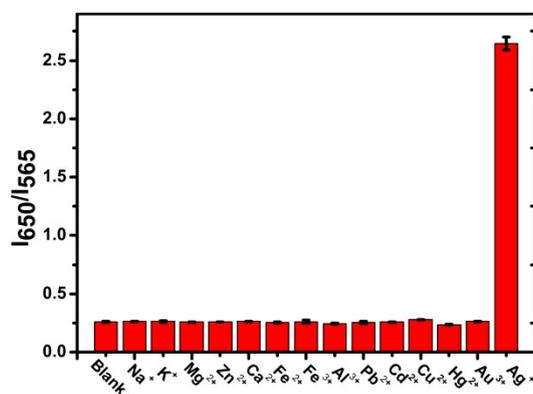
**Table S1.** Decay Parameters for GSH-Au NCs and PEI-Ag(I)/Au NCs.

Names	$\tau_1/\mu\text{s}$	$\tau_2/\mu\text{s}$
GSH-Au NCs	1.7403	0.1648
	68.3%	31.7%
PEI-Ag(I)/Au NCs	1.6477	0.1757
	78.6%	21.4%

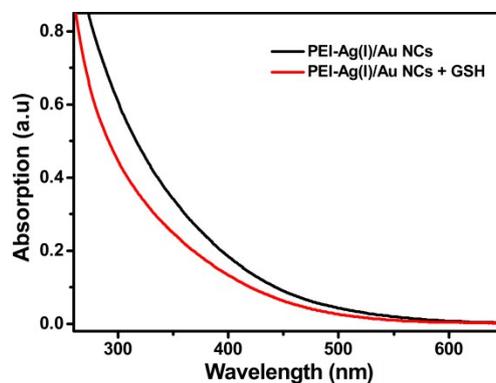
All data were fitted by a bi-exponential decay function. The microsecond-scale lifetime were obviously visible and might be ascribed to phosphorescence generated from the metal-centered triplet states.



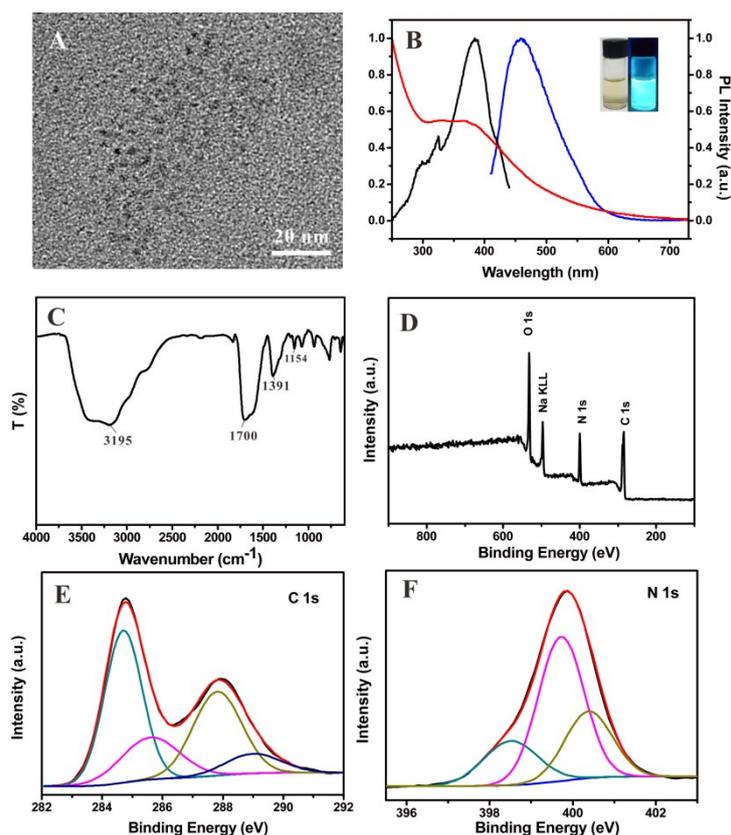
**Figure S5.** Optimization of synthesis conditions of PEI-Ag(I)/Au NCs: (A) concentration of PEI; (B) reaction time.



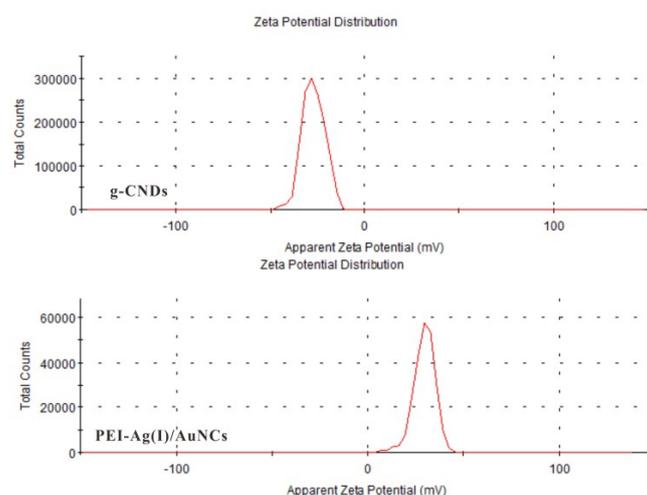
**Figure S6.** Relative photoluminescence intensity ( $I_{650}/I_{565}$ ) of the Au NCs (0.04 mg/mL) after reacting with different metal ions at 70 °C for 40 min. The concentrations of all tested species were 30  $\mu$ M.



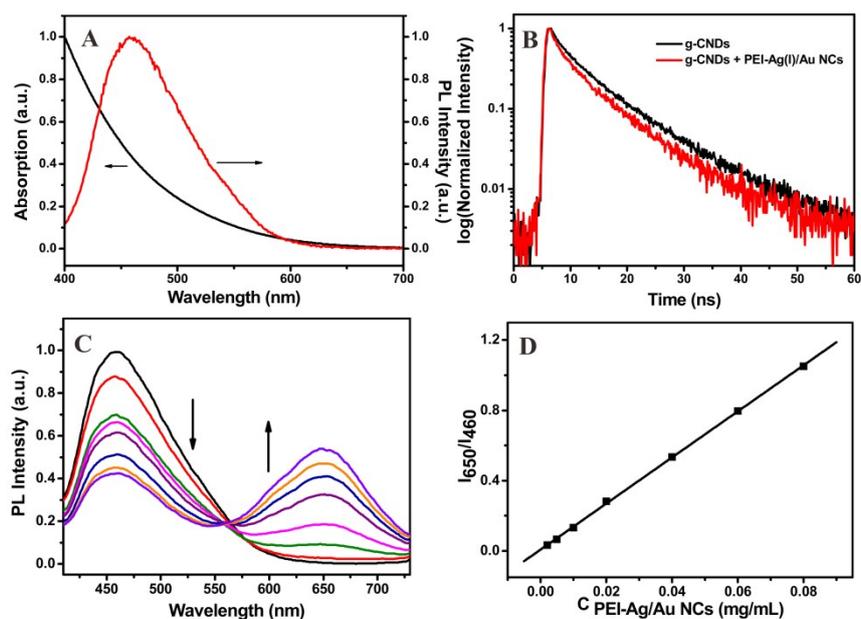
**Figure S7.** UV-vis absorption spectra of PEI-Ag(I)/Au NCs in the absence (black) and in the presence (red) of GSH.



**Figure S8.** (A) TEM image of the as-synthesized g-CNDs. (B) UV-vis absorption (red), photoluminescence excitation (black) and emission (blue) spectra of the g-CNDs. The inset shows the color change of the g-CNDs solution without and with UV irradiation. (C) FT-IR absorption spectra of the g-CNDs. The broad absorption bands at about 3100-3600  $\text{cm}^{-1}$  were corresponded to the stretching vibration of O-H and N-H. The high intensity peak at 1700  $\text{cm}^{-1}$  was ascribed to the stretching vibration of C=O. (D) X-ray photoelectron spectroscopy (XPS) survey spectrum of g-CNDs. (E, F) Expanded spectrum in the C 1s (E) and N 1s (F) region. The C1s spectrum can be deconvoluted into four peaks at 284.6, 286.1, 288.0 and 289.0 eV, which were attributed to  $\text{sp}^2$  C-C bonds,  $\text{sp}^2$  N-C=N bonds, C-O and O-C=O bonds, respectively. The N1s spectrum showed three peaks at 398.6, 399.5 and 400.5 eV, which were associated with C=N-C, C-N-C, N-(C)<sub>3</sub>.



**Figure S9.** Zeta potential of the as-synthesized g-CNDs and PEI-Ag(I)/Au NCs.

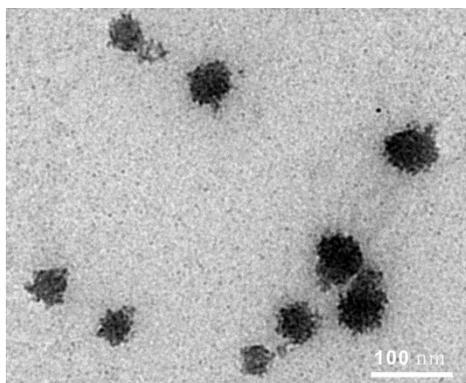


**Figure S10.** (A) The spectral match between the absorption of PEI-Ag(I)/Au NCs (black) and the photoluminescence of g-CNDs (red). (B) Time-resolved photoluminescence decay curves of g-CNDs (0.3 mg/mL) without (black) and with (red) PEI-Ag(I)/Au NCs (0.05 mg/mL). Excitation wavelength: 375 nm. (C) The photoluminescence spectra of the g-CNDs (0.08 mg/mL) in the presence of different concentrations of PEI-Ag(I)/Au NCs (0-0.08 mg/mL). Excitation wavelength: 380 nm. (D) Relative photoluminescence intensity ( $I_{650}/I_{460}$ ) of the g-CNDs in the presence of different concentrations of PEI-Ag(I)/Au NCs.

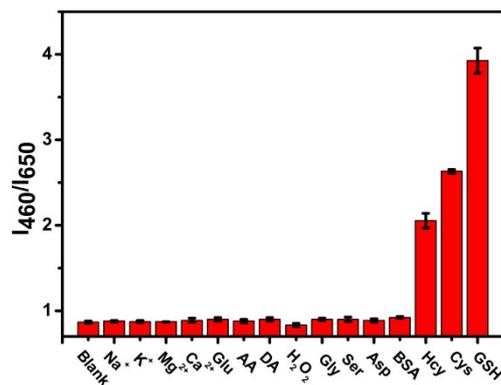
**Table S2.** Decay Parameters for g-CNDs without and with PEI-Ag(I)/Au NCs.

Names	$\tau_1$ /ns	$\tau_2$ /ns
g-CNDs	8.356	1.999
	84.9%	15.1%
g-CNDs + PEI-Ag(I)/Au NCs	7.456	1.717
	80.3%	19.7%

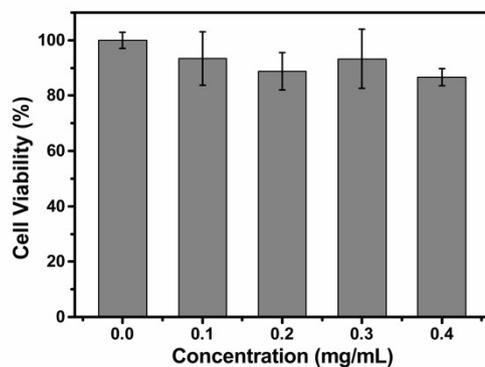
All data were fitted by a bi-exponential decay function.



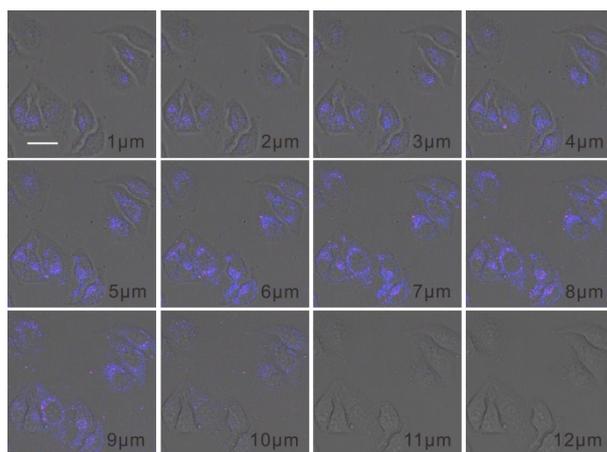
**Figure S11.** TEM image of the g-CNDs and PEI-Ag(I)/Au NCs aggregates.



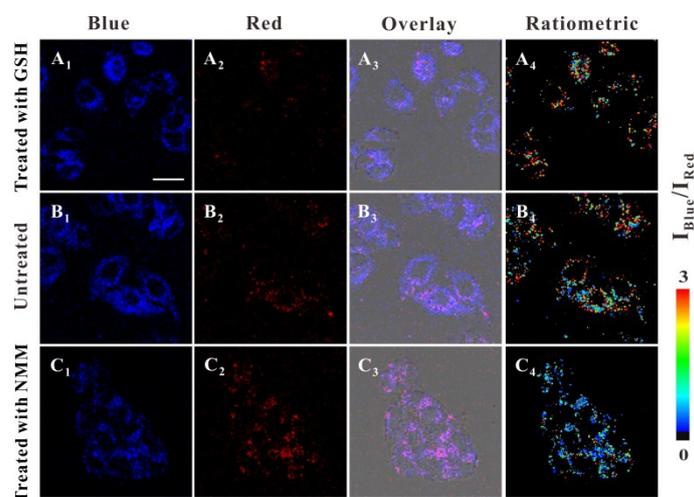
**Figure S12.** Relative photoluminescence intensity ( $I_{460}/I_{650}$ ) of the nanoprobe (0.15 mg/mL) in the presence of different substances. The concentration of BSA was 0.5 mg/mL, and the concentration of other species was 200  $\mu$ M.



**Figure S13.** Cell viability of HeLa cells incubated with nanoprobe at different concentrations for 24 h.



**Figure S14.** Z-direction slices of confocal luminescence imaging for HeLa cells after incubation with nanoprobe (0.4 mg/mL). The images were obtained by scanning the sample along the Z-direction through the focus of the laser beam. Scale bar, 30  $\mu$ m. Excitation wavelength: 380 nm.



**Figure S15.** Confocal microscopic images of HeLa cells incubated with the nanoprobe (0.4 mg/mL). Cells were incubated with 0.5 mM GSH for 1 h before incubation with the nanoprobe (A), with the nanoprobe without pretreatment (B), and with 0.5 mM NMM for 1 h before incubation with the nanoprobe (C). Emission was collected by blue channel at 420-520 nm (A<sub>1</sub>-C<sub>1</sub>) and red channel at 580-680 nm (A<sub>2</sub>-C<sub>2</sub>) under excitation at 380 nm. (A<sub>3</sub>-C<sub>3</sub>) Overlay of brightfield, blue, and red channel images. (A<sub>4</sub>-C<sub>4</sub>) Pseudocolored ratiometric images ( $I_{\text{blue}}/I_{\text{red}}$ ) of HeLa cells. Scale bar (30  $\mu\text{m}$ ).

**Table S3.** Comparison of our sensing system with other approaches for GSH detection.

Strategy	Label	Detection		Liner range	Detection	
		signal	Application		limit	ref
DNA-Hg(II)	Label	single-signal	Solution analysis	5-200 nM	4.2 nM	2
CQDs-Cu(II)	Label	dual-channel	Live cell imaging	0.18-23.3 $\mu\text{M}$	0.05 $\mu\text{M}$	3
Au NPs	Label-free	single-signal	Solution analysis	10-100 and 200-800 $\mu\text{M}$	10 $\mu\text{M}$	4
MnO <sub>2</sub> -Scopoletin	Label-free	ratiometric	Solution analysis	0.02-2 $\mu\text{M}$	6.7 nM	5
Au NCs-TMB	Label-free	single-signal	Solution analysis	2-25 $\mu\text{M}$	0.42 $\mu\text{M}$	6
UCNPs-MnO <sub>2</sub>	Label-free	single-signal	Live cell imaging	Not given	0.9 $\mu\text{M}$	7
CDC-Au(III)	Label-free	single-signal	Solution analysis	0-150 $\mu\text{M}$	2.0 $\mu\text{M}$	8
g-CNDs-NCs	Label-free	ratiometric	Live cell imaging	50-200 $\mu\text{M}$	5.3 $\mu\text{M}$	this work

## References

- [1] Y. Xiao, Z. Wu, K.-Y. Wong, Z. Liu, *Chem. Commun.*, 2014, 50, 4849-4852.
- [2] H. Xu, M. Hepel, *Anal. Chem.* 2011, 83, 813–819.
- [3] Y. Huang, J. Zhou, H. Feng, J. Zheng, H.-M. Ma, W. Liu, C. Tang, H. Ao, M. Zhao, Z. Qian, *Biosens. Bioelectron.* 2016, 86, 748–755.
- [4] F. Ghasemi, M. R. Hormozi-Nezhad, M. Mahmoudi, *Analytica Chimica Acta*, 2015, 882, 58-67.
- [5] D. Fan, C. Shang, W. Gu, E. Wang, and S. Dong, *ACS Appl. Mater. Interfaces.*, 2017, 9, 25870-25877.
- [6] J. Feng, P. Huang, S. Shi, K.-Y. Deng, F.-Y. Wu, *Anal. Chim. Acta*, 2017, 967, 64-69.
- [7] R. Deng, X. Xie, M. Vendrell, Y.-T. Chang, X. Liu, *J. Am. Chem. Soc.*, 2011, 133, 20168-20171.
- [8] J. Gao, D. Hu, W. Wang, Q. Zhang, Z. Meng, X. Jia, K. Xi, *Biosens. Bioelectron.*, 2015, 68, 27-33.