Electronic Supplementary Information

Based on graphene oxide design of a dual-output fluorescent DNA logic gate and detection of silver ions and cysteine

Wan Yi Xie, Wei Tao Huang, Nian Bing Li*, and Hong Qun Luo*

Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P.R. China

* Corresponding authors: linb@swu.edu.cn (N.B. Li); luohq@swu.edu.cn (H.Q. Luo)

1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), fluorescein, amino acids and glutathione (GSH) were purchased from Aladdin Ltd. (Shanghai, China). The used metal salts, i.e. $AgNO_3$, $Bi(NO_3)_3$, $MgCl_2$, $Cu(NO_3)_2$, $Mn(Ac)_2$, $ZnCl_2$, $CrCl_3$, $Pb(NO_3)_2$, $Ni(NO_3)_2$, $CoCl_2$, $Cd(NO_3)_2$, $FeCl_3$, $FeCl_2$, $CaCl_2$, $NaNO_3$, and $Hg(NO_3)_2$ were of analytical grade and used as received without further purification. The oligonucleotides (DNA1: 5'-CTC TCT CTC TCT CTC TCT CTC-FAM-3', DNA2: 5'-CAC ACA CAC ACA CAC ACA CAC-3') were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Stock solutions of DNA1, DNA2, and $AgNO_3$ were prepared in ultrapure water. All working solutions were prepared with HEPES buffer solution (10 mM, pH 7.4, 200 mM $NaNO_3$). The amino acid solutions were prepared freshly on the day of use.

2. Preparation of graphene oxide (GO)

Here we used chemical oxidation of graphite (spectral pure, Sinopharm Chemical Reagent Co., Ltd., China) to obtain hydrophilic graphene oxide (GO) by a modified Hummers method.[1,2] Graphite powder (3.0 g) was oxidized in a hot solution (80°C) containing concentrated H_2SO_4 (12.0 mL), $K_2S_2O_8$ (2.5 g), and P_2O_5 (2.5 g). The mixture was kept at 80 °C for 4.5 h using a hotplate. The mixture was cooled to room temperature and diluted to 300 mL with ultrapure water and left overnight. Then, the mixture was filtered with a filter membrane of 0.22 μm. The product was dried under ambient condition overnight. This pre-oxidized graphite powder was put into cold (0 °C) concentrated H_2SO_4 (120 mL). Then, $KMnO_4$ (15.0 g) was gradually added to the mixture under continuous stirring and the temperature of the mixture was kept to be below 20 °C by cooling. The mixture was further stirred at 35 °C for 2 h, in which ultrapure water (250 mL) was added. After that, 0.7 L of ultrapure water and 20 mL of 30% H_2O_2 were added to the mixture, successively. The product was filtered and the resulting solid was washed with HCl (1:10) aqueous solution (1.0 L) and then with water to remove the acid. The solid was dried in air and then suspended in ultrapure water to obtain a GO dispersion (0.5% w/w). Finally, it was purified by dialysis for one week to remove residual metal ions. 0.0844 g of as-synthesized GO was dispersed in 100 mL of ultrapure water under the aid of magnetic stirring for 2 h, then sonicated for 30 min (100 W) using a KQ 218 probe-type sonicator (Kunshan Ultrasonication Instrument

Co., Ltd, China). The brown homogeneous GO solution (Fig. S1A) had a concentration of about 0.844 mg/mL.

The prepared GO was analyzed by atomic force microscopy (AFM) and the sheet thickness of GO was approximately 1.2 nm (Fig. S1B), illustrating the successful formation of single-layered GO. The chemical structure of GO was characterized by X-ray photoelectron spectroscopy (XPS). The high-resolution XPS spectra presented in Fig. S1C show the details of oxygen binding in GO. Taken together, these data supported the premise that single-layered GO sheets were successfully prepared. AFM imaging was carried out with a Multimode microscope (Veeco, USA) with a Nanoscope IIIa controller, equipped with a Nanoscope Quadrex in tapping mode using a TESP7 Veeco AFM tip. The XPS measurement was carried out with an ESCALAB 250 high performance photoelectron spectrometer (Thermo VG Scientific, U.K.) with an Al Kα (1486.6 eV) radiation. The spectra obtained were calibrated to a C1s peak at 284.6 eV, and fitted by Avantage (a freeware).

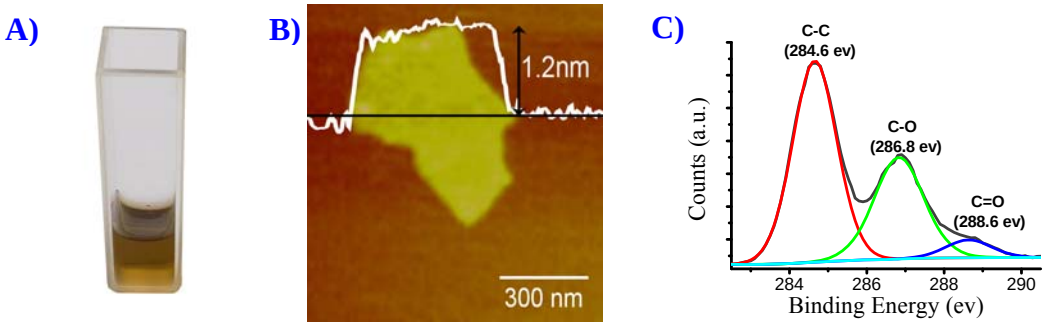

Fig. S1. A) Photograph of the as-prepared GO. B) AFM tapping-mode image of the as-prepared GO sheet. C) High-resolution XPS spectra of GO.

3. Optimization of the variables of the measuring system

For evaluation of validity of the proposed principle, the fluorescence quenching ability of GO was evaluated via fluorescent measurements of the dye molecule of ssDNA in the presence of GO. As expected, the fluorescence of DNA1/DNA2 was decreased rapidly with the addition of GO. In our experiment, more than 99% quenching was observed when the volume of GO solution (0.844 mg/mL) was over 17.5 μL (Fig. S2A). Here, GO acted as an excellent "nanoquencher" for the fluorophore.

We also investigated the effect of the addition order of DNA1, DNA2, Ag^+, and GO of this system on the fluorescence: (1) DNA1/DNA2 was incubated with Ag^+ at room temperature for 30 min firstly, and GO was then added to the mixture before detection. As shown in curve a of Fig. S2B, the fluorescence intensity was decreased at first, while 5 min later the fluorescence intensity no longer gave an obvious change and kept stable. These results implied that double–stranded DNA was formed by C–Ag^+–C coordination chemistry in the presence of Ag^+ and the duplex DNA formed in this way cannot interact with GO effectively. (2) DNA1 was mixed with GO at first and DNA2 and Ag^+ were then added in the DNA1/GO mixture. Importantly, the fluorescence prequenched in DNA1/GO was slowly recovered (see curve b in Fig. S2B). This approach is slow in kinetics due to the competition between DNA1/GO adsorption and the DNA1/Ag^+/DNA2 hybridization; (3) GO was added to the DNA1/DNA2 solution at first and then this solution was incubated with Ag^+. With the addition of Ag^+, the fluorescence intensity exhibited a rapid increase in the first 10 min, after that no obvious emission change could be observed (see curve c in Fig. S2B), but the fluorescence intensity was much lower than that of curve a. Among these three addition strategies we favor the use of the first addition strategy which agrees with Fan and his co-worker's experiment.[3] Therefore, we chose this addition strategy in the following experiment.

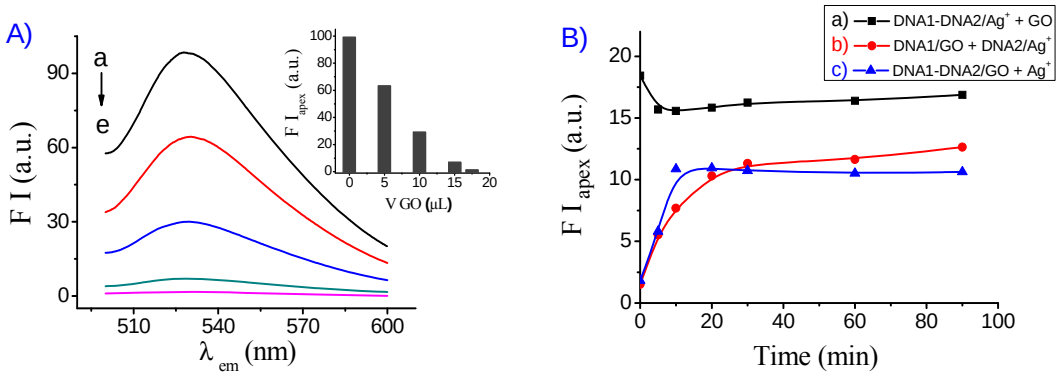

Fig. S2 A) Fluorescence emission spectra of DNA1/DNA2 (50 nM/50 nM) titrated with different volumes of GO (0.844 mg/mL). Volume of GO (from a to e, μL): 0, 5.0, 10.0, 15.0, and 17.5. B) The fluorescence intensity change with the time for the different addition orders of DNA1, DNA2, GO, and Ag^+. (DNA1/DNA2: 50 nM/50 nM, GO: 0.037 mg/mL, λ_{ex} = 480 nm).

4. Fluorescence emission spectra of DNA1/DNA2 (50 nM/50 nM) under different conditions

Fig. S3 shows fluorescence emission spectra of DNA1/DNA2 (50 nM/50 nM) under different conditions. Without Ag^+, GO almost completely quenches FAM fluorescence, even in the presence of its semicomplementary DNA2 (as shown in curve a of Fig. S3A). However, in the presence of Ag^+, FAM fluorescence is greatly increased (curve b in Fig. S3A), although a little quenching of FAM fluorescence is observed upon addition of Ag^+ without GO (curve e in Fig. S3B). In the presence of GO, the addition of Cys to the complex of DNA1/DNA2/Ag^+ leads to a remarkable decrease in FAM fluorescence (curve c in Fig. S3A). As shown in Fig. S3C, this comparison clearly demonstrates that the GO greatly improved the sensitivity for the detection of both Ag^+ and Cys.

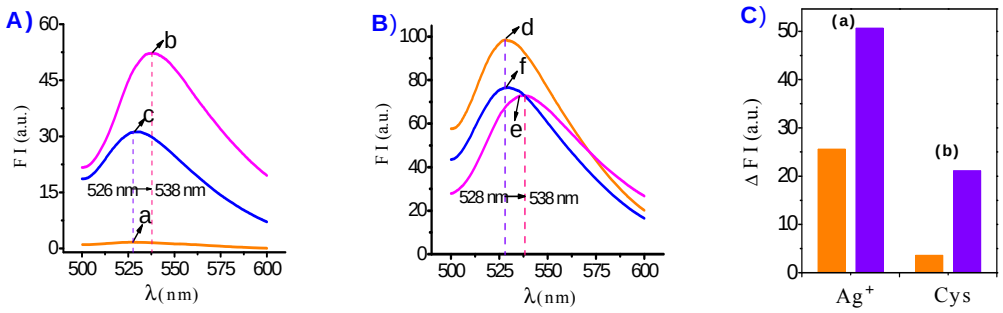

Fig. S3 Fluorescence emission spectra of DNA1/DNA2 (50 nM/50 nM) under different conditions: **A**) (**a**) DNA1/DNA2 + GO; (**b**) DNA1/DNA2 + 1.5 μM Ag^+ + GO; (**c**) DNA1/DNA2 + 1.5 μM Ag^+ + 500 nM Cys + GO. B) (**d**) DNA1/DNA2; (**e**) DNA1/DNA2 + 1.5 μM Ag^+; (**f**) DNA1/DNA2 + 1.5 μM Ag^+ + 500 nM Cys; **C**) Comparisons of the ΔFI (a.u.) of DNA1/DNA2 (50 nM) by Ag^+ (1.5 μM) and Cys (500 nM) in the absence (orange bars) and presence (violet bars) of GO. The concentration of GO was 0.037 mg/mL. Fluorescence intensity was recorded at the apex with an excitation wavelength of 480 nm.

5. Fluorescence spectra of fluorescein

In order to understand whether the interaction between Ag^+ and 6-FAM (6-carboxyfluorescein) causes the red shift, we investigated the fluorescence of fluorescein change with the addition of Ag^+. Before detection Ag^+ was directly added to the fluorescein work solution. The results are shown in Fig. S4.

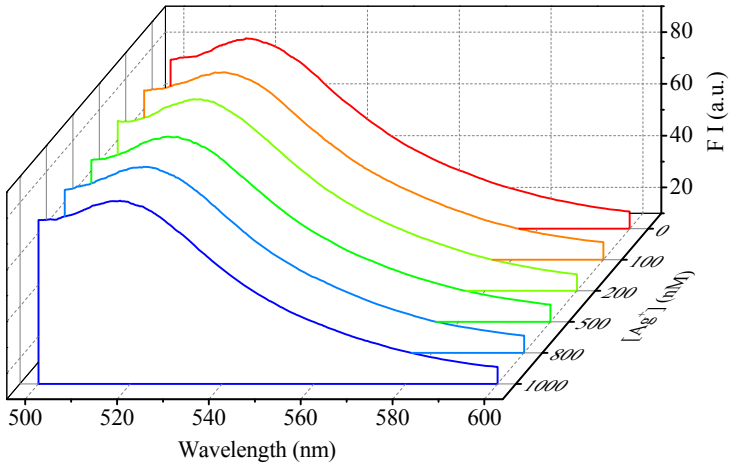

Fig. S4 3D waterfall of fluorescence spectra (λ_{ex} = 480 nm) of fluorescein solution (10 nM) on addition of $AgNO_3$.

6. Selectivity of the Ag^+ sensing system

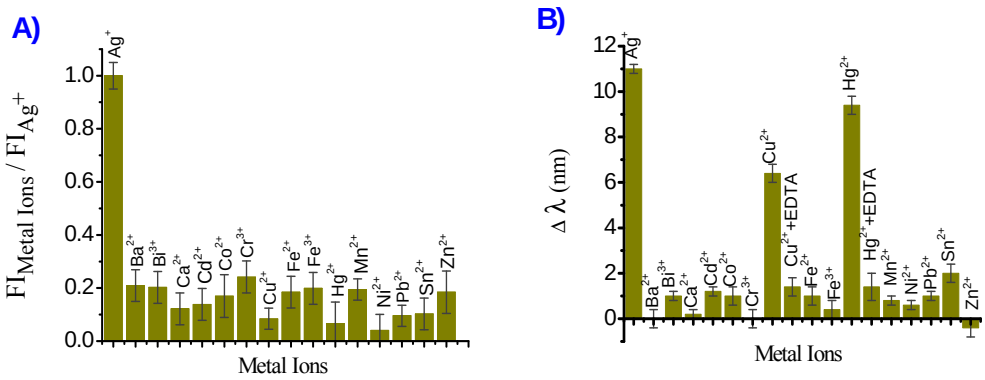

Fig. S5 Selectivity of the Ag^+ sensing system. A) The fluorescence intensity changes at fluorescence emission peak in the presence of Ag^+ and other competing metal ions. B) The fluorescence emission wavelength changes relative to 528 nm in the presence of Ag^+ and other competing metal ions. All competing metal ions were tested at 10 μM. For comparison, the sensor response to 1 μM Ag^+ was also presented. The DNA1/DNA2, EDTA, and GO concentrations were 50 nM, 100 μM, and 0.037 mg/mL, respectively. Fluorescence intensity was recorded at the apex with an excitation wavelength of 480 nm.

7. Selectivity of the Cys sensing system

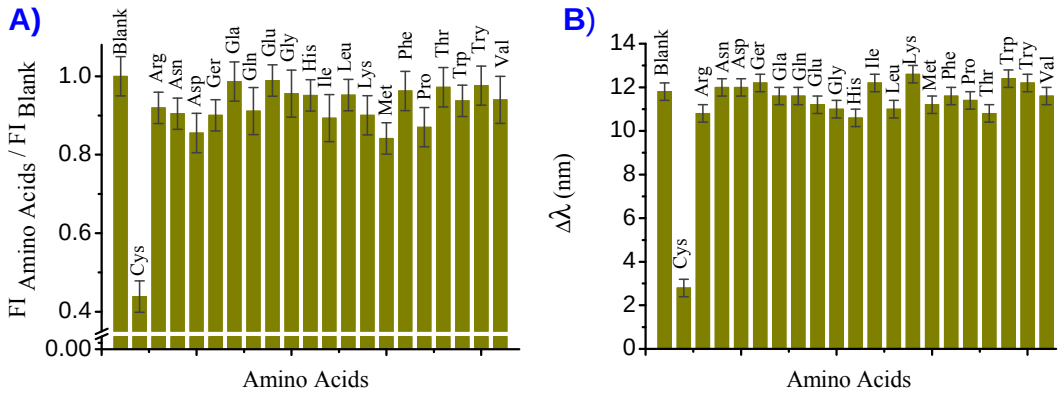

Fig. S6 Selectivity of the Cys sensing system. A) The fluorescence intensity changes at fluorescence emission peak in the presence of Cys and other competing amino acids. B) The fluorescence emission wavelength changes relative to 528 nm in the presence of Cys and other competing amino acids. All competing amino acids were tested at 5 μM. For comparison, the sensor response to 500 nM Cys is also presented. The DNA1/DNA2, Ag^+, and GO concentrations were 50 nM, 1.5 μM, and 4.1μg/mL, respectively. Fluorescence intensity was recorded at the apex with an excitation wavelength of 480 nm.

8. Effect of Hcy and GSH on the DNA1/DNA2/Ag^+/GO system

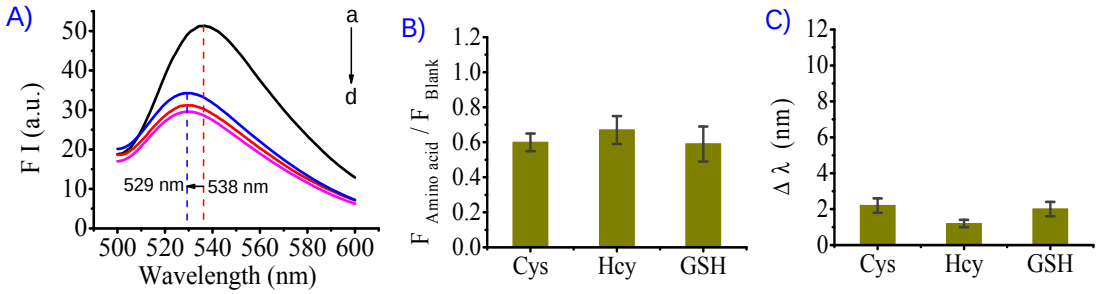

Fig. S7 A) Fluorescence emission spectra of DNA1/DNA2/Ag^+/GO in the absence (a) and presence of Hcy (b), Cys (c), and GSH (d). B) The fluorescence intensity changes at fluorescence emission peak in the presence of Cys, Hcy, and GSH. C) The fluorescence emission wavelength changes relative to 528 nm in the presence of Cys, Hcy, and GSH. (DNA1/DNA2: 50 nM/50 nM, Ag^+: 1.5 μM, Cys: 500 nM, Hcy: 500 nM, GSH: 500 nM, GO: 0.037 mg/mL, λ_{ex} = 480 nm).

9. Determination of Ag^+ in real samples (tap and river water)

For tap water, the sample was collected after discharging tap water for about 20 min and boiled for 5 min to remove chlorine. River water sample was obtained from Chia-ling River. The sample collected was first filtered through a 0.2 μM filter membrane to remove oils.

Table S1. Detection of Ag^+ in water samples using the proposed method (n = 5)

Sample	Background Content/nM	Concentration		Recovery (%)	RSD (%)
		Added/nM	Found/nM		
Tap water 1	ND	100	112	112.0	2.16
Tap water 2	ND	200	218	109.0	1.76
Tap water 3	ND	300	316	105.3	4.12
Chia-ling River 1	ND	100	98	98.0	3.34
Chia-ling River 2	ND	200	190	95.0	2.91
Chia-ling River 3	ND	300	295	98.3	4.31

ND: not detected

Supplementary references

1. W. S. Hummers and R. E.Offeman, *J. Am. Chem. Soc.* 1958, **80**, 1339.

2. Y. X. Xu, H. Bai, G. Lu, C. Li and G.Q. Shi, *J. Am. Chem. Soc.* 2008,**130**, 5856–5857.

3. S. J. He, B. Song, D. Li, C. F. Zhu, W. P. Qi, Y. Q. Wen, L. H. Wang, S. P. Song, H. P. Fang and C. H. Fan, *Adv. Funct. Mater.*, 2010, **20**, 453–459.