Electronic Supplementary Information

A novel graphene oxide based fluorescent nanosensing strategy with hybridization chain reaction signal amplification for highly sensitive biothiol detection

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EXPERIMENTAL SECTION

Reagents and Apparatus. The hairpin probes (HP1 and HP2) and helper DNA1 and DNA2 were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Their sequences are listed in Table S1 (helper DNA2 was used as a control here to demonstrate detection feasibility). HP1 was labeled with FAM. Both HP1 and HP2 have a stem of 18 base pairs (bp) and a loop of 6 nucleotides (nt) with an extra sticky tail of 6 nt (Scheme 1). The sequences of HP1 and HP2 are complementary in a staggered configuration (Table S1), such that they will hybridize when HP1 is opened by helper DNA1. Hg $(NO_3)_2$ was bought from Sigma-Aldrich (Shanghai, China). Cysteine (Cys), glutathione (GSH), bovine serum albumin (BSA), and other amino acids were also obtained from Sigma-Aldrich. All reagents were used as received without further purification. All solutions were prepared using ultrapure water, obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) having an electric resistance >18.2 MΩ.

The fluorescence measurements were carried out on an FL-7000 spectrometer (Hitachi, Japan). The fluorescence emission spectra were collected from 500 nm to 600 nm at room temperature with a 488 nm excitation wavelength.

Synthesis of GO

GO sheets were synthesized according to previous eports.[1] Generally, the preoxidized graphite was mixed with 2 mL of concentrated H_2SO_4 at 0 °C. After this, under the condition of stirring and temperature not more than 20 °C, 1.5 g of $KMnO_4$ was added slowly. Then, the mixture was transferred into 35 °C water bath and

vigorously stirred for 4 h, 100 mL of deionized water was added to dilute the mixture. A volume of 2 mL of 30% H_2O_2 was then added drop by drop. Next, synthesized GO was filtered and washed with 0.1 M HCl and deionized water at least 5 times. The obtained solid was redispersed into deionized water followed by dialysis for 7 days. The resulting solution was filtrated and finally dried in vacuum to obtain GO powder. GO powder (10 mg) was dispersed in 5 mL of deionized water by sonication for 2 h. The mixture was then centrifuged at 5000 rpm for 20 min and the supernatant was diluted further, yielding a stable dark yellow GO dispersion with the concentration about 100 μg mL^{-1} (for characterization see Fig. S1 in ESI).

Optimization of Assay Conditions

It was found that the amount of GO nanosheets used in the assay has substantial effect on the fluorescence quenching responses (Fig. S3 in ESI). The fluorescence responses in the presence of GO nanosheets of different concentrations were examined. It was obvious that the use of more GO nanosheets led to more efficient adsorption of HP1, increasing the quenching efficiency. When concentration of GO nanosheets was 5 μg mL^{-1}, the fluorescence signal from the FAM labeled dsDNA HCR product relative to the fluorescence from the FAM labeled HP1 reached the maximal value. This observation could be ascribed to the weak adsorption of the FAM labeled dsDNA product on GO nanosheet surfaces, which became dominant in the presence of excessive GO nanosheets. As a result, 5 μg mL^{-1} was used as the optimized concentration for GO nanosheets in the assay. In addition, the concentration of Hg^{2+} used in the assay was also optimized. The results showed the maximum

fluorescence enhancement was obtained in the present of 500 nM Hg^{2+} (Fig. S4 in ESI).

Interactions between FAM Labeled DNA and GO Nanosheets

The kinetic behaviors of the newly designed GO nanosheets platform were studied by monitoring the fluorescence intensity as a function of time. The adsorption of HP1 on the surface of GO nanosheets is very fast at room temperature (Fig. S5, curve a in ESI). The process of adsorption reaches equilibrium within 5 min. However, the FAM labeled dsDNA HCR product has much lower binding ability to GO nanosheets, as observed in the time dependent experiments. For FAM labeled dsDNA HCR product, GO nanosheets could not cause a remarkable fluorescence decrease within 30 min (Fig. S5, curve b in ESI). This phenomenon can be explained by the difference in absorption between hairpin DNA and dsDNA at the GO nanosheet surface.

Fluorescence Detection of Cysteine and Glutathione

In an aliquot of 150 μL hybridization buffer containing 6.67 mM Tris-HCl (pH 8.0), 66.67 mM $MgCl_2$, 10 μL of helper DNA1 (1 μM), 10 μL of Hg^{2+} (10 μM) and a certain amount of cysteine or glutathione were incubated at room temperature for 10 min. After adding 20 μL of HP1 (10 μM) and 20 μL of HP2 (10 μM), the mixture was incubated at home temperature for 60 min. Then, 10 μL GO sheets (100 μg mL^{-1}) were added into the reaction mixture, followed by the fluorescence measurement with an excitation wavelength of 488 nm.

Preparation and Analysis of Human Serums Amples

The human serum samples were obtained from the local hospital. For

determination of total thiols, the disulfide bonds were reduced in order to release the protein-bound thiols by addition of triphenylphosphine (PPh3) as reductant.[2] Briefly, 500 mL of samples was first reduced by adding 40 mL of 0.2 M HCl and 20 mL of 0.4 M PPh3 (in water–acetonitrile 20:80 v/v and 2 M HCl). After incubating for 20 min under vigorous stirring, the hydrolyzed serum was mixed with 500 mL of acetonitrile to precipitate proteins and then centrifuged at 4000 rpm for 20 min. The supernatant, which contained the reduced biothiols, was used for further sample analysis. Then before measurement, the serum samples were diluted 800-fold with 25 mM Tris-HCl (pH 7.2) in order to be consistent with the dynamic range of our method. Aliquots were mixed with the Hg^{2+} and helper DNA1, and the other aliquots were pretreated with 1 mM N-ethyl-maleimide as a thiol-blocking compound before reaction with the Hg^{2+} and helper DNA1. Cysteine of known concentrations was added to the sample for recovery studies and the total biothiols concentrations were determined by the standard addition method. The detection procedure was the same as those described in the aforementioned experiment for biothiols detection in clean reaction buffer.

Table S1. Sequences of DNA probes[a]

Name	Sequences（5′-3′）
helper DNA1	CTCTCCTCTCCAAGGTGTGGTGTG
helper DNA2	CACACCACACCAAGGAGAGGAGAG
HP1	FAM-**TCTCCAAGGTGTGGTGTG**CAAAGT **CACACCACACCTTGGAGA***GGAGAG*
HP2	*ACTTTG***CACACCACACCTTGGAGA** CTCTCC**TCTCCAAGGTGTGGTGTG**

[a]Boldface type indicates stem sequences of hairpin DNA probes. Italic type in HP1 and HP2 shows sticky ends. Underlining represents mismatched sites.

Table S2. Determination of biothiols in human serum using graphene oxide nanosheets-based platform couple with hybridization chain reactions.

Biothiols in human serum (μM)	Determined biothiols (μM)	Added cysteine (μM)	Measured cysteine (μM)	Rcovery(%)
325	0.406	1	1.05	105%
		2	1.98	99%

Fig. S1. TEM image of GO nanosheets.

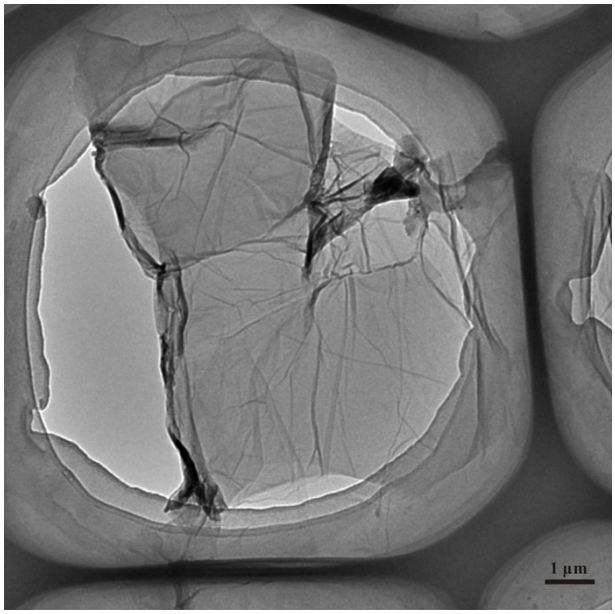


Fig. S2. Agarose gel electrophoresis image of HCR products. Lane 1: H1+H2; Lane 2: Helper DNA1+HP1+HP2+Hg^{2+}; Lane 3: Helper DNA1+HP+HP2+Hg^{2+}+cysteine; Lane M is the DNA size marker. The concentrations for different components are as follows: Helper DNA1, 50 nM; HP1, 1 μM; HP2, 1 μM; Hg^{2+}, 500 nM; cysteine, 2 μM.

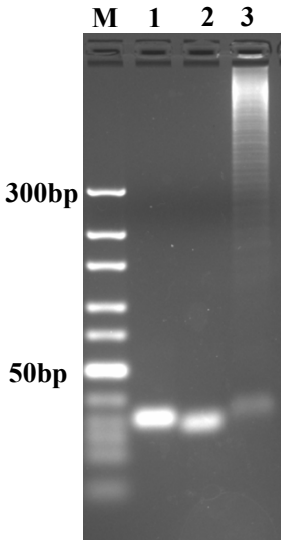


Fig. S3. Fluorescence intensity histogram of Helper DNA1 + HP + HP2 + Hg^{2+}+ cysteine + GO (black) and Helper DNA1 + HP1 + HP2 + Hg^{2+} + GO (gray) in the presence of 2.5, 5, 7.5, 10 and 15 μg mL^{-1} GO nanosheets. Error bars were estimated from three replicate measurements. The concentrations for different components are as follows: Helper, DNA1 50 nM; HP1, 1 μM; HP2, 1 μM; Hg^{2+}, 500 nM; cysteine, 1μM.

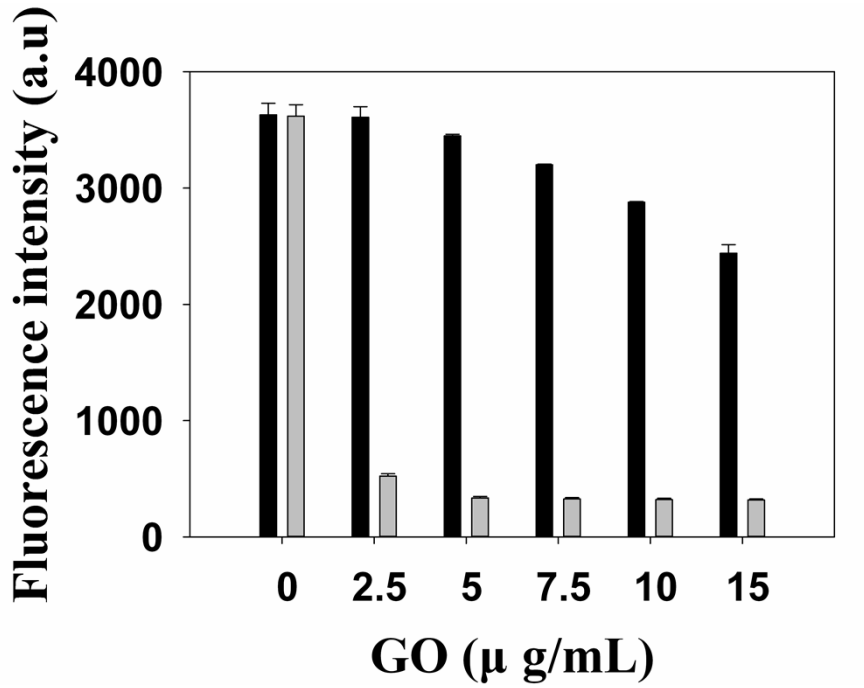


Fig. S4. Optimization of Hg^{2+} concentration. Error bars were estimated from three replicate measurements. The concentrations for different components are as follows: GO, 5 μg mL^{-1}; Helper DNA1, 50 nM; HP1, 1 μM; HP2, 1 μM; cysteine, 1μM.

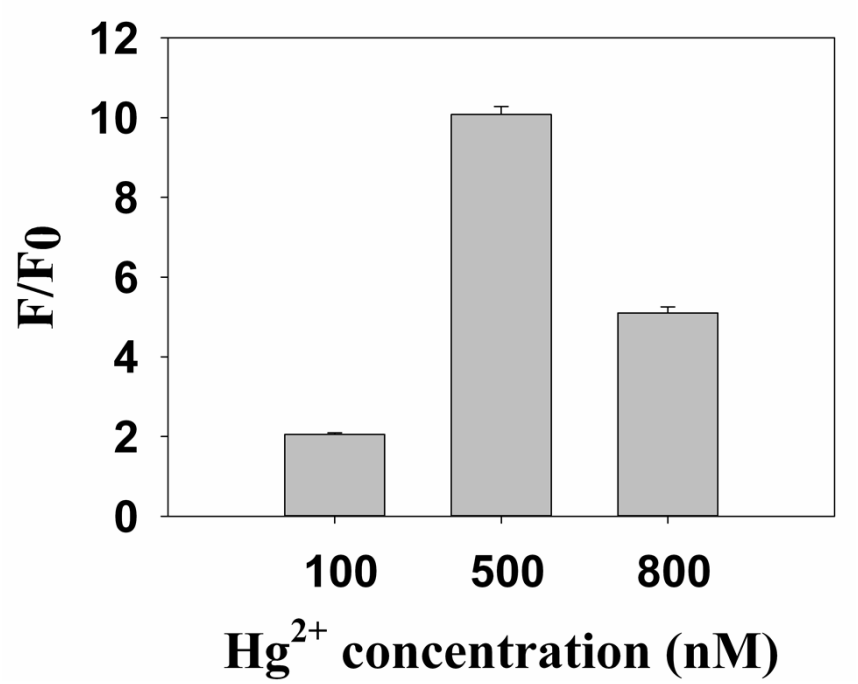


Fig. S5. Fluorescence intensities of (a) Helper DNA1+HP1+HP2+Hg^{2+} and (b) Helper DNA1+HP1+HP2+Hg^{2+}+cysteine via time in the presence of GO nanosheets. The concentrations for different components are as follows: GO, 5 μg mL^{-1}; Helper DNA1, 50 nM; HP1, 1 μM; HP2, 1 μM; Hg^{2+}, 500 nM; cysteine, 1μM.

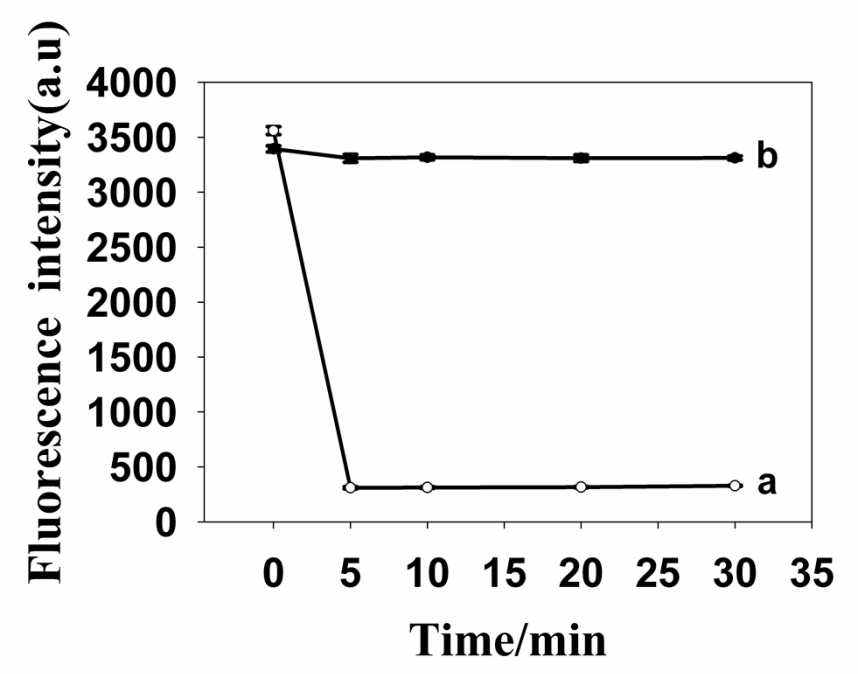
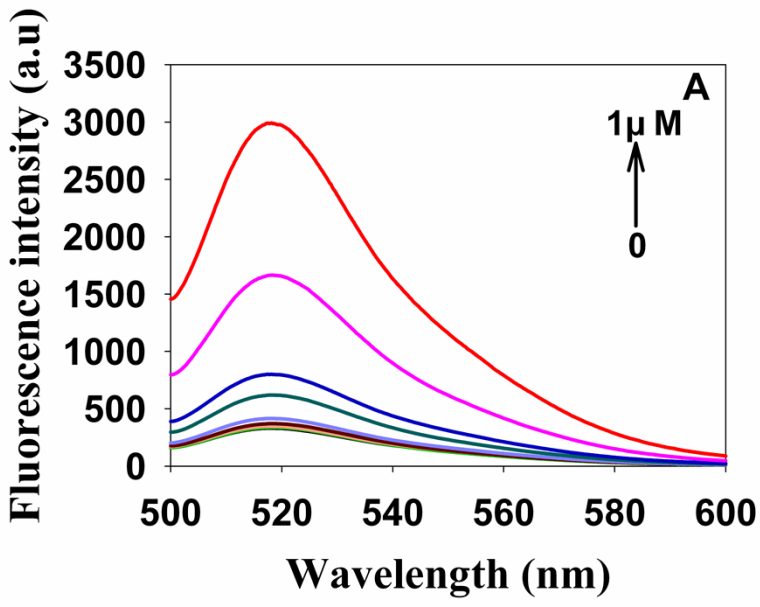


Fig. S6. (A) Fluorescence emission spectra of GO nanosheet platform in the presence of increasing amounts of glutathione, the arrow indicating the signal changes with increases in glutathione (0, 0.1, 0.5, 1, 10, 100, 200, 500 and 1000 nM). (B) Calibration curve of GO nanosheets nanosensor in the presence of increasing amount of cysteine dependence of fluorescence intensity on the concentration of glutathione. The final concentrations of GO, Helper DNA1, HP1, HP2 and Hg^{2+} ions are 5 μg mL^{-1}, 50 nM, 1 μM, 1 μM and 500 nM, respectively.



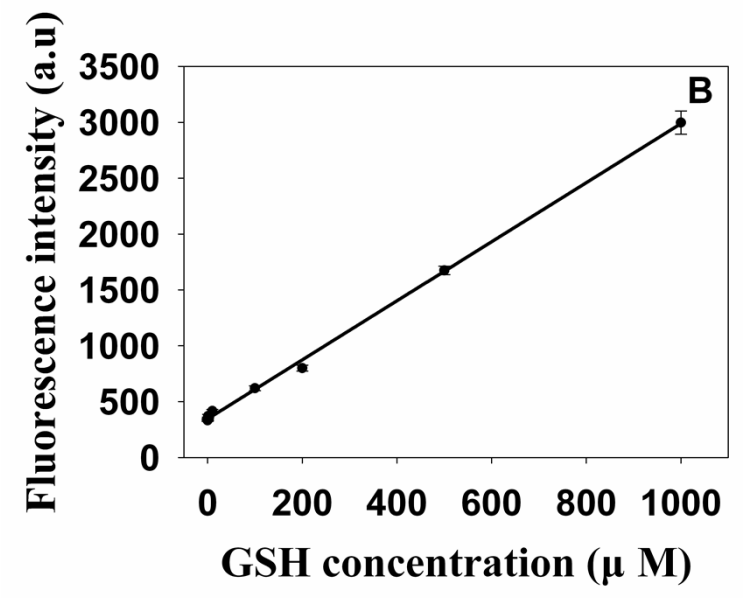
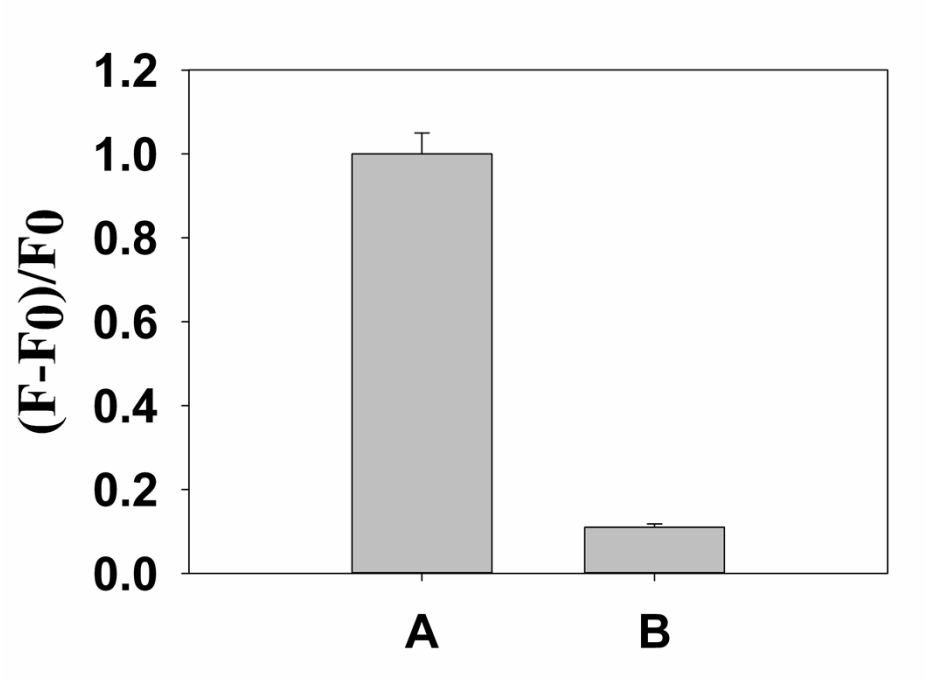


Fig. S7. Fluorescence responses of GO nanosheet platform for a human serum sample before (a) and after (b) using the thiol blocking agent maleimide. F and F_0 are fluorescence intensities of GO nanosheet platform in the presence and absence of human serum. The error bar represents the standard deviation of three measurements.



References:

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

(2) J. V. Ros-Lis, B. García, D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. Soto, F. Gonzalvo and M. C. Valldecabres, *J. Am. Chem. Soc.*, 2004, **126**,4064–4065.