



Short communication

Selective electrochemical detection of cysteine in complex serum by graphene nanoribbon

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ABSTRACT

Selective detection of cysteine in serum samples was achieved on a graphene nanoribbon (GNR) and Nafion nanocomposite modified electrode with high precision. The superior conductivity and abundant amount of active chemical oxygen groups on the edge of GNR led to extremely highly electrocatalytic activity of GNR towards the electrochemical oxidation of cysteine at +0.025 V. The electrocatalytic behavior was further used for sensitive detection of cysteine by differential pulse voltammetry. Under optimized conditions, the calibration curve was linear in the range from 25 nM to 500 μM. The electrochemical sensor showed strong antifouling ability, good stability and selectivity. It could effectively exclude the interferences from other kinds of biothiols and the biological relevant species, thus had great perspective for *in vivo* analysis of biological samples.

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1. Introduction

Low-molecular mass thiols such as cysteine (CySH), homocysteine (Hcy), and glutathione (GSH), have been proven to play an important role in metabolism and cellular homeostasis (Chen et al., 2010; Yonge et al., 2004). The abnormal level of these biothiols is correlated with many diseases (Wang et al., 2005). For example, CySH deficiency is involved in the syndromes of slow growth in children, liver damage, and weakness. An elevated level of Hcy in plasma is an important indicator of Alzheimer's and cardiovascular diseases. The abnormal concentration of GSH is an important signal of oxidative stress change in cells, which is crucial to cell growth and death and would lead to stroke, cancer, and many neurodegenerative diseases. Therefore, the rapid and selective detection of these specific mercapto biomolecules in biological samples could provide the possibility to unravel the complex chemical mechanisms underlying the biological processes, and enhance the diagnosis and the early onset of complications. Many analytical methods, including high performance liquid chromatography (Liu et al., 1996) fluorescence microscopy (Ros-Lis et al., 2004), and chemiluminescence (Wang et al., 2009), have been proposed for the analysis of biothiols, however, they were either inconvenient, unstable, expensive, or have to be conducted in organic systems. Moreover, these

methods could not satisfy the demand of *in vivo* analysis in complex biological systems without the interference from other thiols.

Due to the innate property of low cost, high sensitivity, fast response, and small dimension, electrochemical sensors are more suitable to be applied to *in vivo* and on-site analysis (Spătaru et al., 2001). In last decade, many electrochemical sensors based on novel nanomaterials and mediators have been designed for the detection of biothiols (Terashima et al., 2003; Ahmad et al., 2010; Zen et al., 2001; Inoue and Kirchhoff, 2000). The introduction of these nanomaterials and suitable mediators greatly improved the sensitivity and stability of those devices. However, to our best knowledge, none of them could effectively discriminate one specific biothiol from the others due to the similar reactivity of biothiol molecules. To achieve selective detection, expensive separation instruments such as HPLC and capillary electrophoresis have to be coupled with, which severely limit their further applications to *in vivo* analysis.

Herein, we report an electrochemical method for the selective and sensitive detection of CySH in untreated serum samples by using a nanocomposite of graphene nanoribbon (GNR) and Nafion to modify the electrode. The selection of GNR as a functional nanomaterial for the detection of biothiol is because of its good electronic conductivity (Min et al., 2011), abundant amount of chemical oxygen groups (Cataldo et al., 2010; Dong et al., 2011), and simple preparation procedure. The active chemical oxygen groups, mainly composed of unsaturated carbonyl groups on the edge of GNR, are good candidates for the selective and sensitive detection of thiols, due to their specificities towards the targets based on

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either 1,4 Michael addition or cycling addition reaction (Chen et al., 2010). Here Nafion could not only effectively prevent the GNR from aggregation (Brownson and Banks, 2011), but also increase the anti-interference ability of the nanocomposite from ascorbic acid and uric acid. The resulting electrochemical sensor exhibits wide linear range (from 25 nM to 500 μ M) and good selectivity. Other biological relevant species such as naturally existing amino acids, glucose, ascorbic acid, uric acid, and other co-existing biothiols including Hcy, GSH, the oxidation product of GSH (GSSG), and cystine all do not interfere the detection of CySH.

2. Experimental

2.1. Materials and reagents

L-CySH was purchased from Beijing Chemical Reagent Co. (China). 20 kinds of essential amino acids and Nafion were purchased from Sigma. Multiwalled carbon nanotubes were purchased from Shenzhen Nanotech. Co., China. The other reagents were of analytical grade and used without further purification. 0.1 M phosphate buffer solution (PBS) was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4 . Doubly distilled water was used in all experiments.

2.2. General procedure for synthesis of GNR and graphene oxide (GRO)

GNR was prepared according to the method reported by Sinitskii et al. (2010). GRO and reduced GRO (GRO-re) were prepared according to a modified Hummer's method (Hummer and Offeman, 1958) using graphite as carbon source, the mixture containing H_2SO_4 , $\text{K}_2\text{S}_2\text{O}_8$ and P_2O_5 as strongly oxidative reagents, and N_2H_4 as reducing reagent.

2.3. Fabrication of GNRs-Nafion modified electrode

1.0 mg of the obtained GNR was dispersed in 1.0 mL pH 7.0 PBS containing 1% Nafion and ultrasonicated for 2 h to obtain the homogeneous GNR-Nafion suspension. Then, 3 μ L of the Nafion stabilized GNR was dropped on a cleaned GCE (3 mm). After drying at room temperature, the GNR-Nafion/GCE was obtained. For comparison, MWCNT-Nafion/GCE, GRO-re-Nafion/GCE, and GRO-Nafion/GCE were also prepared based on the same preparation procedure.

2.4. Apparatus

The electrochemical measurements were carried out on a CHI 660C electrochemical working station (CH Instruments Co.). A three-electrode system was employed with GNR-Nafion/GCE as working electrode, a saturated calomel electrode as reference electrode and a platinum wire as counter electrode. FT-IR spectra ($4000\text{--}500\text{ cm}^{-1}$) in KBr were collected on a Nicolet Avatar 360 FT-IR spectrometer. Transmission electron microscopy (TEM) experiments were performed on a JEM-300 CX electron microscope (JEOL, Japan) with an acceleration voltage of 300 kV.

3. Results and discussion

3.1. Characterization of GNR

Fig. S1 shows the TEM images of the GNR and MWCNT. From the TEM images, the clear edge of the MWCNT could be observed and its diameter is about 13 nm, while the edge of the GNR turns to be rough and unclear and the diameter of the GNR increases

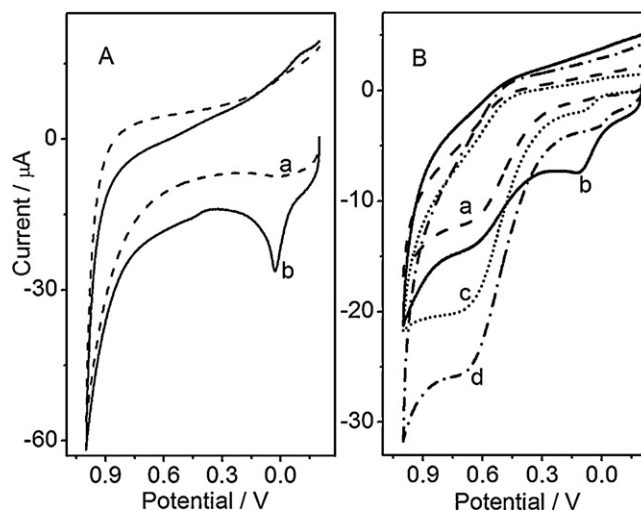


Fig. 1. CVs of (A) GNR-Nafion/GCE in 0.1 M PBS (pH 7.0) in absence (a) and presence (b) of 1.0 mM CySH, and (B) bare GCE (a), GRO-re-Nafion/GCE (b), MWCNT-Nafion/GCE (c) and GRO-Nafion/GCE (d) in 0.1 M pH 7.0 PBS containing 1.0 mM CySH at 50 mV/s.

to 27 nm, which indicate the edge of the GNR may be partially curled. The IR spectrum of GNR (Fig. S2b) shows typical adsorption peaks of carbonyl (carboxylic acid anhydride and/or in aldehyde), α,β -unsaturated ketones, C–O–C, and epoxy group, which were centered at 1730, 1645, 1048, and 880 cm^{-1} (Cataldo et al., 2010; Dong et al., 2011), respectively. However, only two weak adsorption peaks of α,β -unsaturated ketones and C–O–C were observed on the MWCNT (Fig. S2a). All these phenomena indicate the successful unzipping of MWCNT and the formation of GNR.

3.2. Electrocatalytic oxidation of CySH on the GNR-Nafion/GCE

The GNR-Nafion/GCE showed two redox waves with the peak potentials of -0.006 and -0.102 V (Fig. 1A), similar to those obtained from carbon nanotubes (Luo et al., 2001) and carbon nanofiber (Wu et al., 2007). 1.0 mM CySH was added to the PBS, a sharp and well resolved anodic peak centered at +25 mV was observed on the GNR-Nafion/GCE. The anodic peak potential of +25 mV for the irreversible oxidation of CySH was much lower than those reported on other electrochemical sensors, indicating the good electrocatalytic activity of GNR for the oxidation of CySH. For comparison, MWCNT-Nafion/GCE, GRO-Nafion/GCE, and GRO-re-Nafion/GCE were fabricated to evaluate their performances for the electro-oxidation of CySH. As shown in Fig. 1B, the peak potentials obtained from the GRO-Nafion/GCE, MWCNT-Nafion/GCE, and GRO-re-Nafion/GCE were 700, 700, and 130 mV, respectively. These results indicated the best catalytic ability of GNR towards the oxidation of CySH and GRO-re-Nafion/GCE got the second rank, which both effectively decrease the overpotential for CySH oxidation. To investigate this phenomenon, the characters of these four materials were compared. Regarding MWCNT, its conductivity is close to GNR (Fig. S3A), but has very few amounts of active oxygen groups (Fig. S3B). As for GRO, it also contains large amount of active oxygen groups, but with low conductivity (Fig. S3A). The conductivity of GNR and GRO-re is similar; however, the oxygen content of GRO-re is less than that of GNR. Therefore, it could be concluded that the good electrochemical catalytic ability of GNR may be due to the good conductivity and its abundant amounts of oxygen groups. The relatively weaker catalytic ability of the GRO-re-Nafion/GCE than the GNR-Nafion/GCE may be due to the poor dispersion of GRO-re in the aqueous solution and relatively less amount of active oxygen groups (Fig. 3B).

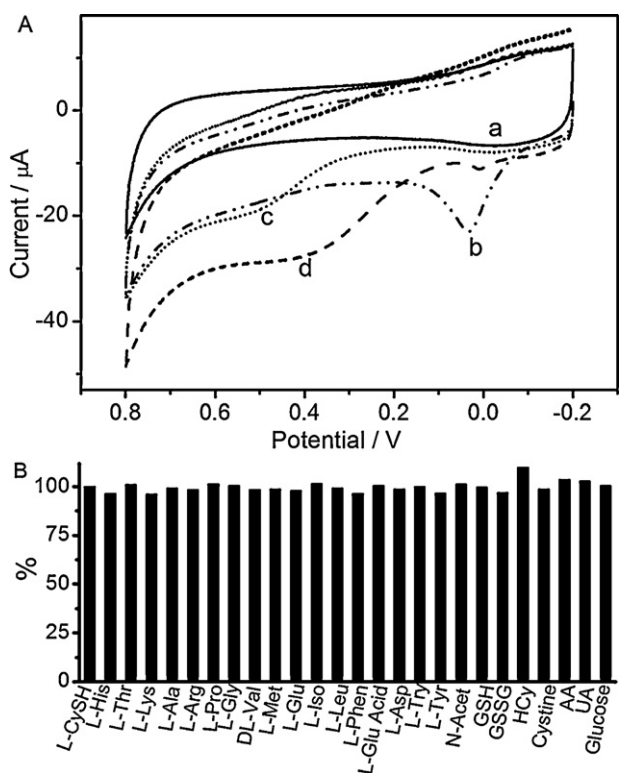


Fig. 2. (A) CVs of GNR-Nafion/GCE in 0.1 M PBS (pH 7.0) in absence (a) and presence of 1.0 mM CySH (b), GSH (c) and Hcy (d) at 50 mV/s, and (B) DPV responses of GNR-Nafion/GCE in 0.1 M pH 7.0 PBS containing 1.0 mM CySH in the absence and presence of different amino acids in saturated concentrations, 1.0 mM GSH, Hcy, GSSG, ascorbic acid, uric acid and glucose, respectively.

3.3. Selective detection of CySH on the GNR-Nafion/GCE

Fig. 2A shows the CVs of the GNR-Nafion/GCE in 0.1 M PBS without and with the presence of 1.0 mM CySH, GSH, and Hcy. In the presence of Hcy, only a very weak oxidation peak centered at +12 mV and a broad oxidation peak centered at +393 mV were observed. In GSH solution, the CV showed only a broad ox-

idation peak centered at +518 mV. The anodic peak potentials for GSH, Hcy, and oxidation were obviously different from that of CySH. These phenomena indicated the GNR-Nafion/GCE had good selectivity for CySH detection. It should be noted that due to the similar reactivity of most biothiols, the discrimination of CySH from Hcy and GSH is always very difficult to achieve. The pioneer work for selective CySH detection was carried out by Strongin et al. using a commercial available unsaturated aldehyde and 3-(4-(dimethylamino) phenyl) acrylaldehyde (Wang et al., 2005). The recognition mechanism was based on a well known cyclization of N-terminal CySH residues reacting with aldehydes to form a stable 5-membered ring heterocycles, thiazolidine. Inspired by this report, we assumed the selective oxidation of CySH at ultralow potential on the GNR-Nafion/GCE was attributed to the formation of some surface complexes between the N-terminal CySH and the active oxygen groups, especially the aldehyde groups. Since the existence of aldehyde groups in the GNR and the crucial role of active oxygen groups have been proved by the IR spectrum and CVs, respectively, it is necessary to clarify the role of $-NH_2$ groups in the redox process to prove this assumption. Therefore, the CVs of MPA and CySH on the GNR-Nafion/GCE were compared under the same condition. The selection of MPA for the control trial is because it also contains three carbon atoms, but lack of $-NH_2$ group. As shown in Fig. S4, no anodic peak was observed with the presence of MPA, while a well resolved anodic peak centered at +25 mV was gotten in the presence of CySH, which indicated that $-NH_2$ group did play an important role in the redox process cooperated with aldehyde groups in the GNR. Therefore, the good selectivity may be due to three factors, the large amount of active oxygen groups of GNR, and the specific structure of CySH, which may form a surface complex during the redox process.

To further verify the selectivity of the GNR-Nafion/GCE for detection of CySH, different interferents were added in the detection solution to perform the differential pulse voltammetric (DPV) measurements. The DPV responses were shown in Fig. 2B. No obvious deviation upon addition of any aforementioned potential interferents was observed. The good selectivity was attributed to the synergistic interaction of GNR and the negatively charged Nafion.

3.4. Antifouling ability of the GNR-Nafion/GCE

Besides the good selectivity, the problem of poor stability arose from the fouling of the electrode surface is also a crucial problem encountered by the electrochemical sensor for thiol detection. Fig. 3A shows the CVs of the GNR-Nafion/GCE and bare GCE in 0.1 M PBS containing 1.0 mM CySH. After successive scan for 20 circles, the peak current of the GNR-Nafion/GCE was almost the same as its initial value, indicating its good antifouling ability. In contrast, the peak current at bare GCE was continually decreased. Another chronocoulometric study was carried out by applying a potential step in the presence and absence of 1.0 mM CySH. The difference between the intercepts of Q_{total} and Q_{dl} (blank) vs $t_{1/2}$ plots was approximately zero (Fig. 3C), indicating the absence of adsorption of reactant within the potential range of CySH oxidation (Safavi et al., 2009).

3.5. Application of GNR-Nafion/GCE: detection of CySH

The proposed method exhibited sensitive response to CySH. Under the optimal conditions (Fig. S5), the DPV peak current of the GNR-Nafion/GCE was proportional to the CySH concentration in the range of 25 nM–500 μ M (Fig. S6). The linear range was much wider than those proposed for detection of CySH in absence of the selectivity. In contrast, the electrochemical responses of the GSH and Hcy were studied by DPV. The linear ranges of GSH and Hcy

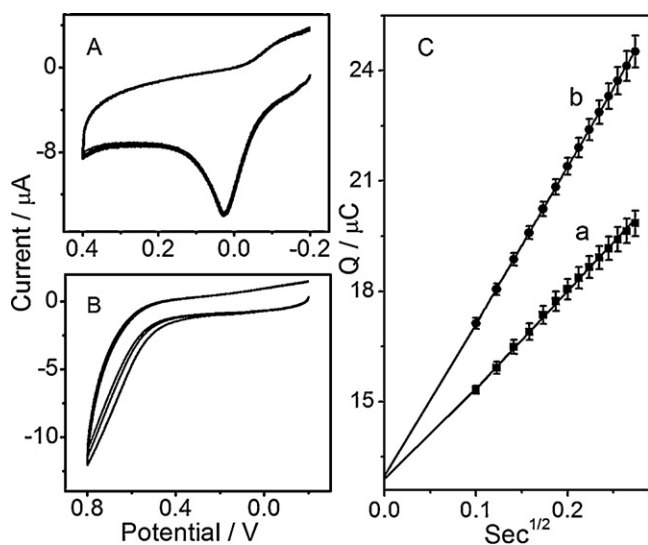


Fig. 3. CVs of (A) GNR-Nafion/GCE and (B) bare GCE at different sweep cycles in 0.1 M pH 7.0 PBS containing 1.0 mM CySH at 50 mV/s; and (C) linear chronocoulometric plots of GNR-Nafion/GCE in 0.1 M pH 7.0 PBS in the absence (a) and presence (b) of 1.0 mM CySH.

were from 300 μM to 1.0 mM, and 300 μM to 2 mM, respectively, and the limits of detection were both 100 μM . These results indicated the better catalysis ability of GNR-Nafion/GCE towards the electrochemical detection of CySH.

3.6. Real sample analysis

To examine the practicality of the presented electrochemical sensor in biological samples, the detection of CySH in real serum was performed. The recovery test was also studied by spiking different amounts of CySH into the 20 times 0.1 M pH 7.0 PBS diluted serum samples. The results were shown in Table S1. The recoveries were from 93.3% to 117%, indicating that the proposed method was highly accurate. The relative standard derivation was from 2.34% to 11.6%, indicating the acceptable reproducibility.

4. Conclusion

In summary, graphene nanoribbon with superior electronic conductivity and large amount of active oxygen groups, especially the unsaturated aldehyde groups, was used for highly sensitive and selective electrochemical detection of CySH with the aid of Nafion. The good selectivity was attributed to the specific cycling addition between the unsaturated aldehyde groups and the N-terminal CySH, which may lead to the formation of a thiazolidine transition state for facilitating the deprotonation and the electrooxidation of CySH. The negatively charged Nafion not only effectively prevent the aggregation of GNR, but also increase the anti-interference ability of the modified electrode from ascorbic acid and uric acid. The sensor also showed excellent antifouling ability and good precision, which could be directly applied for the *in vitro* selective detection of CySH in serum samples without any pre-treatments. By the combination of microelectrode, the strategy may have the possibility to be used for *in vivo* monitoring of CySH in living bodies, thus providing essential information for unraveling the complex physiological processes and reasons of diseases.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.12.006.

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Supporting Information

Selective electrochemical detection of cysteine in complex serum by graphene nanoribbon

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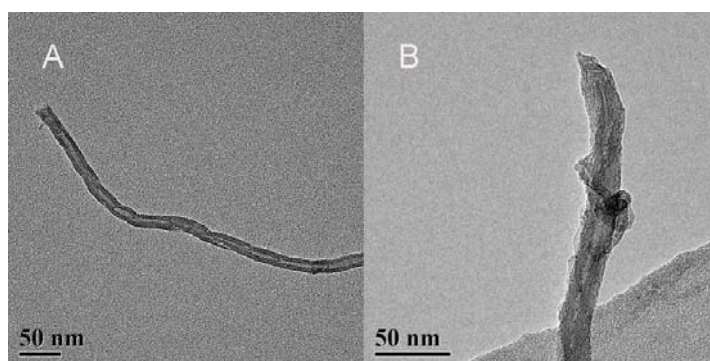


Figure S1. TEM images of (A) MWCNT and (B) GNR.

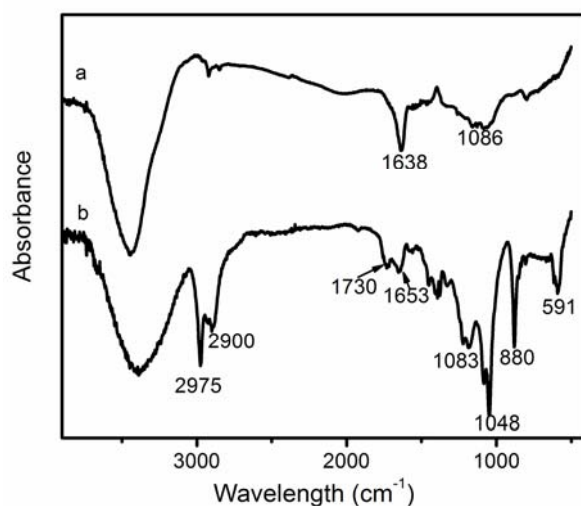


Figure S2. IR spectra of MWCNT (a) and GNR (b).

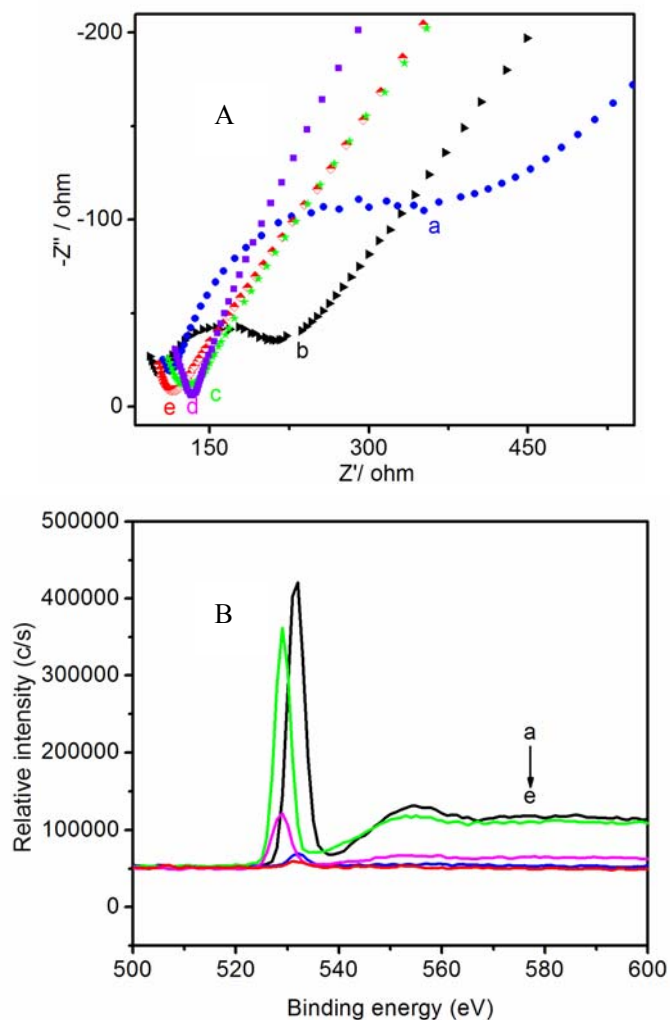


Figure S3. (A) EIS of GRO modified electrode (a), bare GCE (b), GRO-re/GCE (c), GNR/GCE (d), and MWCNT/GCE (e), in 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$. (B) O 1s XPS spectra collected from GNR (a), GRO (b), GRO-re (c), MWCNT-COOH (d), and untreated MWCNT (e).

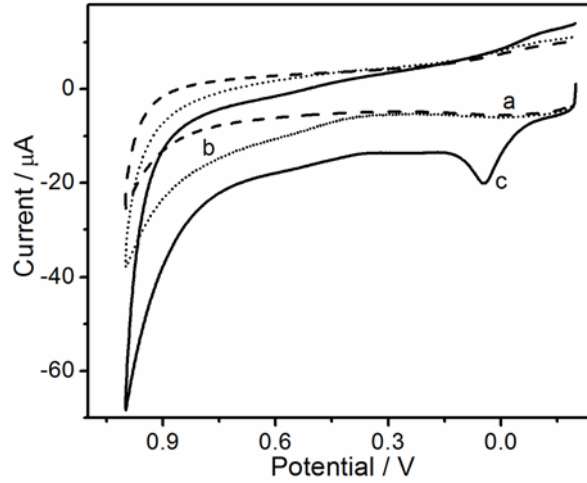


Figure S4. (A) CVs of GNR-Nafion/GCE in 0.1 M PBS (pH 7.0) in absence (a) and presence of 1.0 mM MPA (b), and CySH (c) at 50 mV/s.

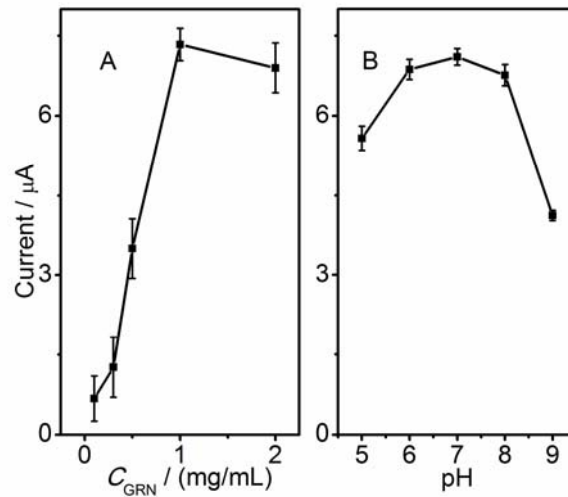


Figure S5. Influence of (A) GNR amount and (B) pH value on DPV response of the electrochemical sensor. The detection was performed in 0.1 M pH 7.0 PBS containing 1.0 mM CySH.

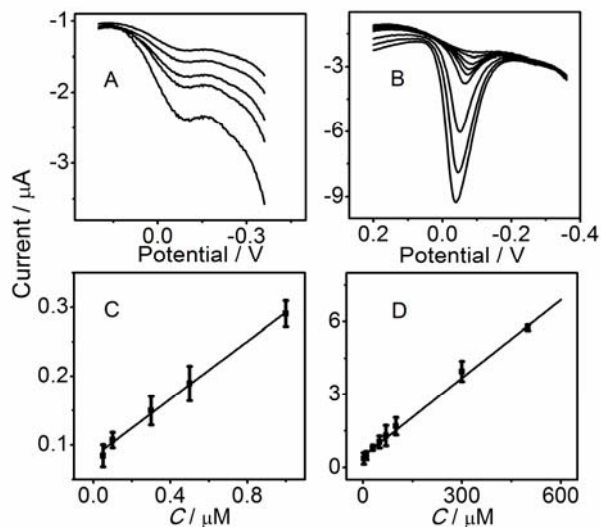


Figure S6. DPV plots of GNR-Nafion/GCE in 0.1 M pH 7.0 PBS containing 0.025, 0.1, 0.3, 0.5 and 1.0 μM (A), and 1, 3, 10, 30, 50, 70, 100, 300 and 500 μM (B) CySH (from higher to lower), and the corresponding calibration curves (C and D).

Table 1. Recovery Studies of CySH in Human Serum (n = 8)

Sample Added		1	2	3	4	5	6	7	8
0 μM	Found/ μM	0.86	0.62	0.90	0.93	0.69	0.501	0.948	0.913
	Recovery/%	—	—	—	—	—	—	—	—
	R.S.D/%	3.25	7.58	2.89	3.11	3.67	5.12	3.99	4.24
1 μM	Found/ μM	1.81	1.64	1.84	2.01	1.73	1.55	2.10	1.86
	Recovery/%	95.0	102	93.3	108	104	109	115	93.9
	R.S.D/%	9.39	12.1	4.66	2.39	5.72	10.1	5.56	3.21
3 μM	Found/ μM	3.83	3.80	3.98	4.01	3.73	3.45	3.93	3.89
	Recovery/%	99.0	106	108	107	104	95.0	98.3	98.0
	R.S.D/%	5.48	11.6	3.56	6.23	4.21	2.55	9.13	5.65
5 μM	Found/ μM	5.98	5.66	6.04	6.09	5.68	5.54	5.99	5.84
	Recovery/%	102	101	115	117	97.7	108	105	91.9
	R.S.D/%	2.34	9.19	5.22	3.46	5.97	4.48	5.56	7.63