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G-quadruplex-based ultrasensitive and selective detection of histidine and cysteine

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Abstract

Histidine and cysteine detection is critically important since an abnormal level of histidine or cysteine is an indicator for many diseases. In this paper, we demonstrated a novel label-free, G-quadruplex-based approach for simultaneous detection of histidine and cysteine. The present assay is based on the highly specific interaction among amino acids (histidine or cysteine), Cu\textsuperscript{2+} and NMM/G-4 (NMM: N-methylmesoporphyrin IX; G-4: G-quadruplex). The fluorescence intensity of NMM was dramatically enhanced in the presence of G-quadruplex formed from 24GT, which can be effectively quenched by cupric ion (Cu\textsuperscript{2+}) due to the chelation of Cu\textsuperscript{2+} by NMM as well as the unfolding of G-quadruplex by Cu\textsuperscript{2+}. The presence of histidine or cysteine will disturb the interaction between Cu\textsuperscript{2+} and NMM/G-4 because of the strong binding affinity of Cu\textsuperscript{2+} to the imidazole group of histidine or the interaction of Cu\textsuperscript{2+} with thiol group in cysteine, leading to distinct fluorescence emission intensity. High selectivity is conferred by the use of
cysteine-masking agent N-ethylmaleimide (NEM), which helps to discriminate histidine from cysteine. At last, a novel and simple approach was developed to determine each precise concentration of histidine and cysteine according to the different response of the system with and without NEM. Importantly, histidine can be also detected even in the presence of a large amount of other amino acids. A detection limit as low as 3 nM for histidine and 5 nM for cysteine was obtained by practical measurement rather than conventional calculation (S/N=3), confirming the high sensitivity of the present approach. Meanwhile, this sensing protocol can determine histidine and cysteine in diluted biological samples such as urine, exhibiting great potential to meet the need of practical application.

**Keywords:** G-quadruplex; cupric ion; fluorescence; histidine detection; cysteine detection
1. Introduction

Histidine and cysteine have draw great attention in recent years due to their crucial role in many biological systems (Holm et al., 1996; Karlin et al., 1997; Roe et al., 1988; Shahrokhian, 2001). Histidine is essential not only for the growth and repair of tissue but also for controlling the transport of metals in biologically important bases and minimizing internal bleeding from microtrauma (Nan et al., 1999). Histidine deficiency usually causes Friedreich ataxia, epilepsy, Parkinson’s disease, and the failure of normal erythropoiesis development (Rao et al., 1993), while ingestion of high levels can result in symptoms of intoxication (Liao et al., 2004).

Cysteine, as a small-molecular-weight biological thiol, can form disulfide bonds and provide a modality for the intramolecular cross-linking of proteins to support their secondary structures and functions (Zhao et al., 2010). Various important biochemical processes are associated with cysteine, including biocatalysis, metal binding, post-translational modifications, and detoxification of xenobiotics (Reddie and Carroll, 2008; Weerapana et al., 2010). Abnormal levels of cysteine are related to many diseases, such as slowed growth, liver damage, skin lesions, Alzheimer’s disease and cardiovascular disease (Lin et al., 2011; Seshadri et al., 2002; Shahrokhian, 2001). Therefore, it is of great significance to determine histidine and cysteine in the fields such as food processing, biochemistry, pharmaceuticals, and clinical analysis (Li et al., 2004b).

Up to date, several methods for histidine detection while lots of strategies for cysteine detection have been developed, including capillary electrophoresis (Inoue and Kirchhoff, 2002; Jalali-Heravi et al., 2005; Jin and Wang, 1997; Tuma et al., 2005; Zhang and Sun, 2004; Zhao and Liu, 2001), mass-spectrometry (Burford et al., 2003; Hovorka et al., 2002; Huang and
Chang, 2006; Miyagi and Nakazawa, 2008; Namera et al., 2002; Yang et al., 2005), flow injection (Batz and Martin, 2009; Zhao et al., 2001), voltammetry (Amini et al., 2003; Ge et al., 2012; Li et al., 2011; Lin et al., 2011; Ndamanisha et al., 2009; Spataru et al., 2001; Wu et al., 2012; Zhang et al., 2010), chromatography (Amarnath et al., 2003; Chwatko and Bald, 2000; Gegg et al., 2002; Katrusiak et al., 2001; Lu et al., 2007; Ruta et al., 2007; Vacek et al., 2006) and spectroscopy (Lim et al., 2008; Rusin et al., 2004; Tanaka et al., 2004; Wang et al., 2001; Wang et al., 2005). These methods suffer from more or less inherent drawbacks, such as complicated operation procedures, expensive instrumentation, chemical modifications, high detection limit, etc. (Li et al., 2004a; Li et al., 2004b; Lin et al., 2011; Nekrassova et al., 2003; Tateda et al., 1998; Tcherkas et al., 2001; Xu and Hepel, 2011; Zhang and Sun, 2004). In most cases, the requirement of large sample volumes as well as eco-unfriendly solvents is the main disadvantage that limits the scope of applications. Recently, highly sensitive electrochemical and fluorimetric methods for histidine detection based on DNAzyme have been reported (Kong et al., 2011; Li et al., 2011; Liang et al., 2011). However, the cost of synthesizing DNAzyme labeled with electrochemically-active molecule or fluorophore/quencher is quite high. Moreover, DNAzyme is prone to degradation and the stability of DNAzyme-based system is a serious concern in practical detection. To the best of our knowledge, simultaneous detection of histidine and cysteine is fewly reported (Pu et al., 2010) and remains a great challenge. Therefore, development of a facile and low-cost method for ultrasensitive, selective and simultaneous detection of histidine and cysteine is of great practical significance.

G-quadruplex (G-4) in combination with small dye molecules, functioning as a fluorescent signaling reporter, has been explored to construct sensors in various applications (Arthanari et al., 1998; Bhasikuttan et al., 2007; Hu et al., 2010; Kong et al., 2009a; Kong et al., 2009b; Qin et
al., 2010a; Ren et al., 2011). G-quadruplex can greatly enhance the fluorescence of small molecules and the fluorescence of G-quadruplex-based complex can be influenced by external stimulus. For instance, the fluorescence of NMM/G-4 (NMM: N-methylmesoporphyrin IX) can be quenched by cupric ion as a result of the chelation of Cu$^{2+}$ by NMM as well as the unfolding of G-quadruplex by cupric ion (Qin et al., 2010b). Considering the strong interaction between histidine (or cysteine) and cupric ion (Jung et al., 2011; Pu et al., 2010; Sarkar and Wigfield, 1967; Taki et al., 2010), it is expected that the presence of histidine or cysteine can influence the fluorescence quenching ability of Cu$^{2+}$ to NMM/G-4 complex. Thus, a label-free approach for histidine detection was developed according to the fluorescence change of NMM/G-4 caused by the interaction among histidine (or cysteine), cupric ion and NMM/G-4. Therefore, simultaneous detection of histidine and cysteine can be realized. In order to achieve high selectivity, histidine can be discriminated from cysteine with the use of N-ethylmaleimide (NEM), which has been widely used as an alkylating agent for biologically important thiols (Giustarini et al., 2011; Zhu et al., 2010). According to the different response of the system with and without NEM, each precise concentration of histidine and cysteine can be determined. Detection limits as low as 3 nM for histidine and 5 nM for cysteine were obtained by practical measurement. Both of the detection limit and linear range are compatible with previous methods. Most importantly, this simple and novel approach can withstand the interference from biological samples such as urine, exhibiting great potential for practical application.

2. Experimental

2.1 Materials

The synthesized oligonucleotide was purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). NMM was purchased from Porphyrin Products (Logan, UT). All the
other chemicals were purchased from Aladin Ltd. (Shanghai, China) and used as received without further purification. The stock solution of NMM (1 mM) was prepared in dimethyl sulfoxide (DMSO) and stored in darkness at -20 °C. The water used throughout all experiments was purified through a Millipore system. The sequence of the used 24GT oligonucleotide is given below: 5’-GGG TTTT GGG TTTT GGG TTTT GGG-3’.

2.2 Instruments

Cary 500 Scan UV/Vis Spectrophotometer (Varian, USA) was used to quantify the oligonucleotide. DNA concentration was estimated by measuring the absorbance at 260 nm. Fluorescence intensities were recorded on a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France). The emission spectra were recorded within the range of 540-720 nm upon excitation at 399 nm. All the experiments were carried out at room temperature (about 25 °C).

2.3 Preparation of NMM/G-4

The desired concentration of NMM and G-quadruplex was achieved by diluting the stock solution of NMM and G-quadruplex with 20 mM HEPES buffer containing 150 mM NaCl and 150 mM KCl (pH: 7.0), respectively. The mixture was incubated for 30 min to allow the formation of NMM/G-4 ensemble.

2.4 Histidine and Cysteine Detection

An optimized concentration of CuCl₂ was achieved by monitoring fluorescence change of NMM/G-4 complex solution against concentration of CuCl₂. For histidine detection, cupric ion (1.5 µM) was premixed with histidine or cysteine for 30 min. Then NMM/G-4 complex was added into the mixture. The final volume of each sample for fluorescence measurement is 400 µL in 20 mM HEPES buffer containing 150 mM NaCl and 150 mM KCl (pH: 7.0). Excitation was at 399 nm, and the emission was monitored at 608 nm. To guarantee the efficient reaction among the components, the fluorescence
intensity was measured after incubation about 1.5 h. In order to differentiate cysteine, 10 µL of 50 mM NEM was incubated with the sample for 40 min in the premixing procedure.

3. Results and Discussion

3.1 Mechanism of Histidine and Cysteine Detection

The fluorescent intensity of some dye molecules can be greatly enhanced by forming complex with G-quadruplex. The structure of G-quadruplex can be influenced by the external stimulus, such as solution pH and metal ions, which exhibits strong effect on the fluorescent intensity of the complex of dye molecules and G-quadruplex. Taking advantage of the tunable conformation, G-quadruplexes have been widely used for constructing nanoscale devices and biosensors, which exhibit excellent stability and reproducibility (Alberti and Mergny, 2003; Miyoshi et al., 2007; Wang et al., 2010; Willner et al., 2008). In our investigations, a novel sensing system was developed by monitoring the fluorescent signal change caused by the tunable conformation of G-quadruplex as shown in Scheme 1. Here, 24GT oligonucleotide is used to form intramolecular G-quadruplex to combine NMM, producing signal indicator NMM/G-4 with strong fluorescent intensity. NMM is a commercially available anionic porphyrin characterized by a pronounced structural selectivity for G-quadruplex but not for duplexes, triplexes, or single-stranded forms (Hu et al., 2010; Oh et al., 2010). One key feature of NMM lies in its two proximal carboxylate groups, which makes it possible to coordinate with Cu$^{2+}$, forming Cu$^{2+}$/NMM complex and resulting in a high fluorescence quenching (as shown in Scheme 1). The unfolding of the G-quadruplex by excess Cu$^{2+}$ can also lead to fluorescence quenching of NMM/G-4 complex (Monchaud et al., 2008). It is known that histidine containing imidazole group can coordinate with cupric ion to form a strong-binding complex (Deschamps et al., 2004; Sarkar and Wigfield, 1967). Upon the presence of histidine (right side of scheme 1), it will
disturb the interaction between Cu$^{2+}$ and NMM/G-4 complex, generating a distinct fluorescence intensity from that of cupric ion alone (left side of scheme 1). Therefore, it is expected that this different fluorescent response between the presence and absence of histidine could find application in histidine detection. Thus a novel label-free approach for histidine detection was developed according to the competitive interaction among histidine, Cu$^{2+}$ and NMM/G-4. Thiol-containing amino acid cysteine can also react with Cu$^{2+}$ (Jung et al., 2011; Taki et al., 2010), which produce similar phenomenon to that of histidine and can also be used for cysteine detection.

Scheme 1

3.2 Fluorescence Quenching of NMM/G-4 by Cupric Ion

Steady-state fluorescence measurements were carried out to serve as a proof of concept to evaluate the feasibility of our design. Fluorescence titration experiments were firstly performed in the presence of NMM at fixed concentration. The fluorescence response of the system increased significantly with increasing the concentration of G-quadruplex (as shown in Fig. 1A). It reached a plateau when the ratio of [NMM]/[G-quadruplex] arrived at 1:1 (as shown in Fig. 1B). Further increase of G-quadruplex did not evidently enhance the fluorescence response of the system. Therefore, the ratio of 1:1 was used in the following experiments to ensure a good signal-to-background ratio. As can be seen from Fig. 1C, the fluorescence intensity is about 30-fold enhanced under the optimized condition, which can improve the detection sensitivity of analyte.

Fig. 1

Considering the quenching ability of Cu$^{2+}$ to NMM/G-4, the amount of Cu$^{2+}$ used is very important. If the cupric ion was superfluous, then more histidine was required to hinder the
fluorescence quenching of Cu\textsuperscript{2+} to NMM/G-4 and that is not in favor of reaching a low detection limit. If the cupric ion used was too less, however, the detection of histidine with high concentration might not be realized, leading to a narrow detection range and a low signal-to-background ratio. Thus, the quenching ability of different amount of Cu\textsuperscript{2+} to NMM/G-4 complex was investigated to find an optimal experimental condition. Fig. 1D exhibits that the fluorescence intensity decreased with the increase of Cu\textsuperscript{2+} concentration due to the coordination of NMM to Cu\textsuperscript{2+} as well as the unfolding of G-quadruplex. According to the result, 88% fluorescence intensity of the system was quenched when the concentration of Cu\textsuperscript{2+} was 1.5 \( \mu \)M, which was used for the following experiments. It should be noted that the amount of Cu\textsuperscript{2+} used for NMM/G-4 fluorescence quenching is not proportional to the amount of NMM/G-4, which can be ascribed to the competitive interaction between Cu\textsuperscript{2+} and NMM/G-4 (Qin et al., 2010b).

3.3 Simultaneous Histidine and Cysteine Detection

Fig. 2

We firstly carried out the performance of histidine detection. Since the detection of analyte was designed according to the competitive interaction among histidine, Cu\textsuperscript{2+} and NMM. It is important to know whether it is true that histidine could snatch Cu\textsuperscript{2+} from the complex of Cu\textsuperscript{2+}/NMM. Hence, histidine was added into the mixture of Cu\textsuperscript{2+} and NMM/G-4. The fluorescence intensity was significantly recovered (Fig. S1 in supporting information (SI)), indicating that the binding ability between histidine and Cu\textsuperscript{2+} is stronger than that between Cu\textsuperscript{2+} and NMM/G-4. Considering that the interaction of histidine and Cu\textsuperscript{2+} would be more sufficient in the absence of NMM/G-4, histidine with different concentration was first mixed with Cu\textsuperscript{2+} for 30 min and then NMM/G-4 complex was added, which might be in favor of reaching a low detection limit as well as saving time. Due to the chelation between histidine and cupric ion, the more histidine was added, the less free Cu\textsuperscript{2+} was left to interact with NMM/G-4. As a result, the
less fluorescence quenching of NMM/G-4 was observed. Learned from Fig. 2A, the fluorescence responses of the system gradually increases with the increase of histidine concentration. To evaluate the repeatability of the sensing system, error bars were calculated by plotting the degree of fluorescence intensity change \((F-F_0)\) against the concentration of histidine (Fig. 2B), where \(F\) and \(F_0\) are fluorescence intensities in the presence and absence of histidine, respectively. The fluorescence intensity change reaches a plateau when the concentration of histidine exceeds 10 \(\mu\)M (as shown in Fig. 2B). A good linear relationship over the range from 3 nM to 10 \(\mu\)M \((R^2=0.98)\) was obtained as shown in the inset of Fig. 2B. A detection limit as low as 3 nM was achieved by practical measurement rather than conventional calculation, which is much lower than the previous reports (Zeng et al., 2009), confirming the high sensitivity of the developed approach. Both the linear range and detection limit for histidine detection are comparable with previous methods, detailed information is available in Table S1. It is reported that cysteine could also react with \(\text{Cu}^{2+}\) due to the existence of thiol group (Jung et al., 2011; Taki et al., 2010), cysteine detection was performed nextly according to the same mechanism of competitive interaction between cysteine, \(\text{Cu}^{2+}\) and NMM/G-4. All procedures were the same to that in histidine detection. Fig. 2C demonstrates the degree of fluorescence intensity changes \((F-F_0)\) plotted against the concentration of cysteine \((F\) and \(F_0\) are fluorescence intensities in the presence and absence of cysteine, respectively). The fluorescence response of the system increases with the increase of cysteine concentration and the reproducibility can be learned from the error bars. The fluorescence intensity change reaches a plateau when the concentration of cysteine exceeds 10 \(\mu\)M (as shown in Fig. 2C). A good linear relationship over the range from 5 nM to 5 \(\mu\)M \((R^2=0.99)\) was obtained as shown in Fig. 2D and a detection limit as low as 5 nM was obtained by practical measurement. Learned from Table S2, the performance in cysteine detection was also comparable with previous methods. It should
be pointed that both of the detection limits here were obtained by practical measurement, indicating the promising potential of the present strategy in practical application.

In above experiments, we have performed a feasibility study of histidine and cysteine detection individually. However, how to determine the fluorescence response of the system to complex mixture is generated from the existence of histidine, or cysteine, or both of them? If both of them were present in the sample, how to determine the individual precise concentration? One solution is to find an agent to mask the fluorescence response of either one. This is the matter of selectivity that needs to be evaluated because high selectivity is essencial to the development of an excellent sensor. The selectivity for histidine and cysteine is mutual, if one of them can be selectively determined, the selectivity for the other one is guaranteed. Both of histidine and thiol-containing amino acid cysteine can also interact with \(\text{Cu}^{2+}\) (Deschamps et al., 2004; Jung et al., 2011; Taki et al., 2010), producing similar phenomenon to the present detection system. However, upon presence of NEM, the alkylated product of cysteine can’t react with \(\text{Cu}^{2+}\) and thus will not disturb the interaction between \(\text{Cu}^{2+}\) and NMM/G-4. Thus, histidine can be discriminated from cysteine due to the help of NEM. Furthermore, by comparing the fluorescent response of the system with and without NEM, the present method could also detect cysteine simultaneously. Considering the structural similarity of amino acids, the potentially competing effects of all the other amino acids on the system were also investigated to further evaluate the selectivity. Adding larger amount of various other amino acids (20 \(\mu\)M), the fluorescence signal of the system is much lower than that of the system in the presence of histidine (8 \(\mu\)M). It can be clearly seen from Fig. 3 that the developed sensing system exhibits excellent selectivity and can discriminate histidine against other amino acids. The influence of cysteine on the selectivity of histidine can be eliminated by introduction of NEM. The presence of NEM in the premixing procedure leads to cysteine alkylation, inhibiting its interaction with cupric ion and generating a different fluorescence response from that of histidine. To further evaluate the selectivity, histidine detection was also conducted in the presence of all other
amino acids (see Fig. S2 in supporting information). The fluorescence response of the system in the presence of all other amino acids containing histidine is much stronger than that of the system in the presence of all the other amino acids without histidine, confirming the good selectivity of the present method. Finally, histidine can be discriminated from cysteine and therefore selectivity for each of them is realized.

Fig. 3

The development of a selective sensor in the physiological condition for histidine and cysteine detection is critically important because a variety of diseases are associated with histidine or cysteine. For this purpose, it is important to take the possible interference from physiological species into consideration. We subsequently investigated the fluorescence assay of histidine and cysteine in the presence of urine. To mimic a biological sample, urine was diluted 100-fold with HEPES buffer. The recovery experiments of histidine and cysteine at different concentrations within the linear range were carried out by applying a standard addition method. The detection was performed according to the procedure mentioned above, and the “Found” concentration was estimated from corresponding fluorescence intensity according to the regression equation. Nice recoveries, 96%—102%, were reached as shown in Table 1, which validate the reliability and practicality of the developed method.

| Table 1 |

4. Conclusions

In summary, we provide a label-free, facile and novel approach for simultaneous histidine and cysteine detection. High sensitivity and selectivity can be achieved with the use of NEM, which is able to mask cysteine. The present assay is based on the highly specific competitive interaction among histidine (or cysteine), Cu$^{2+}$ and the strong fluorescent NMM/G-4 ensemble. The system is simple in design and
fast in operation with the help of premixing strategy. Moreover, the present approach is more convenient and promising than other methods, eliminating the need of organic co-solvents, enzymatic reactions, separation processes, chemical modifications, and sophisticated instrumentations. Detection limits as low as 3 nM for histidine and 5 nM for cysteine were obtained through practical measurement. Both of detection limits and linear range are compatible with the previous methods. Last, but not the least, the protocol offers high selectivity for histidine detection against all the other amino acids with the help of NEM to mask cysteine. Considering that only histidine and cysteine can disturb the interaction between Cu²⁺ and NMM/G-4 in all amino acids, the selectivity for histidine also means high selectivity for cysteine. According to the different response of the system with and without NEM, each precise concentration of histidine and cysteine can be determined if both of them exist. Importantly, the present system can withstand the interference from biological fluids urine. Therefore, the provided approach holds great potential for practical application to meet the requirements in various fields. Exploration of the system in real sample detection and logic gate operation is still under way.

Acknowledgement

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Appendix A. Supplemental data

Supplemental data associated with this article is available online, at doi:
References


Figure captions

Table 1 Analysis of histidine in HEPES buffer containing urine.

Fig. 1 (A) The fluorescence emission spectra of NMM (1 μM) in the presence of different concentrations of G-quadruplex (From bottom to up: 0, 0.06, 0.17, 0.28, 0.39, 0.5, 0.67, 0.83, 1, 1.17 μM). Slit widths for the excitation and emission were 2.5 and 5 nm, respectively. (B) The corresponding fluorescence intensity at 608 nm in (A) plotted against the ratio of [G-quadruplex]/NMM. The error bar represents the standard deviation of three measurements. (C) Fluorescence emission spectra of (a) NMM; (b) NMM/G-4. [NMM]=300 nM, [G-quadruplex]=300 nM. Slit widths for both of the excitation and emission were 5 nm. (D) Fluorescence emission spectra of NMM/G-4 against different concentrations of Cu$^{2+}$: 0 μM, 0.5 μM, 1.0 μM, 1.5 μM, 2.0 μM (From top to bottom). [NMM]=300 nM, [G-quadruplex]=300 nM. Slit widths for both of the excitation and emission were 5 nm.

Fig. 2 (A) Fluorescence emission spectra of NMM/G-4 in the presence of Cu$^{2+}$ and histidine with different concentrations (From bottom to top: 3 nM, 10 nM, 50 nM, 0.2 μM, 0.5 μM, 1.0 μM, 2.0 μM, 4.0 μM, 6.0 μM, 8.0 μM, 10.0 μM, 15.0 μM). (B) The fluorescence intensity change ($F-F_0$) plotted against histidine concentration with error bars, where $F$ and $F_0$ are fluorescence intensities of NMM/G-4/Cu$^{2+}$ in the presence and absence of histidine, respectively. Inset shows the linear relationship between ($F-F_0$) and histidine concentration in the range of 3 nM-10.0 μM. (C) The fluorescence intensity change ($F-F_0$) plotted against cysteine concentration with error bars, where $F$ and $F_0$ are fluorescence intensities of NMM/G-4/Cu$^{2+}$ in the presence and absence of cysteine, respectively. Different cysteine concentrations (from left): 5 nM, 60 nM, 100 nM, 200 nM, 500 nM, 1 μM, 3 μM, 5 μM, 10 μM, 20 μM. (D) The linear relationship between ($F-F_0$) and cysteine concentration in the range of 3 nM-5 μM. [NMM]=300 nM, [G-
quadruplex]=300 nM, [Cu^{2+}]=1.5 μM. Slit widths for both the excitation and emission were 5 nm. The error bar represents the standard deviation of three measurements.

**Fig. 3** Fluorescence intensity change (F-F₀) histograms of NMM/G-4 at 608 nm in the presence of Cu^{2+} and various amino acids with error bars, where F and F₀ are fluorescence intensities of NMM/G-4/Cu^{2+} in the presence and absence of different amino acid, respectively. [NMM]=300 nM, [G-quadruplex]=300 nM, [Cu^{2+}]=1.5 μM, [histidine]=8.0 μM, [every other amino acid]=20.0 μM. In order to discriminate cysteine, 10 μL of 50 mM NEM was incubated with the sample for 40 min in the premixing procedure. Slit widths for both the excitation and emission were 5 nm. The error bar represents the standard deviation of three measurements.

**Scheme 1.** Schematic illustration of the fluorescence change of the NMM/G-4 ensemble under different conditions. The combination of NMM and an intramolecular G-quadruplex generated from 24GT oligonucleotide functions as a signal indicator NMM/G-4 with strong fluorescent intensity. Cupric ion can quench the fluorescence of NMM/G-4 through its coordination with NMM as well as the unfolding of G-quadruplex by Cu^{2+} (as shown in the left side). However, the presence of histidine or cysteine can disturb the interaction between Cu^{2+} and NMM/G-4 complex due to their interaction with Cu^{2+}, generating a distinct fluorescence response from that of cupric ion alone (as shown in the right side).
Table 1

<table>
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<th>Sample</th>
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<th>Found (µM)</th>
<th>Recovery (%)</th>
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<td>3</td>
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Fig. 1
Fig. 2
Fig. 3
Scheme 1
Highlights

- A label-free, G-quadruplex-based approach for histidine and cysteine detection was provided.
- The present fluorescent method is endowed with high sensitivity and selectivity.
- Detection limits 3 nM for histidine and 5 nM for cysteine were achieved by practical measurement.
- Histidine and cysteine can be detected in spite of the interference from urine.
- An IMPLICATION logic operation was realized according to the designed sensing system.
**Fig. S1** Fluorescence emission spectra of (a) NMM/G-4, (b) NMM/G-4/Cu^{2+}, (c) NMM/G-4/Cu^{2+} + histidine. [NMM]=2 μM, [G-quadruplex]=300 nM, [Cu^{2+}]=10 μM, [histidine]=20 μM. All measurements were carried out in a buffer containing 20 mM HEPES, 150 mM NaCl, and 150 mM KCl (pH: 7.0). Excitation was at 399 nm. Slit widths for both the excitation and emission were 2.5 nm and 5 nm, respectively.
Fig. S2 Fluorescence emission spectra of (a) NMM/G-4/Cu²⁺, (b) NMM/G-4/Cu²⁺ + all amino acids except histidine, (c) NMM/G-4/Cu²⁺ + all amino acids. [NMM]=300 nM, [G-quadruplex]=300 nM, [Cu²⁺]=1.5 μM, [histidine]=8.0 μM, [every other amino acid]=20.0 μM. NEM was premixed with amino acids. All measurements were carried out in a buffer containing 20 mM HEPES, 150 mM NaCl, and 150 mM KCl (pH: 7.0). Excitation was at 399 nm. Slit widths for both the excitation and emission were 5 nm.
Table S1. Comparison of the linear range and detection limit for histidine using different methods.

<table>
<thead>
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<th>Method and Reference</th>
<th>Histidine</th>
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<tr>
<td><strong>Method and Reference</strong></td>
<td><strong>Linear range</strong></td>
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<tr>
<td>Disubstituted polyacetylene-based fluorescent method (Zeng et al., 2009)</td>
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<tr>
<td>Lysine-functional silver nanoparticle-based system (Bae et al., 2010)</td>
<td>5 - 30 μM</td>
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<td>DNAzymes and gold nanoparticle–graphene nanosheet composite-based electrochemical method (Liang et al., 2011)</td>
<td>10 pM - 10 μM</td>
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<td>An indicator-displacement assay (Sun et al., 2012)</td>
<td>2.0 - 30 μM</td>
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<td>DNAzyme self-cleavage-based electrochemical method (Li et al., 2011)</td>
<td>1 - 50 nM</td>
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<td>Layer-by-layer assembly electrochemical sensor based on molecular imprinting functionalized indium tin oxide electrode (Zhang et al., 2010)</td>
<td>1 μM - 1 mM</td>
</tr>
<tr>
<td>Metal ion mediated imprinting-based electrochemical sensor (Prasad et al., 2011)</td>
<td>64 pM - 2.21 nM</td>
</tr>
<tr>
<td>Ni^{2+}-modulated homocysteine-capped CdTe quantum dots-based photoluminescent sensor (Wu and Yan, 2010)</td>
<td>1 - 30 μM</td>
</tr>
<tr>
<td>Enzymatic recycling cleavage strategy-based amplified fluorescent sensor (Kong et al., 2011)</td>
<td>2 - 100 μM</td>
</tr>
<tr>
<td>Our method</td>
<td>3 nM - 10 μM</td>
</tr>
</tbody>
</table>
Table S2. Comparison of the linear range and detection limit for cysteine using different methods.

<table>
<thead>
<tr>
<th>Method and Reference</th>
<th>Cysteine</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linear range</td>
<td>Detect limit</td>
</tr>
<tr>
<td>Gold nanoparticle-based near-infrared fluorescent signal (Shang et al., 2009)</td>
<td>4.0 - 250 μM</td>
<td>10 nM</td>
</tr>
<tr>
<td>Poly(methacrylic acid) templated fluorescent Ag clusters (Shang and Dong, 2009)</td>
<td>6.0 - 250 μM</td>
<td>20 nM</td>
</tr>
<tr>
<td>Fluorescent CdTe/CdSe quantum dots (Zhang et al., 2009)</td>
<td>0.2 - 100 μM</td>
<td>131 nM</td>
</tr>
<tr>
<td>Fluorescent CdTe quantum dots-Hg$^{2+}$ system (Han et al., 2009)</td>
<td>2.0 - 20 μM</td>
<td>0.6 μM</td>
</tr>
<tr>
<td>Fluorescein-based fluorescent probe (Chen et al., 2010)</td>
<td>Not given</td>
<td>50 nM</td>
</tr>
<tr>
<td>Oligonucleide-based fluorescent Ag nanocluster (Han and Wang, 2011)</td>
<td>8.0 - 100 nM</td>
<td>4 nM</td>
</tr>
<tr>
<td>Ratiometric fluorescent probe (Yuan et al., 2011)</td>
<td>0 - 900 μM</td>
<td>0.75 μM</td>
</tr>
<tr>
<td>DNA/Ligand/Ion-based fluorescent system (Pu et al., 2010)</td>
<td>2.5 - 110 nM</td>
<td>5.1 nM</td>
</tr>
<tr>
<td>Gold nanoparticle-based resonance light scattering technique (Li et al., 2006)</td>
<td>82.5 nM - 2.06 μM</td>
<td>16.5 nM</td>
</tr>
<tr>
<td>Spiropyran-based ensemble system (Shao et al., 2006)</td>
<td>25 μM - 1 mM</td>
<td>40 nM</td>
</tr>
<tr>
<td>Quinine/Ce(IV)-based chemiluminescent method (Nie et al., 2003)</td>
<td>3.5 nM - 3.5 μM</td>
<td>2.5 nM</td>
</tr>
<tr>
<td>Fluorescent conjugated polymer-stabilized gold nanoparticle-based system (Shang et al., 2007)</td>
<td>50 nM - 4.0 μM</td>
<td>25 nM</td>
</tr>
<tr>
<td>Polythiophene derivative-based colomeric and</td>
<td>0.05 - 0.25 mM</td>
<td>0.1 nM</td>
</tr>
</tbody>
</table>
fluorescent dual probe (Yao et al., 2011)

**FRET-based ratiometric fluorescent probe (Long et al., 2011)**

<table>
<thead>
<tr>
<th>Linear range</th>
<th>Detect limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 - 100 μM</td>
<td>82 nM</td>
</tr>
</tbody>
</table>

**Ag⁺-mediated DNAzyme system (Li et al., 2009)**

<table>
<thead>
<tr>
<th>Linear range</th>
<th>Detect limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 - 110 μM</td>
<td>40 nM</td>
</tr>
</tbody>
</table>

**A reusable DNA single-walled carbon-nanotube-based fluorescent sensor (Zhao et al., 2010)**

<table>
<thead>
<tr>
<th>Linear range</th>
<th>Detect limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 200 nM</td>
<td>17.9 nM</td>
</tr>
</tbody>
</table>

**Graphene oxide and thiol-activated DNA metallization-based fluorescent probe (Lin et al., 2011a)**

<table>
<thead>
<tr>
<th>Linear range</th>
<th>Detect limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1 μM</td>
<td>2 nM</td>
</tr>
</tbody>
</table>

**DNA/gold nanoparticle-based colorimetric competition assay (Lee et al., 2008)**

Not given |

**T-Hg²⁺-T based molecular beacon system (Stobiecka et al., 2012)**

Not given |

<table>
<thead>
<tr>
<th>Method and Reference</th>
<th>Linear range</th>
<th>Detect limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphene nanoribbon-based electrochemical method (Wu et al., 2012)</td>
<td>25 nM - 500 μM</td>
<td>Not given</td>
</tr>
<tr>
<td>Electrochemical biosensor based on graphene oxide and Au nanoclusters composite (Ge et al., 2012)</td>
<td>50 nM - 20 μM</td>
<td>20 nM</td>
</tr>
<tr>
<td>Magnetic FRET nanoprobe (Yang et al., 2012)</td>
<td>1 - 30 μM</td>
<td>0.91 μM</td>
</tr>
<tr>
<td>Graphene oxide/DNA/Ag⁺-based fluorescent probe (Xie et al., 2012)</td>
<td>60 nM - 500 nM</td>
<td>44 nM</td>
</tr>
<tr>
<td>Fluorosurfactant-stabilized pH-sensitive gold colloidal system (Xiao et al., 2011)</td>
<td>1 μM - 5 μM</td>
<td>1 μM</td>
</tr>
<tr>
<td>Gold-5-amino-2-mercapto-1,3,4-thiadiazole core-shell nanoparticles film-based electrochemical method (Kannan and John, 2011)</td>
<td>10 - 140 nM</td>
<td>3 pM</td>
</tr>
<tr>
<td>Method</td>
<td>Sensitivity1</td>
<td>Specificity2</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Electrochemical method based on DNA-templated Ag deposition (Lin et al., 2011b)</td>
<td>0 - 2 μM</td>
<td>6 nM</td>
</tr>
<tr>
<td>Hg&lt;sup&gt;2+&lt;/sup&gt;-mediated G-quadruplex DNAzyme system (Jia et al., 2011)</td>
<td>0 - 1 μM</td>
<td>19 nM</td>
</tr>
<tr>
<td>Molecular beacon-based fluorescent assay (Xu and Hepel, 2011)</td>
<td>6 - 200 nM</td>
<td>4.2 nM</td>
</tr>
<tr>
<td>Our method</td>
<td>5 nM - 5 μM</td>
<td>5 nM</td>
</tr>
</tbody>
</table>