Biosensors and Bioelectronics 43 (2013) 379–383

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A molecular beacon and graphene oxide-based fluorescent biosensor for Cu^{2+} detection

Jiahao Huang, Qingbin Zheng, Jang-Kyo Kim, Zhigang Li *

Department of Mechanical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong,

ARTICLE INFO

Article history:
Received 25 October 2012
Received in revised form
19 December 2012
Accepted 27 December 2012
Available online 4 January 2013

Keywords:
Biosensor
Copper ion
Molecular beacon
Graphene oxide
Fluorescence

ABSTRACT

In this work, we report a "turn-on" fluorescent strategy for the direct detection of Cu^{2+} in solutions using molecular beacons (MBs) and graphene oxide (GO). MBs are special single-stranded DNA and carry fluorescence sources. GO is a new nanomaterial having remarkable physical properties. In the sensing system, GO was used as an efficient fluorescence quencher upon the adsorption of MBs, which reduced the background signal and made the detection method highly sensitive. In the presence of Cu^{2+}, the MBs were cut into short pieces and released by the GO, leading to fluorescence restoration. The detection limit of the sensing strategy was ~50 nM, which is sufficiently sensitive for practical applications. The sensing method also exhibited high selectivity in testing samples containing other metal ions. The application of the method for drinking water is demonstrated.

1. Introduction

Copper is an essential trace element for human body. At an appropriate level, it plays important roles in maintaining normal biological functions. At lower or higher concentrations, however, it can cause health issues. Copper deficiency is responsible for many deceases, such as neutropenia and osteoporosis (Bonham et al., 2002). On the other hand, an accumulation of copper in human body can be highly toxic and cause damages to organs, such as liver and kidney (Hahn et al., 1995; Zietz et al., 2003). High concentration of Cu^{2+} in drinking water has also been proven to be associated with serious neurodegenerative diseases (Barnham and Bush, 2008; Brown and Kozlowski, 2004).

To monitor or control the intake of copper ions from diet and other sources, sensitive and selective detections of Cu^{2+} are highly demanded. In the past decades, Cu^{2+} detection has been a hot topic in scientific research and various techniques have been developed. Conventional methods, including atomic absorption spectrometry (Chen and Teo, 2001; Lin and Huang, 2001) and inductively coupled plasma atomic emission spectroscopy (Abollino et al., 1998; Vaisanen et al., 2002), have been developed for efficient detection of Cu^{2+}. However, these methods usually involve sophisticated equipment and complex sample preparations, which limit their applications in routine Cu^{2+}detections. Fluorescent chemosensors have also attracted considerable attention because they are relatively simple, low-cost, and sensitive (de Silva et al., 1997; Shao et al., 2005; Torrado et al., 1998; Zheng et al., 2001). Most of the previous chemosensors were fabricated to produce a "turn-off" signal arising from the quenching of fluorescence after the binding between fluorescence source carriers and Cu^{2+}. Unfortunately, the "turn-off" mechanism could generate false signals due to the presence of unexpected quenching entities. Recently, the advent of "Click Chemistry" has offered novel and promising routes for the design and development of Cu^{2+} sensors. In spite of the advantages of "Click Chemistry", this technique needs to transform Cu^{2+} to Cu^{+} and therefore is an indirect method (El-Sagheer and Brown, 2010; Hua et al., 2012; Moses and Moorhouse, 2007; Shen et al., 2012; Xu et al., 2010; Zhou et al., 2008). Furthermore, several groups have developed new strategies to monitor Cu^{2+} levels using metallic nanoparticles (e.g. Au and Ag particles), whose fluorescence signals can be turned on/off by Cu^{2+} (Chen et al., 2009; He et al., 2005; Lan et al., 2010; Su et al., 2010). Another approach for detecting Cu^{2+} employs DNAzymes (Sando et al., 2003; Wang et al., 2011, 2012), which are catalytic DNA molecules and can promote the cleavage or ligation of DNA molecules in the presence of specific metal ions. Most DNAzymes are highly stable and inexpensive. They can maintain the binding ability and catalytic activity even undergoing denaturation or renaturation. Different DNAzyme-based biosensors have been developed for Cu^{2+} detection by monitoring fluorescence signals or color changes (Liu and Lu, 2007a, 2007b; Wang et al., 2010; Wu et al., 2010; Yin et al., 2009). For fluorescent detections, usually two or more quenchers labeled at the DNA probes are required to reduce the background signal. For colorimetric assays, the detection limit, usually at micromolar levels, is relatively high due to the difficulty of distinguishing the color changes at low target concentrations although the method is simple and low-cost.

In this work, we propose a "turn-on" fluorescent sensing method for the direct detection of Cu^{2+} in solutions. This strategy employs

* Corresponding author. Tel.: +852 2358 7186.
E-mail address: mezli@ust.hk (Z. Li).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.12.056

molecular beacons (MBs) and graphene oxide (GO). MBs are special single-stranded DNA (ssDNA) labeled with a fluorophore and a quencher at the two ends. They form hairpin structures with the fluorophore and quencher in close proximity in solutions and do not fluoresce in the absence of detection targets, as discussed later. However, if they experience structural changes and the fluorophore is separated from the quencher, fluorescence signals will be generated. MBs have been widely used for detecting DNA, RNA, proteins, and other species (Biggins et al., 2000; Huang et al., 2012; Tyagi and Kramer, 1996; Wang et al., 2009), but have not been used for Cu^{2+} detection. GO is an attractive nanomaterial and possesses unique abilities in DNA adsorption and fluorescence quenching with nanoscale long-range energy transfer properties. This makes GO, in working with MBs, a good candidate for biosensors (He et al., 2010; Lu et al., 2009, 2010). In the proposed sensing system, MBs are adsorbed on the GO sheets in the absence of Cu^{2+}, leading to a very low background signal. When copper ions are introduced with H_2O_2, the MBs are cleaved and detached from the GO surfaces, leading to the generation of fluorescence. In this approach, MBs are used as the molecular recognition unit for generating fluorescence signals. The roles of the GO are twofold. One is to lower the background signal and enhance the signal-to-background (S/B) ratio through the adsorption of MBs on its surface. The other is to control the fluorescence generation through its distinct adsorption abilities to short ssDNA and hairpin-shaped DNA (MBs) (the former can easily desorb from GO surfaces). This "turn-on" fluorescent method has the merits of a very low background signal and hence a high sensitivity. The MBs employed in this strategy do not require a special or complex design, which makes this approach very easy to carry out. The application of the sensing system is demonstrated by detecting the Cu^{2+} levels in drinking water.

2. Materials and methods

2.1. Materials

The MBs (DNA probes) were commercially synthesized by TaKaRa Bio Inc. (Dalian, China). The sequence of the MBs is FAM-TGCGAGTTGAAGTAGTGACATTCATCACGCTCAATCACTACTTCAACT-CGCA-DABCYL (FAM is fluorescein-based dye; DABCYL is a quencher), where the underlined letters denote complementary sequences, which bind together and form the MBs into a stem–loop (hairpin) structure, as shown in Fig. 1a. The GO sheets were synthesized through the oxidation and exfoliation of natural graphite by using a modified Hummers method. The thickness of the GO sheets was ~1.0 nm and the lateral size was 1–5 μm. The detailed information about the GO fabrication can be found in our previous work (Aboutalebi et al., 2011; Zheng et al., 2011). All other reagents were of analytical grade and were used without further purification or modification. All the solutions were prepared with water from a NANOpure Diamond (Barnstead Int., Dubuque, IA) source.

2.2. Fluorescence measurement

Fluorescence measurements were performed using a F4500 fluorometer (Hitachi, Japan). The excitation and emission wavelengths were set at 496 and 522 nm, according to the fluorescent property of FAM labeled on the MBs. The slit width for both excitation and emission was set at 5 nm. To obtain the fluorescence emission spectra, the samples were excited by a 496 nm light and scanned from 510 to 600 nm with a step of 1 nm. All the solutions were of 600 μL and prepared by mixing 83.3 nM MBs and 3.33 μg/mL GO in the reaction buffer (50 mM $MgCl_2$ and 5 mM Tris–HCl, pH = 8.0). All the samples were incubated at 24 °C for at least 10 min before the experiments.

3. Results and discussion

3.1. Detection principle

The related detection mechanisms are illustrated in Fig. 1. Fig. 1a shows a simple method for fluorescence generation. An MB takes a hairpin structure in solutions due to the molecular binding between the complementary sequences, which bring the fluorophore and quencher into close proximity and cause fluorescence quenching. In the presence of Cu^{2+} and H_2O_2, the MB is cleaved into short ssDNA pieces and the fluorophore is separated from the

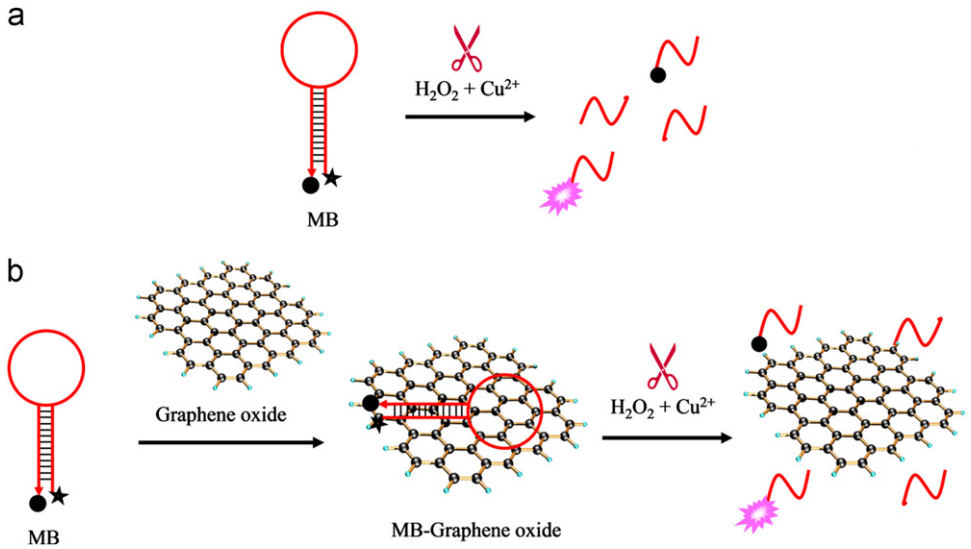

Fig. 1. Principles for Cu^{2+} detection. (a) Detection mechanism based on MBs only. An MB, which assumes a stem–loop structure in the absence of Cu^{2+}, brings the fluorophore and quencher in close proximity and the fluorescence is quenched. With the help of H_2O_2, the addition of Cu^{2+} cuts the MB into pieces and leads to the separation of the fluorophore from the quencher and the restoration of fluorescence and (b) Schematic of Cu^{2+} detection using MBs and GO (for clarity, oxides are not shown). GO absorbs the MB through non-covalent interactions and further quenches the fluorescence of the MB. When H_2O_2 and Cu^{2+} are introduced, the MB is cleaved into short fragments, which are released from the GO, and the fluorescence signal is generated.

quencher, leading to fluorescence generation (Fig. 1a). Although MBs are designed not to fluoresce in their hairpin structures, they usually emit weak fluorescence (Huang et al., 2012), which will increase the background signal and deteriorate the sensitivity of the sensor. In the detection principle in Fig. 1a, if GO is introduced into the MB solutions before detection, the MBs could be adsorbed by the GO sheets through non-covalent pi-stacking interactions between the ring structures in the MBs nucleobases and the hexagonal cells of the GO (He et al., 2010). In this case, the super-quenching capability of the GO can greatly reduce the fluorescence emission of the MBs, improve the S/B ratio, and hence increase the detection sensitivity. When Cu^{2+} appears with H_2O_2, the MBs are cut into pieces, which would desorb from the GO due to the weak binding between short ssDNA and GO (Fig. 1b). Finally, the fluorescence of the fluorophore is recovered. To confirm the principle in Fig. 1b, the fluorescence responses of the two sensing methods in Fig. 1 were measured before and after 23.3 μM Cu^{2+} was added into the corresponding solutions, as shown in Fig. 2. After the addition of Cu^{2+}, the fluorescence intensity of the sample containing 83.3 nM MBs only increased and the S/B ratio was 2.91 at the emission wavelength of 522 nm. For the sample containing 83.3 nM MBs and 3.33 μg/mL GO, as expected, the background signal was greatly reduced due to the adsorption of MBs on the GO surfaces. The S/B ratio was measured as 17.35 (at 522 nm), which was significantly enhanced compared with the method shown in Fig. 1a. The high S/B ratio arising from GO is expected to make the sensing method highly sensitive.

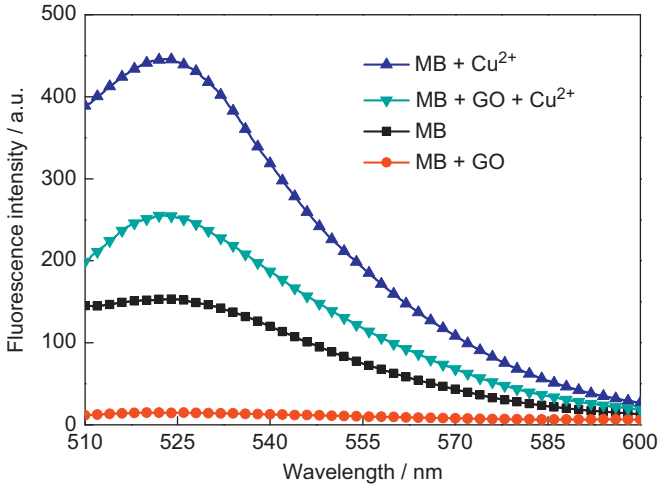

Fig. 2. Fluorescence responses of the systems in Fig. 1. The concentrations of MBs and GO were 83.3 nM and 3.33 μg/mL in the reaction buffer (50 mM $MgCl_2$ and 5 mM Tris–HCl, pH=8.0). All the samples reacted at 24 °C for 1.5 h after the addition of 23.3 μM Cu^{2+} and 2.1 mM H_2O_2.

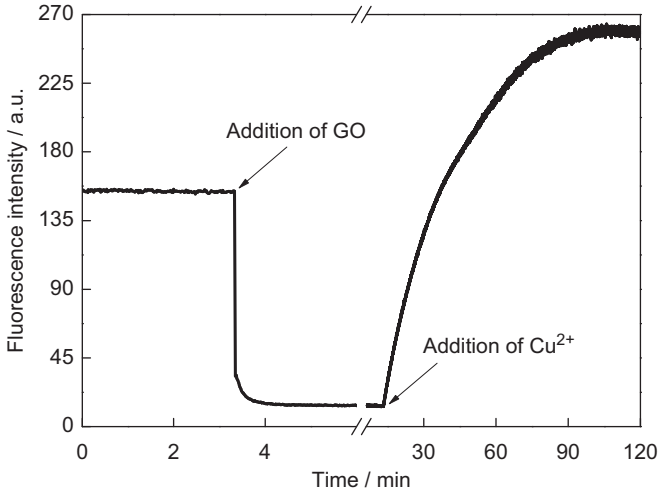

Fig. 3. Fluorescence quenching and restoration of an MB solution in the presence of GO and Cu^{2+}. The solution containing 83.3 nM MBs in the reaction buffer (50 mM $MgCl_2$ and 5 mM Tris–HCl, pH=8.0) was prepared first. Then GO with a final concentration of 3.33 μg/mL was introduced, which was followed by the addition of 23.33 μM Cu^{2+} and 2.1 mM H_2O_2.

3.2. Detection feasibility

The kinetic study of the MB–GO platform was carried out by recording the fluorescence response of an MB solution after the addition of GO and Cu^{2+}. As shown in Fig. 3, the fluorescence intensity of the sample containing 83.3 nM MBs was about 154.3 a.u. After 3.33 μg/mL GO was introduced into the sample, the fluorescence signal was significantly lowered after a few seconds, showing the ultrafast quenching speed and excellent quenching capability of GO. At a later time, when 23.33 μM Cu^{2+} was added, the response signal went up, indicating the cleavage and desorption of the MBs, and became stable after about 80 min (Fig. 3), which was faster than the methods using "Click Chemistry" (El-Sagheer and Brown, 2010; Hua et al., 2012; Moses and Moorhouse, 2007; Shen et al., 2012; Xu et al., 2010; Zhou et al.,

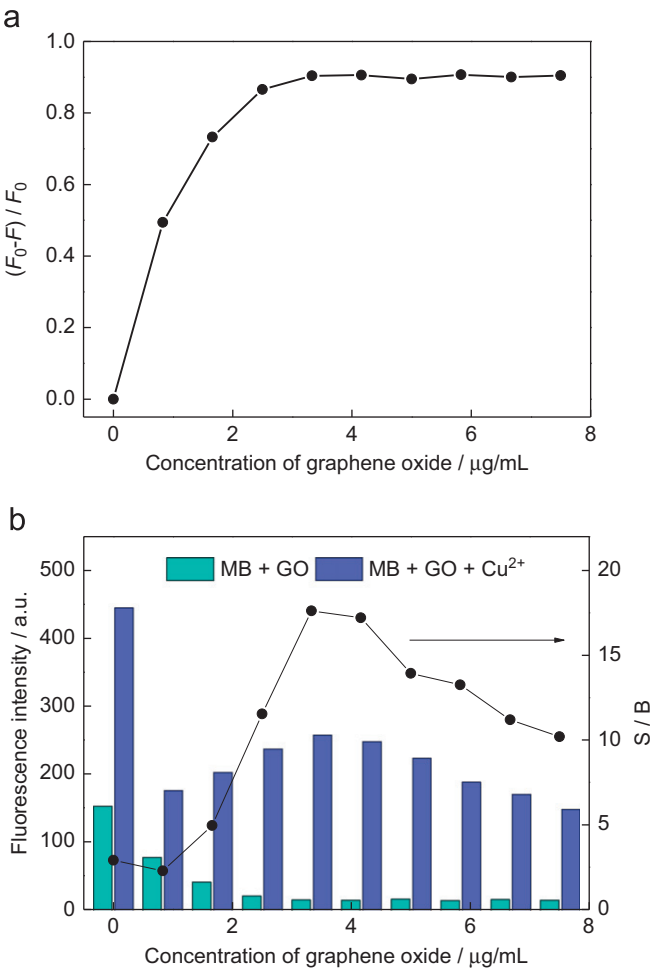

Fig. 4. Effects of GO concentration on the fluorescence response of the sensing system. (a) Quenching efficiency, $(F_0-F)/F_0$, as a function of GO concentration (F and F_0 are the fluorescence intensities of the MB (83.3 nM) solution at the emission wavelength of 522 nm with and without GO, respectively) and (b) Fluorescence intensity and S/B ratio of the sensing system (MB: 83.3 nM) before and after the addition of Cu^{2+} (23.33 μM). and H_2O_2 (2.1 mM) The samples were allowed to react for 1.5 h.

2008). To achieve the best performance of the sensing system, the effect of GO concentration was investigated by considering the quenching efficiency and the S/B ratio. The concentration of MBs was fixed at 83.3 nM and that of GO was varied from 0 to 7.5 μg/mL. The quenching efficiency is defined as $(F_0-F)/F_0$, where F and F_0 are the fluorescence intensities of the MB solution with and without GO. Fig. 4a shows $(F_0-F)/F_0$ as a function of GO concentration at the emission wavelength of 522 nm. It is seen that the quenching efficiency increased and then leveled off when the GO concentration was higher than 2.5 μg/mL. With the presence of 23.33 μM Cu^{2+}, the fluorescence response and the S/B ratio were also obtained as a function of GO concentration (Fig. 4b). Fig.4b shows that the best S/B ratio took place at the GO concentration of 3.33 μg/mL, at which the quenching efficiency also reached the optimal value. Therefore, the GO concentration of 3.33 μg/mL was chosen for most of the experiments in this work.

3.3. Detection sensitivity and selectivity

The sensitivity of the detection method was investigated by changing the concentration of Cu^{2+}. Fig. 5a shows the emission spectra of the sensing system after incubation with Cu^{2+} for 1.5 h. It is seen that the fluorescence intensity increased with increasing Cu^{2+} concentration, indicating the growth of the number of MBs cut by Cu^{2+}. The emission spectra could be clearly identified even for nanomolar Cu^{2+} concentrations, especially in the vicinity of ~520 nm. The dependence of the fluorescence intensity on logarithmic Cu^{2+} concentration, Log C, is plotted in Fig.5b. The signal response was proportional to Log C at low target concentrations (from 53.3 nM to 1.333 μM) and the detection limit was experimentally determined as 53.3 nM, which is lower than those obtained in the colorimetric assays using gold nanoparticles and "Click Chemistry" (Xu et al., 2010; Zhou et al., 2008) and sufficiently sensitive for various practical applications (the level of Cu^{2+} in drinking water set by WHO is 30 μM). It is noted that when the hairpin probes were labeled with dye (FAM) only (without quencher), the quenching efficiency would follow the same fashion as that in Fig. 4 as GO concentration was increased. However, the detection limit was experimentally determined as 1.0 μM, which is much higher than the current sensing method, as shown in Fig. S1 (Supporting information).

The selectivity of the MB–GO platform was also studied by monitoring the fluorescence response when the sensing system was challenged with other metal ions, including Sn^{2+}, Ni^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Hg^{2+} and Fe^{3+}. As shown in Fig. 6, 10 μM Cu^{2+} produced a significant fluorescence signal, while the fluorescence responses for 50 μM other metal ions were significantly lower (black bars). Fig. 6 (red bars) also indicates that the coexistence of Cu^{2+} and other competing metal ions did not affect the detection quality of the sensing system. This is necessary for practical applications since different metal ions may be present simultaneously in real samples. The high selectivity of the sensing system is due to the efficient breakage of DNA strands induced by hydroxyl radicals, which is produced by the Cu^{2+}-dependent reaction ($Cu^{2+}+H_2O_2$).

Finally, to demonstrate that the sensing method is applicable for real samples, recovery experiments for drinking water with different Cu^{2+} concentrations were performed. The drinking water samples were acquired from the tap in the laboratory and the Cu^{2+} concentration was measured as 203.6 nM using our sensing system. Fortification solutions of different Cu^{2+} concentrations were then added to the water samples and the Cu^{2+} levels were obtained again using the sensing system. The results

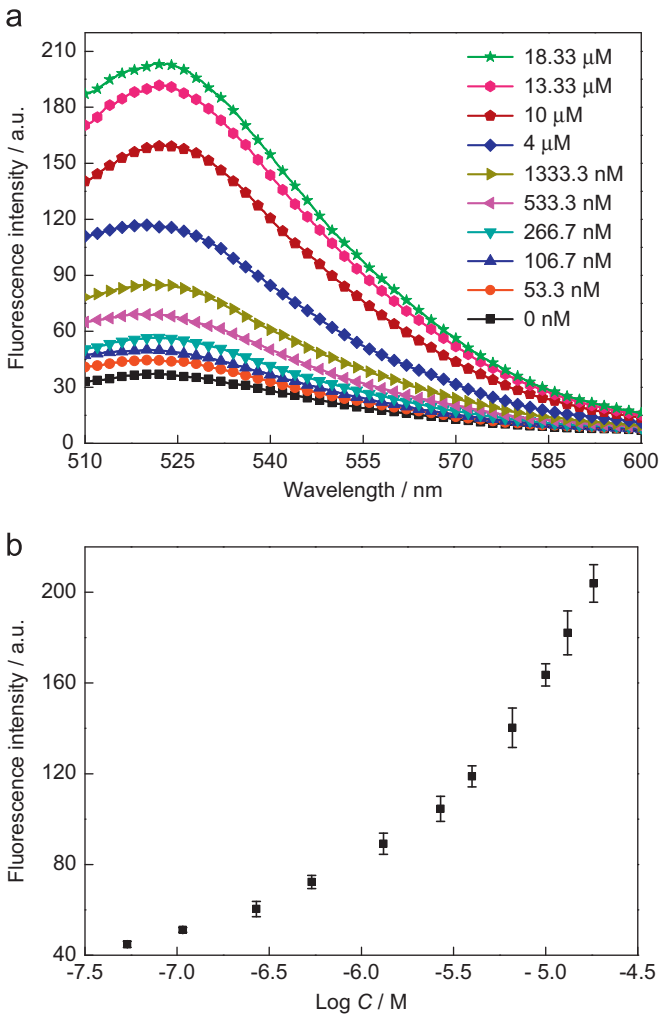

Fig. 5. Fluorescence response of the sensing system (MB: 83.3 nM, GO: 3.33 μg/mL) at different Cu^{2+} concentrations. (a) Fluorescence emission spectra. (b) Relationship between the fluorescence intensity and the Cu^{2+} concentration. All the samples reacted at 24 °C for 1.5 h after the addition of different concentrations of Cu^{2+} and 2.1 mM H_2O_2.

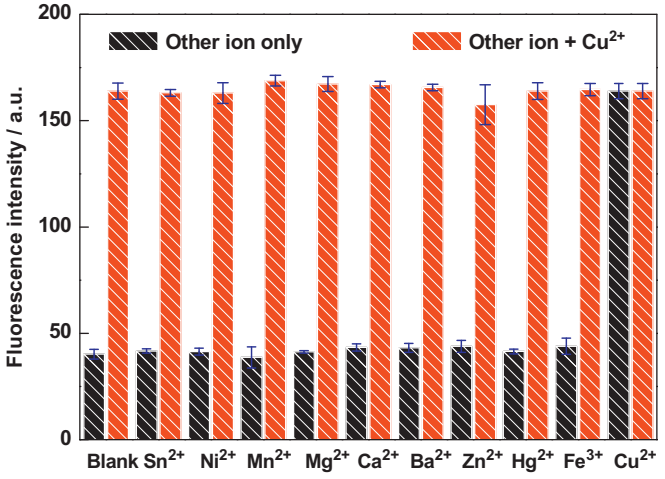

Fig. 6. Selectivity of the sensing system for Cu^{2+} against other metal ions. The concentrations were 10 μM for Cu^{2+} and 50 μM for other metal ions. Black bars represent the fluorescence responses of the sensing system challenged with a specific metal ion. The red bars show the fluorescence responses of the sensing system toward the presence of 10 μM Cu^{2+} and 50 μM other metal ions. All the samples contained 83.3 nM MBs and 3.33 μg/mL GO, and reacted at 24 °C for 1.5 h after the addition of different metal ions and 2.1 mM H_2O_2.

Table 1
Recovery experiments of Cu^{2+} in drinking water samples.

Sample	C1 (nM)	C2 (nM)	C3 (nM)	Recovery (%)
1	203.6	106.7	307.0 ± 10.4	99.6
2	203.6	266.7	478.6 ± 27.7	103.1
3	203.6	533.3	740.3 ± 24.3	100.6
4	203.6	2666.7	2746.5 ± 312.5	95.4

C1: concentration measured in unfortified samples; C2: concentration of fortifications; C3: concentration measured in fortified samples.
Rate of recovery $=(C3-C1)/C2 \times 100\%$

summarized in Table 1 show that the sensing method could accurately measure the Cu^{2+} concentrations.

4. Conclusions

In summary, an MB and GO-based fluorescent sensing approach has been developed for direct detection of Cu^{2+} in solutions. In the sensing system, MBs were used as the molecular recognition unit, which produced signals after being cleaved and detached from the GO surfaces in the presence of Cu^{2+}. GO, a remarkable fluorescence quencher, was employed to reduce the background signal and enhance the sensitivity of the detection. It also acted as a signal controller through the release of short ssDNA fragments (cleaved MBs) from its surface, achieving fluorescence generation. The detection limit of the sensing method was obtained as 53.3 nM. Experiments have also shown that the detection strategy is highly selective and applicable for the detection of Cu^{2+} in real samples.

Acknowledgments

This work was supported by the Research Grants Council of the Hong Kong Special Administrative Region under Grant No. 615710.

Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.12.056.

References

Abollino, O., Aceto, M., Bruzzoniti, M.C., Mentasti, E., Sarzanini, C., 1998. Analytica Chimica Acta 375, 299–306.
Aboutalebi, S.H., Gudarzi, M.M., Zheng, Q.B., Kim, J.K., 2011. Advanced Functional Materials 21, 2978–2988.
Barnham, K.J., Bush, A.I., 2008. Current Opinion in Chemical Biology 12, 222–228.
Biggins, J.B., Prudent, J.R., Marshall, D.J., Ruppen, M., Thorson, J.S., 2000. Proceedings of the National Academy of Sciences 97, 13537–13542.
Bonham, M., O'Connor, J.M., Hannigan, B.M., Strain, J.J., 2002. British Journal of Nutrition 87, 393–403.
Brown, D.R., Kozlowski, H., 2004. Dalton Transactions, 1907–1917.
Chen, J.R., Teo, K.C., 2001. Analytica Chimica Acta 450, 215–222.
Chen, W.B., Tu, X.J., Guo, X.Q., 2009. Chemical Communications, 1736–1738.
de Silva, A.P., Gunaratne, H.Q.N., Gunnlaugsson, T., Huxley, A.J.M., McCoy, C.P., Rademacher, J.T., Rice, T.E., 1997. Chemical Reviews 97, 1515–1566.
El-Sagheer, A.H., Brown, T., 2010. Chemical Society Reviews 39, 1388–1405.
Hahn, S.H., Tanner, M.S., Danke, D.M., Gahl, W.A., 1995. Biochemical and Molecular Medicine 54, 142–145.
He, S.J., Song, B., Li, D., Zhu, C.F., Qi, W.P., Wen, Y.Q., Wang, L.H., Song, S.P., Fang, H.P., Fan, C.H., 2010. Advanced Functional Materials 20, 453–459.
He, X.R., Liu, H.B., Li, Y.L., Wang, S., Li, Y.J., Wang, N., Xiao, J.C., Xu, X.H., Zhu, D.B., 2005. Advanced Materials 17, 2811–2815.
Hua, C., Zhang, W.H., De Almeida, S.R.M., Ciampi, S., Gloria, D., Liu, G.Z., Harper, J.B., Gooding, J.J., 2012. Analyst 137, 82–86.
Huang, J.H., Su, S.F., Li, Z.G., 2012. Analytical Chemistry 84, 5939–5943.
Lan, G.Y., Huang, C.C., Chang, H.T., 2010. Chemical Communications 46, 1257–1259.
Lin, T.W., Huang, S.D., 2001. Analytical Chemistry 73, 4319–4325.
Liu, J.W., Lu, Y., 2007a. Journal of the American Chemical Society 129, 9838–9839.
Liu, J.W., Lu, Y., 2007b. Chemical Communications, 4872–4874.
Lu, C.H., Li, J.A., Lin, M.H., Wang, Y.W., Yang, H.H., Chen, X., Chen, G.N., 2010. Angewandte Chemie International Edition 49, 8454–8457.
Lu, C.H., Yang, H.H., Zhu, C.L., Chen, X., Chen, G.N., 2009. Angewandte Chemie International Edition 48, 4785–4787.
Moses, J.E., Moorhouse, A.D., 2007. Chemical Society Reviews 36, 1249–1262.
Sando, S., Sasaki, T., Kanatani, K., Aoyama, Y., 2003. Journal of American Chemical Society125, 15720–15721.
Shao, N., Zhang, Y., Cheung, S.M., Yang, R.H., Chan, W.H., Mo, T., Li, K.A., Liu, F., 2005. Analytical Chemistry 77, 7294–7303.
Shen, Q.P., Tang, S.Y., Li, W.H., Nie, Z., Liu, Z.L., Huang, Y., Yao, S.Z., 2012. Chemical Communications 48, 281–283.
Su, Y.T., Lan, G.Y., Chen, W.Y., Chang, H.T., 2010. Analytical Chemistry 82, 8566–8572.
Torrado, A., Walkup, G.K., Imperiali, B., 1998. Journal of American Chemical Society 120, 609–610.
Tyagi, S., Kramer, F.R., 1996. Nature Biotechnology 14, 303–308.
Vaisanen, A., Suontamo, R., Silvonen, J., Rintala, J., 2002. Analytical and Bioanalytical Chemistry 373, 93–97.
Wang, F.A., Elbaz, J., Orbach, R., Magen, N., Willner, I., 2011. Journal of American Chemical Society 133, 17149–17151.
Wang, F.A., Elbaz, J., Willner, I., 2012. Journal of American Chemical Society 134, 5504–5507.
Wang, K.M., Tang, Z.W., Yang, C.Y.J., Kim, Y., Fang, X.H., Li, W., Wu, Y.R., Medley, C.D., Cao, Z.H., Li, J., Colon, P., Lin, H., Tan, W.H., 2009. Angewandte Chemie International Edition 48, 856–870.
Wang, Y., Yang, F., Yang, X.R., 2010. Nanotechnology 21, 205502.
Wu, C.S., Oo, M.K.K., Fan, X.D., 2010. ACS Nano 4, 5897–5904.
Xu, X.Y., Daniel, W.L., Wei, W., Mirkin, C.A., 2010. Small 6, 623–626.
Yin, B.C., Ye, B.C., Tan, W.H., Wang, H., Xie, C.C., 2009. Journal of American Chemical Society 131, 14624–14625.
Zheng, Q.B., Ip, W.H., Lin, X.Y., Yousefi, N., Yeung, K.K., Li, Z.G., Kim, J.K., 2011. ACS Nano 5, 6039–6051.
Zheng, Y.J., Huo, Q., Kele, P., Andreopoulos, F.M., Pham, S.M., Leblanc, R.M., 2001. Organic Letters 3, 3277–3280.
Zhou, Y., Wang, S.X., Zhang, K., Jiang, X.Y., 2008. Angewandte Chemie International Edition 47, 7454–7456.
Zietz, B.P., de Vergara, J.D., Dunkelberg, H., 2003. Environmental Research 92, 129–138.