RSC Advances

This article can be cited before page numbers have been issued, to do this please use: H. Wang, H. Zhang, Y. Chen, Y. Li and T. Gan, *RSC Adv.*, 2015, DOI: 10.1039/C5RA14852A.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

View Article Online
DOI: 10.1039/C5RA14852A

H_2O_2-mediated fluorescence quenching of double-stranded DNA templated copper nanoparticles for label-free and sensitive detection of glucose

Hai-Bo Wang*, Hong-Ding Zhang, Ying Chen, Yang Li, Tian Gan

College of Chemistry and Chemical Engineering, Xinyang Normal University, Xinyang 464000, PR China

* Corresponding author. Tel.: +86 376 6391172; Fax: +86 376 6391172.
E-mail address: wanghaibohn@163.com (H.-B. Wang).

Abstract

Blood glucose monitoring has attracted extensive attention because diabetes mellitus is a worldwide public health problem. Here, a novel and label-free fluorescent sensing strategy was reported for simple and sensitive detection of glucose in human serum on the basis of H_2O_2-mediated fluorescence quenching of double-stranded DNA (ds-DNA) templated copper nanoparticles (Cu NPs). In this strategy, the fluorescence intensity of ds-DNA templated Cu NPs was found to be quenched effectively in the presence of H_2O_2. Similarly, glucose could be monitored based on the enzymatic conversion of glucose by glucose oxidase to generate H_2O_2. Under the optimized conditions, the strategy exhibited sensitive and selective detection of glucose in a linear range from 50 nM to 100 μM and with a detection limit of 12 nM. In addition, the method was successfully applied in the detection of glucose in human serum samples with satisfactory results. Furthermore, the strategy was free of any fluorescence dye label, complex DNA sequence design, and sophisticated experimental techniques. Therefore, the proposed approach could hold great potential for diabetes mellitus research and clinical diagnosis.

Keywords: Glucose detection; H_2O_2; Label-free fluorescent sensor; Copper nanoparticles

View Article Online
DOI: 10.1039/C5RA14852A

1. Introduction

As a major component of animal and plant carbohydrates, glucose plays a key role in living systems. It acts not only as an important energy source of the living cells, but also as a metabolic intermediate in the synthesis of other complex molecules. The blood glucose levels are also an important indicator of human health conditions.[1] The deficiency of glucose may result in hypoglycemia. On the other hand, a high level of glucose is connected to many other diseases such as diabetes, hypertension and cardiovascular diseases. About 300 million people suffered from diabetes in the year 2010; and this number is estimated to almost double in 2030 by the statistics of the World Health Organization. Thus, it is of great importance to be able to accurately monitor the blood glucose levels in clinical diagnosis of diabetes. Several conventional methods including spectrophotometry, fluorometry, chemiluminescence and electrochemistry have been developed for the monitoring blood glucose levels.[2-7] Although these methods were quite powerful, they usually suffered from some disadvantage. The spectrophotometry and electrochemistry-based methods were limited by the interference of blood color and contamination of the electrode by the proteins in blood. Among these methods, fluorescent method was used widely due to its operational simplicity and high sensitivity.[3] Quantum dots (QDs) become one of the most popular fluorescence materials in such application.[8-10] However, most of previous strategies usually suffered from complicated modification or harsh detection environment. Furthermore, the cytotoxic effect of the probes could not be neglected.[11, 12] Thus, it is still highly desirable to develop simple, low-cost and sensitive approaches for the determination of glucose.

In contrast to the conventional organic dyes and quantum dots, fluorescent metal nanoclusters or nanoparticles have attracted significant attention in the field of bioanalysis due to their unique electrical, optical properties and low toxicity.[13-17] Among the various reported metal nanoclusters or nanoparticles, DNA or oligonucleotide templated fluorescent copper nanoparticles as a type of newly

emerged functional biochemical probe, have possessed great potential as fluorescent probes for biochemical applications because of their advantages of good biocompatibility, low-cost, ease of preparation, low-toxicity and excellent fluorescence property.[18-22]

Recently, Mokhir et al. reported that ds-DNA could act as an efficient template for the formation of Cu NPs through the reduction of Cu^{2+} by ascorbic acid and the formed Cu NPs exhibited excellent fluorescence, whereas ss-DNA template did not support the formation of Cu NPs.[23] The formation of ds-DNA templated Cu NPs contained two steps. The first step in the reaction was the reduction of Cu (II) to Cu (I) followed by the disproportionation of Cu (I) into Cu (II) and Cu (0). The second step was that the formed Cu (0) was clustered on ds-DNA scaffolds. Furthermore, ds-DNA templated Cu NPs could be facilely prepared within 5 minutes at room temperature. Thus, due to its simplicity, high efficiency, rapidity, and hypotoxicity, the ds-DNA templated Cu NPs have been used as fluorescence probes in some biological assays.[18-20, 24, 25] Chen et al. found that Pb^{2+} could quench the fluorescence of ds-DNA templated Cu NPs.[24] Based on the phenomenon, they have used ds-DNA templated Cu NPs as a novel fluorescence probe for the detection of Pb^{2+} through the $5d^{10}(Pb^{2+})$–$3d^{10}(Cu^{+})$ metallophilic interactions to induce fluorescence quenching.[24] Hu et al. have utilized ds-DNA templated Cu NPs as novel fluorescence probe for label-free detection of biothiols based on the quenching of their fluorescence.[18] The quenching effect was ascribed to the coordination complex formed by the Cu-S metal-ligand bond between the Cu NPs and the biothiols. Our group found that dopamine could also effectively quench the fluorescence of Cu NPs by the formation of photo-induced electron transfer process between dopamine and Cu NPs.[25] However, exploration of fluorescence quenching of ds-DNA templated Cu NPs is still at a very early stage.

Interestingly, we found that the fluorescence intensity of ds-DNA templated Cu NPs could be quenched effectively by H_2O_2 in this study. Moreover, glucose could be oxidized by dissolved oxygen (O_2) in the presence of glucose oxidase (GOD) to produce glucose acid and H_2O_2. Then, the concentration of glucose could be obtained

by detecting the amount of the enzymatically generated H_2O_2, which quenched the fluorescence of Cu NPs. The principle of our proposed fluorescent sensor for hydrogen peroxide (H_2O_2) and glucose detection was schematically represented in Scheme 1. The ds-DNA templated Cu NPs were used as fluorescent indicator. In the absence of hydrogen peroxide, the formed ds-DNA templated Cu NPs exhibited excellent fluorescence intensity. However, it was found that the fluorescence intensity of the ds-DNA templated Cu NPs could be quenched effectively by the presence of H_2O_2 (shown in Scheme 1A). The fluorescence quenching mechanism was discussed in ESI†. Based on the intensive quenching effects, H_2O_2 could be successfully detected through the fluorescence change of ds-DNA templated Cu NPs. In addition, by taking advantage of H_2O_2 as a mediator, this strategy was further exploited for constructing oxidase-based biosensors for glucose detection. As shown in Scheme 1B, glucose was oxidized by dissolved oxygen (O_2) in the presence of glucose oxidase to produce glucose acid and H_2O_2. Consequently, the glucose concentration could be determined indirectly by the amount of enzymatically generated H_2O_2 according to the fluorescence quenching. Hence, a novel and cost-effective fluorescent sensor was constructed for sensitive detection of glucose based on the H_2O_2-mediated fluorescence quenching of ds-DNA templated Cu NPs.

(Scheme 1)

2. Experimental

2.1. Reagents

All oligonucleotides in this work were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China) and used without further purification. The sequences of these oligonucleotides were shown as follows: P1 5'-CAT AGC GGC AGG ATC AGT TAC AGT G-3'; P2: 5'-CAC TGT AAC TGA TCC TGC CGC TAT G-3'. Glucose oxidase, $CuSO_4$ $5H_2O$, H_2O_2 (30%) and ascorbic acid were purchased from Sigma-Aldrich

(USA). All other chemicals were of analytical grade and without further purification. All the water used in this work was obtained from a Millipore Milli-Q water purification system (with an electrical resistance of >18.2 MΩ).

2.2. Apparatus

The fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrometer (Hitachi Co. Ltd., Japan) equipped with a Xenon lamp excitation source. A quartz fluorescence cell with an optical path length of 10 mm was used. The excitation wavelength was set at 340 nm, and the fluorescence emission spectra of Cu NPs were collected from 500 nm to 640 nm at room temperature with both the excitation and emission slit set at 5.0 nm. All fluorescence measurements were carried out at room temperature unless stated otherwise.

2.3. Synthesis of ds-DNA templated Cu NPs

The ds-DNA templated Cu NPs were synthesized according to the literature with a slight modification.[18-20, 25] Briefly, a mixture solution containing 1 μM P1 and 1 μM P2 ss-DNA in MOPS buffer (20 mM MOPS, 300 mM NaCl, pH 7.0) was firstly prepared. Then the mixture was denatured at 95 ^{o}C for 10 min, and subsequently cooled down slowly to room temperature to ensure that P1 and P2 DNA were completely hybridized to form ds-DNA. After that, 10 μL 2.5 mM $CuSO_4$ solutions and 10 μL 30 mM ascorbic acid solutions were added into the mixture solution and kept for 5 minutes at room temperature to form ds-DNA templated Cu NPs. Finally, the fluorescent spectrum of the mixture was recorded by F-7000 spectrophotometer (Hitachi Co. Ltd., Japan) immediately. The morphology of ds-DNA templated Cu NPs was characterized by transmission electron microscope (TEM) and shown in Fig. 1. And the size of ds-DNA templated Cu NPs was about 3-5 nm.

(Fig. 1)

View Article Online
DOI: 10.1039/C5RA14852A

2.4. Fluorescence quenching effect by H_2O_2

In a typical measurement, different concentrations of H_2O_2 were freshly prepared before use. Then, 10 μL different concentrations of H_2O_2 were added into 90 μL as-prepared ds-DNA Cu NPs, and the mixture was incubated at room temperature for 10 min in the dark. After that, the fluorescence intensity of the mixture was immediately measured by F-7000 spectrophotometer with the excitation wavelength of 340 nm.

2.5. Label-free detection of glucose

In a typical assay of glucose, a 10 μL mixture solution (20 mM MOPS, 300 mM NaCl, pH 7.0) containing 0.05 mg/mL glucose oxidase and different concentrations of glucose were incubated at 37 ^{o}C for 30 min. After that, the above mixture was added into 90 μL prepared Cu NPs and incubated at room temperature for 10 min in the dark. At last, the fluorescence intensities of the reaction solution were measured by F-7000 spectrophotometer with the excitation wavelength of 340 nm.

3. Results and discussion

3.1. Evaluation the quenching effect of H_2O_2

In order to evaluate the feasibility of the strategy, the fluorescence intensity of obtained Cu NPs were tested in the absence and presence of H_2O_2. As shown in Fig. 2, it was observed that ds-DNA templated Cu NPs exhibited excellent fluorescence at 565 nm in the absence of H_2O_2 (curve a in Fig. 2). However, the fluorescence intensity decreased significantly (curve b in Fig. 2) after the addition of H_2O_2. These results indicated that H_2O_2 could strongly quench the fluorescence intensity of ds-DNA templated Cu NPs. Based on the quenching effect, a simple and label-free

fluorescent assay could be developed for H_2O_2 detection by using the ds-DNA templated Cu NPs.

(Fig. 2)

It has been reported that the fluorescence intensity of ds-DNA templated Cu NPs was obviously pH dependent and relatively low in acidic solutions. Thus, the effect of pH value on the fluorescence response was studied. The efficiency of fluorescence quenching was calculated by F_0/F, where F_0 and F were the fluorescence intensity of ds-DNA templated Cu NPs in the absence and presence of 10 μM H_2O_2, respectively. As shown in Fig. 3A, the fluorescent quenching efficiency increased gradually in the pH value range from 4.0 to 7.0 and then decreased when the pH value was higher than 7.0. A remarkable response was obtained at pH 7.0. Therefore, pH 7.0 was suitable for this sensing system.

Furthermore, the incubated time of H_2O_2 was another important parameter influencing the fluorescent intensity. So, the effect of incubated time of H_2O_2 was also investigated to improve the sensitivity of this strategy. As shown in Fig. 3B, the fluorescence intensity decreased obviously in the presence of 10 μM H_2O_2. And there was no obvious change in the fluorescence intensity of ds-DNA templated Cu NPs after 10 min. Thus, the incubated time of H_2O_2 was set at 10 min.

(Fig. 3)

To further demonstrate the fluorescence quenching ability of H_2O_2, the fluorescence intensity of ds-DNA templated Cu NPs at different concentrations of H_2O_2 was investigated under the optimized experimental condition. The fluorescence spectra of the ds-DNA templated Cu NPs in the presence of variable concentrations of H_2O_2 were shown in Fig. 4A. It was found that the fluorescence intensity of ds-DNA templated Cu NPs decreased with the H_2O_2 concentration increasing from 10 nM to 50 μM. Fig. 4B depicted the relationship between the H_2O_2 concentration and the fluorescence intensity at the maximum emission wavelength. As shown in inset of Fig. 4B, a good linear relationship was obtained in the concentration range from 10 nM to

2 μM (R = 0.9957). The detection limit (in terms of the 3σ rule) was calculated to be 3 nM. The detection limit was comparable to or better than those of previously reported fluorescence methods.[26-28]

(Fig. 4)

3.2. Fluorescent detection of glucose

The fluorescence quenching of Cu NPs by H_2O_2 enabled the implementation of the ds-DNA templated Cu NPs as versatile fluorescence indicators for sensitive detection of the activity of O_2-dependent oxidases and their substrates. For example, glucose could be oxidized by oxygen (O_2) in the presence of glucose oxidase (GOx) to generate H_2O_2. Thus, it is also possible for the detection of glucose by H_2O_2-mediated fluorescence quenching of ds-DNA templated Cu NPs.

Since glucose and glucose oxidase were all essential to produce H_2O_2, some parameters (such as concentration of glucose oxidase and incubated time of glucose oxidase) were optimized to achieve the sensitive detection of glucose. Firstly, the effect of glucose on the fluorescence intensity was investigated. It was found that no significant change of the fluorescence intensity were observed when Cu NPs only mixed with glucose, indicating that glucose had little effect on the fluorescence intensity of Cu NPs. Secondly, the effect of the concentration of glucose oxidase on the fluorescence quenching was also studied. The efficiency of fluorescence quenching was calculated by F_0/F, where F_0 and F were the fluorescence intensity of ds-DNA templated Cu NPs in the absence and presence of glucose, respectively. As shown in Fig. 5A, the efficiency of fluorescence quenching reached a maximum value when the glucose oxidase concentration was 0.05 mg/mL. Thus, 0.05 mg/mL glucose oxidase was selected as the optimized concentration. Additionally, the incubated time of glucose oxidase was another important parameter influencing the fluorescent intensity. So, the effect of incubated time of glucose oxidase was investigated to improve the sensitivity of this strategy. It could be seen from Fig. 5B that the fluorescence intensity increased rapidly and then approached a plateau after 30 min.

Therefore, 30 min of the glucose oxidase incubated time was used throughout the experiments.

(Fig. 5)

Fig. 6 depicted the typical fluorescent assay of glucose on the basis of H_2O_2-mediated fluorescence quenching of ds-DNA templated Cu NPs. It could be seen from Fig. 6A, the fluorescent intensity decreased gradually with increasing concentrations of glucose range from 50 nM to 500 μM, which suggested that the higher the glucose concentration being added, the more H_2O_2 was generated. Fig. 6B illustrated the relationship between the glucose concentration and the fluorescence intensity. According to fluorescence quenching model of Stern-Volmer,[29, 30] the F_0/F exhibited a good linear relationship with the logarithmic glucose concentrations in the range from 50 nM to 100 μM (shown in the inset of Fig. 6B). Where, F_0 and F were the fluorescence intensity of sensing system in the absence and presence of glucose, respectively. The detection limit was calculated to be 12 nM based on three times the standard deviation rule (3σ), which was comparable to or better than that of most previously reported methods.[31-35] Thus, these results demonstrated that the proposed method could be applied to sensitively determinate glucose.

(Fig. 6)

3.3. Selectivity of glucose

In order to demonstrate the selectivity of the present strategy toward glucose, other possible interfering substances were investigated, such as various saccharides, amino acids, ascorbic acid (AA), and uric acid (UA). As shown in Fig. 7, except for glutathione (GSH) and cysteine (Cys), these substances did not result in obvious interference in glucose detection. This was because the thiol group of GSH and Cys could also quench the fluorescence intensity of Cu NPs.[18, 36] Thus, a masking agent (N-ethylmaleimide, NEM) was introduced into the sensor system to eliminate interference from GSH and Cys.[34] After incubation of GSH or Cys with NEM, a

negligible fluorescence response was observed, whereas glucose detection was unaffected by the introduction of NEM. The results suggested that the proposed assay exhibited high selectivity and could be used for determination of glucose in biological samples.

(Fig. 7)

3.4. Detection of glucose in human serum samples

To verify the feasibility of our new approach for detection of glucose in biological samples, we applied it to analyze glucose in healthy human blood serum samples provided by Xinyang Central Hospital (Xinyang, China). Taking into consideration the normal glucose level in healthy human blood as well as the linear range of our method, the blood serum samples were diluted 20 times. Then, 0.3 mM NEM was added into the samples to eliminate the interference from GSH and Cys in real samples. The results were presented in Table 1. The glucose concentrations of the serum samples were coincided with those provided by local hospital. In order to determine the accuracy and precision of the method, appropriate amounts of glucose standards were added to the human serum sample (shown in Table S1 of ESI†). The results revealed that proposed method was feasible for practical blood glucose monitoring in real samples.

(Table 1)

4. Conclusions

In conclusion, we have developed a label-free and sensitive fluorescent biosensor for glucose detection on the basis of H_2O_2-mediated fluorescence quenching of ds-DNA templated Cu NPs. Due to the excellent quenching ability of H_2O_2, the label-free sensor exhibited sensitive and selective detection of glucose with a detection limit of 12 nM. The method was also applied to monitor glucose levels in

human serum with satisfactory results, suggesting that our approach had great potential for diabetes mellitus research and clinical diagnosis. The strategy was convenient and without complicated preparation procedure. Furthermore, the proposed strategy provided an alternative platform to detect other substrates through oxidation by its O_2-dependent oxidase which could generate H_2O_2. Thus, it could offer a new approach to developing low-cost and sensitive methods for biological and clinical diagnostics applications.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (No. 21305119), Foundation of Henan Educational Committee (No. 13A150768).

View Article Online
DOI: 10.1039/C5RA14852A

References

1 L. H. Jin, L. Shang, S. J. Guo, Y. X. Fang, D. Wen, L. Wang, J. Y. Yin and S. J. Dong, *Biosens. Bioelectron.*, 2011, **26**, 1965-1969.

2 W. Shi, Q. Wang, Y. Long, Z. Cheng, S. Chen, H. Zheng and Y. Huang, *Chem. Commun.*, 2011, **47**, 6695-6697.

3 P. Shen and Y. Xia, *Anal. Chem.*, 2014, **86**, 5323-5329.

4 D. T. Bostick and D. M. Hercules, *Anal. Chem.*, 1975, **47**, 447-452.

5 D. Lan, B. Li and Z. Zhang, *Biosens. Bioelectron.*, 2008, **24**, 934-938.

6 J. Song, L. Xu, C. Zhou, R. Xing, Q. Dai, D. Liu and H. Song, *ACS Appl. Mater. Interfaces*, 2013, **5**, 12928-12934.

7 L. Lin, J. Yan and J. Li, *Anal. Chem.*, 2014, **86**, 10546-10551.

8 D. B. Cordes, S. Gamsey and B. Singaram, *Angew. Chem. Int. Ed.*, 2006, **45**, 3829-3832.

9 B. Tang, L. H. Cao, K. H. Xu, L. H. Zhuo, J. C. Ge, Q. L. Li and L. J. Yu, *Chem. Eur. J.*, 2008, **14**, 3637-3644.

10 R. Freeman, L. Bahshi, T. Finder, R. Gill and I. Willner, *Chem. Commun.*, 2009, **45**, 764-766.

11 A. M. Derfus, W. C. W. Chan and S. N. Bhatia, *Nano Lett.*, 2004, **4**, 11-18.

12 A. Katstumiti, D. Gilliland, I. Arostegui and M. P. Cajaraville, *Aquatic Toxicology*, 2014, **153**, 29-52.

13 X. Jia, J. Li, L. Han, J. Ren, X. Yang and E. K. Wang, *ACS Nano*, 2012, **6**, 3311-3317.

14 M. Z. Fahmi, K. L. Ou, J. K. Chen, M. H. Ho, S. H. Tzing and J. Y. Chang, *RSC Adv.*, 2014. **4**, 32762-32772.

15 M. L. Hong, L. J. Li, H. X. Han and X. Chu, *Anal. Sci.*, 2014, **30**, 811-815.

16 X. Tian, X. J. Kong, Z. M. Zhu, T. T. Chen and X. Chu, *Talanta*, 2015, **131**, 116-120.

17 L. J. Ou, X. Y. Li, L. J. Li, H. W. Liu, A. M. Sun and K. J. Liu, *Analyst*, 2015,

140, 1871-1875.

18 Y. H. Hu, Y. M. Wu, T. T. Chen, X. Chu and R. Q. Yu, *Anal. Methods*, 2013, **5**, 3577-3581.

19 L. L. Zhang, J. J. Zhao, H. Zhang, J. H. Jiang and R. Q. Yu, *Biosens. Bioelectron.*, 2013, **44**, 6-9.

20 L. L. Zhang, J. J. Zhao, M. Duan, H. Zhang, J. H. Jiang and R. Q. Yu, *Anal. Chem.*, 2013, **85**, 3797-3801.

21 H. B. Wang, H. D. Zhang, Y. Chen and Y. M. Liu, *Biosens. Bioelectron.,* 2015, **74**, 581-586.

22 H. Zhang, Z. H. Lin and X. G. Su, *Talanta*, 2015, **131**, 59-63.

23 A. Rotaru, S. Dutta, E. Jentzsch, K. Gothelf and A. Mokhir, *Angew. Chem. Inter. Ed.*, 2010, **49**, 5665-5667.

24 J. H. Chen, J. Liu, Z. Y. Fang and L. W. Zeng, *Chem. Commun.*, 2012, **48**, 1057-1059.

25 H. B. Wang, H. D. Zhang, Y. Chen, K. J. Huang and Y. M. Liu, *Sensor Actuat. B-Chem.*, 2015, **220**, 146-153.

26 Y. C. Shiang, C. C. Huang and H. T. Chang, *Chem. Commun.*, 2009, **23**, 3437-3439.

27 T. Wen, F. Qu, N. B. Li and H. Q. Luo, *Anal. Chim. Acta*, 2012, **749**, 56-62.

28 M. Duan, Y. L. Peng, L. L. Zhang, X. Y. Wang, J. Ge, J. H. Jiang and R. Q. Yu, *Anal. Methods*, 2013, **5**, 2182-2187.

29 S. H. Pawlis, G. Berth, V. Wiedemeier, L. Schmidt, A. Zrenner and H. J. Warneck, *J. Lumines.*, 2010, **130**, 1958-1962.

30 N. Shehata, K. Meehan, I. Ashry, I. Kandas and Y. Xu, *Sensor Actuat. B-Chem.*, 2013, **183**, 179-186.

31 Y. Ling, N. Zhang, F. Qu, T. Wen, Z. F. Gao, N. B. Li and H. Q. Luo, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2014, **118**, 315-320.

32 D. H. He, C. B. Zheng, Q. Wang, C. L. He, Y. I. Lee, L. Wu and X. D. Hou, *Talanta*, 2015, **142**, 51-56.

33 J. L. Liu, L. L. Lu, A. Q. Li, J. Tang, S. G. Wang, S. Y. Xu and L. Y. Wang,

View Article Online
DOI: 10.1039/C5RA14852A

Biosens. Bioelectron., 2015, **68**, 204-209.

34 J. Yuan, Y. Cen, X. J. Kong, S. Wu, C. L. W. Liu, R. Q. Yu and X. Chu, *ACS Appl. Mater. Interfaces*, 2015, **7**, 10548-10555.

35 F. Molaabasi, S. Hosseinkhani, A. A. Moosavi-Movahedic and M. Shamsipur, *RSC Adv.*, 2015. **5**, 33123-33135.

36 H. B. Wang, H. D. Zhang, Y. Chen, L. J. Ou and Y. M. Liu, *Anal. Methods*, 2015, **7**, 6372-6377.

View Article Online
DOI: 10.1039/C5RA14852A

Figure captions

Scheme 1 (A) Schematic illustration of fluorescence quenching of ds-DNA templated Cu NPs by H_2O_2. (B) Schematic illustration of fluorescent strategy for glucose detection based on H_2O_2-mediated fluorescence quenching of ds-DNA templated Cu NPs.

Fig. 1 TEM image of ds-DNA templated Cu NPs.

Fig. 2 Fluorescence spectra of ds-DNA templated Cu NPs in the absence (curve a) and presence of 10 μM H_2O_2 (curve b), respectively.

Fig. 3 (A) The effect of pH values on the fluorescence responses of the sensing system. Where F_0 and F were the fluorescence intensity of ds-DNA templated Cu NPs in the absence and presence of 10 μM H_2O_2, respectively. (B) The effect of H_2O_2 incubation time on the fluorescence intensity.

Fig. 4 (A) Fluorescent spectra of ds-DNA templated Cu NPs in the presence of different concentrations of H_2O_2, the curves from top to bottom, the concentration of H_2O_2 were 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 μM, respectively. (B) The relationship between the fluorescence intensity and H_2O_2 concentration. Inset of (B) is the calibration curve. The error bars represent the standard deviation of three measurements.

Fig. 5 (A) The effect of glucose oxidase on the fluorescence response. Where, F_0 and F were the fluorescence intensity of sensing system in the absence and presence of glucose, respectively. (B) The effect of glucose oxidase incubation time on the fluorescence intensity.

Fig. 6 (A) Fluorescent spectra of sensing system in the presence of different

concentrations of glucose. (B) The relationship between the fluorescence intensity and glucose concentration. Inset of (B) is the calibration curve. The error bars represent the standard deviation of three measurements.

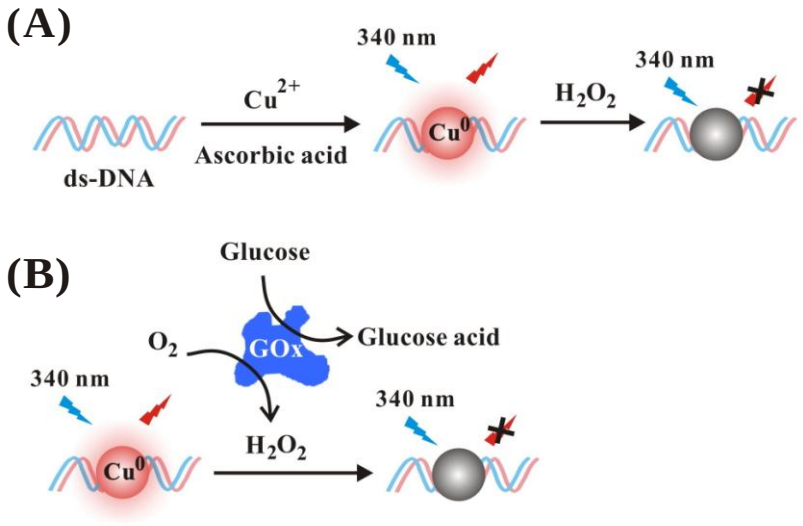
Fig. 7 The selectivity of H_2O_2-mediated fluorescence quenching for glucose assay. Where, F_0 and F are the fluorescence intensity of sensing system in the absence and presence of glucose and other analytes, respectively. The error bars represent the standard deviation of three measurements.

View Article Online
DOI: 10.1039/C5RA14852A

Table captions

Table 1 Determination of glucose levels in the human serum samples (n = 3).

View Article Online
DOI: 10.1039/C5RA14852A



Scheme 1

View Article Online
DOI: 10.1039/C5RA14852A

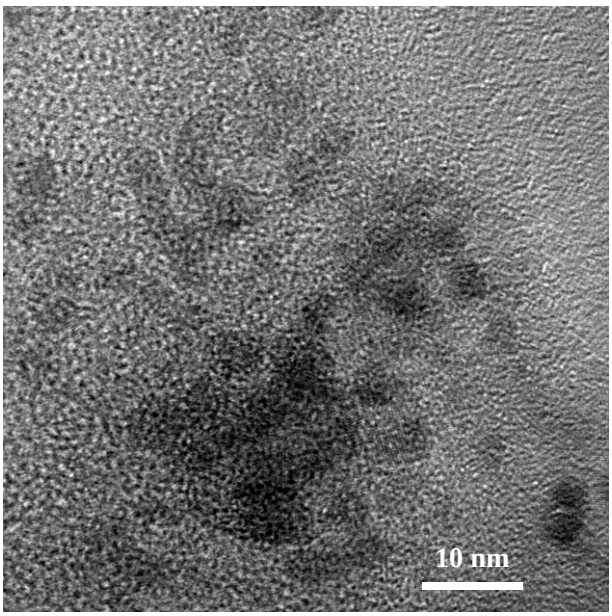


Fig. 1

View Article Online
DOI: 10.1039/C5RA14852A

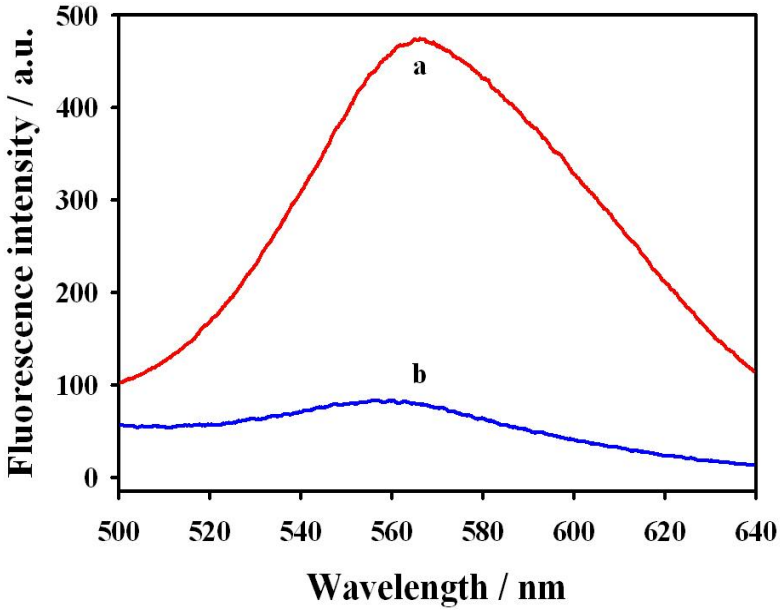


Fig. 2

View Article Online
DOI: 10.1039/C5RA14852A

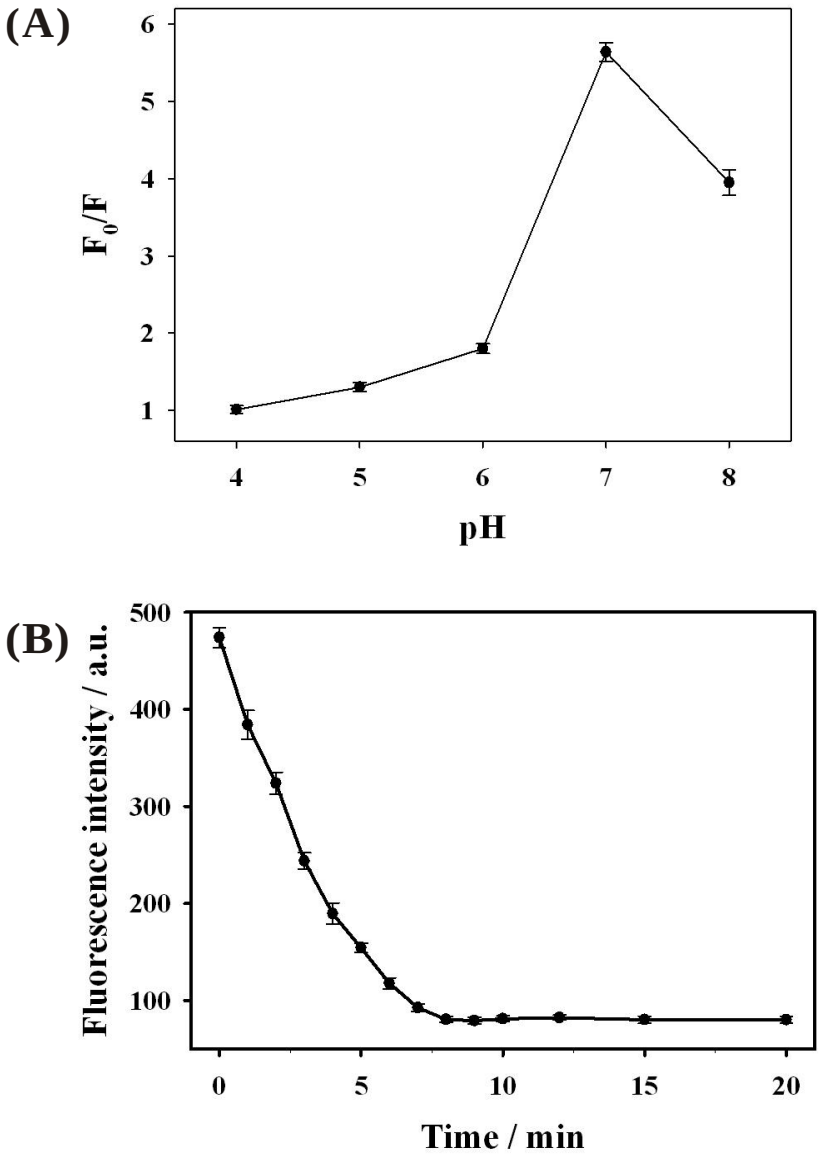


Fig. 3

View Article Online
DOI: 10.1039/C5RA14852A

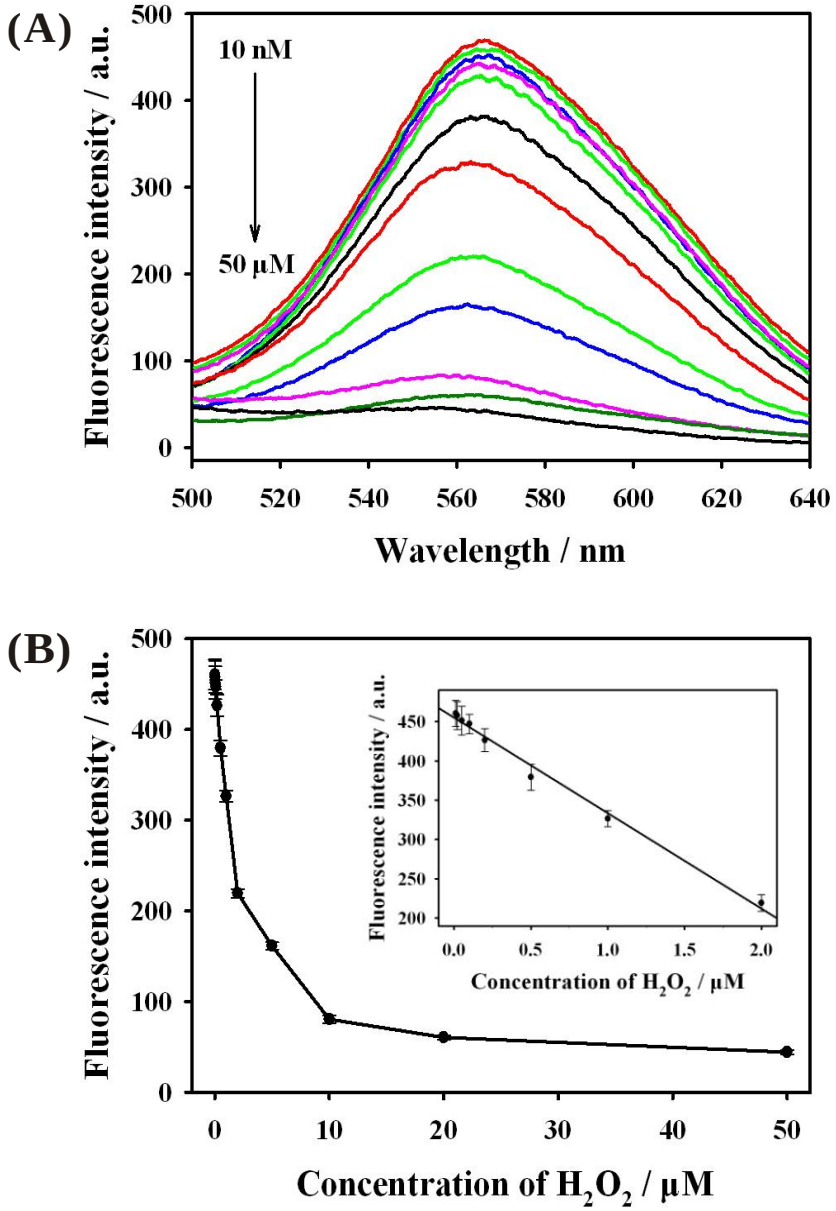
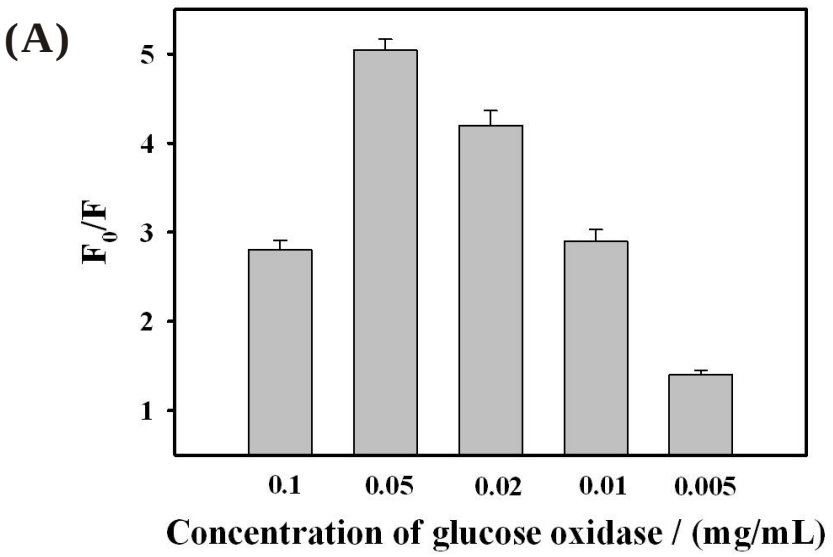


Fig. 4

View Article Online
DOI: 10.1039/C5RA14852A



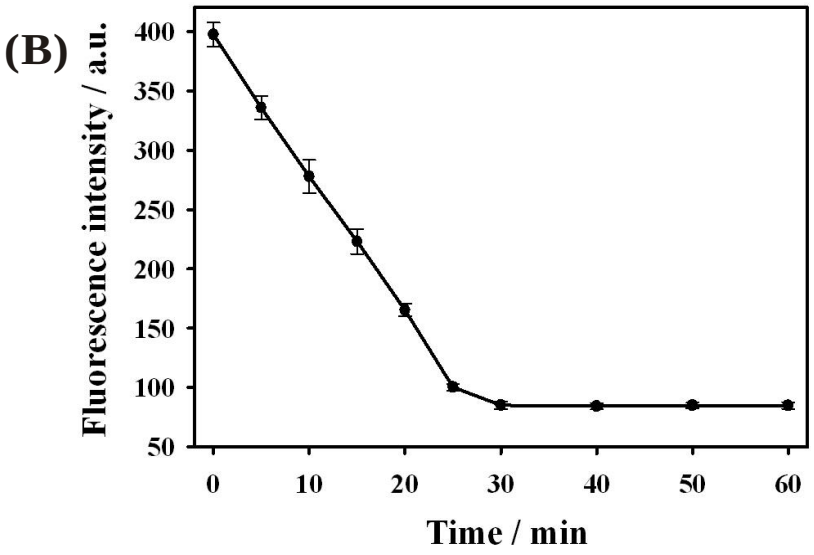


Fig. 5

View Article Online
DOI: 10.1039/C5RA14852A

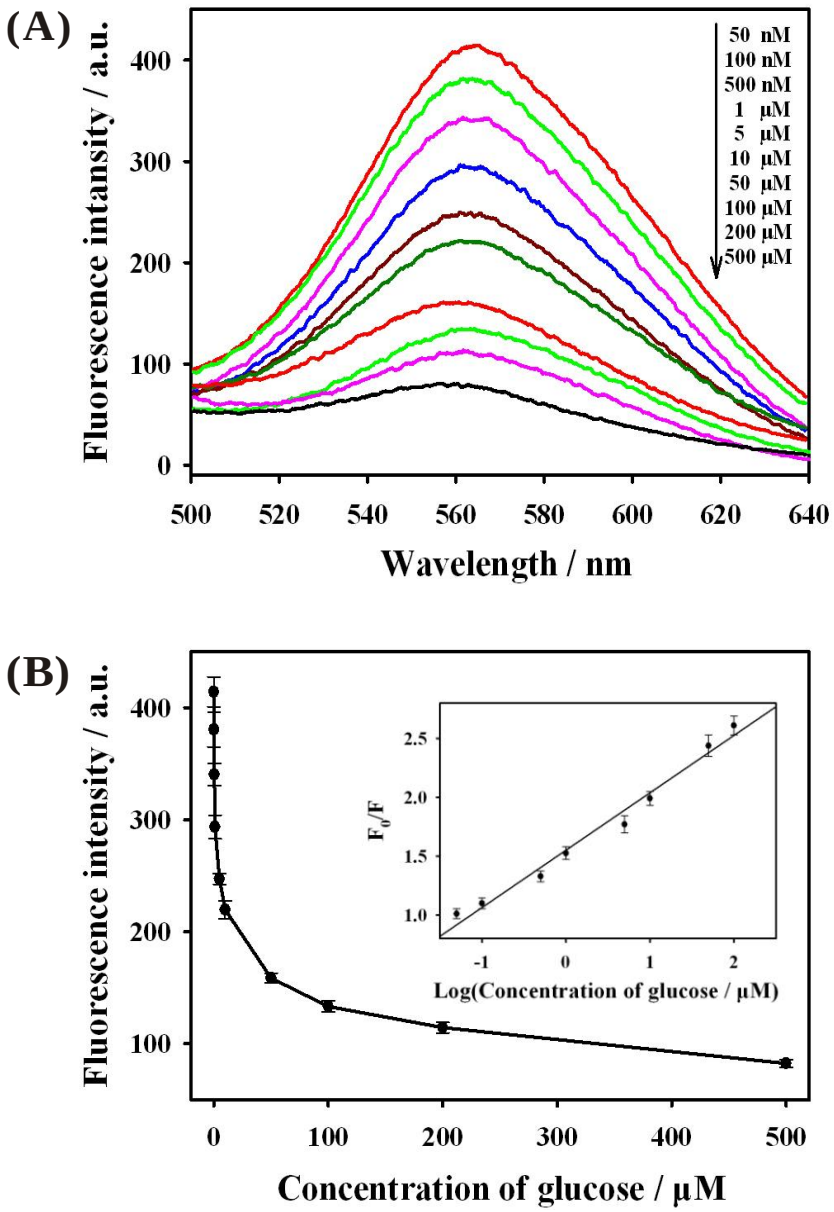


Fig. 6

View Article Online
DOI: 10.1039/C5RA14852A

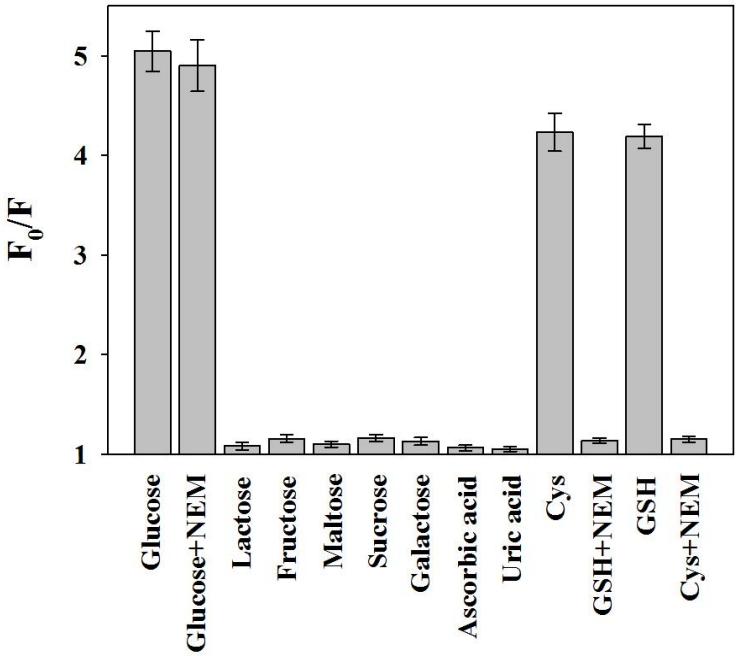


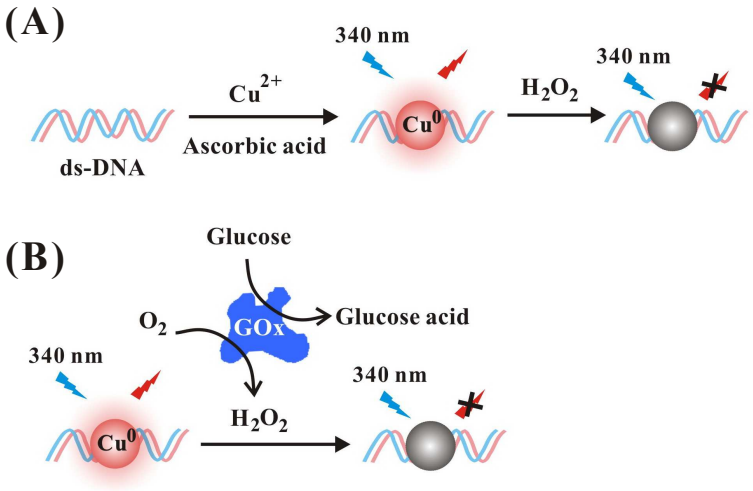
Fig. 7

View Article Online
DOI: 10.1039/C5RA14852A

Table 1

Samples	Proposed method (mM)	RSD (%)	Local hospital (mM)	Relative deviation (%)
1	4.51	2.8	4.66	-3.2
2	5.34	3.5	5.20	2.7
3	6.28	2.3	6.55	-4.1

View Article Online
DOI: 10.1039/C5RA14852A



Scheme 1

View Article Online
DOI: 10.1039/C5RA14852A

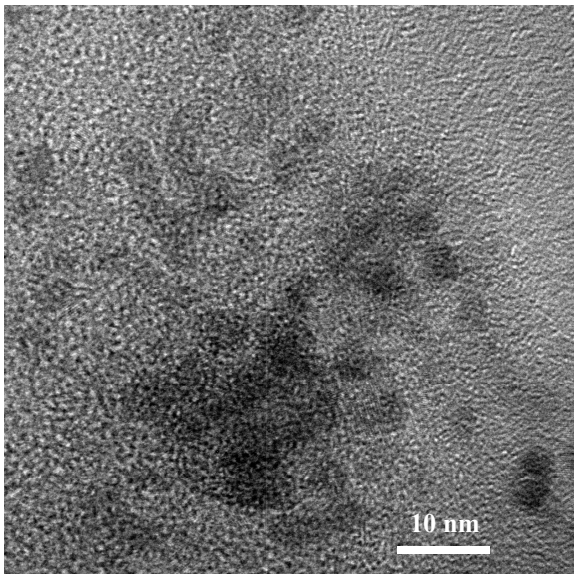


Fig. 1

View Article Online
DOI: 10.1039/C5RA14852A

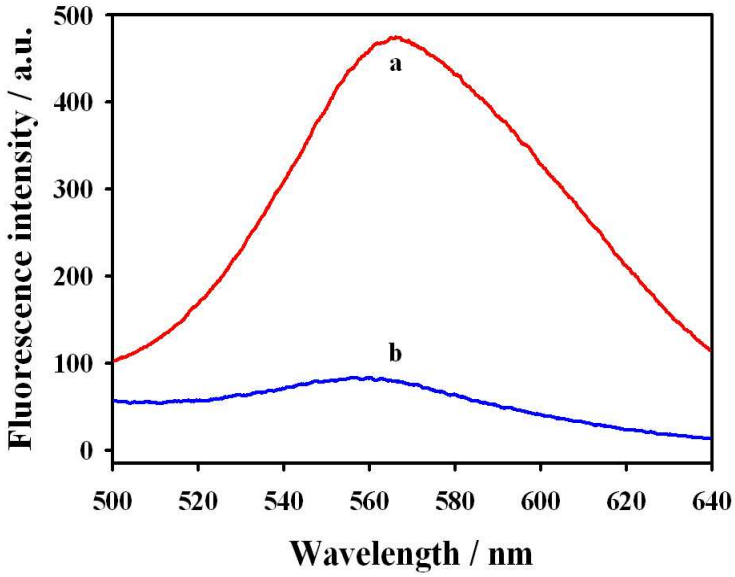


Fig. 2

View Article Online
DOI: 10.1039/C5RA14852A

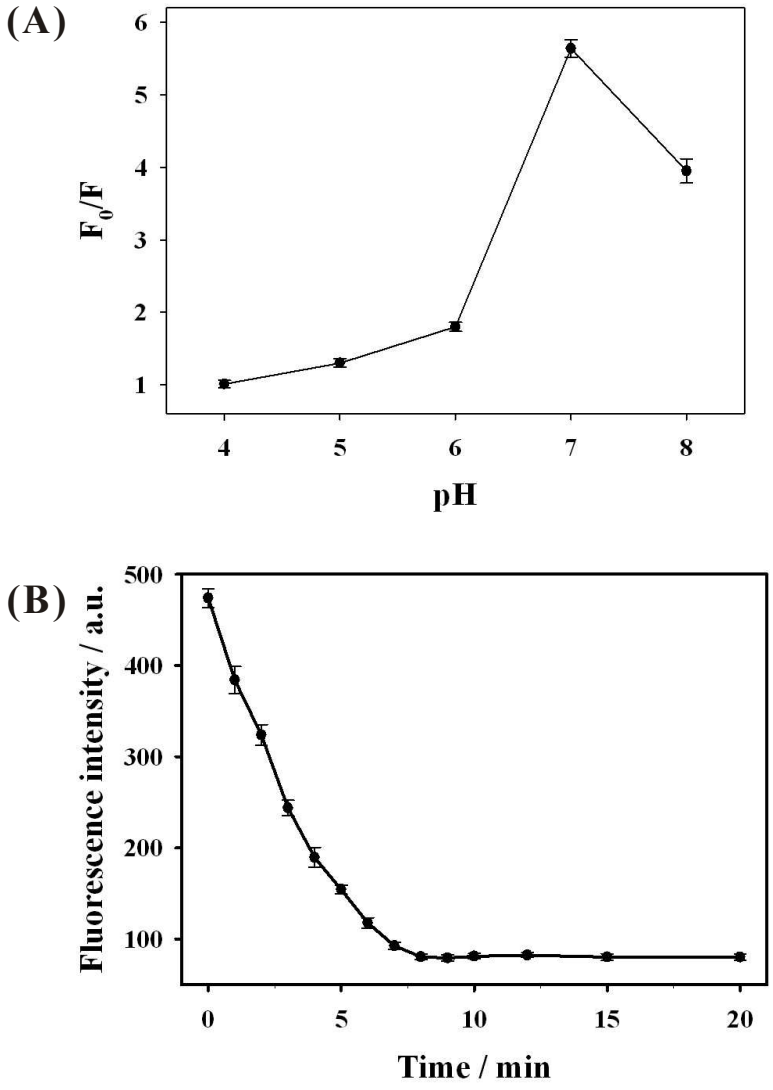


Fig. 3

View Article Online
DOI: 10.1039/C5RA14852A

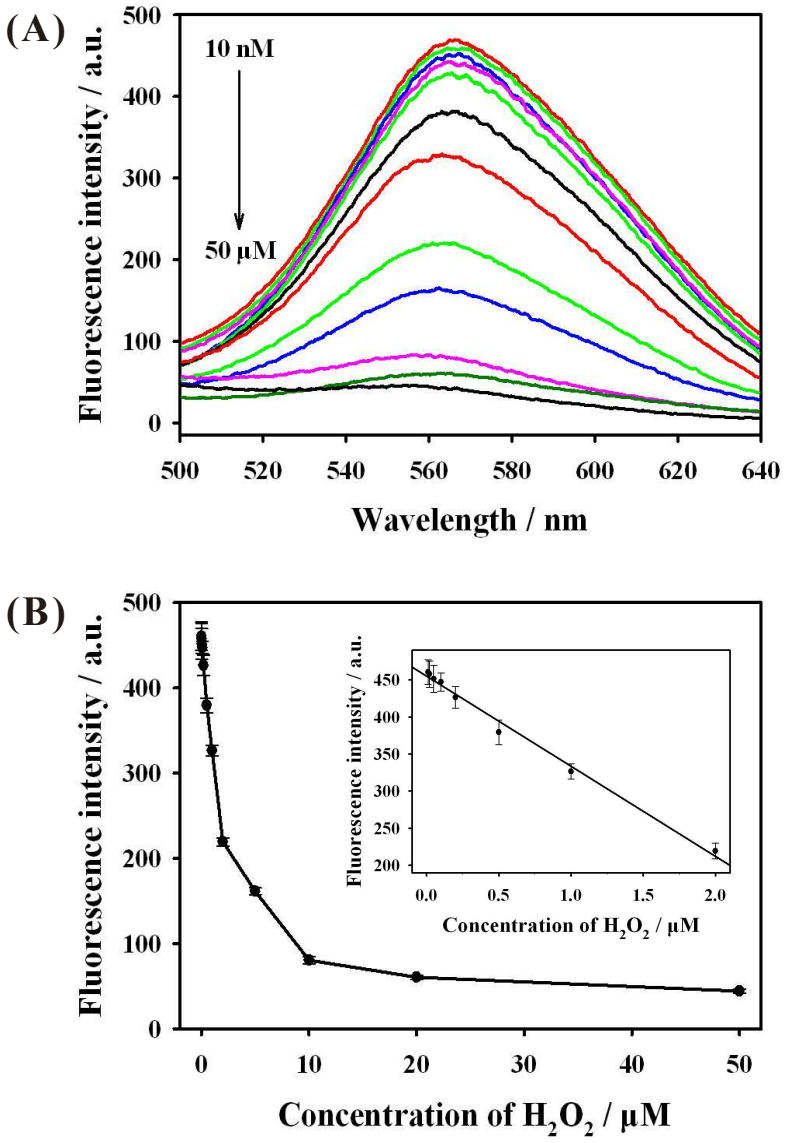


Fig. 4

View Article Online
DOI: 10.1039/C5RA14852A

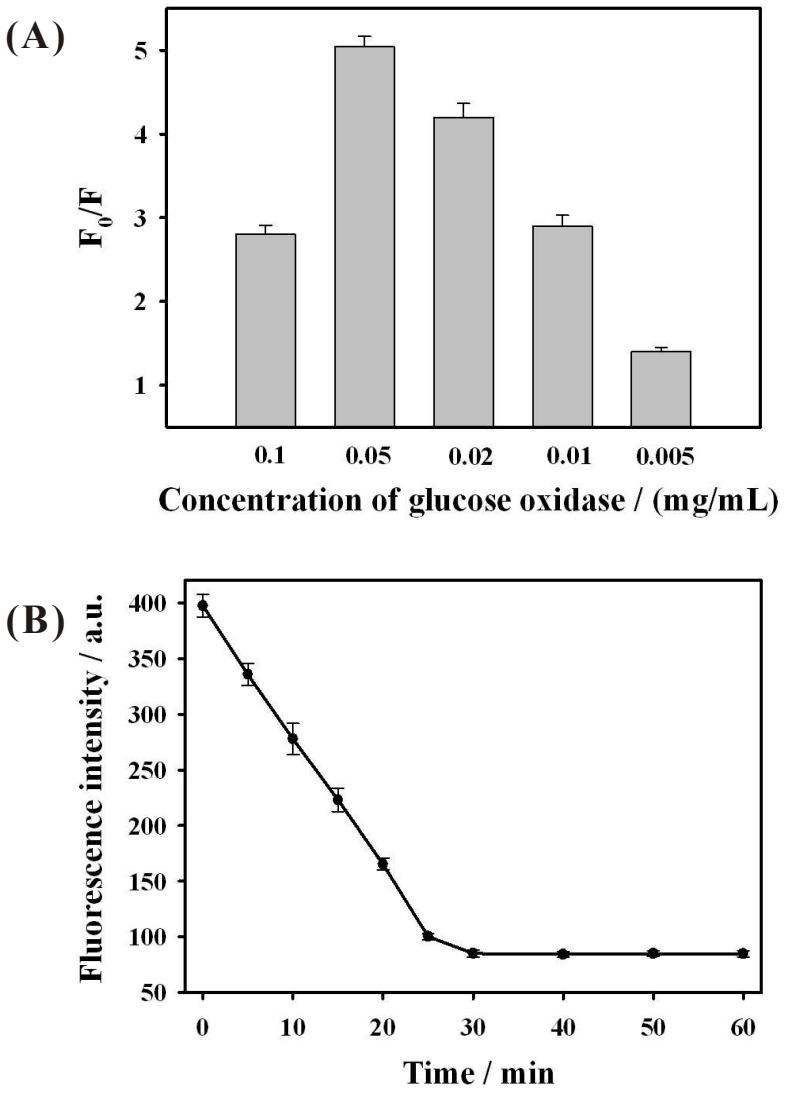


Fig. 5

View Article Online
DOI: 10.1039/C5RA14852A

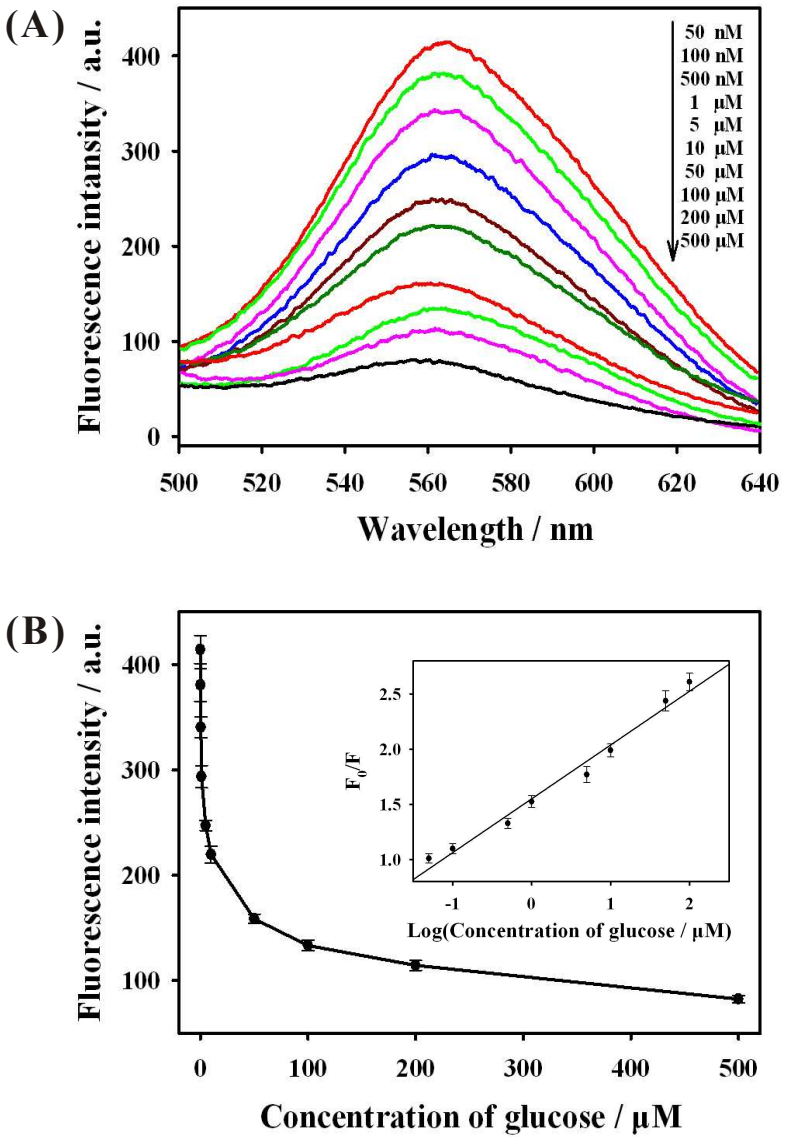


Fig. 6

View Article Online
DOI: 10.1039/C5RA14852A

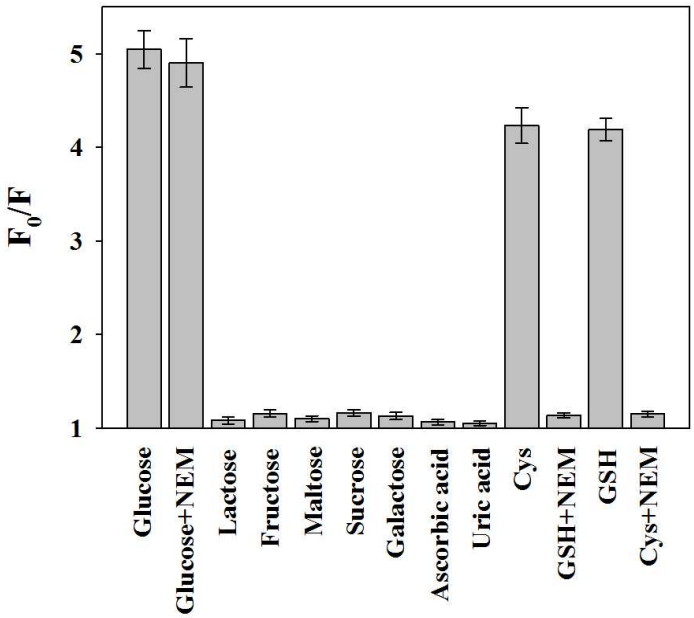


Fig. 7

View Article Online
DOI: 10.1039/C5RA14852A

Table 1

Samples	Proposed method (mM)	RSD (%)	Local hospital (mM)	Relative deviation (%)
1	4.51	2.8	4.66	-3.2
2	5.34	3.5	5.20	2.7
3	6.28	2.3	6.55	-4.1